2003 National Fusarium Head Blight Forum Proceedings

Holiday Inn Select
Bloomington, MN
December 13-15, 2003

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Sample Reference:

> Buerstmayr, H., Steiner, B., Hart, L., Griesser, M., Angerer, N., Lengauer, D. and Lemmens, M. 2002. "Molecular Mapping of QTLs for Fusarium Head Blight Resistance in Spring Wheat." In: Canty, S.M., Lewis, J., Siler, L. and Ward, R.W (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2002 Dec 7-9; Erlanger, KY. East Lansing: Michigan State University. pp. 22-25.

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Organized by:

U.S. Wheat & Barley
Scab Initiative

Proceedings compiled by: Susan M. Canty, Janet Lewis and Richard W. Ward

Michigan State University

Printed in the United States by Kinko's, Okemos, MI 48864.

Copies of this publication can be viewed at http://www.scabusa.org.

We would like to acknowledge
the following companies and organizations
for their sponsorship of the
2003 National Fusarium Head Blight Forum.

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IMPACT OF INCREASED DON LEVELS ON THE MILLING AND BAKING INDUSTRY

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ABSTRACT

Currently the Milling and Baking Industry operates under a Federal Advisory that "re-commends" that wheat flour produced for human consumption contain < 1.00 ppm DON (vomitoxin). There is currently no guidance level for the raw wheat from which the derivative flour is generated. Milling companies have found over the years that there is a "milling loss" of DON associated with the milling of wheat into flour. Typically this "loss" was on the order of 50%. Therefore, if your raw wheat contained 2.00 ppm of DON, the derivative flour of this wheat would typically test at 1.00 ppm of DON or less. With this historical data in hand, the Milling Industry would specify DON levels of 2.00 ppm or less in their grain contracts in order to meet the Federal Advisory Level of 1.00 ppm or less in flour intended for human consumption.

With this crop year, particularly in the Soft Red Winter Wheat of the Mid-Atlantic States, this "milling loss" appears to have dropped from 50% to 20% or less. Specifically, if the raw wheat tests at 2.00 ppm DON, the derivative flour will test at 1.80 ppm DON or higher. When DON reaches levels such as these in wheat flour products, not only is the Milling Industry unable to satisfy Advisory Levels issued by Federal Regulatory Agencies, it us unable to deliver flour to its customer base since they are unwilling to process flour into baked goods if the flour does not meet regulatory guidelines.

Investigation by various Milling Industry entities has revealed that there probably never was a "milling loss." What was actually happening is that the wheat cleaning equipment in the flour mills was taking out the diseased shriveled kernels. With this crop year, we are seeing kernels that are "infected" and are still plump and therefore not removed by the cleaning process. Hence the higher DON loads.

The purpose of this panel discussion is to give the audience to direct specific questions to the panel as to the impact of increased DON loads in the grains they purchase, and the strategies that are being pursued to deal with this issue.

SELECTED GENES FROM THE FUSARIUM HEAD BLIGHT RESISTANT CULTIVAR FUNDULEA 201R

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ABSTRACT

The development of wheat varieties with resistance to Fusarium head blight (FHB) is slowly occurring through breeding techniques. The varieties so far developed provide improved resistance to the spread of infection within the spike (Type II resistance). Immunity to FHB (Type I) has not yet been reported, however, the correlation between the known components of resistance and toxin decontamination is not well-defined. Chinese germplasm has served as the source of resistance genes in the majority of varietal development. However, it is necessary to diversify the sources of resistance to FHB in order to increase the success rate of developing wheat varieties with good resistance. F201R, developed at A.R.D.I-Fundulea as result of complex crosses, is a FHB-resistant Romanian winter wheat whose resistance is not related to the Chinese resistant germplasm. F201R carries the FHB resistance associated with QTLs (quantitative trait loci) located on chromosomes 1B, 3A, 3D and 5A, unlike the Sumai3 resistance QTL which is associated with chromosome 3BS. In an effort to isolate individual genes involved with FHB resistance, particularly the reaction to the presence of the toxin DON, we have used a suppressive hybridization cDNA subtraction method (Clontech) to obtain differentially expressed messages. Libraries were made from F201R inoculated with water (control) to obtain plant immune response genes; inoculated with *F. graminearum* to obtain fungal genes, as well as plant response genes; and inoculated with DON (deoxynivalenol) to obtain plant genes that are turned on in response to toxin. Results indicate that libraries containing genes from water-inoculated and DON-inoculated F201R contained many of the same genes and, therefore, selection of specific genes turned on in the presence of DON should be accessible. BLAST comparison of sequences has found a hypervariable sequence from rice, a UDP-galactose 4-epimerase-like protein, a carbamoyl phosphate synthase, unidentified rice BAC clones, and some sequences that are unique. Further comparison of sequences from the libraries with publicly available sequences should lead to the identification of plant genes involved with resistance to DON and/or fungal invasion.

IMPROVEMENT OF FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY THROUGH *IN VITRO* SELECTION

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ABSTRACT

Infection of barley (*Hordeum vulgare* L.) by *Fusarium graminearum* (Schwabe) is associated with accumulation of mycotoxins such as deoxynivalenol (DON) which play a significant role in Fusarium head blight (FHB) pathogenesis. A study was conducted to determine the effectiveness of using such mycotoxins in anther culture system for doubled haploid (DH) production to select mycotoxin tolerant barley plants with improved FHB resistance in the field. Twelve crosses varying in FHB resistance were subjected to *in vitro* selection (IVS) using a mixture of 2 or 3 mycotoxins. All fertile IVS and control DH lines from 7 crosses involving "exotic" FHB resistance sources were evaluated for FHB resistance in the Brandon nursery in 2001and 2002, while 5 standard breeding crosses were evaluated in 2002. DON content was determined by the ELISA technique at Ottawa. Of 7 exotic crosses, only the two-row sub-group of Chevron/CDC Fleet cross showed significantly lower DON content of IVS vs. control group in 2001. Among the 5 standard crosses, only IVS lines from Rivers//Rivers/SB93806 cross had significantly lower DON content than control lines. Several IVS lines from both populations had substantially lower DON content than their parents. In conclusion, *in vitro* selection was effective in improving FHB in only some crosses but further testing is needed.

TRANSCRIPTION PROFILING OF WHEAT GENES FOR RESISTANCE TO *F. GRAMINEARUM* USING CDNA MICROARRAYS

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ABSTRACT

Fusarium head blight (FHB), primarily caused by *Fusarium graminearum* Schw., is a destructive disease of wheat (*Triticum aestivum* L.). Although several genes related to FHB resistance have been reported, global analysis of gene expression in response to FHB infection remains to be explored. To characterize differences in gene expression between FHB resistant and susceptible wheat cultivars with a view toward gaining insight into the genetic mechanism of Type II resistance in wheat, cDNA microarrays with 2306 ESTs from wheat subtraction libraries were used to determine Fusarium induced and differentially expressed cDNA in wheat resistant cultivar Ning 7840 and susceptible cultivar Clark. The subtraction libraries were made from *F. graminearum*-infected spikes of bulked resistant and bulked susceptible Ning 7840 x Clark F_{12} recombinant inbred lines. The dynamic change of wheat spike transcriptomes was monitored at a series of time courses (0h, 3h, 6h, 12h, 24h, 36h, 48h, and 72h) after imposition of pathogen stress. Microarray analysis with cDNAs from *F. gaminearum*-inoculated Ning 7840 and Clark as target revealed 170 ESTs with at least two-fold difference in gene expression level. There were more differentially induced genes than repressed genes in the resistant genotype during the first 24h after inoculation with the pathogen, but more significantly down-regulated genes were observed from treatments of 36h and onward. DNA sequencing to putatively identify the ESTs and real time PCR to confirm the result from microarray analysis is underway.

TRANSPOSONS AND MERISTEMATIC CULTURES: TOOLS TO IMPROVE TRANSGENE STABILITY, AGRONOMIC PERFORMANCE, AND CONSUMER ACCEPTANCE

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ABSTRACT

The production of transgenic barley plants expressing antifungal proteins (AFPs) is a potential tool for the introduction of novel sources of resistance to Fusarium head blight (FHB). For biotechnological approaches to be applied to practical production problems—such as fighting FHB—it is imperative that researchers keep in mind that the ultimate residence of their technology will be commercially useful germplasm. In deference to such needs, we are utilizing technological improvements in the production of transgenic barley that improve transgene expression and transgenic plant performance, and which also may facilitate consumer acceptance of transgenic crops.

The maize *Ac-Ds* transposable element system has been introduced into barley and has been shown capable of delivering transgenes into single copy regions which support stable transgene expression. Essential elements of the system are: 1) expression cassettes in which the AFP gene is flanked by the *Ds* terminal inverted repeat (TIR) sequences, and 2) transgenic lines expressing the maize *Ac* transposase (*AcTPase)* which mediates transposition of the *Ds*-flanked AFP. Hybridization of *AcTPase*-expressing lines with *Ds*-AFP lines mediates transposition of the *Ds*-AFP cassette, in some cases to unlinked locations which enable the segregation of *Ds*-AFP from the original site of insertion, thus eliminating bacterial and selectable marker sequences present in the original transformant. Studies of *bar* (herbicide resistance) expression have shown that transposition stabilizes and elevates transgene expression.

Meristematic cultures have been developed which, relative to standard embryogenic cultures, are characterized by greater levels of differentiation, long-lived regeneration, and a reduced level of somaclonal variation in regenerated plants. We are targeting our transformation efforts to meristematic tissues of the cultivar Drummond, a 6-rowed malting cultivar recently released by North Dakota State University. Direct transformation of such germplasm will facilitate the production of commercially competitive cultivars for the midwest because backcrossing from unadapted germplasm is obviated. A more immediate benefit is that such transformants, relative to those produced from unadapted cultivars such as Golden Promise, will possess morphological features similar to the midwestern cultivars in which FHB resistance is most needed, and which have been shown to influence FHB infection and severity.

To date, *Ds*-flanked AFP expression cassettes have been constructed for two oat-derived thaumatin like proteins (*tlp1* and *tlp4*), and two trichothecene pathway genes from *F. sporotrichioides, TRI101* and *TRI12*, driven either by maize *ubiquitin* or rice *actin* promoters. Antibodies have been prepared against both *tlp* genes and from *TRI101*. Because working with meristematic cultures of Drummond is technically difficult, preliminary experiments were conducted in which these constructs were introduced into scutellar cells of Golden Promise. The experiments have resulted in plants from 3 transgenic lines containing *tlp1* and

tlp4 which have been confirmed to be transgenic via PCR. Additional lines putatively encoding these genes, and also *TRI101* and *TRI12*, are awaiting additional molecular characterizations.

Subsequent transformation efforts have resulted in the production of three transgenic Drummond lines containing *tlp1*, *tlp4*, or *TRI101*. Additional lines putatively encoding these genes are awaiting additional molecular characterizations. We have also produced Drummond-derived plants expressing *AcTPase*. To our knowledge, these are the first 6-rowed barley plants produced which have been confirmed to contain AFP genes introduced via transformation.

T_1 plants derived from both Drummond and Golden Promise transformants will be developed into homozygous lines and characterized for protein and/or mRNA production. Promising lines which produce measurable transgene products will be tested for FHB resistance by collaborating pathologists. In addition, they will be crossed to *AcTPase*-expressing plants to initiate transposition of the *Ds*-AFP cassettes. Ultimately, we intend to provide comprehensive characterizations of these materials with respect to AFP production, FHB resistance, the inheritance of these transgene-encoded traits, and the agronomic performance of lines derived from these materials.

MOLECULAR MAPPING AND MARKER ASSISTED SELECTION OF QTLS FOR FUSARIUM HEAD BLIGHT RESISTANCE IN CHINESE WHEAT LINE W14

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OBJECTIVES

Objectives of the current study were to identify QTLs in addition to 3BS in Chinese wheat line W14 and to evaluate contribution and feasibility of selecting these QTLs in a marker assisted selection (MAS) assay.

INTRODUCTION

Fusarium head blight (FHB) is one of the most destructive diseases of wheat causing significant reductions in grain yield and quality. Deployment of resistant varieties is an effective, economical and environmentally safe way to control FHB in wheat. Development of commercially viable FHB resistant varieties via traditional breeding methods has been hindered because resistance of currently used sources is quantitative in nature and incomplete. In addition, most resistance sources are not adapted, susceptible to other prevalent diseases and have poor combining ability. Significant environmental effects and laborious disease screening techniques also impede progress. Use of molecular markers offers an efficient alternative to phenotypic selection for FHB resistance. A QTL on 3BS has been identified as being a robust QTL for FHB resistance in mapping studies of several related sources including Sumai 3 (Anderson et al., 2001), Ning7840 (Zhou et al., 2002), and CM-82036 (Buerstmayr et al., 2002, 2003), but only recently has it been used for MAS in breeding programs. Minor QTLs on other chromosomes also have been postulated (Zhou et al., 2002; Otto et al., 2002; Buerstmayr et al., 2003; Shen et al., 2003; Steiner et al., 2003), yet their significance needs validation as results vary among labs, genetic background, and environments.

MATERIALS AND METHODS

Genetic populations - W14, an improved type II resistance source developed by recurrent selection (Jiang, 1997), was crossed with two FHB susceptible soft red winter (SRW) wheat cultivars Pioneer 2684 and Madison. Two doubled haploid populations (DH1: 96 lines, and DH2: 62 lines) were developed using wheat by maize hybridization method (Chen et al., 2001). Floret inoculation method was used to assay disease severity and type II resistance in greenhouse tests. Severity (percentage of infected florets) was assessed 7, 14 and 21 days after inoculation. Severity ratings made on 21st day were used in mapping analyses. For both DH populations, two independent greenhouse experiments were conducted, and entry means of DH lines from the two experiments were used in subsequent analyses. W14 and another related source Futai8944 were backcrossed four times to adapted FHB tolerant cultivars Roane and Ernie. The BC_4F_1 and BC_4F_2 generations were used to develop NILs via MAS.

SSR mapping – Linkage groups previously postulated as possessing QTLs for FHB resistance include 1B, 2B, 3B, 3A, 5A, 6A, 6B, 6D, and 7B. Therefore, 308 SSRs known to be located on these chromosomes

were selected and used to survey DNA polymorphism among parents W14, Pion2684 and Madison as well as four extreme bulks using BSA analysis (Michelmore et al., 1991). Sequence information of 100 SSR primers was available from Röder et al. (1998), and the remaining 208 were kindly provided by P. Cregan, USDA-ARS, Beltsville, MD. DNA extraction, PCR amplification and SSR assays were conducted as previously described (Saghai Maroof et al., 1994; Bryan et al., 1997; Röder et al., 1998). Simple and multiple regression analysis were conducted using Agrobase (Agronomic Software, INC. 1999). Linkage maps were constructed using MAPMAKER 3.0b for MS-DOS (Lander et al., 1987). Markers were grouped using a threshold LOD > 3.0.

RESULTS AND DISCUSSION

QTLs detected in W14 mapping populations (data and maps will be presented at the meeting) – While 71% (218 out of 308 SSRs) of the primers were polymorphic among parents W14, Pioneer 2684 and Madison, only 18 SSRs showed putative association with FHB resistance in one or both of the DH populations based on BSA analysis. Twenty-six pairs of primers, including the 18 primers above were mapped in DH1 and DH2 populations. Five QTLs were detected on 1BL, 2BS, 3BS, 5AL, and 7AL chromosome regions. Eighteen markers in these regions were significantly ($P < 0.05$) associated with FHB resistance. These markers explained 42% and 62% of the total phenotypic variation in DH1 and DH2, respectively. According to the peaks of the LOD profiles, the QTL on 3BS had a much larger effect than the 5AL and 2BS QTLs in both DH populations, and therefore confirmed as being a major QTL explaining 29% and 42% of phenotypic variation in DH1 and DH2, respectively. QTL position of 3BS is in agreement with Anderson et al. (2001), Zhou et al. (2002), and Buerstmayr et al. (2002 & 2003). QTL position of 5AL is similar to Buerstmayr et al. (2003). QTL position of 2BS is in disagreement with Zhou et al. (2002). Two minor QTL regions (1B and 7A) were detected in DH2 and are being saturated with more markers.

Application of MAS for FHB QTLs in Backcrosses - Eighteen SSRs were used for MAS in BC_4F_1 and BC_4F_2 generations. A total of 84 recombinants were selected in four BC_4F_2 populations having allele or allele combinations of the two resistant parents (RP) at four SSR loci (BARC75-Xgwm533a-BARC133-Xgwm533b) in 3BS QTL region, two SSR loci (BARC18 and BARC91) in 2BS QTL region, and two SSR loci (BARC100 and Xgwm156) in 5AL QTL region (Table 1). These recombinants were also identified as having alleles of the two recurrent parents (RCP) for the other ten SSRs (data not presented in Table 1). The 3BS QTL was found to be more frequently transferred into recurrent backgrounds than the 2BS and 5AL QTLs using phenotypic selection for type II resistance; however, recurrent parent background did affect efficiency in selection of this QTL. The 3BS QTL spans a relatively large interval of five SSR markers (BARC75-Xgwm533a-BARC133-Xgwm493-Xgwm533b) in our map. The intervals in this QTL region have been divided into smaller segments in a Roane backcross population in which 23 homozygous NILs having different allelic combinations in this region have been derived. The precise location and composition of the 3BS QTL will be further delineated via evaluation of these NILs for resistance to FHB compared to their marker composition.

ACKNOWLEDGMENTS

Funding was provided by U.S. Wheat and Barley Scab Initiative, Virginia Agricultural Council and Virginia Small Grain Board.

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Table 1. Haplotypes and mean disease severity (%) of 84 selections among 213 plants in four BC_4F_2 populations (Total plants in the four BC_4F_2s were 91, 58, 32, 32, respectively. The number in parenthesis represents the total number of plants having same allele type).

		BC_4F_2 populations (No. of plants)			
Haplotypes*	Allele type**	W14 x Roane	Futai8944 x Roane	W14 x Ernie	Futai8944 x Ernie
1	3, 3, 3, n, 1, 1	21.7 (2)	19.3 (4)		
2	2, 2, 2, n, 1, 1	18.4 (3)	18.2 (10)		
3	1, 1, 1, n, 1, 1	19.7 (2)	16.3 (3)		
4	2, 3, 3, n, 1, 1		36.1 (1)		
5	1, 2, 2, n, 1, 1		20.6 (1)		
6	2, 2, 1, n, 1, 1		10.5 (1)		
7	3, 3, 1, n, 1, 1	50.0 (1)			
8	1, 1, 2, n, 1, 1	47.4 (1)			
9	1, 1, 3, n, 1, 1	17.5 (1)			
10	1, 3, 3, n, 1, 1	32.4 (1)			
11	1, 3, 3 ,3, 1, 1	10.5 (1)			
12	1, 2, 2, 3, 1, 1	33.3 (1)			
13	1, 1, 1, 3, 3, 1	17.2 (5)			
14	1, 1, 1, 3, 2, 1	18.8 (2)			
15	1, 1, 1, 3, 1, 1	18.1 (6)			
16	1, 1, 1, n, 3, 1	47.6 (7)			
17	1, 1, 1, n, 2, 1	21.1 (1)			
18	3, 3, 3, n, n, 1				15.3 (8)
19	3, n, 3, n, n, 1			14.9 (12)	17.4 (9)
20	3, n, 3, n, n, 2				32.1 (1)
21	3, n, 3, n, n, 3				33.3 (1)

*Haplotypes were characterized based on allele and allele combinations of two parental lines for six SSR marker loci ordered by BARC75, Xgwm533a, BARC133, Xgwm533b, BARC18, and BARC100. ** Allele types are scored as 3 = W14 and/or Futai8944 type, 1 = Roane and/or Ernie type, n = null allele type of Roane and/or Ernie, 2 = heterozygous type of two parental alleles. Haplotype of W14 and Futai8944 = 3,3,3,3,3,3, Roane = 1,1,1,n,1,1, Ernie = 3,n,3,n,n,1. Disease severity of the four parental lines were: 12.0, 13.5, 31.6 and 33.4%, respectively.

SATURATION MAPPING OF A MAJOR FUSARIUM HEAD BLIGHT RESISTANCE QTL REGION IN TETRAPLOID WHEAT

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ABSTRACT

Previous screening for Fusarium head blight (FHB) resistance identified a *Triticum dicoccoides* accession carrying FHB resistance genes. Using *T. durum* cv. 'Langdon'-*T. dicoccoides* chromosome 3A recombinant inbred chromosome lines (RICLs), a major quantitative trait locus (QTL) *Qfhs.ndsu-3AS*, that explains 55% of the genetic variation for FHB resistance, and a microsatellite locus, *Xgwm2*, tightly linked to the highest point of the QTL peak have been identified (Otto et al. 2002). This QTL region spanned a 29.3cM interval on chromosome 3A. The objective of this study is to saturate the QTL *Qfhs.ndsu-3AS* region and identify recombinants for a smaller donor chromosomal segment carrying this QTL.

Screening of the *T. monococcum* bacterial artificial chromosome (BAC) library with the DNA-based probe *NDSU.fhb.3A* derived from the microsatellite marker *Xgwm2* has identified 15 BAC clones. We are isolating low or single copy sequences from these BACs to generate more markers for saturating the QTL region. A novel marker technique Target Region Amplification Polymorphism (TRAP) has also been used to generate markers in this study (Hu et al. 2003). We designed 50 fixed primers based on the ESTs mapped on the short arm of chromosome 3A (3AS) and the conserved domain leucine rich repeat (LRR) of disease resistance genes. Nine polymorphic markers were generated with these fixed primers in combination with random primers. All these 9 markers were mapped on chromosome 3A. In addition, we have designed 9 pairs of microsatellite primers based on the 3' and 5' sequences of the ESTs mapped on 3AS and have been trying to generate more markers within the QTL region. Two microsatellite markers, *Xgwm493* and *Xgwm389*, mapped in a major FHB resistance QTL *Qfhs.ndsu-3BS* region, showed no polymorphism between the two parents of the RICLs. Fourteen out of 28 STS primer pairs developed from the ESTs mapped on chromosome 3B by Dr. J. A. Anderson (Liu et al. 2003) showed polymorphism between the two parents of the RICLs. Six STS markers generated through this approach have been mapped on chromosome 3A. Based on microcolinearity between wheat homoeologous group 3 chromosomes and rice chromosome 1, we have been identifying the rice genomic sequences in the collinear region and using these sequences to screen the ESTs that have not yet been mapped in the wheat genomes. Positive ESTs from this screening will be used to develop STS markers for saturating the QTL region. Concurrently, a large F_2 population has been developed by crossing Langdon with a RICL carrying a smallest *T. dicoccoides* chromosomal fragment spanning the *Qfhs.ndsu-3AS*. This population is being employed to identify more recombinants within the QTL region for fine mapping.

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TRANSFORMATION OF BARLEY WITH TWO ANTIFUNGAL GENES

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ABSTRACT

Insertion and expression of multiple antifungal genes has the potential of increasing resistance to a variety of fungal diseases. Using particle bombardment and bialaphos selection for the *bar* gene, multiple barley plants containing both a rice thaumatin-like protein (*tlp*) gene and a rice chitinase (chi) gene were regenerated from three transformation events. Southern analysis confirmed integration of the transgenes into the barley genome. Northern analysis of T_0 plants indicated that event 1 did not contain RNA from either gene. Plants from event 2 expressed both genes at high levels, while plants from event 3 showed small amounts of *tlp* RNA but no chi RNA. Western analysis of T_1 progeny confirmed that event 2 lines expressed both genes and event 3 lines expressed only *tlp*. Homozygous T_2 lines have been identified and are being examined for gene expression levels for both transgenes.

EXPRESSION OF THE YEAST L3 AND THE POKEWEED ANTIVIRAL PROTEIN GENES CONFERS RESISTANCE TO TRICHOTHECENE MYCOTOXINS

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ABSTRACT

Trichothecenes are a highly diverse class of toxic, sesquiterpenoid secondary metabolites that are produced mainly by plant pathogenic fungi. The contamination of important agricultural products, such as wheat, barley or maize with the trichothecene mycotoxin, deoxynivalenol (DON) due to infection with *Fusarium graminearum* and *F. culmorum* is a worldwide problem. Trichothecene mycotoxins interact with the peptidyltransferase site of eukaryotic ribosomes and inhibit eukaryotic protein synthesis. Ribosomal protein L3 (*RPL3*) participates in the formation of the peptidyltransferase center. Mutations in the *RPL3* gene (called *TCM1*) were initially identified by conferring resistance to trichodermin, a trichothecene mycotoxin that inhibits the peptidyltransferase reaction.

To determine if expression of the yeast *RPL3* gene will confer resistance to trichothecene mycotoxins, we generated transgenic tobacco plants expressing either the wild type or mutant forms of the yeast *RPL3* alone or together with pokeweed antiviral protein (PAP), a ribosome inactivating protein that inhibits viral and fungal infection. Transgenic plants containing the wild type yeast *RPL3* and PAP or a mutant form of the yeast *RPL3* and PAP were phenotypically normal. Similarly, transgenic lines expressing the yeast *RPL3* genes alone were indistinguishable from wild type plants. To determine if transgenic tobacco plants expressing the yeast *RPL3* genes are resistant to trichothecenes, seeds from transgenic and wild type plants were germinated on MS medium, containing 1 μM DAS or 10 μM of DON and their root length was measured at the end of six weeks. Plants from all transgenic lines showed resistance to DAS and DON compared to the wild type plants. However, the highest level of resistance was observed with transgenic plants expressing the yeast *RPL3* genes together with PAP. To confirm that yeast *RPL3* genes are expressed in these plants, we carried out real time PCR analysis using primers specific for the yeast *RPL3* genes, which do not hybridize to the tobacco L3 genes. The results confirmed the expression of the yeast *RPL3* genes in the transgenic lines. These results demonstrate that we can obtain phenotypically normal transgenic plants that show high levels of resistance to DON by coexpressing the wild type or mutant forms of the yeast *RPL3* together with PAP in transgenic tobacco plants.

WANGSHUIBAI: A HEXAPLOID WHEAT RESISTANT TO THE SPREAD OF FUSARIUM HEAD BLIGHT

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INTRODUCTION

Fusarium head blight (FHB) or scab has become one of the most serious diseases of wheat in North America, and around the world. In North Dakota alone, it is estimated that the impact of FHB on the state economy exceeded 6 billion US$ in the last ten years.

Scab is caused by different species of *Fusarium*. In North America, the predominant species is *Fusarium graminearum*. Infection takes place during anthesis; ideal conditions are warm temperatures (25-30°C) and high humidity. The disease reduces yield through floret sterility and poor seed filing. Quality losses are also important due to reductions in storage proteins, cellulose, and amylose (Boyacioglu and Hettiachchi, 1995). Additional economical impact is due to the accumulation of a vomitoxin (deoxynivalenol, or DON) in the seed, which makes it unsuitable for human and animal consumption.

Several types of resistance have been described. Three of these types are commonly accepted as Type I, resistance to the initial infection, Type II resistance to the spread of the pathogen through the spike, and Type III, resistance to the accumulation of DON. Type II resistance is most frequently measured and used in breeding programs.

The most predominant source of type II resistance is the Chinese cultivar 'Sumai 3'. Several QTLs for type II resistance have been identified on the genome of this cultivar. A major QTL has been identified on 3BS (Waldron et al., 1999, Anderson et al., 2001, and Del Blanco et al., 2001). Additional QTLs have been identified on chromosome 5A (Buerstmayer, et al., 2002), 6B and 6A (Anderson et al., 2001) and 7D (Sneller et al., 2001).

Other sources of type II resistance to FHB have been studied and several QTLs have been identified on chromosome 3A of *Triticum dicoccoides* (Otto et al., 2001), chromosome 2D of 'Wugham', 3BS of 'Maninga' (Somers et al., 2003), and chromosomes 1B, 3A, 3D and 5A of 'Fundulea 201R' (Shen et al., 2003).

Other potential sources of type resistance to FHB are Wangshuibai (the subject of this paper) from China, cltr9445 from China, PI157593 from S. Korea, PI362463 from Yugoslavia, and cltr9429 from China.

Previous genetic diversity studies suggested that Wangshuibai (a landrace from the Chinese provice of Jiangsu) may have different resistance genes than that in Sumai 3. Bai et al. (2003) showed that Wangshuibai is not closely related to Sumai 3. In a more recent study looking at the genetic diversity of the short arm of chromosome 3B, Liu and Anderson (2003) found that Wangshuibai has no alleles in common with Sumai for any of the molecular markers around the QTL found on chromosome 3BS of Sumai 3. Given

these data and the high level of resistance it is logical to contemplate the possibility of Wangshuibai carrying different resistance genes than Sumai 3.

In this study our objectives were to 1) identify the chromosomal location of genes responsible for the resistance to FHB of Wangshuibai, 2) estimate the effect of these genes, both in single locus and epistatic models, and 3) identify PCR markers closely linked to these genes, so they can be used in a marker assisted selection (M.A.S.) breeding scheme to develop resistant cultivars.

MATERIALS AND METHODS

An F_6 derived population consisting of 388 recombinant inbreed lines (RIL) was developed by SSD from the cross between Wangshuibai and ND671 (an elite HRSW line from the NDSU-HRSW breeding program). A random subset of 88 lines was used in this study.

Phenotyping of these lines was done in 3 greenhouse replicated trials during the years 1999, 2000, and 2001. Lines were grown in 36X21 cm buckets with two rows of five plants/bucket. At the time of anthesis, heads were single point inoculated in a floret in the middle of the spike with 10 ¼l of a suspension with 50000 spores/ml. After inoculation, the spikes were covered with plastic bags and misted for 3 days to ensure high humidity conditions. Temperature in the greenhouse was kept between 25 and 30°C. Disease scores were taken 14 and 21 days after inoculations. The NDSU variety 'Alsen' (derived from Sumai 3) was used as a resistant check.

Genotyping of these lines was done using 2 sets of SSR PCR primers, GWMs (Röder et al., 1998), and BARCs (http://www.scabusa.org/pdfs/BARC_SSRs_011101 .html). Amplification was done in accordance to those described by the developers of both sets. Amplified products were separated using either 6% acrylamide non denaturing gel, or 6% acrylamide denaturing gel visualized with silver staining. Several STS markers developped by Liu and Anderson (2003) were also included in the linkage map. In addition, several Target Region Amplified Polymorphisms (TRAPs) were developed from wheat EST sequences. In total, the linkage map contains 185 loci across the genome.

The linkage map was constructed using MAPMAKER v 2.0 (Lander et al.. 1987). The genome was scanned for QTLs using NQTL (Tinker and Mather 1995). Important regions were analyzed for epistatic interactions. Analysis of variance to estimate the effect of epistatic interactions was done with SAS system for Windows v. 8.01 (SAS Institute).

RESULTS AND DISCUSSION

The population segregated for the spread of FHB both at14 and 21 days after infection (DAI). The range of the scores 14 DAI were between 0% and 15%. Wanguibai showed an average infection of 3.8%, and the average infection level of ND671 was 7%. Alsen had the same level as Wangshuibai. The range of score at 21 DAI (Fig 1) was between 2.5% and 50%. The average infection in Wangshuibai was 3.8%, no change from the scores at 14 DAI, for ND671, the average was 22 %, a three fold increase from 14 DAI. Alsen had an average infection of 10%, almost a 3-fold increase from 14 DAI.

After scanning the genome for significant QTL peaks, we found a major peak on chromosome 3B, directly over *Xsts138* (Fig 2). This peak explains 17% of the total phenotypic variance for 14 DAI, and 31% 21

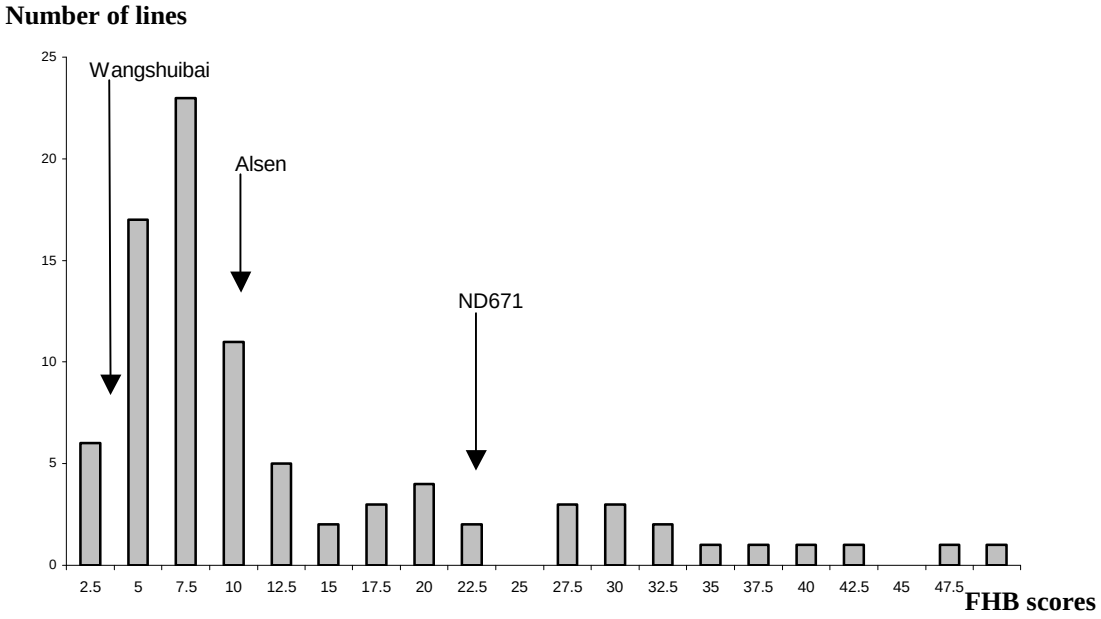

Fig.1: Frequencies histogram for FHB 21 DAI.

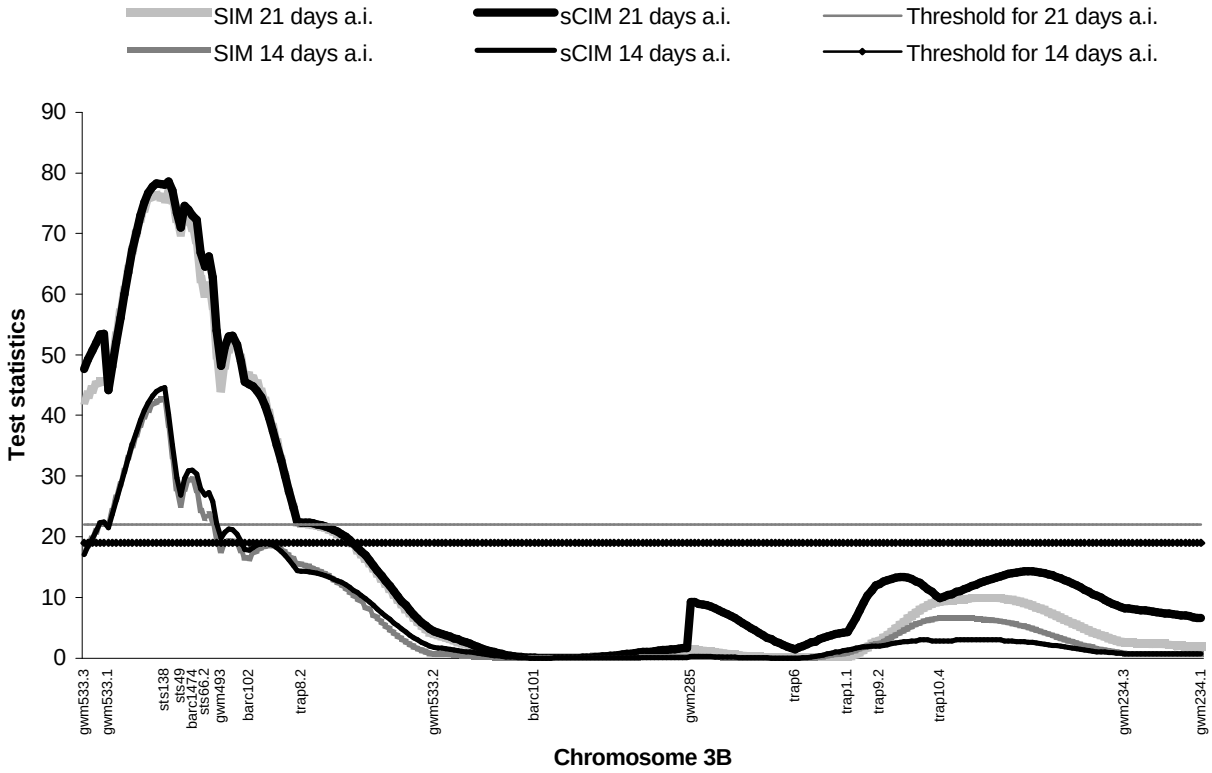

Fig.2: Simple interval mapping (SIM) and simplified composite interval Mapping (sCIM) graphs on chromosome 3B for readings 14 and 21 DAI.

In the case of 14 DAI interactions were identified between *Xsts138(3B)* and *Xgwm304(5A)*, *Xbarc117(5A)* and *Xgwm192(4B)*. The estimates of the effects of the interactions ranged between 40 to 53%, compared to a 17% of single locus model with *Xsts138*. In the case of 21 DAI interactions were identified between *Xsts138(3B)* and *Xgwm2(3A)*, *Xgwm333(7B)*, *gwm165(4B)*, *Xbarc101(3B)*, *Xbarc1033(6B)*, and *Xtrap9.2(3B)*. The estimates of the effect of these interactions ranged between 47 to 67%. Given the population size, only one-way interactions were considered, however the data suggests the possibility of 3-way interactions. This will be tested with the remaining 300 individuals.

Table 1: Loci found to have epistatic interactions with locus *Xsts138*.

Locus	PhenotypicVariance explained (%)	Time of observation (DAI)
Xgwm2 (3A)	67	21
Xgwm333 (7B)	47	21
Xgwm156 (4B)	55	21
Xbarc101 (3B)	51	21
Xtrap9.2 (3B)	59	21
Xbarc1033 (6B)	55	21
Xgwm304 (5A)	53	14
Xbarc117 (5A)	42	14
Xgwm192 (4B)	40	14

The results of this study clearly indicate the significance of the 3BS QTL. Additionally, epistatic interactions seem more critical than previously believed in determining strong resistance to FHB. An important region to note in this regard is that on chromosome 3A, which together with that on 3BS could explain up to 67% of the phenotypic variance. The results of this study further confirm field data on the degree of the 3BS QTL effectiveness.

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PLANT AND FUNGAL GENOMICS OF FHB/GIBBERELLA EAR ROT

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ABSTRACT

In order to further develop robust disease resistance to *F. graminearum* (*Fg*) in wheat and maize, we are using genomic and proteomic approaches to investigate the host/pathogen interaction. High and low density maize arrays (6.6K unigene or targeted arrays) are being used to establish how susceptible and resistant maize inbreds respond to attack by *Fg*, by comparing gene expression between fungal-inoculated or mock-inoculated kernel and silk tissues. Many genes, including PR proteins, genes from the terpene biosynthesis pathways, and genes with unknown function, are being induced or up regulated by *Fg*. Proteomic analyses by 2-D gel separation are being carried out in parallel on the same tissue samples. Comparison of the kernel tissues as identified a novel 35 kDa protein which was unique to infected tissues from the susceptible hybrid. Analyses with the silk tissues are in progress. Our collection of *Fg*-challenged wheat ESTs has been combined with a unigene set of about 1500 rye ESTs (enriched in genes induced under cold stress) to produce a "5K stress" small grain chip and array hybridizations are underway to compare gene expression in susceptible and resistant wheat cultivars during *Fg* infection.

We have released the majority of our ~7400 *Fg* ESTs to assist the annotation of the *Fg* genome sequence (Gb#AACM00000000). Electronic northerns have been conducted using in-house and public *Fg* EST databases to identify *Fg* pathogenicity gene candidates. We are collaborating with the USDA to determine *Fg* gene function through directed gene disruption. Disruption of the gene represented by contig Fg1A2287 has demonstrated that this sequence encodes a cytochrome P450 responsible for oxygenation at carbons 8 and possibly 7 in the trichothecene mycotoxin biosynthetic pathway.

IDENTIFICATION OF GENES UPREGULATED IN BARLEY IN RESPONSE TO INOCULATION WITH *FUSARIUM GRAMINEARUM*

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ABSTRACT

We used the barley Affymetrix GeneChip to examine transcript profiles in barley in response to inoculation with *Fusarium graminearum* (*Fg*). The barley GeneChip contains over 22,800 unique barley transcripts. Three replicate experiments were performed to compare transcript accumulation between *Fg*-inoculated and mock-inoculated spikes of the susceptible cultivar Morex at 24, 48, 72, 96 and 144 hours after inoculation. Barley plants were grown in six-inch pots in a growth chamber with 16h light, and 8h dark at 20°C and 18°C, respectively. Plants were inoculated by spraying conidiospores (100,000 spores/ml) onto spikes 2-3 days after they emerged from the boot. Total RNA for each time point/treatment was extracted from 8 spikes and checked for quality on an Agilent Bioanalyzer before labeling and hybridization to the Gene Chip. Comparisons between transcript levels in *Fg*-inoculated and mock-inoculated plants from each time point were performed using Gene Data Expressionist software. Transcripts showing differential accumulation were not detected at 24 h after inoculation, but were initially detected at 48 h, and also at 72, 96 and 144 h. The detection of transcripts with differential accumulation at 48 h coincides with the timing of a change in *Fg* pathogenicity from hemi-biotrophism to necrotrophism, which was observed by others. The induction of some classes of genes has been confirmed by RNA blot analysis. A description of these genes, their expression profiles and their possible role in defense will be presented.

EXPRESSION OF ANTI-APOPTOTIC GENES IN SPRING WHEAT CONFER RESISTANCE TO NECROTROPHIC PATHOGENS (*FUSARIUM GRAMINEARUM*) BY INHIBITING HOST-CELL DEATH

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ABSTRACT

Fusarium graminearum, a necrotrophic fungal pathogen of cereals, has caused severe damage to crops during the last decade. In particular, Fusarium head blight epidemics have caused an estimated $3,000 million in damages throughout the North-Central United States during the 1990s (Windels, 2000). As *Fusarium graminearum* resistant germplasm is limited, traditional breeding practices used to confer resistance have resulted in limited progress. Recombinant DNA technology for *Agrobacterium*-mediated wheat transformation has become sufficiently developed to be a practical means for introgression of genes conferring beneficial agronomic traits. We have previously demonstrated the efficacy of using a trans-kingdom approach for conferring resistance to necrotrophic pathogens, by generating tobacco plants harboring animal anti-apoptotic genes and showing that these transgenic plants are resistant to necrotrophic pathogens (Dickman et al. PNAS 2001) as well as abiotic stresses (unpublished). Apoptosis is a genetically regulated process that results in the decomposition of non-essential and non-functional cells and tissues during the growth and development of organisms. Importantly, during these fungal diseases of tobacco, markers associated with mammalian apoptosis were observed, but absent from transgenic resistant plants. We therefore explored the use of these cell survival genes in wheat. We have previously reported greenhouse and field trial evaluations of advanced-homozygous events expressing significant increases in resistance. Wheat plants expressing heritable resistance to necrotrophic pathogens through animal anti-apoptotic genes; Bcl-xL (chicken), CED-9 (nematode) and Op-IAP (baculorvirus) have demonstrated resistance/reduced PCD in various independent events (>10/transgene). We then evaluated whether scab disease exhibited characteristics associated with mammalian-programmed cell death. Following head inoculation, wild type (Bob White) wheat showed DNA fragmentation, DNA laddering and TUNEL positive staining cells, indicative of an apoptotic-like response. Scab tolerant transgenic wheat expressing anti-apoptotic genes, when inoculated resulted in minimal DNA fragmentation and nuclear staining, suggesting that the apoptotic-like response was inhibited. As reduced levels of apoptosis during head inoculation were detected, effects of the anti-apoptotic genes in transgenic wheat were subsequently studied under other conditions. Due to reports showing increase resistance to abiotic stresses in tobacco expressing anti-apoptotic genes, we also tested for increased resistance in the transgenic wheat. When placed under conditions of high salinity, Op-IAP transformed wheat did not show characteristic DNA laddering while all transgene-containing events demonstrated a reduced level of TUNEL positive nuclei. These results demonstrate that expression of anti-apoptotic genes in spring wheat results in broad-spectrum resistance to abiotic and biotic stresses.

GENETIC STUDIES OF SCAB RESISTANCE IN THE SOFT RED WINTER WHEAT 'ERNIE'

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OBJECTIVES

This research was designed to: (1) identify QTL associated with type II resistance in Ernie and to determine if these QTL differed from those in Sumai 3; (2) map the QTL for other agronomic traits and to study the associations between scab resistance and these traits; and (3) estimate the gene number and genetic effects conditioning scab resistance in Ernie.

INTRODUCTION

Fusarium head blight (FHB), also called scab, caused by *Fusarium graminearum* Schwabe [telomorph: *Gibberella zeae* Schw. (Petch)], is a disease that affects wheat (*Triticum aestivum, and T. durum*) and barley (*Hordeum vulgare* L.) in warm, humid areas of the world. Genetic studies on host plant resistance using both molecular and traditional methods have been emphasized (Kolb et al., 2001). Research to date has mainly focused on resistance in the Chinese cultivar Sumai 3 and its derivatives, and the Brazilian cultivar Frontana (Zhou et al., 2002), which have been widely used in the breeding programs globally. More recently, scab resistance in cultivars from Europe, such as Fundulea 201R, have also been studied (Shen et al., 2003).

Incidental sources of scab resistance have been identified from routine screening in U. S. breeding programs. The soft red winter wheat Ernie, released in 1995 by the University of Missouri (McKendry et al., 1995) has a moderately high level of scab resistance and is now used in U. S. breeding programs as a complementary source of resistance to the Sumai 3 source; however, the genetics of its scab resistance have not been studied. This information should enable breeders to more efficiently exploit this source of resistance.

MATERIALS AND METHODS

A set of 244 F_8 recombinant inbred lines (RILs) was developed for QTL analysis from the cross Ernie / MO 94-317. Eight plants/RIL were planted in a greenhouse environment, arranged in a randomized complete block design with three replications. For conventional genetic analyses, F_1 (and reciprocal, 50 plants/generation/replication), F_2 (200 plants/replication) and backcross generations (120 plants/generation/replication) were also developed from the same cross. Plants were planted in the greenhouse, arranged in a completely random design with 3 replications. Phenotypic data for *F. graminearum* type II resistance and related traits were collected. Data collected included disease spread (the number of diseased spikelets on the inoculated head), spread with wilt (the number of diseased spikelets plus those spikelets wilted due to disease), spike length (the total number of spikelets on the inoculated head), Fusarium head blight index (FHBI) (spread/spike length), FHBI with wilt (spread with wilt/spike length).

AFLP procedures followed manufacturer's recommendations from the AFLP System I Kit from Invitrogen (Carlsbad, CA). Sixty-four EcoRI/MseI primer pairs and 420 SSR primers were used to screen parents for polymorphisms. Sequence information of SSRs was from Roder et al. (1998) and Q. J. Song and P. Cregan USDA-ARS, Beltsvile, MD (personal communication). The chromosome locations of these SSR markers were from Roder et al. (1998) and JR. Shi and R. Ward, Michigan State University (personal communication). Analyses of variance and QTL multiple regressions were done using SAS Version 8.0. MapMaker 3.0 was used to construct the linkage maps. Composite interval mapping was done using QTL Cartographer 1.16 model 6. Generation means analyses were conducted according to Mather and Jinks (1977).

RESULTS AND DISCUSSION

A total of 139 markers including 94 SSR and 45 AFLP markers were mapped on 19 chromosomes. Two chromosomes, 4D and 6D had only one marker. The order and distance of most mapped markers were consistent with the reference map (Roder et al., 1998; Shi and Ward, personal communication). Based on composite interval mapping at LOD =3.0, five QTL were identified associated with both FHBI and FHBI with wilt on chromosomes 2B, 3B, 4B, 5A, and 5D (Table 1; Figure 1). Among them, the QTL on 5A was also associated with spread and spread with wilt. The major QTL for scab resistance in Ernie were located on 4B, 5A, and 5D, and explained 9.7 to 33.3% of phenotypic variation. All resistance alleles were from Ernie. Most Chinese cultivars have the 3BS major QTL from Sumai 3, which is located on telomeric region of 3BS (Zhou et al., 2002). The minor QTL on 3BS in Ernie was located near the centromere. We concluded that the 3BS QTL identified in Ernie differed from that in Sumai 3. Compared with mapped QTL in other resistant sources, the QTL on 2B and 5D appeared to be new QTL. QTL identified on 3B, 4B, and 5A need further study to determine if they differ from those identified in Wuhan-1 and Fundulea 201R (Somers et al. 2003; Shen et al. 2003). Digenic interactions were found for spread with wilt, FHBI, and FHBI with wilt that explained additional phenotypic variation.

Conventional genetic analysis agreed with molecular analysis. Four genetic factors were estimated for FHBI while 2 factors were associated with disease spread. Gene action conditioning scab resistance was primarily additive, however, a small but significant dominance effect was detected. Additive x dominance epistasis was also significant for both disease spread and FHBI. These data suggest recurrent selection could be important in pyramiding genes for scab resistance in breeding programs utilizing Ernie.

Table 1. QTL associated with type II scab resistance the soft red winter wheat cross Ernie x MO 94-317.

Traits	Chromosome location	Markers	QTL peak position	LOD score	R^2 (%)	Additive effect	Source of alleles
Spread	5A	Xbarc056	38.67	4.1	10.4	-0.77	Ernie
Spread with			36.67				Ernie
wilt	5A	Xbarc056		5.2	12.5	-1.37	
FHBI	2B	Xgwm319	118.62	3.9	5.0	-3.98	Ernie
	3B	E8M1_1	123.71	4.3	10.3	-5.00	Ernie
	4B	Xgwm495	0.01	10.3	14.7	-6.02	Ernie
	5A	Xbarc165	42.67	5.8	13.9	-5.71	Ernie
	5D	Xgwm182	27.84	3.3	16.8	-6.28	Ernie
FHBI with			118.45				Ernie
wilt	2B	Xgwm319		4.0	5.4	-5.60	
	3B	Xgwm077	98.80	3.0	5.1	-4.95	Ernie
	4B	Xgwm495	0.01	6.7	9.7	-6.69	Ernie
	5A	Xbarc165	40.67	8.7	23.5	-10.10	Ernie
	5D	Xgwm182	32.84	3.0	33.3	-12.10	Ernie

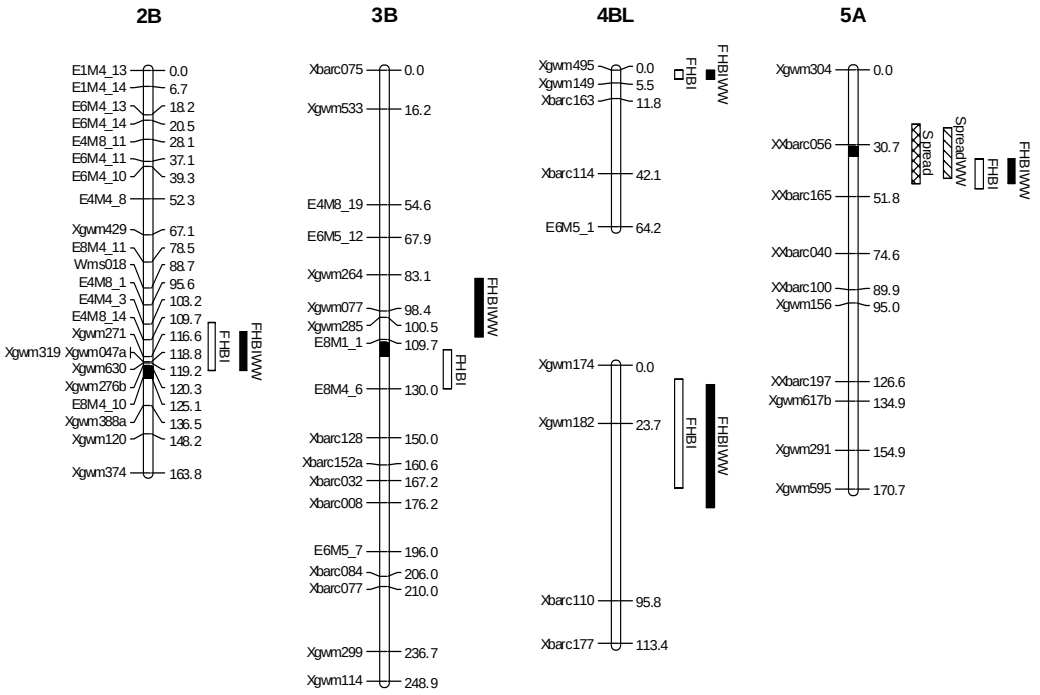

Figure 1. QTL associated with type II scab resistance in the soft red winter wheat cross Ernie x MO 94-317. Abbreviations are defined as follows: FHBIWW and FHBI = Fusarium head blight resistance index with and without wilted spikelets; SpreadWW and Spread = number of diseased spikelets with and without wilted spikelets, in the inoculated head.

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COMPLEX MICROSYNTENY AMONG WHEAT, RICE AND BARLEY AT THE *QFHS.NDSU-3BS* REGION

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ABSTRACT

A major wheat QTL, *Qfhs.ndsu-3BS*, for resistance to Fusarium head blight (FHB) has been identified and verified by several research groups. The objectives of this study were to construct a high resolution map of the *Qfhs.ndsu-3BS* region, and to examine the microsynteny among wheat, rice and barley at this genomic region. Sixteen hundred F_2 plants derived from a single F_7 plant heterozygous for the *Qfhs.ndsu-3BS* region were screened for recombinants with two SSR markers, gwm533 and gwm493, and 192 recombinants were identified. Two additional SSR markers and six STS (sequence-tagged site) markers developed from wheat ESTs were used to genotype the 192 recombinants, and a fine genetic map was constructed. Except for two STS markers that cosegregate, the genetic distance between adjacent markers ranges from 0.2 to 1.5 cM. An inversion was revealed by comparing the order of STS markers and their counterpart genes on three overlapped rice PAC clones. It was previously reported that the microsynteny at this genomic region between rice and barley was interrupted by insertion of six additional barley genes. Six STS markers were developed from wheat ESTs homologous to each of the six barley genes. One STS marker was placed on the high resolution map, and an inversion between wheat and barley was revealed. Therefore, microsynteny among wheat, rice and barley at the *Qfhs.ndsu-3BS* region is complicated by microrearrangements such as inversions and insertion/deletions.

A MODEL CULTIVAR FOR TRANSFORMATION OF WHEAT TO IMPROVE RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Transformation of wheat is proving to be an effective method of introducing new sources of scab resistance into existing germplasm. However, transformation is a time-consuming process and transgenic lines require at least three greenhouse screens before it is prudent to field test the lines. Thus, the time taken to develop, test and move a transgenic line into a breeding program is several years. The cultivar Apogee is a fast-growing dwarf wheat. It flowers significantly faster than Bobwhite, the current cultivar of choice for wheat transformation. We have tested the regenerability of Apogee and have produced transgenic plants with the pAHC25 construct, carrying the *uidA* and bar genes under the control of the maize ubiquitin promoter. Moreover, we have shown that Apogee is susceptible to Fusarium head blight. Histochemical analyses of the expression of the *uidA* gene throughout our transgenic plants are presented along with data relating to the growth of Apogee in growth chambers and the greenhouse. Our data show that Apogee is a model cultivar for developing and testing transgenic wheat for scab resistance.

A TRANSGENIC APPROACH TO ENHANCING THE RESISTANCE OF WHEAT TO FUSARIUM HEAD BLIGHT

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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 chitinases, thaumatin-like proteins (tlps) and ribosome-inactivating proteins (RIPs) are known to inhibit fungal growth via different mechanisms. Chitinases degrade fungal cell walls, tlps degrade fungal cell membranes and RIPs inhibit fungal protein synthesis. Transgenic wheat over-expressing these AFPs were generated using micro-projectile bombardment. We developed 17 and 7 lines carrying a barley chitinase and a barley RIP, respectively. In addition, we developed 4, 11 and 5 lines expressing a combination of chitinase/RIP, chitinase/tlp-1 and RIP/tlp-1, respectively. These combinations each employ two of the three different mechanisms of fungal growth inhibition. We screened these lines for FHB resistance in the greenhouse 2-3 times. Results of these screens are discussed.

OVER-EXPRESSION OF ANTIFUNGAL PROTEINS INCREASES THE RESISTANCE OF WHEAT TO FUSARIUM HEAD BLIGHT

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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, thaumatin-like proteins (tlps) and thionins are known to inhibit fungal growth via different mechanisms. Glucanases degrade fungal cell walls while tlps and thionins degrade fungal cell membranes. Transgenic wheat over-expressing these AFPs were generated using micro-projectile bombardment. We developed 25, 25 and 31 lines carrying a wheat a-puro-thionin, a barley tlp-1 and a barley ß-1,3-glucanase respectively. Three to five independent glasshouse screens were conducted to assess these lines for FHB resistance. Two tlp-1 lines, four glucanase lines and one a-puro-thionin line consistently performed well. Molecular characterization of our lines has shown that they are genetically independent and that they accumulate the appropriate AFP. These 7 lines will be screened in the field in the summers of 2004 and 2005.

NPR1: A CANDIDATE TO ENHANCE BROAD SPECTRUM SCAB RESISTANCE IN WHEAT

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ABSTRACT

In recent years, Fusarium head blight (FHB) or scab has re-emerged as a devastating disease of wheat and barley, severely limiting productivity. Breeding has been the mainstay in developing wheat with improved resistance to scab. Biotechnology offers an alternative approach for rapidly developing scab resistant wheat. The *NPR1* gene which coordinates expression of several defense-associated genes in *Arabidopsis thaliana* is a regulatory gene that offers promise in developing plants with broad-spectrum resistance to scab and other fungal diseases. Overexpression of the Arabidopsis *NPR1* gene confers enhanced resistance in transgenic Arabidopsis and rice plants. We have generated transgenic wheat plants which overexpress the Arabidopsis *NPR1* transcript from the ubiquitously expressed maize Ubi1 promoter. Currently T_2 progeny derived from some of these transgenic lines are under evaluation for resistance to scab.

In parallel, we have identified three BAC's that contain the wheat homolog (WhNPR1) of the Arabidopsis and rice NPR1 gene. WhNPR1 has been mapped to chromosome 3. In addition, we have cloned a partial cDNA for WhNPR1 from a rust-infected Lr21 wheat cDNA library. The predicted WhNPR1 protein exhibits 80% similarity to the rice NPR1 protein. Expression of WhNPR1 is elevated in the flag leaves of scab-inoculated plants. We are presently reconstructing the full-length WhNPR1 cDNA by RACE. Since, interaction with other plant proteins is essential for the NPR1-mediated resistance, we hypothesize that in comparison to the Arabidopsis NPR1, overexpression of the WhNPR1 may confer higher level of disease resistance. To test this hypothesis we will generate transgenic wheat plants, which overexpress the WhNPR1 protein. We will present the current status of our research with the WhNPR1 gene and the transgenic wheat plants expressing the Arabidopsis NPR1 gene.

REGENERATION AND GENETIC TRANSFORMATION OF DURUM WHEAT

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ABSTRACT

Durum wheat (*Triticum turgidum* L.) is an important cereal crop used for making pasta and semolina. Efforts are in progress to improve durum wheat through gene transfer technology for characteristics such as disease resistance, especially for Fusarium head blight (FHB) caused by *Fusarium graminearum* (Schwabe). A major constraint is the lack of an efficient, reproducible and reliable method of genetic transformation of durum wheat. We have established an efficient and reproducible regeneration system with the cv. Monroe. Murashige and Skoog (MS) medium with different concentrations (1.0, 1.5, 2.0, 2.5 and 3.0 mg/L) of picloram (4-amino-3,5,6-trichloropicolinic acid) or 2 mg/L 2, 4, 4-dichlorophenoxy acetic acid (2,4-D) were used to culture immature embryos for their morphogenetic response. Embryogenic calli proliferated on 2.0 mg/L picloram but was less frequent on 2,4-D containing media. Picloram at 2.0 mg/L also regenerated more plants than either 2,4-D or the other picloram concentrations. For genetic transformation, the calli were bombarded with the pathogenesis-related gene thaumatin-like (*tlp*) from rice, and a modified *Tri101* gene, along with the *bar* gene for selection. PCR and Southern analysis indicated the regenerated plants contained the transgenes and the western analysis confirmed the expression of the *tlp* in the durum wheat cv. Monroe.

FINE MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE AND HEADING DATE QTL IN BARLEY

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ABSTRACT

Previous mapping studies in barley using populations derived from the Fusarium head blight (FHB) resistant cultivar Chevron have positioned a QTL for FHB resistance on chromosome 2 (2H) in a genomic region (~20 cM) flanked by SSR markers *Bmag0140* and *Ebmac0521a*. A coincident QTL for heading date (HD) is also located in this region creating uncertainty as to whether this association is due to linkage or pleiotropy. We are fine mapping the target QTL region using a population of five hundred and thirty two F_2 plants derived from a cross between a BC_5 line carrying the Chevron alleles in this region and the recurrent parent M69. Forty-four recombinants identified in this population were genotyped with 13 SSR markers previously mapped between *Bmag0140* and *EBmac0521*. A linkage map was constructed using GMendel v3.0 software. An average marker interval of 1.3 cM was observed. The 44 recombinants were advanced to the $BC_5F_{2:4}$ and evaluated in replicated field trials for FHB and HD at St. Paul and Crookston, MN, in the summer of 2003. Using trait means from individual locations, data were analyzed for presence of QTL via simple interval mapping (SIM) model (PlabQTL v1.0 software) and single marker analysis of variance. A QTL for HD was detected between markers *Bmac0093* and *EBmac0558* (0.2 cM apart) at St. Paul (LOD=6.7; R^2=85.6) and at Crookston (LOD=6.7; R^2=85.4). An FHB resistance QTL was found to be closely associated with EBmac0521b (located 4.7 cM away from the HD QTL) both in St. Paul (*P*=0.03) and Crookston (*P*=0.05). These preliminary results suggest that FHB resistance and late HD are closely associated due to linkage rather than pleiotropy.

INVESTIGATING THE GENETICS OF RESISTANCE TO FHB IN SIX-ROWED, HULLESS, BARLEY ACCESSION HOR211

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ABSTRACT

Development of Fusarium head blight (FHB) resistant barley varieties will require breeding with diverse sources of resistance that carry different resistance genes. All FHB mapping studies in barley to date have identified a significant quantitative trait locus (QTL) for FHB on chromosome 2(2H) in many cases linked to heading date. On the basis of cluster analyses and selective genotyping analyses, we identified the Ukrainian line Hor211 (six-rowed, hulless) as a genotype that is genetically dissimilar from other known sources of FHB resistance. The overall objective of this study is to identify QTLs for FHB resistance and to determine the genetic relationship between FHB and other associated traits (heading date, plant height, DON concentration, and presence of a hull). Hor211 was crossed with Lacey (University of Minnesota cultivar) to develop a $F_{6:8}$ mapping population. The Hor211/Lacey population was evaluated in three field environments (St. Paul 2002, Hangzhou, China 2002, St. Paul, 2003). We found significant variation among the lines for FHB severity, plant height and heading date. Thus far, linkage maps have been created for chromosome 1(7H) and 2(2H) using SSR markers. QTL analysis has identified a QTL for heading date on chromosome 1(7H) (R^2 = 14.5%, 24.5%) and on chromosome 2(2H) (R^2 = 12.7%, 13.0%) detected in two environments. No QTLs for FHB resistance were found on either chromosome 1(7H) or 2(2H). This suggests that Hor211 likely carries resistance that is not associated with the major QTLs that have been previously identified. Continued mapping in this population should yield new information about FHB resistance in barley.

IN VITRO REGENERATION OF COMMERCIAL DURUM CULTIVARS AND TRANSFORMATION WITH ANTIFUNGAL GENES

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OBJECTIVES

To standardize an efficient *in vitro* regeneration protocol for commercial durum wheat cultivars and to incorporate antifungal genes to produce resistance to Fusarium head blight.

INTRODUCTION

Durum wheat (*Triticum turgidum* L., $2n = 4x = 28$; AABB genomes) is an important cereal used for human consumption worldwide. Scab or Fusarium head blight (FHB), caused primarily by the fungus *Fusarium graminearum* Schwabe, is a devastating disease inflicting heavy losses to wheat growers especially in the Northern Plains of the United States (Windels, 2000). There is no reliable source of FHB resistance in current durum cultivars. Through sexual hybridization with wild grasses, coupled with manipulation of chromosome pairing, we have produced FHB-resistant durum germplasm (Jauhar and Peterson, 2001; Jauhar, 2003). Although such chromosome-mediated gene transfers have resulted in genetic improvement of both bread wheat and durum wheat (Friebe et al., 1996; Jauhar and Chibbar, 1999), this technique of germplasm enhancement is often tedious and time-consuming. Genetic engineering provides an efficient means of directly incorporating disease resistance genes into the wheat genome. FHB resistance in wheat was achieved by expressing antifungal genes, including *TRI101*, *PDR5*, and thaumatin-like protein (TLP) genes that degrade structural components of the fungus and/or interfere with biochemical and metabolic processes in the pathogen (Chen et al., 1999; Anand et al., 2003).

A major impediment to durum wheat transformation has been the lack of an efficient method of *in vitro* regeneration. Earlier, we standardized a rapid regeneration protocol for four durum cultivars (Bommineni and Jauhar, 1996) and using this regeneration system, we produced the first transgenic durum wheat (Bommineni et al., 1997). Since we showed that durum is amenable to genetic transformation, there are a few more reports of production of transgenic durum (He et al., 1999; Pellegrineschi et al., 2002). Incorporation of the gene(s) of interest directly into modern cultivars will facilitate development of new germplasm in a relatively short time. Because of genotypic differences in regenerability, it is important to determine optimal *in vitro* culture conditions for current commercial cultivars before selecting one for transformation. Therefore, we studied the effects of three growth regulators (2,4-D, picloram, and dicamba) on callus induction and plant regeneration from scutellum cultures of four commercial durum cultivars, Ben, Maier, Munich, and Lebsock. Having selected the best responding cultivar, Maier, we are incorporating antifungal genes into it.

MATERIALS AND METHODS

Plant material - Four agronomically superior cultivars, Ben, Maier, Munich, and Lebsock, were selected to study their *in vitro* culture abilities.

Callus induction and plant regeneration - Scutella were isolated from the four cultivars and regenerated on Murashige and Skoog medium according to the method described by Bommineni and Jauhar (1996). Four different concentrations (0.5, 1.0, 2.0, and 2.5 mg L^{-1}) of each of 2,4-D, picloram, and dicamba were used in the modified MS medium. The experiment was replicated three times with 20 scutella per concentration. The scutella were incubated in the dark at 25±2°C for a period of 4 weeks for callus induction. Four week-old calli were transferred to auxin-free MS medium and incubated at 25°C with a 16-h photoperiod. The regenerated plants were transferred to peat pellets and, when established, were grown to maturity in the greenhouse.

The data gathered on the number of explants callusing and the number of calli showing green shoot buds were analyzed (4 cultivars × 3 growth regulators × 4 concentrations) using a logistic regression model (Hosmer and Lemeshow, 1989). For plant regeneration, a Poisson Regression Model was used. SAS (version 8.2, 1999-2001) procedures were used for statistical analyses.

Chromosomal studies - Root tips from regenerated plants were fixed and somatic chromosomes were studied using conventional and fluorescent genomic *in situ* hybridization (fl-GISH) techniques standardized earlier (Jauhar et al., 1999).

Transformation - The most regenerable cultivar Maier was selected for transformation experiments. Two-week old calli cultured on 2.0 mg L^{-1} dicamba-containing medium were subjected to microprojectile bombardment. The plasmids (pAHC25/pAHRC-TLP) containing *bar* alone or both *bar* and rice *tlp* were co-bombarded with the pUBK-Bgl containing modified *TRI101* gene. All the genes are under the control of ubiquitin promoter and nos terminator. Transformations were done according to Bommineni et al. (1997).

Preliminary screening of transformants was done using PCR analysis. Genomic DNA was isolated from leaves of putative T_0 and T_1 transformants as well as from non-transformed (control) plants using the method of Dellaporta et al. (1983). PCR analyses were carried out using *bar* and *TRI101* gene-specific primers. For Western blot analysis, a polyclonal anti-TLP-antibody was used as a primary antibody (Chen et al., 1999) for confirming the expression of *tlp* gene. For Southern hybridization, genomic DNA (15 µg) was digested with enzymes *Eco*RI or *Hin*dIII and electrophoresed following standard procedures. The *bar* gene fragment (1.4 kb) released from pAHC25 was used as a probe. The *bar* gene was biotin-labeled using random priming method. Hybridization and detection of the signal were carried out according to Boehringer Mannheim manual instructions.

RESULTS AND DISCUSSION

Initiation of callus was apparent as a white translucent tissue within 3-7 days in all four cultivars. The callus induction rates among cultivars varied from 13-93%. The embryogenic calli differentiated into somatic embryos within 3-4 weeks on auxin containing medium. On transfer to media devoid of growth regulators, the embryos differentiated as green shoot buds and developed into plantlets when exposed to light, as observed earlier by Bommineni and Jauhar (1996).

The logistic regression model showed significant effects of cultivar, growth regulator, and concentration of growth regulator on callus induction ($p < 0.001$) and shoot bud formation ($p < 0.05$). Poisson regression analysis revealed significant differences among cultivars, growth regulators, and their concentrations for plant regeneration.

Among the four cultivars, Lebsock showed the highest callus induction, while Maier gave highest number of plantlets per explant. We found dicamba to be more effective for callus induction and subsequent plant regeneration compared to other two growth regulators, 2,4-D and picloram. Dicamba has been reported to equal or surpass 2,4-D in inducing shoot formation in many cereals including wheat, maize, and barley (Mendoza and Kaeppler, 2002).

All of the regenerated plantlets grew to maturity without any apparent morphological changes and had the expected chromosomal number of $2n = 28$. Fluorescent GISH proved that the chromosome complement of regenerants was intact, with 14 A- and 14 B-genome chromosomes.

Two week-old calli of Maier bombarded with *bar* or *tlp* along with *mTRI101* gene were selected on medium containing 5 mg L^{-1} bialaphos. About 50% of the calli turned either brown or remained watery and did not grow further. From the 650 scutella co-bombarded with genes *bar* and *mTRI101*, 117 plantlets (18%) were regenerated, and out of 720 scutella co-bombarded with *tlp* and *mTRI101*, 120 plantlets(16.7%) were derived.

Preliminary screening of the transformants was done by PCR analyses using *bar* and *TRI101*-specific primers. Western blot analysis using specific antibody to Tlp protein showed an expected protein band (~25 kDa) in some of the transformants that was absent in the untransformed control. By over-expressing a rice *tlp* gene in a transgenic wheat line, Chen et al. (1999) observed moderate resistance to scab.

We have raised 20 T_1 progeny from each T_0 putative transformant. Southern hybridization is being done to confirm the inheritance of the transgene and estimate the copy number.

CONCLUSION

To standardize the *in vitro* regeneration protocol, we studied the effect of three different growth regulators on callus induction and subsequent plant regeneration in four commercial durum wheat cultivars. Overall, the results showed dicamba to be most suitable for callus formation and plant regeneration across the four cultivars. However, Maier offered the best choice for use in genetic transformation experiments. Using microprojection, we have incorporated antifungal genes into Maier and inheritance of the transgene in the progeny is being studied. We are also standardizing an *Agrobacterium*-mediated transformation system for durum cultivars. We believe that chromosome-mediated gene transfers (involving sexual hybridization with potential gene donors in the primary and secondary gene pools) as well as transgenic approaches should be adopted to combat Fusarium head blight, a ravaging disease of cereal crops.

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SATURATION MAPPING OF A MAJOR FUSARIUM HEAD BLIGHT QTL ON BARLEY CHROMOSOME 2H

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OBJECTIVES

To dissect the barley chromosome 2(2H) major Fusarium Head Blight (FHB) Quantitative Trait Locus (QTL) by genetic and physical mapping and development of substitution lines containing small CI 4196 chromosome 2H fragments in a susceptible cultivar background.

INTRODUCTION

In every cross investigated in barley, chromosome 2(2H) has been identified to harbor a strong FHB resistance QTL. De la Penna et al.. (DE LA PENA *et al.* 1999) working with the Chevron x M69 cross, reported 3 QTL which we have designated FHBqtl1, 2, an3. Chr. 2(2H) FHBqtl1 extends from ABC311 to MWG858 (Bins 3 and 4). FHBqtl2 extends from ABG459 to MWG520A (Bin 5). FHBqtl3 extends from MWG887 to ABG619 (Bins 6 to 9). This region is just above and may include the 2 vs. 6-rowed gene *Vrs1*. Another study involving Chevron crossed with Stander (Ma *et al.* 2000) identified 2 chromosome 2(2H) QTL, one from BCD307D to CDO1407 (Bins 6 to 9). This QTL seems to correspond to the FHBqtl3 reported by de la Pena. Another QTL was reported between the markers BCD307B and CDO684B (Bins 12 to 15). This QTL was not previously reported and I have designated it FHBqtl4. In the above cases Chevron, a 6-rowed barley, contributed the resistant alleles. Zhu et al. (Zhu *et al.* 1999) working with 2-rowed genotypes Gobernadora x CMB643 reported a single major FHB QTL on chromosome 2(2H) for Type I and II resistance centered on marker MWG503 (Bin 11). This QTL overlaps with FHBqtl3 and may be the same. This work is reviewed in (Kolb *et al.* 2001). More recently, two other studies have identified FHB QTL on chromosome 2H (Mesfin *et al.* 2003 and Dahleen *et al.* 2003). Although the exact borders of the QTL vary slightly and it is sometimes difficult to make exact comparisons due to the use of different markers, these studies indicate that there are important FHB resistance QTL located on barley chromosome 2H. A FHB QTL mapping study in wheat reported a QTL centered near ksuH16 (Bin 15) (Waldron *et al.* 1999). This QTL may correspond to the barley FHBqtl4. All of the barley QTL were associated with DON accumulation, heading date, and plant height.

In the Foster x CI4196 (FosCI) cross, a very strong FHB resistance QTL was detected on chromosome 2(2H) extending from ABG005 (Bin5) to MWG882 (Bin12). This QTL was identified in all 3 locations and all 4 years that it was tested in North Dakota. The highly significant QTL appearing in all locations and all years extended from ABC306 (Bin8) to MWG503 (Bin11). Thus it appears that this chromosome 2(2H) region is extremely important for FHB resistance and the variation in the size and exact location of this QTL depends on the experimental design, the quality of the maps used, and environmental conditions. To more precisely identify smaller chromosome regions for their role in FHB resistance we need to develop isolines of this chromosome fragment from CI4196 into a uniform, susceptible genomic background. The FHB resistance QTL region on chromosome 2H is also involved in DON accumulation, heading date, and height.

Rice synteny with barley in this region is known. Rice chromosomes 4 and 7 appear to be involved (Van Deynze *et al.* 1995). The barley marker ABG716 (Bin 7) was mapped in rice on chromosome 7 at 83.8 cM, while the marker ABG356 (Bin 8) was mapped on rice chromosome 4 at 65.6 cM. A more detailed synteny is presented in Fig. 1.

MATERIALS AND METHODS

Saturate the target region with molecular markers - Rice PAC or BAC clone sequences are blasted (blastn) against the Triticeae EST database. One barley EST with the highest S-value from each contig within a PAC/BAC was selected. Rice PAC/BAC sequences were obtained from the Rice Genome Research Program website (http://rgp.dna.affrc.go.jp/) and/or the International Rice Genome Sequencing Project website (http://rgp.dna.affrc.go.jp/IRGSP/). Additionally, ESTs mapped to wheat group 2 deletion lines were used to identify homologous barley ESTs by blastn analysis. The identified ESTs are mapped in the Foster x CI4196 recombinant inbred population or, if not polymorphic, in one of the many other populations available to us.

The mapped EST clones are hybridized against the barley cv. Morex BAC library and the positive clones identified. Once the BAC addresses are identified, the BAC clones are picked, grown in 96-well plates, and colony blotted on filters using a 96-pin hand replicator.

Isoline development - Two selected recombinant inbreds from the FosCI population were crossed to Morex to initiate the isoline development. Morex is being used as the recurrent parent because we have a Morex male sterile line, thus facilitating backcrossing. The F_1 lines will be backcrossed to Morex, progeny with the chromosome 2(2H) FHB QTL region fragments selected and backcrossed to Morex again.

Develop a physical map of the chromosome 2(2H) FHB QTL region - BAC clones from the chromosome 2H FHB resistance QTL identified from the Morex BAC library are sent to Dr. Mingcheng Luo who is conducting the fingerprinting of the barley BAC clones under an NSF grant to Tim Close.

RESULTS AND DISCUSSION

The 2H FHB QTL region spans ~40cM and ~30cM on the FosCI and the Steptoe/Morex (SM) genetic maps, respectively. A total of 28 rice BAC clones, comprising ~2.5Mbp, span the rice chromosome 4 region with synteny to the barley chromosome 2H QTL region. The rice chromosome 4 BACs assemble into two contigs, BAC clones OSJNBb0091E11 to OSJNBa0029H02 (~365kb) and OSJNBa0014K14 to OSJNBa0010H02 (~2.2Mbp). There is a gap of unknown distance between OSJNBa0029H02 and OSJNBa0014K14. Ninety-seven unique barley ESTs were identified from the 28 rice BACs. Fourty-seven have been tested for polymorphism against several barley cultivars. Twenty-six of these 47 have been mapped to the 2H FHB QTL region. The rest either mapped elsewhere in the genome or were non-polymorphic. These results give a 55.3% efficiency of barley ESTs identified with homology to rice that map in syntennous positions. One EST mapped to the 2H QTL region that has homology to rice chromosome 7 PAC clone P0022E03.

Wheat ESTs mapped to group 2 deletion lines were used to identify 39 homologous barley ESTs that were screened for polymorphisms. Only four of these mapped to the 2H FHB QTL region. Due to the relatively large distances between deletion line breakpoints that delimit each wheat chromosome bin, several ESTs

were identified that mapped outside the FHB QTL region on 2H. Other probes were either non-polymorphic or mapped to other chromosomes.

A total of 34 probes (including genomic clones) have been mapped to the QTL region to date (Fig.1). Nineteen have been mapped in the FosCI population, three in SM DHLs, 10 in both FosCI and SM, one in Harrington x Morex DHLs, one in Harrington x TR306 DHLs, and one in a Chebec x Harrington population. All 34 probes have been used to screen the 6x cv. Morex barley BAC library, 17 of which have been confirmed as identifying 98 individual BAC clones.

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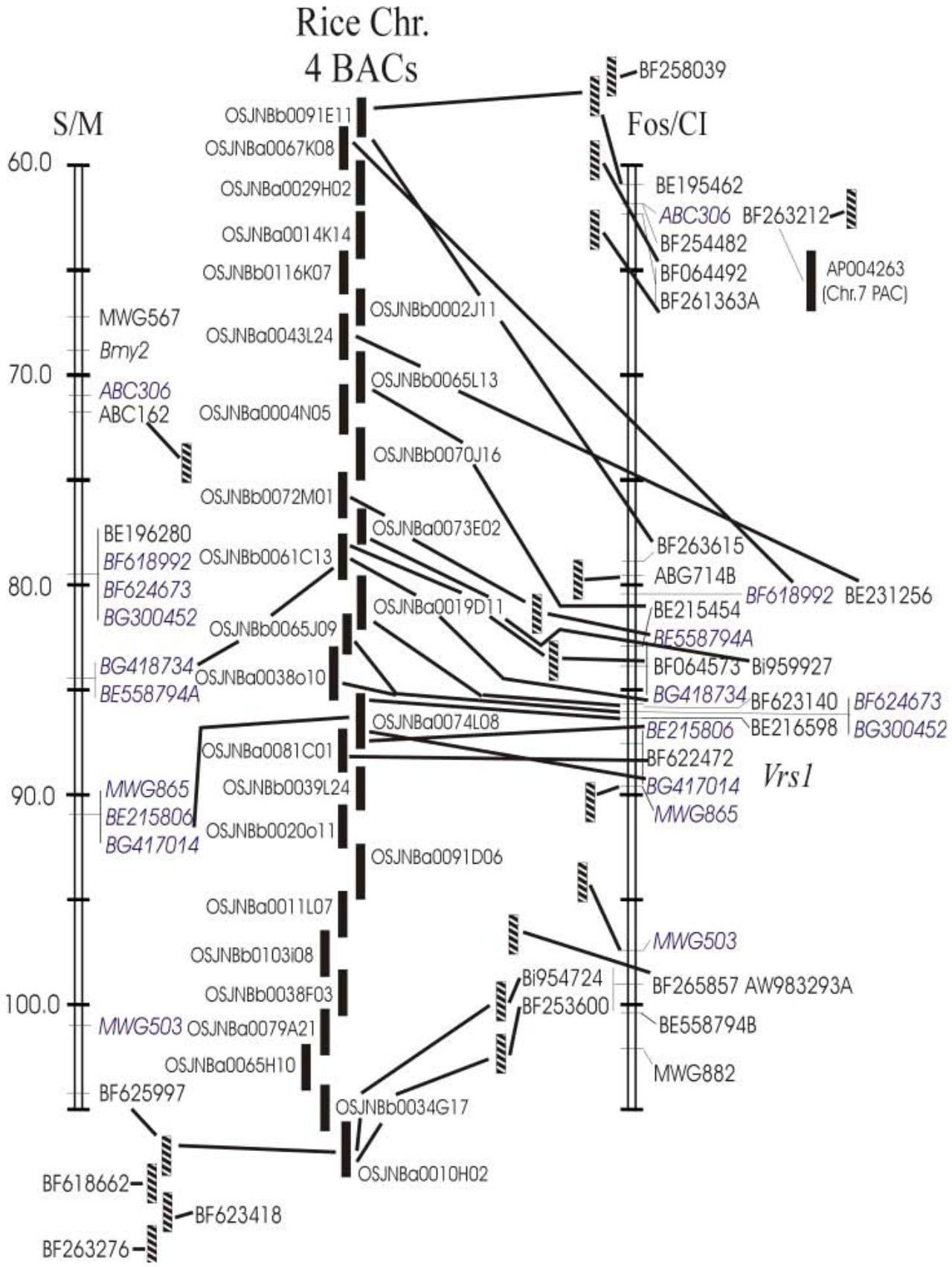
Fig.1 (Right). The Fusarium head blight (FHB) chromosome 2H major QTL region is depicted from the Steptoe x Morex (SM) 150 DHL map, the Foster x CI4196 (FosCI) 144 RIL map, and the syntennous rice chromosome 4 BACs. The SM map is shown on the left and the FosCI map on the right. Approximate centiMorgan values are given to the left of the SM map and correspond to the FosCI map. The rice chr. 4 BACs are between the two barley genetic maps. Rice BACs are represented by a black box, while barley BACs are represented by a striped box. Lines connecting ESTs to BAC clones show which ESTs have confirmed barley BACs and which rice BACs they are homologous to. The approximate position of the *Vrs1* locus is shown to the right of the FosCI map.


Rice Chr.
4 BACs
S/M
Fos/CI
60.0
70.0
80.0
90.0
100.0
MWG567
Bmy2
ABC306
ABC162
BE196280
BF618992
BF624673
BG300452
BG418734
BE558794A
MWG865
BE215806
BG417014
MWG503
BF625997
BF618662
BF623418
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OSJNBa0067K08
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OSJNBa0014K14
OSJNBb0116K07
OSJNBb0002J11
OSJNBa0043L24
OSJNBb0065L13
OSJNBa0004N05
OSJNBb0070J16
OSJNBb0072M01
OSJNBa0073E02
OSJNBb0061C13
OSJNBa0019D11
OSJNBb0065J09
OSJNBa0038o10
OSJNBa0074L08
OSJNBa0081C01
OSJNBb0039L24
OSJNBb0020o11
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OSJNBa0011L07
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OSJNBb0038F03
OSJNBa0079A21
OSJNBa0065H10
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ABC306
BF263212
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BF064492
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AP004263
(Chr.7 PAC)
BF263615
ABG714B
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BE231256
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BF624673
BE215806
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Vrs1
BG417014
MWG865
MWG503
Bi954724
BF253600
BF265857
AW983293A
BE558794B
MWG882

CONTROLLING SCAB WITH PUROINDOLINE-EXPRESSING WHEAT AND BARLEY

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ABSTRACT

The wheat puroindolines (PINA and PINB) are small (ca. 120 a.a.), basic proteins that are normally expressed only in the endosperm. They have been shown to contribute to grain texture, with the presence of PIN leading to softer grain. The puroindolines have also been shown to have *in vitro* and *in vivo* anti-fungal properties. Analyses of the anti-fungal activity have been extended to wheat Fusarium scab. The growth of *F. graminearum* and *F. culmorum* was inhibited by PIN in *in vitro* bioassays. Control and transgenic HiLine wheat varieties that over-express the *pinB* gene driven by the constitutive maize ubiquitin promoter or by the endosperm-specific glutenin-promoter, were inoculated with *F. graminearum* or *F. culmorum* in field and greenhouse studies that have been replicated numerous times. Generally, Hi-Line and transgenic (only contain the selectable marker) control plants showed between 20-50% severely infected spikelets/head (over 40% infected spikelets). *PinB*-transgenic lines often showed a dramatic reduction in plants with severe infections, with a concomitant increase in heads with lesser infection. The transgenic plants showed a decrease of the percentage of tombstones, when compared to the control. There were no significant differences in toxin levels of heads with similar levels of infection, regardless of the plant. Thus, *pin*-expressing plants would have a deceased total level of toxin, since they have lower levels of infection overall. These data suggest that PIN proteins may provide protection to wheat and barley, which we are currently transforming with *pinA*, against *Fusarium* scab.

ACKNOWLEDGEMENT

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 58-5442-2-314. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative.

GENETIC AND PHYSICAL MAPS OF *XBARC* SSR LOCI IN WHEAT

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INTRODUCTION

Genetic maps saturated with informative markers are of great importance for localizing and manipulating important genes and QTLs. In recent years, microsatellite loci, also referred to simple sequence repeats (SSRs) have proved to be a valuable source of highly polymorphic DNA markers. SSR polymorphisms are based on differences in the length of simple sequence repeats at loci defined by locus-specific PCR primers flanking the microsatellite. Currently, approximately 350 publicly available wheat microsatellite primer pairs have been reported in the peer reviewed literature (Röder *et al.* 1998; Korzun *et al.* 1997; Devos *et al.* 1995; Pestsova *et al.* 2000; Salina *et al.* 2000). Here we display our latest version of a genetic/physical map containing over 1400 total loci including 367 new SSR loci. Curated, interactive maps and primer (probe) details are available with GrainGenes Web site (http://wheat.pw.usda.gov).

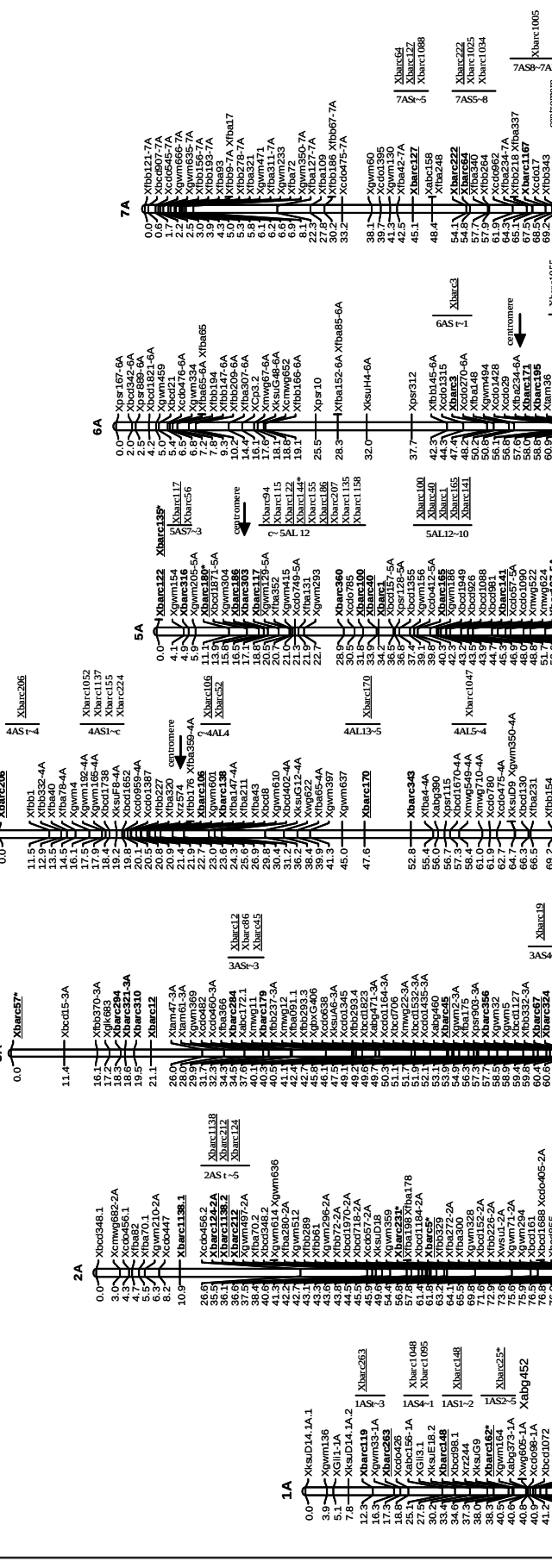
METHODS

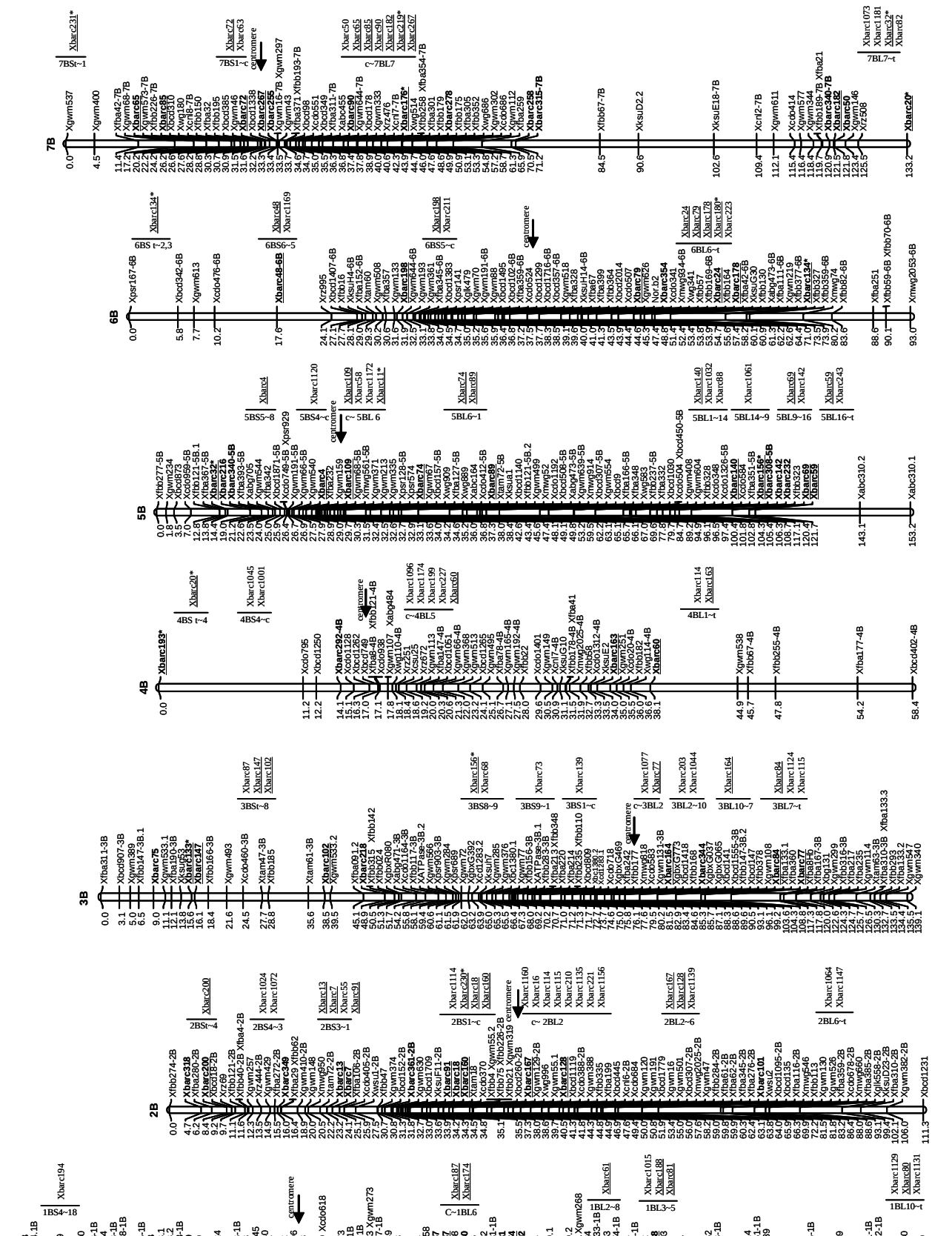
Over 700 "BARC" primer sets that amplify SSR loci were developed in P. Cregan's lab at USDA-ARS's Beltsville Agricultural Research Center (BARC) (Song *et al.*, 2000). The general genetic segregation analysis was conducted at MSU and physical (aneuploid and deletion) mapping was conducted at KSU and MSU. For primer pairs that were polymorphic between the ITMI population parents W7984 and Opata 85, the first 83 or 94 recombinant inbred lines (RILS) of the population were used for segregation analysis. PCR reactions and gel system were described by Shi *et al.* (2001). Linkage analysis and map construction were performed using MAPMAKER 3.0b (Lander *et al.* 1987) and Joinmap 3.0 (Van Ooijen *et al.* 2001). For primers which were not polymorphic in the ITMI population, we used 48 nullitetrasomic and ditelosomic lines to assign the markers to chromosome arms. Different numbers of single-break deletion stocks on each chromosome were then used for sub-arm localization of SSR loci.

RESULTS AND DISCUSSION

A data set containing 1469 markers, including newly developed microsatellites and 1232 markers previously mapped across 21 chromosomes downloaded from GrainGenes website, and 115 progenies of the ITMI population was used for analysis. Two hundred twenty-five Xbarc loci were assigned to linkage map. Another 142 Xbarc SSR loci (amplified by 137 primer pairs) were localized to chromosome arms with deletion mapping (Endo and Gill, 1996).

The final map combined genetic/physical line maps of all 21 chromosomes of bread wheat. Correspondence of physical and genetic positions of Xbarc loci is not perfect. Out of 102 Xbarcs which are mapped both genetically and physically, 82 Xbarcs are confirmed on both maps, 4 Xbarcs on same chromosome but different region, and 16 Xbarcs on totally different chromosomes.





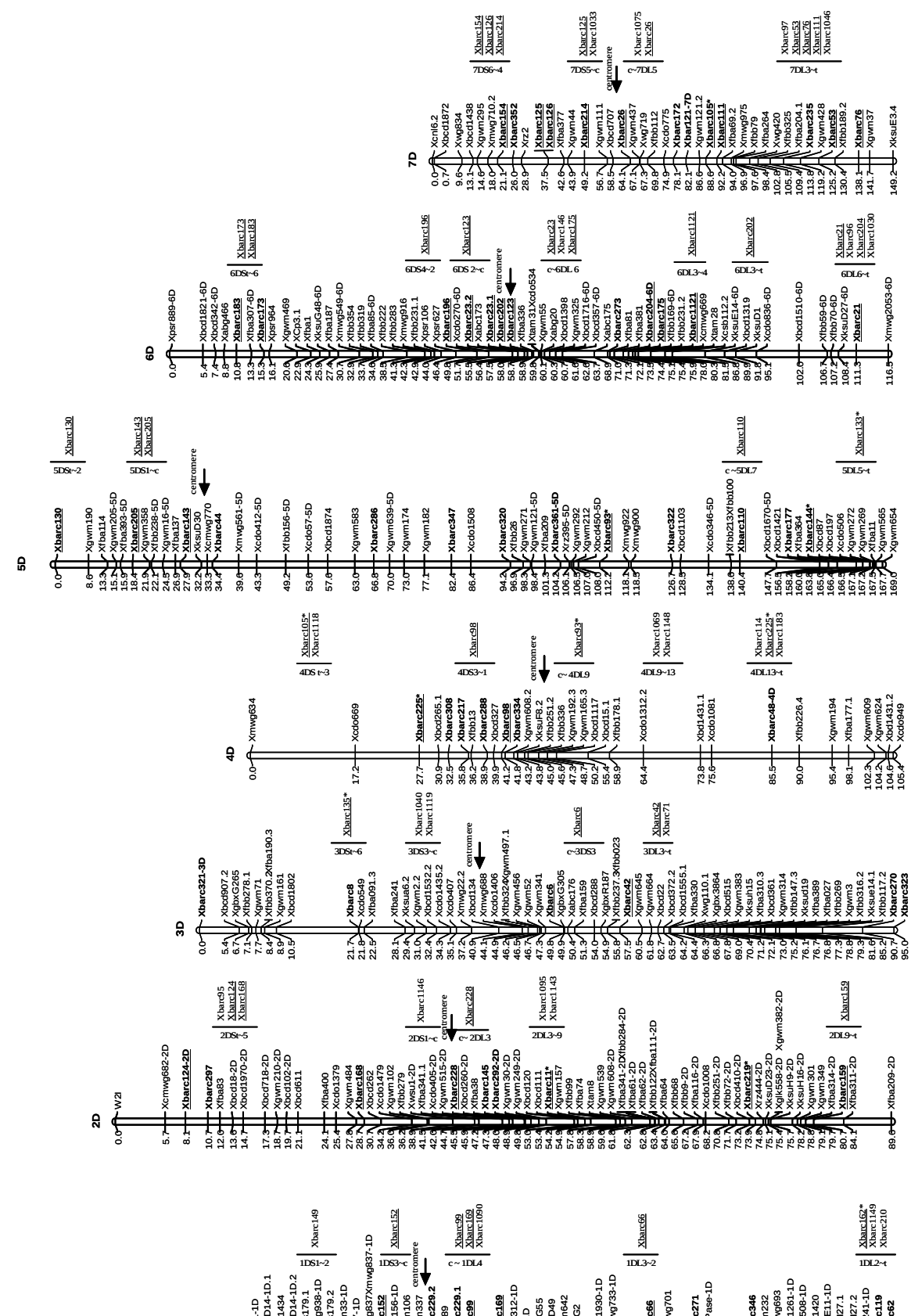


Figure 1. Genetic and Physical Maps of Wheat Xbarc SSR Loci. Genetic line maps were produced by JoinMap3.0. Cumulative Centimorgan (cM) distances are indicated on the left side of each chromosome. The short arm of each chromosome is on top. Loci genetically mapped are listed immediately to the right of the chromosome. Loci derived from GrainGenes data are in normal font. Xbarc loci are in Bold. Physical locations are indicated in BIN order. All loci mapped physically are listed according to the region in which a Xbarc marker is localized – i.e. 1AS-2~1 is the region between deletion breakpoints 2 and 1. For physical locations, "t" and "c" refer to the telomere and centomere, respectively. Centromeres are indicated with arrows. For the Xbarcs mapped both physically and genetically, the marker's name is underlined. Xbarc loci which mapped to different locations on the physical and genetic maps are denoted with asterisks.

ACKNOWLEDGMENTS

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 58-1275-0-024. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative.

Data for ITMI RILs for 1232 loci were obtained from the USDA-ARS GrainGenes website. The generosity of the various authors contributing that data is gratefully acknowledged.

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ASSESSING GENETIC DIVERSITY OF FHB RESISTANCE IN BARLEY USING MOLECULAR MARKERS

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ABSTRACT

Developing sufficient genetic resistance to FHB to keep barley a sustainable crop in the Upper Midwest is a substantial challenge. Research to date indicates that significant levels of resistance can be obtained only through the accumulation of resistance alleles at multiple loci derived from diverse sources of resistance. We are using molecular markers to characterize the diversity of FHB resistance and identify useful alleles for breeding. We are in various stages of mapping, validating, and fine mapping QTL for FHB in four different sources of resistance. Most studies conducted to date indicate that chromosome 2 carries at least 2 important QTL for FHB. Unfortunately, each of these QTL is coincident with other confounding traits (heading date and 2-rowed/6-rowed spike morphology). Another important QTL has been validated on chromosome 6 and the FHB resistance allele is linked to resistance to kernel discoloration and high grain protein concentration. We have used selective genotyping to identify new sources of FHB that will likely carry different genes for resistance. Preliminary evidence suggests that both sources Atahualpa and Hor211 carry FHB QTL not located on chromosome 2. In an effort to identify diverse genes for resistance, we have studied resistance to DON accumulation after point inoculation in the mapping population Frederickson x Stander. A single QTL on chromosome 3 accounts for significant variation for this trait and is not associated with FHB severity suggesting a resistance mechanism for accumulation of DON that is independent of resistance to infection. It is our hope that the development of genetic stocks that carry useful alleles for FHB resistance and the accompanying molecular marker information will provide substantial assistance to efforts to breed FHB resistant varieties.

CHARACTERIZATION OF ORGAN-SPECIFIC PROMOTERS FROM MAIZE AND BARLEY IN TRANSGENIC WHEAT

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ABSTRACT

Genetic engineering is a promising approach to increase plant resistance to fungal pathogens, including *Fusarium*. At present, constitutive promoters are widely used to achieve high expression levels of antifungal genes throughout most tissues of the plant. If only some tissues need to be protected, the use of specific promoters is desirable. Because the glume and lemma comprise the protective barrier encasing the reproductive organs, expression of anti-*Fusarium* genes in these outer floral structures is required to make either wheat or barley resistant to FHB. In order to identify promoters suitable for targeting anti-*Fusarium* gene expression to wheat tissues surrounding the developing seed, we have characterized the organ- and developmental specificity of the promoters from a maize glutamine synthase gene, $GS_{1\text{-}2}$, and from a barley floret-expressed gene, *Lem1*, in stable hexaploid wheat transformants. The plasmids pGS176 and pGS177 (GS:GUS) and pBSD5sGFP (Lem1:GFP) were introduced into immature embryos of *cv*. Bobwhite by particle bombardment. The expression of the reporter genes was monitored in the T_0 and T_1 plants. We found that the maize $GS_{1\text{-}2}$ promoter is expressed in the pericarp and in the scutellum of mature embryos. Thus, this promoter is not suitable for use in anti-*Fusarium* constructs. Monitoring GFP fluorescence in primary transformants revealed that *Lem1* promoter drove the highest *gfp* expression in the lemma, palea, glume, awns, and rachis at anthesis when the anthers first become visible outside the glumes. *Lem1* did not function in developing wheat florets and the surrounding tissues before anthesis when the young ovary and anthers are completely covered by the outer floral organs. After pollination, GFP fluorescence was restricted to a few cells of the lemma and palea and was not detectable in these organs at later stages of seed development. No *gfp* expression was detected in any vegetative organs. Thus, *Lem1* activity in transgenic wheat is identical to its pattern in its native context in barley. Transient assays indicate that *Lem1* is about 4-5 times less active than UBI, one of the strongest of cereal promoters characterized to date. The tissue specificity and moderate strength of the barley *Lem1* promoter suggest that it would be an excellent choice to target anti-*Fusarium* gene expression to wheat tissues surrounding the developing seed. Two cloning vectors have been constructed: pBGS9Lem1 carrying the *Lem1* promoter and the NOS 3' terminator sequence and pBGS9Lem1ADHi1, in which the first intron of the maize *ADH1* gene was added after the *Lem1* promoter, where it may serve as a quantitative element to raise expression levels. Cloning of candidate anti-*Fusarium* genes into these vectors is in progress.

DETERMINATION OF *FUSARIUM GRAMINERAUM* CHEMOTYPE BASED ON UPSTREAM SEQUENCES OF THE *TRI5* GENE

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ABSTRACT

The *Tri5* gene which encodes trichodiene synthase, the first step in the trichothecene biosynthetic pathway, is reported to co-segregate with the locus governing the type of trichothecene produced. Sequence analysis of 26 isolates with known chemotype, representative of the global lineages of *F. graminearum*, revealed that all deoxynivalenol (DON) chemotypes displayed characteristic deletions in a region in the upstream sequences of the *Tri5* gene. The distinct length polymorphisms in this region between the DON and nivalenol (NIV) chemotypes allowed a PCR assay to be developed in this study to distinguish between these chemotypes. Six *F. graminearum* isolates from southern NSW in Australia and twenty overseas isolates were analysed using this technique and compared with published assays utilising polymorphisms in the *Tri7* and *Tri13* genes to distinguish DON and NIV chemotypes. Results demonstrated the potential for reliable use of the molecular tool targeting the upstream sequences of the *Tri5* gene to differentiate NIV and DON chemotypes. Two of the isolates from southern NSW were of the DON chemotype while the other four were of the NIV chemotype. Further research is required to establish the relative distribution of DON and NIV chemotypes in the NSW and Australian grain-belt.

MOLECULAR, PATHOLOGICAL AND TOXICOLOGICAL EXAMINATION OF THE HUNGARIAN *FUSARIUM GRAMINEARUM* POPULATION COMPARED TO MOLECULAR LINEAGES OF THE WORLD-WIDE POPULATION

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ABSTRACT

Fusarium head blight is the most important disease of wheat in Hungary. The main causative agents of this disease are *Fusarium graminearum* and *F. culmorum*. Mycotoxin contamination is the most serious effect of ear fusariosis, since the mycotoxins produced are harmful both to humans and animals. We examined the mycotoxin producing abilities, aggressiveness and molecular variability of *Fusarium graminearum* isolates using different techniques. Altogether 27 Hungarian, 3 Austrian isolates and representatives of the 8 lineages identified by O'Donnell et al. (2000) were involved in this study. Mycotoxin producing abilities of the isolates were tested by thin layer chromatography. The mycotoxins tested included deoxynivalenol and its acetylated derivatives, nivalenol, zearalenone and fusarenone X. Most of the isolates produced zearalenone. All Central-European isolates were found to belong to chemotype I (producing deoxynivalenol). Most *F. graminearum* isolates were found to be highly pathogenic in *in vitro* aggressiveness tests. In our studies, the aggressiveness of *F. graminearum* isolates belonging to chemotype I was in general higher than that of isolates belonging to chemotype II, in accordance with previous observations. Phylogenetic analysis of random amplified polymorphic DNA (RAPD) profiles of the isolates obtained by using 40 different random decamers let us cluster the Central-European isolates into 10 haplotypes. The three Austrian isolates formed a distinct clade on the tree. We also examined the variability of the intergenic spacer region (IGS) of the ribosomal RNA gene cluster using IGS-RFLP. The Central-European isolates belonged into 9 haplotypes on the tree based on IGS-RFLP data. Representatives of the *F. graminearum* lineages formed distinct branches on both trees. When RAPD and IGS-RFLP data were combined, almost every single Central-European *F. graminearum* isolate could be differentiated from each other (27/30 haplotypes). Such a lack of strict correlation between trees based on different data sets indicates that recombination took place in the examined population due to frequent outcrossing. Based on RAPD and IGS-RFLP data, most Central-European isolates most probably belong to lineage 7 characteristic to the Northern hemisphere, with the exception of two Hungarian isolates. One of these was most closely related to lineage 6 originated from Asia, while the other isolate was not closely related to any of the other examined *F. graminearum* isolates. Correlation was not observed between mycotoxin producing abilities of the isolates and their position on either trees. Double-stranded RNA elements were observed in a single isolate came from South-Africa (lineage 3), but in none of the Hungarian or Austrian isolates. Sequence analysis of a putative reductase gene fragment and a translation elongation factor gene fragment of some of the isolates is in progress to clarify the assignment of them to lineages. Further work is also in progress to examine the role and organization of the dsRNA elements, and to compare the pathogenicity of the isolates belonging to different lineages in field tests.

ALTERNATIVE TRANSCRIPTION OF A PUTATIVE LONG-CHAIN ACYL-COA BINDING PROTEIN GENE POSSIBLY REGULATES THE PATHOGENIC STRENGTH OF *FUSARIUM GRAMINEARUM* IN RESPONSE TO CHANGING PATHOGENETIC-ENVIRONMENT

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ABSTRACT

Expression of a host resistance responder gene (designed as *HRR-2*) of *Fusarium graminearum* was found up-regulated during the Fusarium head blight (FHB) pathogenesis in FHB-resistant wheat. Two alternative *HRR-2* transcripts of different size (601 nt and 630 nt, respectively, without the poly A tail) were discovered. The two transcripts are basically identical except that the 630-nt transcript has an additional 29-nt fragment at its 5' end containing a 25-nt CT track. Analysis of the upstream regulatory region of the cloned *HRR-2* gene revealed two alternative promoter sites: TATA/CAAT motifs and a CT motif. It seems that the CT motif would initiate the high-level transcription of the 630-nt transcript in response to the encountered host resistance. The TATA/CAAT motifs should be responsible for the basal-level expression, producing the 601-nt transcript. Sequence analysis suggested that both the 601-nt and the 630-nt transcripts code for a putative long-chain acyl-CoA binding protein of 105 amino acid residues with a calculated molecular mass of 11.5 kD. It is possible that *HRR-2* regulates the pathogenicity of *F. graminearum* by participating in the acyl-CoA-mediated gene expression regulation and/or in trichothecene biosynthesis.

CONVERSION OF AFLP MARKERS ASSOCIATED WITH FHB RESISTANCE IN WHEAT INTO STS MARKERS WITH AN EXTENSION-AFLP METHOD

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ABSTRACT

Amplified fragment length polymorphism (AFLP) has been proven to be a powerful tool for tagging genes or QTLs of interest in plants. However, conversion of AFLP markers into sequence-tagged site (STS) markers is technically challenging in wheat due to the complicated nature of its genome. In this study, we developed an 'extension-AFLP' method to convert AFLP markers associated with Fusarium head blight (FHB) resistance into STS markers. When an AFLP marker of interest was detected with an *Eco*RI+3/*Mse*I+4-selective primer combination, the PCR product was used as a template for an additional selective amplification with four primer pairs in which one additional selective base (either A, C, G, or T) was added to the 3'-end of one of the two primers. The extended primer-pair that produced the targeted band was further extended by adding each of the four selective nucleotide bases for the next round of selective amplification. Extension selective amplification was performed until the target bands became clear enough for subsequent cloning and sequencing. By using the extension-AFLP method, we successfully converted two AFLP markers, which are located in chromosome 3BS and were associated with FHB resistance, into STS markers. Our results indicated that the extension-AFLP method is an efficient approach to converting AFLP markers into STS markers in wheat. The developed STS markers might be used for marker-assistant selection (MAS) for FHB resistance in wheat breeding programs.

HIGH THROUGHPUT GENOTYPING FACILITY FOR MARKER-ASSISTED BREEDING AND MOLECULAR MARKER DEVELOPMENT

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ABSTRACT

The USDA Genotyping Center in Manhattan, Kansas is one of the three newly established USDA regional genotyping centers. Its mission includes developing high throughput molecular markers for agronomically important traits of cereal crops and application of molecular markers in marker-assisted selection for breeding programs. A genotyping protocol has been optimized to improve throughput and automation of marker analysis. By grinding plant tissue in a Mixer Mill from Rebtosh, handling liquid with a robotic system and isolating DNA using NaOH method, more than one thousand samples of DNA can be isolated from fresh tissue in a single day. The quality of DNA isolated with this method is good enough for PCR to be analyzed in a Li-Cor DNA Sequencer. With this method, about 500mg of fresh leaf tissue can provide DNA for more than 50 reactions without damage of original plants. To improve resolution of PCR analysis, PCR products are analyzed in either Li-cor 4200 or ABI 3100 DNA Analyzer. Both systems can detect single nucleotide polymorphism and score data automatically. To reduce cost and time for PCR analysis, multiplex PCR is used to analyze several markers simultaneously. This method has been successfully used in MAS of 3BS major QTL for scab resistance. To construct a new map to identify new QTL, AFLPs and SSRs in coupling with bulk segregant analysis strategy are used. More than 100,000 marker data points can be collected in less than six months. In addition, an Odyssey Image System from Li-Cor is used for quick cloning of AFLP markers to develop breeder-friendly STS markers. With the high throughput protocol, it is feasible for the center to do marker-assisted genotyping for multiple breeding programs. Analysis of FHB resistant breeding materials from USWBSI with SSR markers linked to 3BS QTL is the first service provided by the center.

GENETIC RELATIONSHIP AMONG ASIAN WHEAT GERMPLASM RESISTANT TO FUSARIUM HEAD BLIGHT ASSESSED ON THE BASE OF MOLECULAR MARKERS

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ABSTRACT

The major QTL (quantitative trait locus) on 3BS from 'Sumai 3' and its derivatives has been used as a major Fusarium head blight (FHB) resistant source worldwide, but more resistant genes are needed to avoid complete dependence on single source. In Japan and China, many wheat cultivars and landraces were reported to have a high level of resistance to FHB. But their genetic relationships have not been documented because pedigree information is not available for many landraces. The objectives of this study were to evaluate Type II FHB resistance of 59 wheat landraces and cultivars from Asia and genetic relationships among these accessions based on amplified fragment length polymorphism (AFLP) and simple sequence repeats (SSRs) from chromosome 3BS. The cluster and principal coordinate analysis (PCA) demonstrate that maker data are consistent with the existing geographic distribution and/or available pedigree information of these wheat accessions. Genetic diversity within Chinese resistant landraces is broader than that of accessions from different Asian countries. The haplotypes on 3BS were determined based on five SSR markers (Xgwm389, Xgwm493, Xgwm533, Xbarc133, and Xbarc102) associated with the 3BS QTL. Among the 59 accessions, five of them have at least four of the five Sumai 3 SSR alleles, and hence, are assumed to carry the 3BS major QTL. Twenty-two accessions didn't carry any Sumai 3 alleles on the five SSR loci and additional 25 accessions carried no more than two Sumai 3 alleles, suggesting that these lines may carry different QTL for FHB resistance, and are worth further study.

INTERPRETIVE SUMMARY

One major gene for scab resistance has been identified in the Chinese cultivar Sumai 3 and used in breeding programs worldwide. However, more genes for scab resistance are needed to enhance genetic diversity of the resistance genes. 59 old wheat cultivars with resistance to scab were collected from China and Japan and tested for scab resistance. They were also characterized with molecular markers to evaluate their genetic relationship. Result indicated that most of these accessions have resistance to scab in greenhouse experiment and may not have the major resistance gene from Sumai 3. Molecular data coincided with their geographic distribution or pedigree information.

INTRODUCTION OF PUTATIVE ANTIFUNGAL GENES INTO TWO-ROW AND SIX-ROW BARLEY THROUGH GENETIC ENGINEERING

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ABSTRACT

Fusarium head blight is a major fungal disease for barley and wheat throughout the world; its incidence in the upper midwestern regions of the U.S has reached epidemic proportions. Yield and quality losses have caused economic hardships for producers and users. Introduction of antifungal proteins into barley by genetic engineering offers the potential to suppress pathogen infection and growth. We are using the *Ac/Ds* gene delivery system to produce marker- and plasmid-free transgenic plants containing single insertions that will lead to stable expression of genes encoding putative antifungal proteins to improve Fusarium head blight resistance. Two pathogenesis-related genes from oat, *tlp1* and *tlp4*, and two trichothecene pathway genes from *F. sporotrichioides, TRI101* and *TRI12*, were put into expression cassettes driven by the maize *ubiquitin*- or rice *actin*-promoter-driven genes flanked by ~200 bp of *Ds* inverted repeat DNA that serves as the recognition site for transposition. An expression cassette with *Ac*-driven *Ac* transposase, which is necessary for the movement of *Ds*, was also used. The *Ds*-AFP and *AcTPase* expression cassettes were introduced separately via particle bombardment into scutellar tissues of immature embryos of Golden Promise (GP, a 2-rowed malting variety), and into highly regenerative, green tissues of Drummond (an elite 6-rowed malting variety). Also introduced with the expression cassettes were plasmids encoding either bialaphos (GP) or hygromycin (Drummond) resistance, to enable selection of transformed tissues. Plants derived from 3 DsUbiTlp1 and 3 DsUbiTlp4 transgenic GP lines were *bar*-positive and *tlp* positive. Plants were regenerated from bialaphos-containing medium from four putative DsActTri101 and one DsActTri12 GP lines. Two DsActTlp1, 2 DsActTlp4 and 4 DsActTri101 lines were generated from hygromycin-resistant green tissues of Drummond; seeds have been obtained from 2 DsActTri101 and 1 DsActTlp1 Drummond plants. PCR tests confirmed the presence of the AFP transgene for 1 DsActTlp1, 1 DsActTlp4 and 1 DsActTri101 line.This is the first confirmed report of the introduction of an AFP into a six-rowed barley variety. In addition, Drummond plants have been recovered which contain the *Ac*-driven *Ac*-transposase (*AcTPase*) gene, as confirmed by hygromycin resistance and PCR assays for *AcTPase*. The *AcTPase* gene is also being introduced into the Drummond background via backcrossing from *AcTPase*-positive transgenic GP lines. To assist in characterizing the level of transgene expression, antibodies to the TLP1, TLP4 and TRI101 proteins were developed. Further characterization of the transgene insertions, expression and inheritance of transgenic progeny is ongoing.

MOLECULAR MAPPING OF SCAB RESISTANCE QTL IN WANGSHUIBAI

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ABSTRACT

Wheat scab, or Fusarium head blight, is a destructive disease that can reduce both yield and quality in many regions of the world. Growing scab resistant varieties is an effective, economical, and environmentally sound way to reduce economic losses caused by this fungal disease. Evaluation of scab resistance is costly and laborious. Identification of scab resistance QTL and marker-assisted selection of identified scab resistance QTL will aid in the development of scab resistant cultivars by increasing selection efficiency and reducing the amount of phenotypic evaluation required. However, most reported scab resistance QTL are from a single source: Sumai 3 and its derived lines. To broaden the genetic base of scab resistance, it is important to identify new scab resistance QTL. Wangshuibai is a scab resistant landrace that originated from the Jiangsu province of China. It was selected and planted by farmers many years before Sumai 3 was bred and released in Jiangsu. To identify new scab resistance QTL from sources other than Sumai 3, F_5 derived recombinant inbred lines (RILs) were developed from a cross between Wangshuibai and Wheaton, a susceptible variety. This set of RILs has been evaluated for Type II scab resistance three times (in 2002 and 2003). SSR markers and AFLP markers associated with QTL for scab resistance in Wangshubai were mapped in this population. A major QTL is located on chromosome 3BS in Wangshuibai. It is most likely that Wangshuibai and Sumai 3 have the same major FHB resistance QTL on 3BS. Besides the 3BS major QTL, three minor QTL for Type II FHB resistance were located on chromosomes 7AL, 1BL, and 3BS (near the centromere). These QTL may be novel FHB resistant QTL because none of the resistance QTL in these chromosome regions has been reported from Sumai 3 and its derived lines. One of the objectives of mapping FHB resistance QTL with PCR based molecular markers is to improve the efficiency of selecting FHB resistant varieties. Novel FHB resistance QTL identified from this study may be useful for stacking these QTL with FHB resistance QTL from other sources to develop breeding lines with transgressive scab resistance.

SEGREGATION OF AN SSR ASSOCIATED WITH A QTL FOR FHB RESISTANCE ON CHROMOSOME 7A IN HEXAPLOID WHEAT

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OBJECTIVE

Our objective was to map SSR markers associated with a putative scab resistance QTL on chromosome 7A from Sumai 3.

INTRODUCTION

Wheat scab is a destructive disease that can reduce both yield and quality in many regions of the world (Bai and Shaner, 1994). Growing scab resistant varieties is an effective, economical, and environmentally sound way to reduce economic losses caused by this fungal disease (McMullen et al., 1997). Evaluation of scab resistance is laborious, costly, and time consuming. Identification of scab resistance QTL and marker-assisted selection of identified scab resistance QTL will aid in development of scab resistant cultivars by increasing selection efficiency and reducing the amount of phenotypic evaluation required (Zhou et al. 2002a, 2003). Molecular mapping has been used to tag scab resistance QTL since Bai first tagged scab resistance QTL with RAPD markers in 1995 (Bai, 1995).

A study on marker-assisted selection for the 3BS QTL showed that phenotypic selection among plants carrying the 3BS QTL was still necessary to identify lines with resistance similar to Sumai 3 and Ning7840 (Zhou et al., 2003). Identification of other scab resistance QTL should increase selection efficiency and enhance the implementation of marker-assisted selection.

In a recent study on the chromosome effect of Sumai 3 on Type II resistance and reduced DON accumulation, chromosomes 2B, 3B, 6B, and 7A from Sumai 3 were shown to carry scab resistance (Zhou et al., 2002b). In chromosome substitution lines where Sumai 3 chromosomes were substituted into a Chinese Spring background, chromosome 7A had the largest effect on both Type II FHB resistance and reduced DON levels (Zhou et al., 2002b). In that study, two sets of substitution lines were developed by crossing individual monosomic lines of Chinese Spring (recipient) with scab resistant cultivar Sumai 3 (donor). The monosomics were then used as the recurrent male parent for four backcrosses. Chromosome specific SSR markers were analyzed for polymorphism between Sumai 3 and Chinese Spring. Polymorphic markers were used to verify chromosome substitution of individual Sumai 3 chromosomes in all substitution lines. SSR markers on chromosome 7A verified that the chromosome substitution in the most resistant substitution line was authentic (Zhou, 2002b).

MATERIALS AND METHODS

Plant materials: Chinese Spring was crossed with a Chinese Spring (Sumai 3) 7A substitution line, F_1 plants were selfed, and F_2 seeds were harvested. About 300 F_2 seeds were germinated, and the seedlings were vernalized at 4^{o}C in a cold chamber for two months before transplanting into the greenhouse. The pots

were arranged randomly on benches in the greenhouse. Plant tissue for DNA isolation was harvested from individual plants three weeks after transplanting. At least one head from each plant was inoculated for evaluation of Type II scab resistance using the single floret inoculation method (Bai et al. 1999). Percentage of scabbed spikelets (PSS) on inoculated heads was determined 21 days after inoculation. PSS values recorded from inoculation of more than one head on the same plant were analyzed as sub-samples.

Quick DNA isolation: To isolate DNA, a 2-cm long piece of leaf tissue was inserted into a 1.5 ml centrifuge tube containing 100 µl 0.5 mol NaOH solution. The tissue was ground with a small plastic pestle. Ten µl of the liquid were pipetted into a new centrifuge tube containing 90 µl 0.1 mol Tris-HCL solution. Two µl of the DNA solution were used as the DNA template for polymerase chain reaction (PCR) with a total reaction volume of 20 µl.

PCR reaction and data analysis: PCR reactions were performed as described by Röder et al. (1998) in a Genius Thermal Cycler (Techne Ltd.) starting with 3 min at 94°C, then 40 cycles of 30 sec at 94°C, 30 sec for annealing, and 30 sec at 72°C, with a final extension of 5 min at 72°C. PCR products were separated on 2.5-3.0% agarose gel at 180 V for 1-2 hours. Gels were stained with ethidium bromide, and visualized and photographed under UV light. Linkage of the SSR markers was analyzed by using Mapmaker, version 3.0 for the PC (Lander et al. 1987). SAS V8.0 (SAS Institute Inc, NC 27513, USA. 2000) was used for variance and regression analysis.

RESULTS AND DISCUSSION

1) The frequency distribution of PSS values for the 196 F_2 plants is shown in Figure 1.

2) Preliminary mapping of the scab resistant gene(s) on Chromosome 7A of Sumai 3:
We genotyped 196 F_2 plants from the cross Chinese Spring/ Chinese Spring (Sumai 3, 7A) with *Xbarc49*, which is polymorphic between Sumai 3 and Chinese Spring. This marker separated the196 F_2 plants into three genotypic groups: 48 plants were homozygous for the allele from Sumai 3, 100 plants were heterozygotes, and 48 plants were homozygous for the allele from Chinese Spring. There were significant differences among average PSS values of the three groups as shown in Tables 1, 2 and 3. Based on the preliminary mapping data the F_2 plants derived from a cross between Chinese Spring and Chinese Spring (Sumai 3, 7A) segregated in a typical ratio of 1:2:1. Significant differences among the three genotypes indicated a possible scab resistance gene on chromosome 7A from Sumai 3. We are developing a set of recombinant inbred chromosome lines from the described F_2 populations and mapping more 7A SSR markers for a better molecular characterization of the scab resistance gene.

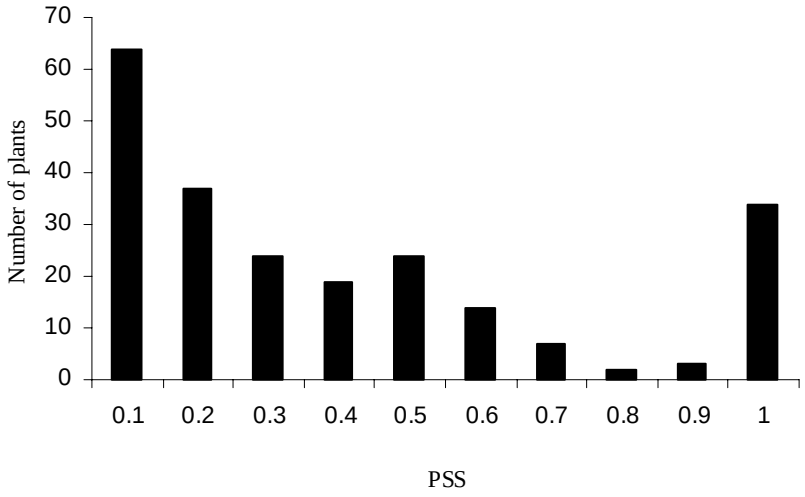

Figure 1. Frequency distribution of PSS values of 196 individual F_2 plants derived from Chinese Spring and Chinese Spring (Sumai 3, 7A).

Table 1. Analysis of variance table for average PSS values of three genotype groups based on marker Xbarc49.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	1.22374464	0.61187232	6.07	0.0028
Error	193	19.45271631	0.10079128		
Corrected Total	195	20.67646094			

Table 2. Means and standard deviation of PSS values of three genotypes.

barc49 genotype[§]	N	Mean	Std Dev
AA	48	0.3142	0.2808
BB	48	0.5088	0.3819
AB	100	0.3314	0.2997

§ A: allele from Sumai 3; B: allele from Chinese Spring

Table 3. Comparisons of means of PSS values of three genotypes

barc49 Comparison	Difference Between Means	95% Confidence Limits		
BB - AB	0.17745	0.06749	0.28740	*
BB - AA	0.19465	0.06683	0.32247	*
AB - BB	-0.17745	-0.28740	-0.06749	*
AB - AA	0.01720	-0.09275	0.12716	
AA - BB	-0.19465	-0.32247	-0.06683	*
AA - AB	-0.01720	-0.12716	0.09275	

Comparisons significant at the 0.05 level are indicated by *.

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LABORATORY STUDIES WITH PURIFIED ITURIN A, AND WITH *BACILLUS SPP.* GROWN IN COMPLEX AND DEFINED GROWTH MEDIA, TO ASCERTAIN THE IDENTITY AND ABILITY OF COMPOUNDS THAT INHIBIT *FUSARIUM GRAMINEARUM*

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ABSTRACT

Certain endospore-forming bacteria in the genus *Bacillus* are able to antagonize *Fusarium graminearum* in laboratory, greenhouse, and field-plot studies. We have worked with four strains of *Bacillus spp.* (likely related to *Bacillus amyloliquefaciens* and/or *Bacillus subtilis*) that have demonstrated ability to antagonize this wheat pathogen. Research has not yet entirely elucidated the mechanism of the antagonism, but it is probably due at least in part to bacterial antibiotics, such as cyclic lipopeptides in the iturin family. It is known from the literature that iturin production is enhanced in some growth media and suppressed in others. We have cultured these bacterial strains in potato dextrose broth (PDB), a complex medium containing glucose which may suppress iturin production to some degree. We have also cultured the *Bacillus spp.* in the defined broth medium of Besson et al. lacking glucose, containing mannitol, glutamic acid and inorganic salts. Bacterial cell numbers in this original formulation were lower than desirable for application of cells to wheat plants, so a modification of the original medium was used increasing the mannitol by 2.3 times, and increasing the glutamic acid by 2.1 times. After 10 days of growth in the modified broth medium with increased carbon and nitrogen sources, bacterial strain 1BA grew to over 10 times the optical density it achieved in the initial medium formulation. Plate count data also showed better growth of 1BA in the modified defined medium having elevated carbon and nitrogen, with plate counts of 10^9 CFU/ml or greater in the richer formulation, and plate counts that were orders of magnitude lower in the original growth medium formulation. Higher numbers of cells in the modified defined growth medium should allow better coverage of bacterial cells sprayed onto wheat surfaces when these bacteria are used in biocontrol trials. In addition, plate assays were done to see whether pure iturin would antagonize *F. graminearum*, and if the defined broth media in solidified form allowed the bacteria to antagonize the fungus. Purified iturin A was found to inhibit *F. graminearum* at a concentration of 40 µg/ml applied to a paper disk, challenging growth of the fungus on Potato Dextrose Agar. Analysis of extracts of broth cultures in defined media by absorption spectroscopy and HPLC indicated that iturin-like compounds were produced by all four *Bacillus* strains we have studied. Better understanding of the productioin of iturin and other compounds that might act in concert with iturin would allow better understanding and use of these and related bacteria as biocontrol agents to control FHB.

CONTROL OF FUSARIUM HEAD BLIGHT WITH FUNGICIDES IN INDIANA, 2003

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OBJECTIVES

To identify fungicides that are effective for control of Fusarium head blight.

INTRODUCTION

Until wheat cultivars with a high and consistent degree of resistance to Fusarium head blight become available, fungicides may be a useful tool for maintaining yield and grain quality under conditions conducive for the disease. Fungicides currently registered for use on wheat in the U.S. are not highly effective against head blight nor for prevention of accumulation of deoxynivalenol (DON) in grain. This study was part of a cooperative effort to identify effective fungicides that will both reduce severity of head blight and accumulation of DON.

MATERIALS AND METHODS

Fungicide trials were conducted at 2 locations in Indiana during 2003. At the Purdue Agronomy Center for Research and Education (ACRE) near West Lafayette, IN, wheat cultivar Patterson, treated with DividendExtreme and Reldan 4E seed treatment, was drilled at 7.5-in. row spacing on 26 Sep 02. At the Southeast Purdue Agricultural Center (SEPAC), near North Vernon, IN, wheat cultivar Patterson was drilled at 7.5-in. row spacing into disked corn stalks on 21 Oct 02. Before seeding at ACRE, 50 lbs /A of N was broadcast and incorporated. Plots were top-dressed on 22 Mar at 90 lb/A of N. Prior to planting at SEPAC, the field was fertilized with 524 lb/A of 7-17-38. Plots were top dressed with ammonium sulfate on 10 Mar at 90 lb/A of N. Harmony Extra at 0.5 fl oz/A was applied in early spring at both locations for weed control. Corn stalks were spread at ACRE between rows of wheat prior to heading to provide inoculum of *Gibberella zeae*. Plots at ACRE and SEPAC were 20 ft long and 5.5 ft wide. An intermittent mist-irrigation system was operated at ACRE in the plots from 13 May, when heads were in the boot stage, through the mid dough stage. Both experiments were randomized complete blocks with 4 replications. Rainfall at ACRE for the week prior to heading and 2 weeks past flowering was 1.7 in. Average high temperature for the same period was 69°F and average low temperature was 50°F. Rainfall at SEPAC was 3.1 in. during the same period, average high temperature was 72°F, and average low temperature was 54°F. Disease developed from natural inoculum. Fungicides were applied with a CO_2-pressurized back-pack sprayer and hand-held boom fitted with TJ 8001VS nozzles (12-in. spacing) that delivered 332 gpa at 40 psi. There were 2 nozzles at each drop, that directed the spray from the sides of the heads. Fungicides were applied at early boot stage (GS 45) on 7 May, head emergence (GS 59) on 10 May, beginning of anthesis (GS 61) on 15 May, and full anthesis (GS 63) on 22 May at ACRE. Fungicides were applied at SEPAC on 14 May at mid flowering (GS 63). Intensity of foliar disease was visually estimated on a whole-plot basis. Incidence of Fusarium head blight (FHB) was estimated by counting the blighted heads in 10 arbitrarily selected 1-ft lengths of row. We estimated severity of blight as the average percentage of spikelets

blighted on those heads that showed any blight. From the product of incidence and severity, we calculated an FHB index. Plots were harvested with a plot combine, on 14 Jul at ACRE and on 26 Jun at SEPAC. Grain was dried to 13 % moisture after which yield and test weight were measured. The frequency of *Fusarium*-damaged kernels (FDK) was determined by counting the number of such kernels in a sample of 100 kernels from each plot. We also determined the frequency of infection in kernels that were visually sound. We surface sterilized 25 healthy-looking kernels from each plot for 2 min in 5% bleach solution (0.2625 % sodium hypochlorite) and then rinsed them in sterile water for 1 min. These seeds were placed on Komada's medium, incubated at 25°C and examined for *F. graminearum* growth after 5 days. We ground 100 g of kernels from each plot and sent the coarse flour to Dr. Pat Hart at Michigan State University for DON analysis.

RESULTS AND DISCUSSION

Weather conditions at ACRE were conducive to a moderate development of FHB. The supplemental mist irrigation promoted severe blight in this trial. All fungicide treatments, even those applied at the boot stage, reduced incidence (Table 1). Only the JAU6476 and V-10116 treatments applied during flowering reduced severity of blight. Most treatments reduced the number of FDK. Several treatments applied at flowering reduced DON level in grain. Three treatments increased yield significantly. Test weights were low in this trial and not improved by any treatment. Lodging was severe, and this, as much as head blight, contributed to low test weight. At SEPAC, all treatments reduced FHB incidence, but as at ACRE, JAU6476 and V-10116 were most effective (Table 2). By the time of the second assessment, incidence and severity had increased in all treatments, but several treatments were still superior to the unsprayed control.

Harvest was interrupted by rain at SEPAC, and some plots were never harvested. We were able to obtain samples from each treatment for analysis of FDK and DON. FDK values and DON were quite high at SEPAC, but 2 treatments reduced DON by more than 34%. Folicur did not reduce DON at either location.

Table 1. Control of Fusarium head blight on mist-irrigated wheat at the Purdue Agriculture Center for Research and Education (ACRE).

Product, rate per acre, growth stage at application	Leaf blotch[1] 20 Jun	FHBI[2]	FHBS[2]	FHBX[2]	FDK[3] %	FHBC[4]	DON[5] ppm	Yield bu/A	Test weight lbs/bu
Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 59	27	32	47	15	10.3	35	1.7	63.6	45.2
Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 61	14	28	45	13	11.5	48	2.1	66.2	46.0
Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 63	22	35	42	15	7.8	53	1.7	65.9	46.9
JAU6476 480SC 5 fl oz + Induce 0.125% v/v, GS 61	8	22	20	5	4.8	21	0.8	73.5	49.3
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v, GS 59	6	24	29	7	5.3	35	1.1	72.1	49.6
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v, GS 61	15	25	39	10	10.5	41	1.6	73.1	45.8
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v, GS 67	29	25	37	10	6.8	34	1.3	64.1	47.2
JAU6476 480SC 3.6 fl oz + Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 61	6	21	21	6	7.8	28	1.1	69.2	46.4
V-10116 1.67 SC 6 fl oz + Induce 0.063% v/v, GS 61	7	30	34	10	8.8	30	0.9	63.7	47.0
V-10116 1.67 SC 8 fl oz + Induce 0.063% v/v, GS 63	37	29	40	12	6.3	29	0.8	59.3	45.4
Tilt 3.6 EC 4 fl oz, GS 45	35	36	41	15	8.5	38	2.5	61.5	43.6
Quadris 9.2 fl oz, GS 45	8	33	45	15	13.8	54	2.8	61.4	42.4
Stratego 250 EC 10 fl oz, GS 45	12	30	41	13	9.5	37	2.4	69.1	45.3
Folicur 3.6 F 4 fl oz, GS 45	22	31	45	14	13.8	46	2.7	68.1	44.9
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v, GS 45	17	36	45	16	8.5	46	2.3	64.8	44.4
Untreated	51	44	52	24	17.0	59	2.4	60.5	46.3
LSD (0.05)	15	13	2	2	4.3	20	0.6	9.3	3.5

[1] Leaf blotch was caused by both *Septoria tritici* and *Stagonospora nodorum*. Severity was rated as the percentage of flag leaf area affected.

[2] FHBI = FHB incidence, the percentage of heads showing blight symptoms. FHBS = FHB severity, the percentage of spikelets on affected heads that were blighted. FHBX = FHB index, the product of incidence and severity expressed as percent.

[3] FDK = *Fusarium*-damaged kernels, determined by examining 25 kernels from each plot.

[4] FHBC= *Fusarium* contamination of apparently sound grain.

[5]DON (deoxynivalenol) content was determined by Dr. Pat Hart at Michigan State University.

Table 2. Control of Fusarium head blight on wheat at the Southeast Purdue Agricultural Center (SEPAC).

Product, rate per acre, growth stage at application	Leaf blotch[1]	FHBI[2] 4 Jun	FHBS[2] 4 Jun	FHBI[2] 13 Jun	FHBS[2] 13 Jun	FHBC[4]	FDK[3] %	DON[5] ppm
Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 63	32	13	19	45	50	6	14 ab	14 cd
JAU6476 480SC 5 fl oz + Induce 0.125% v/v, GS 63	17	5	14	30	49	3	12 a	7 a
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v, GS 63	38	7	12	30	49	6	13 a	10 b
JAU6476 480SC 3.6 fl oz + Folicur 3.6 F 4 fl oz + Induce 0.125% v/v, GS 63	32	6	12	31	46	6	17 ab	11 bc
V-10116 1.67 SC 6 fl oz + Induce 0.063% v/v, GS 63	10	7	11	23	45	6	12 a	9 ab
V-10116 1.67 SC 8 fl oz + Induce 0.063% v/v, GS 63	15	5	10	25	44	8	14 ab	11 bcd
Untreated	60	20	17	57	58	11	20 b	13 cd
LSD (0.05)	23	4	4	21	13	6		

[1] Leaf blotch was caused by both *Septoria tritici* and *Stagonospora nodorum*. Severity was rated as the percentage of flag leaf area affected.

[2] FHBI = FHB incidence, the percentage of heads showing blight symptoms. FHBS = FHB severity, the percentage of spikelets on affected heads that were blighted. FHBX = FHB index, the product of incidence and severity expressed as percent.

[3] FDK = *Fusarium*-damaged kernels, determined by examining 25 kernels from each plot.

[4] FHBC= *Fusarium* contamination of apparently sound grain.

[5]DON (deoxynivalenol) content was determined by Dr. Pat Hart at Michigan State University.

POPULATION DYNAMICS OF THE FUSARIUM HEAD BLIGHT BIOCONTROL AGENT *CRYPTOCOCCUS NODAENSIS* OH182.9 ON WHEAT

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ABSTRACT

Cryptococcus nodaensis OH182.9 is a naturally occurring wheat anther colonist. Application of OH182.9 to wheat at anthesis reduced Fusarium head blight (FHB) severity by 56% and doubled 100-kernel weight in field trials. Confirmation of the ability of OH182.9 to survive on wheat heads prior to anthesis would support the possibility of applying OH182.9 earlier in crop development without sacrifice to efficacy. Biomass of OH182.9 was produced in liquid culture and applied to field and greenhouse grown wheat just prior to and during early anthesis. Populations of OH182.9 on extruded anthers were monitored for 10-12 d after application and on kernels harvested from treated heads. Significant populations of OH182.9 were recovered from all treated plants indicating that OH182.9 is able to survive in the absence of anthers. A significant increase in OH182.9 populations 4-8 d after application was observed suggesting that OH182.9 reproduced on the head. OH182.9 was recovered on kernels of treated wheat. Results of studies will be presented.

2003 UNIFORM FUNGICIDE PERFORMANCE TRIALS IN SOUTH DAKOTA

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ABSTRACT

Fusarium head blight (FHB – scab) has been a serious concern for wheat producers in South Dakota for ten years. 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, Oxen and Ingot, were planted at three South Dakota locations (Brookings, Groton, and South Shore/Watertown). Data were collected from two of three spring wheat study sites, Groton and Brookings, SD. Conditions were extremely dry at the third site and little disease developed. The winter wheat study site South Shore, was lost due to dry conditions at seeding. Trial treatments were all from the Uniform Fungicide Trial treatments list for the suppression of FHB and included an untreated check, Folicur at 4 fl oz/A, JAU6476 at 5.7 fl oz/A and 5.0 fl oz/A, JAU6476 at 3.6 fl oz/A + Folicur at 4 fl oz/A, and V-10116 at 6 or 8 fl oz/A. Trials were planted in a factorial randomized complete block design with six replications. Trial treatments were applied at anthesis. The following day, the crop was challenge inoculated with 10^4 macroconidia/ml of *Fusarium graminearum* 'Fg4'. The plots were misted for five minutes out of every twenty over a 14 days period. 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), deoxynivelanol (DON), grain yield, test weight, and protein. At Brookings, all treatments significantly reduced leaf disease as measured by a whole plot, green leaf rating. When assessed by percent necrotic leaf tissue, only the low rate of JAU6476 was inferior. Similarly, leaf rust was significantly reduced by all treatments except JAU6476. While head severity of FHB was not reduced by any treatment, incidence and total disease were significantly reduced by all treatments. FDK was generally not affected. Yield and test weight were increased by all treatments. Similar trends resulted at Groton. All treatments significantly reduced leaf disease as measured by a whole plot, green leaf rating or percent necrotic leaf tissue. Leaf rust was only controlled by V-10116 at the high rate. While head severity of FHB was not reduced by any treatment, incidence and total disease were significantly reduced by all treatments.

2003 UNIFORM TRIALS FOR THE PERFORMANCE OF BIOLOGICAL CONTROL AGENTS IN THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA

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INTRODUCTION AND OBJECTIVES

Biological control agents (BCAs) have several advantages in the suppression of Fusarium head blight (FHB or scab). When organic crops are grown, fungicide options are not available and crops such as barley are susceptible over a long period of time following head emergence and before maturity. As such, biological control has a good fit for FHB management under those conditions.

The objectives to this study were to evaluate the efficacy of various BCAs relative to the standard fungicide comparisons for the suppression of Fusarium head blight on wheat and barley.

MATERIALS AND METHODS

'Robust' barley was planted in a randomized complete block design with six replications and 'Oxen' hard red spring wheat were planted in a factorial randomized complete block design with four replications, both at Brookings, SD. Both spring wheat and barley were protected with isolates of *Bacillus subtilus*-type isolates SDSU-1BA and SDSU-1BC each of which were grown in either a potato dextrose broth or a defined medium intended to increase the production of iturin while reducing factors that might support growth of *Fusarium* on the plant surface; *Cryptococcus nodaensis* OH 182.9; *Bacillus*-type isolate TrigoCor 1448, and; *Lysobacter sp*. strains 'C3' and '7A4'. The BCAs were compared to a standard chemical treatment of Folicur (4 fl/oz/A) applied with Induce non-ionic surfactant (0.125%).

At initial anthesis of wheat or full head emergence of barley, the BCAs were applied to the heads as a spray of the growth medium + agent and allowed to dry. The following day, the plants were challenge inoculated with 10^4 macroconidia/ml of *Fusarium graminearum* 'Fg4'. The plots were also treated with Fg4 grown on corn grain inoculum.

Following challenge inoculation, a misting cycle was started for 5 minutes out of every 20 minutes, 24 hours a day for 14 days.

RESULTS AND DISCUSSION

During the inoculation period, the environment was very hot and dry, but the moisture was retained on the heads between misting cycles. No misting was done before heading, so the corn grain inoculum had a minimal influence. No differences were detected among the FHB disease measurements in either study

(Table 1 and 2). Similarly yields were not impacted by the treatments, including the fungicide treatment. However, DON was significantly reduced by the addition of TrigoCor 1448 as compared to the untreated control. For some reason, Folicur resulted in a significantly higher DON content and OH 182.9, SDSU 1BA and 1BC from either growth media resulted in significantly lower DON content as compared to the Folicur treatment.

Table 1. Disease control and yield components on spring wheat at Brookings[1].

Treatment	Fusarium Head Blight (FHB)			DON (ppm)	FDK (%)	Yield (bu/A)	Test Wt (lb/bu)	Protein (%)
	Incidence[2] (%)	Head Severity[3] (%)	Index[4] (%)					
Untreated	13.0	8.4	1.1	1.1	5	34.7	46.8	16.0
Folicur + NIS	14.0	8.9	1.2	1.5	5	34.6	47.3	15.9
7A4	15.0	9.1	1.4	1.4	4	34.3	46.3	16.1
C3	18.0	10.3	1.8	1.2	4.5	33.8	46.5	15.9
Trigo Cor 1448	21.3	9.2	2.1	0.5*	5.3	34.1	44.0	16.1
1 BA (defined)	19.0	9.6	1.9	1.1	4	34.0	45.1	16.1
1 BC (PDB)	12.5	8.6	1.0	1.0	4.5	34.6	47.4	15.9
1 BA (PDB)	12.5	7.3	0.9	1.0	4	35.8	46.0	16.0
1 BC (defined)	14.0	13.0	1.7	0.9	3.5	36.1	48.2	15.9
OH 182.9	11.0	8.4	0.9	1.0	4	34.9	46.0	16.0
LSD (P=0.05)	NS	NS	NS	0.4	NS	NS	NS	NS
CV	29.5	35.5	47.2	7.4	22.5	7.4	3.9	1.2

[1] Measurements of leaf disease were not significant.
[2] Incidence indicates the percentage of heads with any level of infection.
[3] Head severity indicates the percentage of disease on infected heads.
[4] Index indicates Incidence * Severity.

Table 2. Disease control and yield components on barley at Brookings 2003[1].

Treatment	Fusarium Head Blight (FHB)			DON[1] (ppm)	Yield (bu/A)	Test Wt (lb/bu)	Protein (%)
	Incidence[1] (%)	Head Severity[2] (%)	Index[3] (%)				
Untreated	69.5	9.5	6.5	3.0	85.6	40.0	12.2
Folicur + NIS	64.0	8.5	5.7	3.4	89.0	40.4	12.4
7A4	66.5	7.0	4.8	3.7	96.1	39.1	12.9
C3	64.0	11.3	7.5	2.9	88.2	40.1	12.3
Trigo Cor 1448	75.5	8.1	6.2	3.8	88.5	40.0	12.3
1 BA (defined)	71.5	8.9	6.6	2.8	89.3	40.2	12.7
1 BC (PDB)	67.0	10.2	6.9	2.9	90.7	39.1	12.5
1 BA (PDB)	64.0	8.0	5.1	3.3	88.2	40.7	12.7
1 BC (defined)	67.5	12.0	8.3	3.2	87.1	41.0	12.5
OH 182.9	62.5	7.0	4.5	2.9	87.9	40.1	13.0
1 BA	68.5	9.1	6.1	2.7	93.5	39.0	12.8
Untreated	67.0	9.0	6.0	4.1	86.7	39.5	12.3
Untreated	62.0	7.2	4.3	2.8	83.8	40.7	12.4
LSD (P=0.05)	NS	NS	NS	0.9	NS	NS	0.5
CV	14.8	33.0	40.7	21.1	6.5	3.6	27

[1] Measurements of leaf disease were not significant.
[2] Incidence indicates the percentage of heads with any level of infection.
[3] Head severity indicates the percentage of disease on infected heads.
[4] Index reflects Incidence * Severity.

GROUND SPRAY SYSTEMS AND SPRAY PARAMETER EVALUATION FOR CONTROL OF FUSARIUM HEAD BLIGHT ON A FIELD SCALE BASIS

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OBJECTIVES

Evaluate field scale spray systems with differing application technologies for enhanced control of Fusarium head blight (FHB).

INTRODUCTION

The production industry, growers and commercial pesticide applicators, often question the validity of research using small scale equipment. In the scientific community the validity of research methods receive constant review and revision. Reasons for using small scale equipment include a) parameter control, including reducing variance attributed to soil difference, b) the extensive time necessary to calibrate large spray booms, and c) the cost of the equipment necessary for conducting a research trial. At the request of the chemical and biological committee, and in response to the production industry, a trial was implemented at the Langdon Research Center to compare field scale spray systems and address the research from an interdisciplinary approach with collaboration between the agricultural engineering community and the plant pathology community.

MATERIALS AND METHODS

A study was planned as a randomized complete block design (RCBD) arranged as a factorial replicated six times to determine differences between spraying systems with differing application technologies with emphasis on droplet size. Due to limitations among the spray systems and time constraints to equate droplet size parameters necessary for the factorial, the data was analyzed as a RCBD. A foundation seed production field, planted to Alsen, was selected for the suitability of plot layout to the spray systems, soil uniformity (Cavour-Cresbard loam), and the site's proximity to the mixing and other facilities of the station. Four replicates had previous crop small grains and two replicates were previous crop potatoes. The site was partitioned into 72 plots 35 x 60 feet to accommodate the field scale equipment. All units sprayed each individual replicate and did an end run around the trial to the front to align for the subsequent replicate and to minimize potential of drip contamination to other plots. About 20 ft from the front and backside of each plot was swathed and discarded from all disease assessment and harvest results. Harvest data was collected from the interior 20 feet of the spray area from each respective unit with a Hege small plot combine. Recommended, NDSU Extension Service, HRSW production practices for the northeast North Dakota were followed. A Fusarium spawn was hand broadcast on each individual plot three weeks prior to flowering at about 300 grams per plot. FHB was visually assessed by sampling 20 spikes per plot and counting the spikes per head and the infected kernels per spike 20 days after spray application. Data is reported as

incidence and (field) severity. Yield, test weight, and protein were determined on all plots and the percent deoxynivalenol (DON) was determined on three replicates after it was determined from the untreated plots that DON was present. Coverage parameters were assessed by including a Day Glo orange fluorescent dye mixed at 3% v/v with the spray solution. Twenty spikes were collected from the first replicate of each treatment, transported to North Dakota State University in Fargo, photographed under incandescent and UV light on both sides of the spike to determine the area covered by the spray solution from each spraying system. Prior to spraying three sampling stands, with water and oil sensitive paper at grain heading height (1" x 2.75" cards, Spraying Systems Co.), were oriented horizontal and vertical back to back east-west and north-south and spaced to collect spray pattern samples from each spray unit. DropletScan, a product of WRK, Inc. and Devore Systems Inc., was used to analyze the water sensitive cards and generate a report. The field scale sprayers, technical assistance, and operation were provided by AGCO Corporation (Chris Mohning) and Hardi Inc. (Richard Hundt). Technical assistance and operation of a plot scale sprayer previously provided by Spray-Air Technologies Ltd. was provided by Bob Dawes of Degelman Industries. A fourth unit utilized a Proptec system (Ledebuhr Industries Inc.-WWW.PROPTEC.COM) on one boom and a Spraying systems hydraulic flat fan nozzle system mounted on a double swivel with nozzles angled 30 degrees downward from horizontal and oriented forward and backward on the opposite boom. The unit was provided and operated by Dr. Gary Van Ee, Agricultural Engineer from Michigan State University. The five spray units each sprayed two treatments and were compared to two untreated checks for a total of 12 treatments. The technical representatives were encouraged to operate their respective system as close to optimum performance conditions for one spray treatment and readjust the equipment to change droplet size as much as possible within the limitations of each spraying system. Dye and water were provided for testing prior to the trial initiation. A CCD camera was available for visual assessment of the coverage. Sprayers and parameters included:

1) AGCO's ESP (Energized Spray Process). The spray solution is delivered through a hydraulic spray boom mounted on a Spray-Coupe. The spray solution is energized with a negative electric charge (40,000 volts) that is attracted to the positively charged wheat spike. The charge creates a high-intensity electrostatic field between the nozzle and the plant that increases spray velocity and attracts the solution to the plant. The ESP sprayed fungicide at 10 gpa through XR8003 nozzles operating at 60 psi and XR8002 nozzles operating at 110 psi. No adjuvant was added to the spray solution by recommendation of the company representative.

2) Hardi Commander Plus 1200 80 ft Twin Force with Mustang 3500 Rate Controller. The spray solution is delivered by hydraulic nozzles, ISO yellow #2 110° tips, which spray into an air stream that delivers the solution to the target. The air stream, generated by a centrifugal fan and dispersed through a bag type manifold, was angled forward 30 degrees. Spray solution was delivered at 15 gpa to both treatments, nozzles spaced 20 inches 22 to 24 inches above the canopy. One treatment was sprayed at 45 psi with fan speed operating at 1750 rpm which would deliver a droplet size of between 160-240 volume mean diameter (VMD). The second treatment sprayed at 100 psi with fan speed of 2300 rpm which would produce a VMD of 160-240 VMD. Greater pressure should produce the droplets in the lower end of the VMD range.

3) Spray-air Technologies Ltd. The spray solution was delivered by CO_2 pressurized system. The solution was dispersed through a metering orifice at 27 psi spaced 10 inches apart angled 15 degrees forward from vertical at 9 gpa. The droplet is formed by wind shear picking the drop off the end of an orifice in the center of the air stream. The speed of the wind stream determines the droplet size with greater wind speed (increased static pressure) producing smaller droplets. The air stream is generated by a centrifugal fan and dispersed by a manifold to individual orifices.

4) Ledebuhr Industries Proptec. This system produces an air stream generated by hydraulic driven axial fans spaced 48 inches apart. Blade pitch is adjustable. The spray solution is delivered by hydraulic pump through a metering orifice. The droplet is generated by a rotary atomizer spinning at approximately 5400 rpm. The Proptec atomizer was operated at approximately 2000 psi with a 4 gpm hydraulic flow rate delivering spray solution at 10.4 gpa. This system generates an extremely fine droplet (approximately 125 micron VMD) in a 40 to 50 mph air stream.
5) Conventional F+B. The control system utilizes hydraulic nozzles, XR8001, angle 30 degrees downward and oriented to deliver the spray solution forward and backward to spray both sides of the spike. The nozzles were operated at 40 psi delivering 10.4 gpa spray solution.

The spray solution consisted of Bayer's experimental fungicide JAU 6476, prothiaconazole. This fungicide was selected because of its linear disease reduction as rate increases. A rate of 2.85 oz/acre, half the recommended rate, was used to measure differences between the spraying systems and application technology parameters. Induce adjuvant was mixed with all solutions at 0.125% v/v except the ESP sprayer. A mixing error reduced the fungicide rate in both treatments of the Hardi system by approximately 20% to a rate of approximately 2.3 oz/acre. 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

Spray application began at about 1:15 p.m. after the foliage had dried sufficiently to permit data collection. The final treatments were concluded by 6:00 p.m. Average wind speed ranged from 8.3 to 10.2 mph with occasional gusts exceeding 15 mph. Wind direction was WSW. Spray application commenced traveling from east to west minimizing drift between plots. Air temperatures ranged from 72 to 78° F and R.H. decreased from 65 to 55 % over the application period. FHB incidence was reduced by several of the treatments including both Spray-air and conventional treatments, the ESP with XR8003 nozzles with the coarse droplets, the Hardi with the fine droplets, and the Proptec angled at 45egree angle compared to the untreated (Table 1). Field severity and leaf disease was reduced by all fungicide applications compared to the untreated. The recommended conventional system had smaller leaf disease levels than the other conventional treatment. The Spray-air (fine droplet), both Hardi treatments, and the Electrostatic with coarser droplets had smaller leaf disease levels compared to the conventional system 36 inches above target, and the Proptec system angled 45 degrees downward. Both Spray-air systems had increased yield over the untreated, the conventional 36 inches above the target, and the Proptec angled 70 degrees downward. No significant differences were measured in test weight or protein. Deoxynivalenol (DON) levels were reduced below 0.5 ppm by all fungicide applications.

Total spike coverage was reduced by about ¼ when the conventional sprayer was operated above recommended height and by ½ when the angle of the Proptec was increased from 45 to 75 degrees (Table 1). Spike coverage on the front side ranged from a high of 63% with the conventional unit at recommended height to 13% on the Proptec angled 70 degrees downward. The greatest backside coverage was also the conventional at recommended height at 25% and the smallest coverage on the conventional 36 inches above target (3%) and Proptec 70 degrees downward.

CONCLUSION

This trial demonstrated the three major spray solution delivery systems, electrostatic, air stream, and hydraulic and further demonstrated three principal methods of spray atomization: hydraulic, wind sheer, and the rotary. The study indicates that operation and adjustment of each of the spray systems can affect one or more disease components and yield parameters. Water sensitive paper analysis indicated less but similar coverage compared to spike measurement (Table 2). The varying VMD indicates a wide range of choices between spraying systems and some variability when parameters of each individual spraying system changed. In most treatments a smaller droplet size was deposited on the back side of the deposition card compared to the front side indicating that a factor other than inertial impact contributed to deposition on the backside of the papers. Each of the spraying systems offers latitude to change application parameters as shown by GPA determinations from the conventional and Proptec systems (Table 2). Similar assessment can be made from the horizontal placed water sensitive paper which would affect leaf coverage and possibly leaf disease control (Table 3). Proper adjustment and operation is imperative to maximize the efficiency of all the systems. Coverage on the untreated indicates minimal intra plot spray drift.

One must conclude that all sprayers in this test successfully delivered the necessary fungicide dose to minimize disease infection. In the future, additional reductions or a range of fungicide rates may be necessary to measure sprayer differences. Measurement of the spray parameters indicates that further study to identify optimal adjustment factors to maximize spray coverage and fungicide efficacy should be undertaken. Evaluation on the appropriate fungicide rate for spray system and spray system parameter study should also be undertaken.

ACKNOWLEDGEMENTS

The study group wishes to acknowledge funding support of the USWBSI, and the cooperating companies, AGCO Corporation, Hardi Inc., Degelman Industries, Spray-Air Technologies Ltd. and company representatives Chris Mohning, Rich Hundt, and Bob Dawes. This material is based upon work supported by the U.S. Department of Agriculture, under Agreement Nos. 59-0790-3-079 and 59-0790-9-072. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

Table 1. FHB Incidence and field severity, leaf disease, yield, test weight, protein, and DON by spray systems, Langdon, 2003.

Spray System	Spray Parameters	FHB Incidence	FHB Field Severity	Leaf Disease	Yield	Test Weight	Protein	DON
		%	%	%	bu/acre	lb/bu	%	ppm
Conv. F+B	10” above target	23.3	0.8	10.2	60.6	60.8	14.6	<0.5
Conv. F+B	36” above target	22.5	0.8	15.9	60.0	60.4	14.4	<0.5
Electrostatic	Twinjet XR8003 @ 60 psi	20.0	0.8	5.7	62.1	61.1	14.4	<0.5
Electrostatic	Twinjet XR8002 @ 110 psi	36.7	1.4	10.4	60.4	60.5	14.8	<0.5
Hardi Twin	45 psi 2200 rpm	39.2	1.6	7.0	61.5	60.7	15.2	<0.5
Hardi Twin	95 psi 1800 rpm	23.3	0.8	8.3	62.5	60.6	14.2	<0.5
Proptec	Angled 45° down	28.3	1.0	13.0	63.5	60.7	14.8	<0.5
Proptec	Angled 70° down	32.5	1.2	9.1	59.1	60.5	14.7	<0.5
Spray Air	Static Pressure 15	23.2	0.8	10.1	64.6	60.9	14.5	<0.5
Spray Air	Static Pressure 25	29.2	0.9	6.7	64.0	60.9	15.1	<0.5
Untreated		50.8	2.7	23.0	60.3	60.2	14.6	0.8
Untreated		45.8	3.0	27.5	57.3	60.1	14.3	1.0
LSD*		14.2	1.0	5.6	3.8	NS	NS	0.3
% CV		39	64	40	5	1	6	28

* Significant at 0.05 probability level for mean comparisons

Table 2. Back, front, and total spike coverage and mean area, VMD, and GPA for front side and backside coverage placed vertically as measured on water sensitive paper by spray system, Langdon 2003.

Spray System	Spray Parameters*	Mean Spike Coverage**			WS Paper Front side Mean			WS Paper Backside Mean		
		Back	Front	Total						
		%	%	%	Area	VMD	GPA	Area	VMD	GPA
Conv. F+B	10” above target @10.4 gpa	24.91	63.18	44.04	38.7	577	7.9	18.2	481	6.0
Conv. F+B	36” above target @10.4 gpa	3.13	16.98	10.05	2.7	219	1.0	0.4	184	0.1
Electrostatic	Twinjet XR8003 @ 60 psi @ 10 gpa	12.62	28.02	20.32	15.3	478	5.2	6.7	472	2.6
Electrostatic	Twinjet XR8002 @ 110 psi @10 gpa	11.35	44.39	27.87	18.3	391	6.5	3.4	300	1.3
Hardi Twin	45 psi 2200 rpm @ 15 gpa	16.14	38.99	27.56	22.5	378	8.3	8.5	439	3.0
Hardi Twin	95 psi 1800 rpm @ 15 gpa	10.02	32.03	21.03	30.5	500	10.2	6.3	285	1.9
Proptec	Angled 45° down @ 10.4 gpa	7.36	21.52	14.44	22.4	270	7.6	2.5	136	0.7
Proptec	Angled 70° down @ 10.4 gpa	1.15	12.55	6.85	5.6	146	1.5	0.9	118	0.2
Spray Air	Static Pressure 15 @ 9 gpa	5.91	36.64	21.27	14.2	353	4.0	4.0	254	1.4
Spray Air	Static Pressure 25 @ 9 gpa	9.3	26.22	17.76	22.2	398	7.0	5.0	170	1.5
Untreated				0.07				0.03	87	.005
Untreated				0.25				0.01	121	.003

*Increased water volumes result in increased coverage parameters when other parameters remain constant.

**20 spike sample

Table 3. Mean area, VMD, and GPA for coverage as measured on water sensitive paper placed horizontally by spray system, Langdon 2003.

Spray System	Spray Parameters*	WS Paper* Horizontal Mean		
		Area	VMD	GPA
Conv. F+B	10” above target @10.4 gpa	30.5	474	10.5
Conv. F+B	36” above target @10.4 gpa	19.3	354	7.5
Electrostatic	Twinjet XR8003 @ 60 psi @ 10 gpa	55.4	614	11.7
Electrostatic	Twinjet XR8002 @ 110 psi @10 gpa	46.7	562	11.1
Hardi Twin	45 psi 2200 rpm @ 15 gpa			
Hardi Twin	95 psi 1800 rpm @ 15 gpa	73.0		
Proptec	Angled 45° down @ 10.4 gpa	21.6	260	7.6
Proptec	Angled 70° down @ 10.4 gpa	3.8	155	1.0
Spray Air	Static Pressure 15 @ 9 gpa	32.4	448	9.7
Spray Air	Static Pressure 25 @ 9 gpa	37.5	398	11.7
Untreated		<0.1	64	<0.1
Untreated		<0.1	61	<0.1

*Missing values due to coverage limitations of the water and oil sensitive paper.

ANALYSIS OF 2003 UNIFORM WHEAT FUNGICIDE TRIALS ACROSS LOCATIONS AND WHEAT CLASSES

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OBJECTIVE

The objective was to evaluate a common set of foliar fungicide treatments, across a range of environments, for effectiveness in managing Fusarium head blight (FHB) and deoxynivalenol (DON) accumulation in wheat.

INTRODUCTION

FHB is a potentially devastating disease that can result in serious economic losses for wheat producers, millers and endusers of wheat products. In addition, grain contaminated with DON, a mycotoxin frequently associated with FHB, can cause health problems in both humans and livestock. Thus, identifying fungicides that significantly reduce FHB incidence and severity in the field, and DON accumulation in harvested grain, would have widespread benefits to growers and endusers of all market classes of wheat. The Uniform FHB Fungicide Trials were established as a means of evaluating fungicide treatments that may be useful in FHB management programs, nationwide.

MATERIALS AND METHODS

Scientists from 11 states conducted 24 trials across a range of wheat classes (**Table 1**). Six fungicide treatments and a non-treated check where evaluated in each trial. All treatments were applied at early flowering (Feeke's stage 10.51) using a CO^2-pressurized sprayer, equipped with Twinjet XR8001 nozzles mounted at a 60^0 angle backward and forward. Many of the trials were inoculated with *Fusarium graminearum* and mist-irrigated in order to promote sufficient disease pressure and allow for discrimination among treatments. Plot size, crop husbandry, spray volume and pressure, sprayer type, and number of replications varied by location. Consult individual state trial reports for details. For all trials, FHB incidence, severity, index (i.e., plot severity), and *Fusarium*-damaged kernels (FDK) were measured using a more or less standardized protocol. DON accumulation was measured by one of the two USWBSI-funded DON Testing Laboratories.

Data were grouped and statistically analyzed according to whether they involved spring or winter wheat. Locations were treated as replications in the analysis. In a few instances, more than one wheat class or variety where grown at the same location. These were treated as separate experiments for the purposes of this summary. The non-treated control was not included in analyses for percent control in order to determine if there were significant differences among fungicide treatments.

RESULTS AND DISCUSSION

Spring Wheat- Data from eleven spring wheat trials are summarized in **Table 2**. FHB pressure was highly variable across locations. All treatments significantly lowered FHB incidence, severity, index and FDK compared to the non-treated control. Conversely, DON values were only reduced for the JAU6476 + Folicur combination treatment and both treatments of V-10116. Generally, Folicur applied at 4.0 fl oz /A was the least effective

fungicide treatment tested. However, no fungicide treatment consistently provided greater than 50% control of FHB or DON accumulation. Percent control values ranged from 32.1% to 66.5% across fungicide treatments and FHB parameters.

Winter Wheat- Data from nine winter wheat trials are summarized in **Table 3**. FHB pressure was heavy in most trials. All treatments significantly lowered FHB incidence, severity and index compared to the non-treated check. Conversely, no treatment resulted in significant control of FDK and only JAU6476 applied at 5.7 fl oz significantly lowered DON compared to the check. There were minimal performance differences among fungicide treatments for any parameter except DON accumulation, where significant differences were noted among treatments. In particular, Folicur at 4 fl oz provided significantly less DON control than other treatments, except for V-10116 at 8 fl oz. No fungicide treatment consistently provided greater than 40% control of FHB or DON accumulation. Percent control values ranged from 6.8% to 48.1% across fungicide treatments and FHB parameters.

Spring and Winter Wheat Comparison- When data were averaged by spring or winter growth habit, fungicide efficacy expressed as percent control, was significantly greater in spring wheat than in winter wheat (**Table 4**). The reason for this difference is unknown. It could be an artifact of the higher disease pressure in winter wheat trials, or it may be due to the longer grain-filling period associated with winter wheat crops. A longer gain-filling period would require fungicides to control FHB over a longer period of time. In any event, the possibility that fungicides are more effective in spring wheat should be monitored as it could play a significant role in fungicide label activities and/or the development of FHB management programs.

SUMMARY

Six fungicide treatments and a non-treated check were evaluated in 24 trials across 11 states as part of the 2003 FHB Uniform Fungicide Trials. Generally, all fungicides reduced FHB and DON compared to the check, but only minimal differences were detected among fungicide treatments. No fungicide treatment was found to be "head and shoulders" above the rest, and no active ingredient, fungicide combination, or rate consistently provided greater than 50% control of FHB or DON accumulation. Fungicides were significantly more effective managing FHB and DON in spring wheat than they were in winter wheat.

Table 1. States, principal investigator, institution, wheat class evaluated, and number of tests conducted, 2003 Uniform Fungicide Trial.

State	PI	Institution	Wheat class*	No. trials
AR	Gene Milus	Univ. of Arkansas	SRRW	1
IN	Greg Shaner	Purdue Univ.	SRRW	2
MD	Arv Grybauskas	Univ. of Maryland	SRRW	1
MI	Pat Hart	Michigan State Univ.	SRRW	1
MO	Laura Sweets	Univ. of Missouri	SRRW	2
MN	Char Hollingsworth	Univ. of Minnesota	HRSW	1
ND	Marcia McMullen	North Dakota State Univ.	Durum	2
			HRSW	4
NY	Gary Bergstrom	Cornell Univ.	SWWW	1
OH	Pat Lipps	Ohio State Univ.	SRRW	1
SD	Martin Draper	South Dakota State Univ.	HRSW	6
			Durum	1
VA	Erik Stromberg	VPI and State Univ.	SRWW	1

*SRWW = Soft red winter wheat
SWWW = Soft white winter wheat
HRSW = Hard red spring wheat

Table 2. FHB and DON results from eleven spring wheat trials, 2003 Uniform Fungicide Trials.

	% FHB Incidence		% FHB Severity		% FHB Index		% FDK		ppm DON	
Treatment	Mean	% Control	Mean	% Control	Mean	% Control	Mean	% Control	Mean	% Control
Non-treated……..	41.5a*	–	22.7a	--	9.2a	–	5.5a	–	4.3a	--
Folicur 3.6F…….. 4.0 fl oz + 0.125% induce	29.4b	32.1b	15.6b	35.3b	4.5b	49.1b	3.1b	33.0a	2.9ab	34.9a
JAU6476 480SC..… 5.7 fl oz 0.125% induce	23.9c	45.8a	11.1bc	54.4a	2.8c	68.3a	3.0b	40.0a	2.9ab	32.8a
JAU6476 480SC..… 5.0 fl oz + 0.125% induce	26.9bc	37.0ab	12.2bc	48.6a	3.2bc	62.8a	2.3b	47.2a	2.6ab	39.8a
JAU6476 480SC….. 3.6 fl oz + Folicur 3.6F 4.0 fl oz + 0.125% induce	23.7c	46.3a	10.4c	55.3a	2.8c	66.5a	2.2b	46.0a	2.3b	46.6bc
V-10116 1.67SC….. 6.0 fl oz + 0.125% induce	27.8bc	38.1ab	10.9bc	50.0a	3.2bc	65.1a	2.6b	38.9a	2.0b	54.3c
V-10116 1.67SC….. 8.0 fl oz + 0.125% induce	25.5bc	42.6a	10.7bc	53.2a	3.1bc	67.9a	2.5b	43.3a	2.1b	52.0bc
CV	19.3	21.5	29.5	24.2	62.0	15.9	52.4	29.9	43.3	34.4

*Means followed by a common letter are not significantly different (P=0.05, Student-Newman-Keuls); except for DON ppm, statistics were performed on arcsine-transformed data.

Table 3. FHB and DON results from nine winter wheat trials, 2003 Uniform Fungicide Trial.

	% FHB Incidence		% FHB Severity		% FHB Index		% FDK		ppm DON	
Treatment	Mean	% Control	Mean	% Control	Mean	% Control	Mean	% Control	Mean	% Control
Non-treated……..	57.9a*	--	38.9a	--	22.8a	--	23.9a	–	4.4a	--
Folicur 3.6F…….. 4.0 fl oz + 0.125% induce	50.3b	20.4b	32.7b	17.2a	16.1b	33.4a	19.9a	18.9a	4.1a	6.8b
JAU6476 480SC….. 5.7 fl oz + 0.125% induce	45.8b	29.1ab	31.3b	21.4a	13.2b	42.7a	14.5a	30.4a	2.5b	43.0a
JAU6476 480SC….. 5.0 fl oz + 0.125% induce	45.7b	29.3ab	29.0b	21.9a	13.1b	43.5a	18.4a	33.4a	3.0b	31.8a
JAU6476 480SC….. 3.6 fl oz + Folicur 3.6F 4.0 fl oz + 0.125% induce	44.7b	30.7a	30.1b	24.8a	12.7b	45.4a	18.0a	27.4a	3.0b	31.8a
V-10116 1.67SC….. 6.0 fl oz + 0.125% induce	48.1b	24.4ab	30.2b	24.6a	15.6b	36.8a	16.9a	26.4a	3.2ab	27.3a
V-10116 1.67SC….. 8.0 fl oz + 0.125% induce	46.7b	25.4ab	30.4b	27.4a	12.7b	40.8a	15.4a	31.2a	3.7ab	15.9ab
CV	9.9	28.9	19.1	62.4	31.2	29.0	17.3	28.0	39.8	35.5

*Means followed by a common letter are not significantly different (P=0.05, Student-Newman-Keuls); except for DON ppm, statistics were performed on arcsine-transformed data.

Table 4. Percent control of FHB and DON for combined fungicide treatments from eleven spring wheat and nine winter wheat trials, 2003 Uniform Fungicide Trials.

	% CONTROL RELATIVE TO NON-TREATED CHECK				
Wheat Type	**Incidence**	**Head Severity**	**Index**	**FDK**	**DON**
Spring Wheat	40.3a*	49.5a	63.3a	41.6a	37.9a
Winter Wheat	26.2b	22.9b	40.4b	28.0b	26.1b
Spring Advantage (%)	+14.1	+26.6	+22.9	+13.6	+5.9

*Means followed by a common letter are not significantly different (P=0.05, Student-Newman-Keuls); statistics were performed on arcsine-transformed data.

PERFORMANCE OF FOLICUR IN FUSARIUM HEAD BLIGHT UNIFORM FUNGICIDE TRIALS, 1998-2003

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OBJECTIVE

The objective of this report was to summarize the performance of Folicur in FHB Uniform Fungicide Trials since 1998.

INTRODUCTION

Folicur 3.6F (38.7% tebuconazole, manufactured by Bayer CropScience, Kansas City, MO) has been granted an emergency exemption (section 18) to manage Fusarium head blight (FHB) and deoxynivalenol (DON) in one or more states since 1999. In 2003, Michigan, Minnesota, Montana, North Dakota and South Dakota applied for, and were granted, an emergency exemption for this use. A significant FHB outbreak in several mid-Atlantic and Midwest states has resulted in additional states evaluating the possibility of applying for a Folicur section 18 in an attempt to manage future FHB/DON problems. This summary is a direct response to requests for information on how Folicur has performed in research plots since 1998. This report IS NOT a summary of all available data on the use of Folicur to manage FHB/DON; however, it is felt that a sufficient quantity of data has been summarized to accurately indicate the past and probable future effectiveness of Folicur for FHB/DON management.

MATERIALS AND METHODS

Data used in this summary were gleaned from 1998-2003 Proceedings of the National Fusarium Head Blight Forum, and from various individual reports where Folicur was evaluated for FHB management. For each report, percent control of FHB variables was calculated as follows:

% Control = non-treated mean – Folicur mean / non-treated mean x 100

Percent control for each variable in each test was classified into one of ten incremental ranges, and an overall percent control by Folicur was calculated.

RESULTS AND DISCUSSION

Folicur applied at early flowering suppressed FHB symptoms and DON accumulation in wheat (**Table 1**). Overall, Folicur reduced FHB field symptoms (i.e., disease index) by about 40%. Lower levels of control (~30% control) were associated with the grain quality variables, Fusarium damaged kernels (FDK) and DON. When data were classified into incremental ranges of percent control (**Table 2**), it was clear that efficacy results were highly variable across tests and years. This variability may be due to overwhelming disease pressure in some trials, and possibly greater efficacy in spring wheat than winter wheat (see 2003 Uniform FHB Fungicide Trials report). Suppression of FHB and DON by Folicur is well below the industry standard for fungicide efficacy of 90+%.

Nonetheless, due to the lack of a more effective fungicide for FHB management, Folicur is considered by some individuals to be a valuable FHB/DON management tool.

SUMMARY

In research plots, Folicur suppressed, but did not control (by industry standards), FHB symptoms and DON accumulation in wheat when applied at early flowering.

Table 1. Percent control of Fusarium head blight and DON by Folicur compared to the non-treated check as gleaned from spring and winter wheat research reports, 1998 - 2003*****

Average % control for Folicur relative to the non-treated check wheat

Incidence**	Severity	Index	FDK	DON
19.7	22.5	39.4	26.7	27.4

* Data summarized primarily from 1998-2003 Proceedings of National Fusarium Head Blight Forums.
**Incidence = proportion of heads with any FHB symptoms
Severity = % florets diseased for heads showing FHB symptoms (excludes heads without FHB symptoms).
Index = FHB plot severity = incidence x severity
FDK = Fusarium damaged kernels

Table 2. Distribution of percent control by Folicur relative to the non-treated check for FHB variables among the tests summarized in this report.

	Number of individual test means in each range				
Range of Control	Incidence	Severity	Index	FDK	DON
0-10%	24	22	5	8	10
11-20%	9	8	4	10	2
21-30%	13	5	6	5	11
31-40%	12	16	5	0	9
41-50%	3	3	11	7	6
51-60%	4	2	7	4	5
61-70%	0	2	7	0	2
71-80%	1	0	2	1	0
81-90%	0	0	1	1	2
91-100%	0	0	0	0	0
Number of Tests	66	58	48	36	47

*Data summarized primarily from 1998-2003 Proceedings of National Fusarium Head Blight Forums.
**Incidence = proportion of heads with any FHB symptoms
Severity = % florets diseased for heads showing FHB symptoms (excludes heads without FHB symptoms).
Index = FHB plot severity = incidence x severity
FDK = Fusarium damaged kernels
DON = Deoxynivalenol

PERFORMANCE EVALUATION OF AERIAL APPLIED FUNGICIDES

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ABSTRACT

Fusarium head blight has caused considerable economic damage to small grain crops in the U.S. and Canada. This has caused producers to spray their fields with fungicide, which has produced mixed results over the years by both aerial and ground application. About 50% of the fungicide is being applied by air, and aerial applicators are looking for answers to improve application methods.

Two aerial application trials were completed in eastern North Dakota during the 2003 growing season. These trials were completed in producer fields near St. Thomas and Hunter, ND. Replicated spray applications were completed at the growth stage (Feekes 10.51) recommended for optimum fungicide efficacy. Plots were 100 ft. or more in width and either 850 or 1000 ft. in length. Four treatments were compared to an untreated check at both locations. The treatments included applying fungicide in one application and making two passes across a field in opposite directions at one half fungicide rate and at one half the amount of carrier. Both spray applications were conducted with large and small droplets. Multiple applications were theorized to improve the coverage to both sides of the grain head. Folicur fungicide was applied at 4 fl.oz./ac. in all treatments in both trials. A harvest sample was obtained by cutting one combine header width near the center of the plot over the length of the plot. Yield data was computed from plot weights recorded from a weigh wagon. A sub-sample was taken from the weigh wagon to test for deoxynivalenol (DON), test weight, and protein. No significant differences in FHB incidence, field severity, DON level, leaf disease, yield, test weight, and % protein were measured at St. Thomas. Significant differences in FHB incidence, yield and test weight were measured at the Hunter site. Coverage data was obtained by adding a food grade fluorescent dye to the spray tank and using an ultra-violet light to illuminate the dye on the grain heads. The spike coverage data did show a significant difference at Hunter but not at the St. Thomas site. Spray drop size information was obtained by using WRK DropletScan . This gives the VMD (volume median diameter) drop size, area of coverage and an estimate of the gallons per acre applied based upon the spray drops on water sensitive cards. In 2003, FHB infection was very low.

UNIFORM FUNGICIDE TRIALS ON FHB OF WHEAT AND BARLEY IN MINNESOTA

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OBJECTIVE

To provide a non-biased evaluation of experimental Fusarium head blight (FHB) chemical control products for hard red spring wheat and spring barley. Cooperatively, the multi-state uniform fungicide trial effort will indicate which fungicide compounds are most effective in reducing disease severity across diverse environments.

INTRODUCTION

Fusarium head blight was originally described more than a century ago (Stack, 2000). Since that time the disease has been responsible for severe and repeated epidemics on small grain crops (Sutton, 1982; McMullen et al., 1997; Steffenson, 1998; Windels, 2000) that have resulted in billions of dollars in crop losses (McMullen et al., 1997; Wood, 2002). The disease is 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 infection of FHB pathogens is largely dependent on weather conditions. Cultural disease management strategies (i.e.: crop rotation, tillage and field sanitation) have offered producers partial control. Likewise, moderate disease control has been achieved with fungicide application. Ongoing research of new, experimental fungicides is needed to further reduce small grain crop yield and quality losses from FHB.

MATERIALS AND METHODS

Wheat and barley experiments were planted in randomized complete block designs with four replicates at the Northwest Research and Outreach Center in Crookston on 28 April 2003. Cultivars 'Oxen' hard red spring wheat and 'Robust' spring barley were seeded at 1.25 mil. live seed/acre and 1.375 mil. live seed/acre, respectively. Plots were inoculated with 250 g of *Fusarium graminearum* infested corn grain five weeks after planting. Night-cycle mist irrigation was initiated the day after inoculation and continued until plants began to senesce. Misting was discontinued temporarily during the growing season when weather events caused standing water at the testing site. Weeds were controlled as needed.

Treatments were applied to wheat nine weeks after planting [Feekes 10.51 growth stage (early flowering)] and to barley eight weeks after planting [Feekes 10.4 (early heading)]. Foliar applications were made with a CO_2 backpack type sprayer adjusted to 40 psi at 18-20 gpa with forward and backward facing nozzles. Disease severity responses were noted 22 and 25 days after treatment application from wheat and barley, respectively. Tests were harvested 15 weeks after planting.

Fusarium head blight severity was estimated based on the visual scale from Stack and McMullen (1995), while percent visually scabby kernels (VSK) was estimated using a set of standards provided by R. Jones

based on his recent publication (Jones and Mirocha, 1999). Percent leaf disease was estimated using James (1971). Grain sample 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

Hard Red Spring Wheat: Weather conditions during anthesis were conducive for *F. graminearum* infection and disease progression. As expected, the nontreated control had the most severe disease response for all categories tested (Table 1). In seven of the nine categories noted, the JAU6476 + Folicur treatment significantly reduced FHB and leaf disease. Compared with other treatments, V-10116 6 fl. oz. had most reduced VSK and DON levels and most increased 1000-kernel weights. Those treatments controlling disease to a lesser extent were V-10116 8 fl. oz., JAU6476 5.0 fl. oz., and JAU6476 5.7 fl. oz. The least effective fungicide treatment at controlling FHB was Folicur 4 fl. oz.

Spring Barley: Severe weather conditions during Feekes growth stage 10 (booting) caused plants in all replicates to lodge. Plant lodging occurred approximately five days before treatments were applied. Therefore, data must be 'weighed' against the 'percent plot lodged' rating (Table 1) before assessing treatment effectiveness. Control plots had the least plants lodged (58.8%) which resulted in reduced disease severity compared with Folicur 4 fl. oz. treated plots (81% lodged). In four of nine parameters noted, the three JAU6476-containing treatments (JAU6476 5.0 fl. oz.; JAU6476 5.7 fl. oz.; and JAU6476 + Folicur) significantly reduced FHB and leaf disease. Compared with other treatments, V-10116 8 fl. oz. controlled FHB head severity, FHB field severity, and DON levels to the greatest extent in spite of its 80% lodging score.

ACKNOWLEDGEMENTS

We would like to thank the U.S. Wheat and Barley Scab Initiative and the Northwest Research and Outreach Center for supporting these studies, as well as Bayer CropScience (Folicur and JAU6476) and Valent U.S.A. (V-10116) for providing fungicide materials.

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Table 1. FHB and leaf spot disease responses from 'Oxen' hard red spring wheat in Crookston, MN.

	Fusarium Head Blight							
Treatment[1]	*HS*[2] *(%)*	*I (%)*	*FS (%)*	*VSK (%)*	*DON (ppm)*	*LDS*[3] *(%)*	*Yield (bu/A)*	*Test Wt. (lb/bu)*
Nontreated control	5.9	98.5	35.7	17.5	15.0	3.4	52.0	53.8
Folicur 3.6F 4 fl oz	4.3	91.5	18.9	8.0	11.2	2.1	65.9	55.4
JAU6476 480SC 5.7 fl oz ...	3.6	73.0	13.9	10.0	12.9	2.4	64.4	55.5
JAU6476 480SC 5.0 fl oz ...	3.6	85.0	11.9	5.0	11.3	2.0	72.0	57.3
JAU6476 480SC 3.6 fl oz + Folicur 3.6F 4 fl oz	3.1	73.5	8.7	5.0	9.1	1.7	73.9	57.3
V-10116 1.67SC 6 fl oz	3.5	84.0	11.8	4.5	6.7	2.2	73.1	56.9
V-10116 1.67SC 8 fl oz	3.8	83.0	14.0	5.0	7.4	1.8	74.0	56.5
$LSD_{0.05}$	*0.3*	*16.5*	*10.1*	*4.8*	*3.2*	*0.3*	*8.4*	*1.2*

[1]Each fungicide treatment included 0.125% Induce. Treatment abbreviations are HS: head severity; I: incidence; FS: field severity; VSK: visually scabby kernels; LDS: leaf disease severity.
[2]Square root transformation.
[3]Log transformation. Foliar diseases consisted of Stagonospora blotch (*Stagonospora nodorum*) and Tan spot (*Pyrenophora tritici-repentis*)

Table 2. FHB and leaf spot disease responses from 'Robust' spring barley in Crookston, MN.

		Fusarium Head Blight							
Treatment[1]	*% Plots Lodged*[2]	*HS*[3] *(%)*	*I (%)*	*FS (%)*	*DON (ppm)*	*LDS*[4] *(%)*	*Plump Kernels*	*Yield (bu/ac)*	*Test Wt (lb/bu)*
Nontreated control	58.8	4.8	100	24.7	36.2	2.7	73.2	88.4	41.6
Folicur 3.6F 4 fl oz	81.3	5.6	100	35.4	37.8	2.9	71.9	90.4	41.2
JAU6476 480SC 5.7 fl oz	86.3	4.6	100	24.1	25.5	2.7	76.6	96.1	42.6
JAU6476 480SC 5.0 fl oz	77.5	4.1	100	19.1	28.2	2.7	78.1	104.1	42.5
JAU6476 480SC 3.6 fl oz + Folicur 3.6F 4 fl oz	86.3	4.3	100	21.7	23.1	2.5	76.2	95.4	42.1
V-10116 1.67SC 6 fl oz	63.8	4.6	100	23.7	28.0	2.7	76.8	93.5	41.8
V-10116 1.67SC 8 fl oz	80.0	4.0	100	17.1	22.5	2.6	75.9	99.8	42.3
$LSD_{0.05}$		*0.4*	*NS*	*NS*	*8.0*	*0.4*	*NS*	*NS*	*NS*

[1]Each fungicide treatment included 0.125% Induce. Treatment abbreviations are as follows: HS: head severity; I: incidence; FS: field severity; VSK: visually scabby kernels; LDS: leaf disease severity.
[2]Severe weather caused plants to lodge between 21-24 June, 2003; approximately five days before treatments were applied. Plot lodging assessments were taken six weeks after plants went down.
[3]Square root transformation.
[4]Log transformation. Foliar diseases consisted of Septoria speckled leaf blotch (*Septoria passerinii* and *Stagonospora avenae* f. sp. *triticea*), net blotch (*Pyrenophora teres*) and spot blotch (*Cochliobolus sativus*).

DIFFERENTIAL RESPONSE OF BARLEY, HARD RED SPRING WHEAT, AND DURUM WHEAT TO MULTIPLE FHB INFECTIONS AND FUNGICIDE TREATMENTS

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ABSTRACT

Wheat is most vulnerable to infection by *Fusarium graminearum* during anthesis, while spring barley becomes vulnerable to infection at early head emergence. If prolonged periods of favorable weather for infection occur after these growth stages, multiple infections may occur, resulting in lower yield and quality and higher deoxynivalenol (DON) than a single infection at optimum infection timing. Greenhouse studies in Fargo in 2001-2002 showed that with multiple infections, split applications of reduced rates of Folicur (tebuconazole) across multiple growth stages in spring wheat and durum did not significantly improve disease control over a single treatment of the full label rate applied at anthesis; ie. one optimum timing of fungicide may be sufficient in these two crops. However, in these trials, FHB field severities were only moderate for spring and durum wheat (untreated 13.3%, 34.1%, respectively), and very low in barley (untreated 2.8%). Additional tests were needed to determine response under more severe disease pressure.

In 2003, hard red spring and durum wheat, and spring barley were exposed to single or multiple (2 to 3) inoculations and fungicide applications in the greenhouse: prior to flowering (Feekes 10.3); early flowering (Feekes 10.51); or kernel watery ripe (Feekes 10.54). *F. graminearum* (10,000 spores/ml) was atomized onto grain heads at the test growth stage. For fungicide treatments, Folicur or AMS21619 (prothioconazole, Bayer experimental) was applied approximately four hours before inoculation, either at full rate or half rate, using a track sprayer equipped with XR8001 flat fan nozzles oriented forward/backward at 60^0 from the vertical. After inoculation and fungicide applications, plants were placed in a mist chamber for 48 hours and then returned to the greenhouse. Disease severity was evaluated at the soft dough stage.

FHB field severity in 2003 was greater (untreated up to 77%) than in the 2001-2002 tests. The higher level of disease provided greater separation of treatment effects. For hard red spring and durum wheat, a single full rate application of either fungicide at anthesis, in combination with three separate inoculations, provided similar FHB control to the two half rate applications of either fungicide in combination with two inoculations (58% vs 61% reduction). However, in barley, if three inoculations were applied, a single fungicide application applied at early full head emergence could not control the disease; disease levels were 26-38% higher than the untreated check exposed to two inoculations. Thus, multiple infection events in barley may have to be controlled with split applications of fungicide at multiple timings, whereas in spring wheat and durum, a single application of the full rate of fungicide may be adequate for FHB reduction.

ACKNOWLEDGEMENT

Funding was provided through USDA-ARS upon the recommendation of the U.S. Wheat & Barley Scab Initiative

ALTERNATIVES TO INCREASE AERIAL SPRAY DEPOSITS FOR FHB CONTROL

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OBJECTIVE

Assess promising aerial application practices and methods that could increase spray deposits on wheat heads.

INTRODUCTION

Fusarium head blight (FHB) has emerged as a major disease of wheat and barley in several major small grain production regions in the U.S. Cultural practices, resistant cultivars, and fungicides have made only limited impact on managing the disease (Parry et al. 1995). General observations from the literature show limited effectiveness and erratic results from fungicide applications to control FHB (Milus and Parsons 1994, Wilcoxson 1996, Shaner and Buechley 1999, and Gilbert and Tekauz 2000). This suggests that there are a number of factors that influence effectiveness of fungicidal applications that must be more thoroughly understood and coordinated before producers can rely on chemical control as an effective tool in FHB management. More detailed studies of these factors are needed to provide producers with options for effectively managing this disease of wheat and barley.

In the absence of robust disease control measures such as resistant cultivars and effective cultural or biological control practices, it is likely that some of the more conventional approaches could be optimized to help provide producers with technologies for FHB management or control. Spraying and dusting pest and disease control agents have been practiced in many crops for many years. Aerial application is an economical and effective means of applying materials to large acreages in a timely manner. Access and crop damage from other distribution means can also necessitate aerial application. Timeliness of application of chemical control materials is often a factor in effectiveness of the measures undertaken. Aerial application methods have been optimized and used more extensively in insect and weed control practices than in disease management. It is reasonable to expect that aerial application methods can be optimized for application of fungicides to wheat and barley heads for control of FHB. Numerous studies have been reported on optimization of aerial application practices for pest control in cotton, corn, and weeds and brush (Bouse et al. 1992, Carlton et al. 1995, Hoffmann et al. 1998, and Kirk et al. 1988, 1992, 1998 and 2001). Similar approaches were proposed to optimize aerial application technologies for improved deposition of disease control materials on wheat heads.

MATERIALS AND METHODS

Based on experience with aerial application and consultation with FHB researchers, aerial application methodologies were selected to assess technologies that could offer improved spray deposits on wheat heads. These methodologies were implemented in three field studies in 2003. The aerial treatments were applied with an Air Tractor AT-402B (Figure 1) in 3-replication randomized blocks, with individual treatment plots of 3 to 5 acres. Aircraft hardware and spray performance variables were documented based on

ASAE Standards (2003), S327.2 FEB03 and S572 AUG99. These standards define Volume Median Diameter, ($D_{V0.5}$), Droplet Spectra Classification (DSC), and other pertinent spray parameters. Weather parameters were monitored and recorded during all spray applications with a Gill 27005 UVW Anemometer, Young 43372VC Relative Humidity and Temperature Probe, and a Campbell 21-X data logger. Water-based spray mixes contained equal per-acre rates of the fluorescent tracer, Caracid Brilliant Flavine FFN, and a surfactant, Triton X-100, to monitor spray deposits and simulate a reasonable spray mix. Five ten-head wheat samples were taken at two locations in each treatment replication. Mylar plate samplers, soda straw samplers, and Water-Sensitive-Paper (WSP) samplers in two orientations were each placed in five positions across the center swath at two locations in each treatment replication (Figure 2). Spray mix samples, mylar plate samplers, and wheat head samples were analyzed spectrofluorometrically for tracer content and deposition. WSP samples were analyzed by computerized image analysis to characterize spray deposits. Deposit data were analyzed statistically to determine treatment effects.

Spray rate and droplet size with conventional aerial hydraulic nozzles to optimize deposits on wheat heads – A range-finding study was conducted to determine the influence of spray rates of 2, 5, and 10 gpa and spray droplet sizes of 250 and 400 µm volume median diameter ($D_{V0.5}$) on spray deposits on wheat heads. These parameters describe the general range of normal aerial application practice. The 250 µm droplet sizes were applied with CP-03 nozzles, DSC = F; and the 400 µm droplet sizes were applied with disc orifice straight stream nozzles, DSC = M. Study protocols similar to those of Bouse et al. 1992 and Kirk et al. 1992 were used in the study.

Spray rates with aerial rotary atomizers to optimize deposits on wheat heads – Previous research has shown that small droplets deposit more effectively on small targets and large droplets deposit more effectively on large targets (Kirk et al. 1992). A study with Curtis Dyna-Fog ASC rotary atomizers that produce a relatively narrow range of small droplets was conducted to exploit the small droplet – small target phenomenon. The rotary atomizers were operated at blade setting 6 for maximum no-load rpm of 9000 at 130 mph. The three treatments for the study were 20, 40, and 60 psi (flow-control orifice removed) for spray rates of approximately 3, 5, and 7 gpa, respectively, and DSC's of VF, F, and F, respectively. A study protocol similar to that used by Kirk et al. 1993 was used for the study.

Multi-direction flight paths to increase deposits on both sides of wheat heads – Several FHB researchers indicated that fungicidal sprays need to be deposited on all sides of wheat or barley heads to maximize protection of the head. Limited small-plot research with ground sprays reported by Hart et al. 2001 indicate that sprays from both sides of the plots reduced the severity of FHB and reduced levels of deoxynivalenol. Four treatments: 5 gpa in 2 spray passes in opposite directions on same swath = 10 gpa, DSC = VF; 5 gpa, 1 pass, DSC = VF; 5 gpa, 1 pass, DSC = F; and 10 gpa 1 pass, DSC = F.

Figure 1. AT-402B applying treatments to wheat.

Figure 2. Artificial samplers for deposit analysis.

RESULTS AND DISCUSSION

Aerial treatments, spray rates, and estimated spray droplet size spectrums are detailed in Table 1 along with measured deposits on wheat heads, mylar plates, and soda straws. Mylar plates were oriented horizontally at the top of the crop canopy and the soda straws were inclined at 45º and also placed at the top of the canopy, Figure 2. The primary objective of these studies was to assess various aerial technologies that would give highest spray deposits on wheat heads. Consequently, the brief discussion presented here will primarily highlight those treatments, along with data from artificial samplers that support observations of increased deposits on wheat heads. The highest observed deposits on wheat heads and artificial samplers were from rotary atomizers with a 5 gpa spray rate. The Fine and Very Fine sprays gave higher deposits than the Medium sprays; the lowest observed deposits on wheat heads were for the Medium DSC sprays, regardless of spray rate. The effects of spray rate on deposits on wheat heads are not particularly definitive, except that Medium sprays at 2 gpa resulted in lower deposits than the other treatments. Two aerial spray passes in opposite directions over the same swath did not give as much total deposit on wheat heads as the same amount of tracer applied in a single pass. A more detailed analysis of the spray droplet spectra as measured from wind tunnel studies and from deposits on water-sensitive paper samplers may provide a better understanding of reasons for the observed treatment differences in deposits on wheat heads. However, the more important issue is whether the aerial treatments that give higher spray deposits will also give improved control of FHB.

Table 1. Aerial spray deposit parameters for three field studies, Buffalo Ranch, Burleson County, TX, 2003

Treatment	Spray Rate, gal/acre	Droplet Size, $D_{V0.5}$, µm, (DSC)	[a]Deposits on Wheat Heads, µg/cm^2	[a]Deposits on Mylar Plates, µg/cm^2	[a]Deposits on Soda Straws, µg/cm^2
Spray rate and droplet size with conventional aerial hydraulic nozzles					
CP-03, 55º, 0.061 , 40 psi, 130 mph	2	249 (F)	0.25 bc	0.21 ef	0.12 de
CP-03, 90º, 0.125 , 20 psi, 130 mph	5	231 (F)	0.17 ef	0.23 def	0.16 e
CP-03, 90º, 0.171 , 40 psi, 130 mph	10	249 (F)	0.25 bc	0.30 bcd	0.26 cd
D-8 SS, 20º, 50 psi, 130 mph	2	415 (M)	0.14 fg	0.17 f	0.12 e
D-8 SS, 20º, 50 psi, 130 mph	5	415 (M)	0.17 ef	0.33 bc	0.18 de
D-8 SS, 20º, 50 psi, 130 mph	10	415 (M)	0.18 def	0.35 b	0.16 e
Spray rates with aerial rotary atomizers					
ASC, No Disc, 20 psi, 130 mph	3	220 (VF)	0.24 bcd	0.26 cde	0.27 cd
ASC, No Disc, 40 psi, 130 mph	5	240 (F)	0.36 a	0.47 a	0.42 a
ASC, No Disc, 60 psi, 130 mph	7	261 (F)	0.21 bcde	0.27 bcde	0.20 de
Multi-direction flight paths					
CP-03, 90º, 0.078 , 54 psi, 100 mph	5	304 (VF)	0.21 bcde	0.22 def	0.37 ab
CP-03, 90º, 0.078 , 54 psi, 100 mph	[b]2@5 =	304 (VF)	0.10 g	0.16 f	0.31 bc
CP-03, 90º, 0.171 , 40 psi, 130 mph	10	249 (F)	0.27 b	0.26 cde	0.47 a
CP-03, 90º, 0.125 , 20 psi, 130 mph	5	231 (F)	0.24 bcd	0.20 ef	0.42 a

[a] Deposit measurements are amounts of the tracer dye deposited per unit of projected area on the respective samplers. Deposits in a single column followed by the same letter or same group of letters are not significantly different, á = 0.05.

[b] Treatment composed of two 5 gpa spray passes over the same swaths in opposite directions for 10 gpa.

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RESULTS OF THE UNIFORM FUSARIUM HEAD BLIGHT FUNGICIDE TEST ON WINTER WHEAT IN OHIO, 2003

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OBJECTIVE

Evaluate a common set of fungicide treatments, as determined by the Chemical and Biological Control committee of the Wheat and Barley Scab Initiative, for efficacy against Fusarium head blight in Ohio. Additionally, to evaluate the effect of varying rates and application timing on efficacy of certain fungicides.

INTRODUCTION

Fusarium head blight, caused by *Fusarium graminearum* and other *Fusarium spp*. occurs in most spring and winter wheat growing regions of the world. Although progress is being made in the development of resistant cultivars, most cultivars grown in the US are either susceptible or have a degree of susceptibility that permits yield loss and/or accumulation of DON in the grain. Wheat producers are interested in chemical control options as an additional tool for preventing yield losses. The Chemical and Biological Control Committee of the Wheat and Barley Scab Initiative establishes protocols for evaluating a set of fungicides each year. These materials are evaluated by researchers in a number of different states on several different classes of wheat in order to develop a data base for possible federal registration of the fungicide and to develop data on which recommendations for their use can be made. Ohio State University has been a cooperator in these evaluations since 1998 and this is a report of our results for 2003.

MATERIALS AND METHODS

Seeds of Elkhart wheat, treated with Raxil-Thiram (tebuconazole 0.6% and thiram 20%, 3.5 fl. oz./cwt), were planted at the rate of 24 seeds/ft of row on 18 October, 2002 in Ravenna silt loam at the Ohio Agricultural Research and Development Center near Wooster, OH. Prior to planting, the field was moldboard plowed, then 75 lb/A of ammonium nitrate was broadcast over the field and incorporated with a disc. The experimental treatments were arranged in a randomized block design with five replicate blocks. Each experimental unit consisted of a 7-row plot, 15 ft long with 7 in. between rows. Additional nitrogen was applied on 27 Mar 03 as 240 lb of ammonium nitrate. Plots were inoculated by broadcasting *F. graminearum* colonized corn kernels (0.12 oz/sq ft) over the plot surface on 6 May. The plot was mist irrigated using NAAN 7110 series bridge with mist sprayer head 327122 fitted with nozzles having 0.35 in. openings that provided 10.2 GPH. Plots were misted each day from 1 wk prior to flowering to 2 wk after flowering (23 May to 12 Jun). The mist irrigation operated for 2.5 min out of each 10 min from 6:00 to 10:00 a.m. and from 8:00 to 10:00 p.m. each day. Fungicides were applied as sprays with an adjuvant (Induce, 0.125% v/v) in 33.4 gal water/A with a CO_2-pressurized back pack sprayer with a constant boom pressure of 40 psi and 15 in. between twinjet XR8001VS nozzles mounted at a 60 degree angle forward and backward. Sprays were applied on 27 May, 30 May or 2 June at heads one-fourth emerged, heads three-quarters emerged and flowering growth stages corresponding to Feekes' growth stage (GS) 10.2, 10.4 and 10.5.1, respectively. Plots were assessed for Fusarium head blight (FHB) on 20 June by determining the percentage of spikelets affected per head of 20 heads in each of five locations in each plot.

Severity was calculated as the average percentage of affected spikelets per head and incidence was calculated as the percentage of heads with disease. Plots were harvested on 30 Jul with a Hege 140 plot combine. Yield (bu/A) was determined from harvested grain adjusted to 13.5% moisture. Harvested grain was visually assessed for the percentage of Fusarium damaged kernels.

RESULTS AND DISCUSSION

Daily mist irrigation favored disease development in spite of relatively cool temperatures during and 1 wk following anthesis (mean daily temperature 58.8° F) resulting in very high disease incidence (mean treatments ranged from 83% to 100%) (Table 1). Rain occurred on 19 of the 40 days between when disease assessments were made and plots were harvested. Frequent rain prevented harvest and kept the heads almost continuously wet during grain maturation. By harvest the grain was severely deteriorated resulting in very low yields (range 17.8 to 36.1 bu/A) and test weights (range 26.6 to 34.9 lb/bu) (Table 1).

Based on analysis of variance, the effect of treatment was significant for all disease assessments, percentage damaged kernels, yield and test weight. Results of the DON analysis of the grain is not yet available. All treatments, except Folicur 3.6 EC alone, had significantly lower FHB severity and FHB index, as well as significantly higher yield and test weight than the untreated control.

The impact of fungicide treatment on yield and test weight was difficult to determine due to the severe deterioration of the grain while in the field before harvest. Regardless, those treatments that had lower FHB severity levels and lower FHB index, generally also had statistically lower percentage of damaged kernels, and higher yield and test weight. Both the JUA6476 and V10116 materials appeared to be superior to Folicur in reducing the effects of Fusarium head blight in this experiment. Additionally, there did not appear to be a difference among the rates used for either material or among different application timings for JUA6476. Greater differences in percentage damaged kernels, yield and test weights may have occurred had protracted wet conditions not prevail prior to harvest. This was the first year we had seen this level of grain deterioration in over 20 years of conducting fungicide trials on wheat in Ohio.

Table 1. Efficacy of fungicides for control of Fusarium head blight of wheat in Ohio, 2003.

Treatment, rate/A	Application timing*	FHB Incidence (%)	FHB Severity (%)	FHB Index**	Damaged kernels (%)	Yield*** (bu/A)	Test weight (lb/bu)
Folicur 3.6 EC, 4.0 fl oz	GS10.5.1	100	36	36	50	21.4	28.5
JUA6476 480 SC, 5.7 fl oz	GS10.5.1	99	20	20	19	27.0	31.8
JUA6476 480 SC, 5.0 fl oz	GS10.5.1	95	26	25	56	28.0	31.2
JUA6476 480 SC, 3.6 fl oz + Folicur 3.6 EC, 4.0 fl oz	GS10.5.1	96	26	25	42	30.2	31.1
V10116 1.67 SC, 6.0 fl oz	GS10.5.1	94	23	22	32	31.1	33.0
V10116 1.67 SC, 8.0 fl oz	GS10.5.1	95	19	18	23	34.1	33.8
V10116 1.67SC, 4.0 fl oz	GS10.5.1	97	29	29	45	27.4	31.3
JUA 6476 480 SC 5.7 fl oz	GS10.2	100	20	20	25	32.9	34.9
JUA6476 480 SC 5.7 fl oz	GS10.4	95	20	20	21	36.1	33.9
JUA6476 480SC, 2.85 fl oz Then JUA6476 480 SC, 2.85 fl oz	GS10.4 GS10.5.1	94	21	21	41	29.0	32.0
JUA6476 480SC 3.56 fl oz + Follicur 43 SC 3.98 fl oz	GS10.5.1	83	20	17	28	31.5	33.4
Untreated		100	39	39	60	17.8	26.6
LSD (P =0.05)		13	10	10	29	5.1	4.2

* Treatments applied at Feekes' growth stage (GS) 10.2 (June 27), 10.4 (June 30), or 10.5.1 (30 May)

** Fusarium head blight (FHB) index = (FHB incidence*FHB severity)/100.

*** Yield based on 13.5% moisture at 58 lb/bu.

COMPARISON OF AERIAL APPLICATION WITH GROUND APPLICATION OF FOLICUR FUNGICIDE FOR THE CONTROL OF FUSARIUM HEAD BLIGHT (FHB) IN DURUM WHEAT

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ABSTRACT

Fusarium head blight has caused severe economic losses in durum wheat in north central North Dakota in recent years. Decreases in yield and quality have resulted in millions of dollars of lost income. At this time, adapted durum cultivars are very susceptible to FHB. Cultivars with tolerance to FHB are not available, so efficient fungicide application is essential to control the disease.

Both aerial and ground applicators are interested in improving application, as acres protected by fungicides are increasing. Due to the importance of both ground and aerial application to North Dakota growers, further studies on the efficiency of both methods in applying fungicides to durum heads for FHB control was completed.

During the 2003 growing season, a trial was conducted comparing a ground sprayer and a spray plane in a commercial durum field north of Kenmare, N.D. A ground sprayer applying 18 GPA with twin jet nozzles was compared to an airplane applying 5 GPA with CP nozzles. The spray strips were 120 ft. wide by 1000 ft. long. All treatments, including the untreated check, had three replications. For each replicate, one pass was made with the ground sprayer applying a 96 ft. wide swath and the airplane made two passes with each pass being 45 ft. wide. Fungicide treatments included one application of 4 fl oz/acre of Folicur, both by ground and airplane, plus a split application of 2 fl oz/acre of Folicur by ground and air, applied 4 days apart. The full rate and first split application were made at Feekes 10.51, anthesis, while the second split application was made 4 days later at the end of flowering. FHB field severity was evaluated at soft dough stage. FHB did not develop in this field because of arid conditions following anthesis. Durum yields were determined by harvesting a 30-foot strip from each plot with a combine and weighing in a weigh wagon. A sub-sample was taken from the weigh wagon for deoxynivalenol (DON) analysis. All fungicide treatments increased yield by 2 to 4 bushels per acre compared to the untreated check. However, an ANOVA showed no significant difference in yield between the treatments or the untreated check. The DON levels for all treatments were below 0.5ppm.

WHEAT UNIFORM FUNGICIDE TRIALS, ND, 2003

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OBJECTIVE

To evauate experimental fungicides for control of Fusarium head blight (scab) and leaf diseases 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). 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. North Dakota continues to participate in these trials and tests fungicides at several locations across grain classes and cultivars.

MATERIALS AND METHODS

A uniform set of six fungicide treatments were evaluated on hard red spring and durum wheat in ND in 2003 (Table 1). Fungicides tested included Folicur (tebuconazole), which had a Section 18 exemption for use on wheat in ND in 2003, JAU6476 (prothioconazole), an experimental fungicide from Bayer CropScience, and V-10116, an experimental product from Valent. 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 Langdon 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. Plots were in a Randomized Complete Block design and data were statistically analyzed across locations using ANOVA.

The uniform trial was done 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 were evaluated over two wheat classes and six cultivars: Fargo, Oxen hard red spring wheat; Carrington, Russ hard red spring wheat and Mountrail durum wheat; Langdon, Alsen hard red spring wheat, Grandin hard red spring wheat, and Lebsock durum; Minot, Mountrail durum.

RESULTS AND DISCUSSION

All sites, except for the Minot location, had some level of FHB infection, from a low of 5.1% field severity in Alsen at Langdon, to a high of 10.2% in Oxen at Fargo. Because of the absence of FHB at Minot, the Minot data is not included in the summary Table 1. All fungicide treatments significantly reduced FHB field severity over the untreated check. The higher application rate of the JAU6476 product resulted in the lowest FHB field severity. All fungicide treatments also significantly reduced DON and FDK. All fungicide treatments significantly reduced the level of leaf disease from the untreated check. Yields were significantly increased by fungicide treatments, from 9.6 to 13.0 bushels, increases that are economic. Test weights also were significantly increased by fungicide treatments.

Table 1. Effect of fungicides on fungal leaf disease and FHB field severity, DON, FDK, yield and test wt., averaged across locations and wheat classes, North Dakota 2003.

Treatment and rate/acre[1]		FHB FS[2] %	DON[3] ppm	FKD[4] %	Leaf disease[5] % severity	Yield Bu/A	Test wt Lbs/bu
Untreated check		8.6 a	2.7 a	5.9 a	47.5 a	60.0 c	59.5 b
Folicur 3.6F	4.0 fl oz	3.7 b	1.5 b	3.1 b	14.3 b	70.0 b	60.9 a
JAU6476 480SC	5.7 fl oz	2.0 c	1.0 b	2.5 b	12.1 b	72.2 ab	61.4 a
JAU6476 480SC	5.0 fl oz	2.6 bc	0.9 b	2.4 b	11.0 b	69.9 b	61.4 a
JAU6476 480SC 3.6 fl oz + Folicur 3.6F	4.0 fl oz	2.7 bc	0.7 b	1.9 b	9.7 b	73.1 a	61.3 a
V-10116 1.67SC	6.0 fl oz	3.1 bc	1.2 b	3.2 b	19.2 b	69.7 b	60.9 a
V-10116 1.67SC	8.0 fl oz	2.5 bc	1.2 b	2.5 b	13.8 b	70.4 b	61.1 a

Numbers followed by different letters are significantly different at the 95% confidence level, using LSD analysis.

[1] All fungicide treatments had 0.125% Induce added; JAU6476 (= AMS21619) is an experimental fungicide from Bayer; V-10116 is an experimental fungicide from Valent

[2] FHB FS = Fusarium head blight field severity; field severity = incidence x head severity; ratings from all sites except Minot which did not have visible FHB

[3] DON (deoxynivalenol = vomitoxin) levels were only available from Carrington and Fargo at time of this report; All DON levels at Minot were <0.5 ppm and were not included in the summary

[4] FDK = Fusarium damaged kernels; data from Fargo and Langdon sites, only

[5] Leaf spot diseases primarily tan spot and Septoria leaf spot complex

All fungicides tested were effective in controlling FHB and leaf diseases and in improving yield and quality. Differences among fungicides generally were not significant, however. The high rate of JAU6476 did result in the lowest FHB field severity, while the combination treatment of JAU6476 and Folicur resulted in the lowest DON and significantly higher yield than some treatments.

ACKNOWLEDGEMENT

Funding was provided through USDA-ARS upon the recommendation of the U.S. Wheat & Barley Scab Initiative.

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INFLUENCE OF METHODICAL AND TECHNOLOGICAL ASPECTS ON THE CONTROL OF FUSARIUM HEAD BLIGHT IN WHEAT

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OBJECTIVES

The tests were made at full coverage of the heads by fungicides. In these tests based on latest experiments the effect of epidemic severity and cultivar resistance will be studied on the efficacy of fungicides. A way will be presented, how we can use the same experiment to demonstrate the efficacy of the same fungicides against leaf diseases. An attempt will be made to see, how the yield loss from these micro-pot test can be transferred to larger experimental plots. We would give further details on AMS 21619 and its combinations.

INTRODUCTION

Among the fungicides used for the control of FHB until now the tebuconazole, metconazole and bromuconazole were identified with larger effect against the disease (Mesterházy 1996, 1997, 2001). However in our tests the tebuconazole containing fungicides with higher rate were the most effective, bromuconazole and metconazole were only of medium effect at lower rates applied in Hungary. Bromuconazole was only medium, but from metconazole (Caramba) also higher rates were tested. A part of the results was made public last year (Mesterházy and Bartók 2001, 2002). In the last years extensive investigations were made with the new Bayer experimental fungicide, signed as AMS 21619 in US or JAU 6476 in Europe, now the DON data are also available. Besides its efficacy the question was also what would be the best formulation and rating of the product. For this reason also leaf rust was rated, this was the most abundant, leaf spots and powdery mildew were only in traces registered.

MATERIAL AND METHODS

Experimental design, spraying and inoculation technique and way of evaluation for traits and don were described by Mesterházy et al. 2002 and 2003. From 2001 48 hrs wet period by bagging was given as under dry conditions the 24 hrs coverage was not always suitable. 2001, 2002 and 2003 were very dry and hot, rain was only 30-50 % of the usual amount following inoculation. No additional mist irrigation was given. After harvesting the groups of heads, the ten heads for a group were separated and the not used heads were collected for the whole plot and threshed. Their mass was given to the plot yield harvested by combine. By this way comparable yield data were gained as from every plot 150 heads were separated for the Fusarium evaluation. Earlier results showed that efficacies for different traits can be different, therefore the mean efficacy characterizes better the fungicide than any of them separately. For this reason the mean efficacies (FHB, FDK, DON, and Yield loss) will be given. Active ingredients of the fungicides used for a L product: Folicur Solo: 250 g tebuconazole, Falcon 465 EC: 167 g tebuconazole, spiroxamine 250 g + triadimenole 43, Kolfugo Super carbendazime 200, Caramba SL metconazole 72 (1.2 l/ha) and 90 (1.5 l/ha), Juwel: Kresoxym-methyl 125 + epoxyconazole 125, Stratego: trifloxystrobin 125 + propiconazole 125EC, Sphera: trifloxystrobin 188 + cyproconazole 080 EC, Prosaro (AMS 21619 125 EC + HWG 125EC 1.0.

RESULTS AND DISCUSSION

In 2001 the results (Table 1) the efficacies are very good, their mean is 91 % for the best fungicide Prosaro that is a composite of AMS 21619 and tebuconazole. This was necessary as AMS 21619 has lower efficacy against leaf rust. The results show that Prosaro is excellent also against leaf rust. Its general efficacy (91 %) is significantly better than that of the Folicur Solo, the best fungicide against FHB until now. Falcon 0.8, the most used fungicide in Hungary against FHB has 68 % mean efficacy. The newer fungicide are more effective, the 22 % efficacy difference to Prosaro 1.0 l/ha is very convincing. As cultivar resistance improves only slowly, the new fungicide will be important for the farms where fungicide application is a general practice. For longer time strobilurines were low rated against FHB. Their combinations with more effective active agents brought improvement also in this respect, like HEC combinations. The average efficacy for different traits is divergent, best is for FHB and yield loss, less for DON and FDK. The correlations between FHB traits are very close, all are significant at P = 0.1 % indicating that improvement in FHB control is proportional with the response of other traits.

The means, however, overlap significant response differences. For this reason the data of the susceptible Zugoly are presented for FDK in Table 2. It is remarkable that three isolates have near the same FDK values, but the fungicide had significant differences in effect. At the 12551 isolates the FDK could be decreased to 1 % from about 60 %, but the picture at 44 F. graminearum isolate was different. Here Caramba and Falcon become ineffective, only the Prosaro variants, Folicur Solo and AMS 21619 could significantly reduce the FDK. This is important; because here we had a very severe epidemic situation and Prosaro 1.0 and AMS 21619 could decrease FDK lower than 20 %. This shows clearly that for the most sensitive cultivars at very heavy epidemic a really good solution has not been found. When we think on the recent devastating epidemics in US and Canada, the lesson is clear. And Zugoly is not the most susceptible cultivar ever seen. In a two years study (Mesterházy, unpublished) mean DON contamination for Zugoly was 45 ppm, but for the German Contra 247 ppm. At more resistant cultivars we do not have this problem in FHB control.

In 2002 we had similar results as ranks, but the efficacy become higher, the less effective fungicides had in this year higher values. We introduced first the Folicur Solo karbendazim mixture that contains the double amount of tebuconazole we had in the Falcon mixture. The result was very similar to Prosaro 1 l/ha, but somewhat weaker. The infection severity was lower than in 2001. The most distinct effect was measured at DON. In the plot yield data the total yield is included except the 150 heads used for FHB evaluation. Leaf rust was significant only at the Fusarium check and Kolfugo, the others gave excellent protection. As leaves were healthy the yield differences between fungicides are not only consequence of the control of leaf diseases. Folicur Solo caused 3 % yield increase; with limitation we can consider this the result of the disease control on the leaves. Caramba, Juwel and Folicur Solo+Kolfugo caused more than 10 % that significantly differs from the Folicur Solo yield. We think that this is a regulator effect that occurs at many triazole fungicides with the positive alterations in cytokinin metabolism. When this is consequent, it can increase the profitability of fungicide use in farms, even diseases occur at low severity.

From 2003 the DON data are not yet available; data are shown in Table 4. Again, the two Prosaro treatments are the best, the Folicur Solo + carbendazime mixture is not better, but in this year it is not always better than Folicur Solo alone. As Prosaro is better, it has no reason to test further this mixture. Until Prosaro does not come to market, the carbendazime mixtures might have some advantages.

CONCLUSIONS

The AMS 21619 containing fungicides are superior to all other fungicide used to control FHB. Their efficacy is about 90 % or more, but in more humid years the efficacy will be possibly lower. It seems that the Fusarium tests allow the measuring of yield response of the wheat also for leaf diseases, independently from Fusarium. By this way the economics of testing fungicides can be increased. It seems also that several fungicides have significant regulator effect (yield increase) than can make the use of some fungicides more profitable when it is stable.

Table 1. Fungicide tests in wheat, summary for 2001, general means across cultivars and isolates (12 situations)

Treatment and rates/ha	FHB %	DON ppm	Yield loss %	FDK %	Mean FHB efficacy %	Leaf rust %	Eff. leaf rust %
Prosaro 1.0	0.60	1.66	3.9	5.61	90.81	3.00	95.95
AMS 21619 125 EC 0.8	0.92	1.88	3.5	6.59	88.68	32.52	56.10
Prosaro 0.8	0.99	2.29	1.1	7.44	86.96	2.56	96.55
Folicur Solo	1.14	4.09	4.8	9.08	80.99	1.74	97.65
HEC+AMS21619 1.0	1.43	3.17	9.5	9.93	81.95	10.96	85.20
Caramba 1.2	2.28	3.94	7.7	12.94	75.58	6.70	90.95
Falcon 0.8+Kolf. S.1.5	1.39	6.16	6.5	13.80	72.41	3.59	95.15
HEC+HWG 1.5	1.93	5.81	5.8	14.47	71.13	2.26	96.95
Falcon 0.8	2.91	5.55	12.3	14.91	68.34	5.37	92.75
Stratego 1.0	3.08	5.58	8.8	18.81	65.69	21.78	70.60
Kolfugo S 1.5	5.71	7.74	17.0	22.56	49.42	58.15	21.50
Fusarium check.	12.02	15.58	17.2	38.54	0.00	74.07	0.00
Mean	2.87	5.3	8.17	14.56		18.56	81.76
LSD 5 %	1.82	0.49	2.05	2.97	5.58	3.49	
Mean fung. efficacy %	83.08	72.07	83.07	67.89	75.63		81.76

Correlations for Table 1

Traits	FHB %	DON ppm	Yield loss %
DON ppm	0.9529		
Yield loss %	0.8244	0.7933	
FDK %	0.9714	0.9793	0.8438

Table 2. Epidemic situations for the susceptible cultivar Zugoly, FDK values, 2001.

Fungicide	Zugoly				
	12551 FcA	12551FcB	44FgA	44FgB	Mean
Prosaro 1.0	0.56	1.11	0.00	14.44	4.03
Jau 0.8	0.00	2.56	0.33	8.44	2.83
Prosaro 0.8	1.33	1.44	0.22	34.44	9.36
Folicur Solo	0.50	2.00	0.67	24.44	6.90
Hec+Jau 1	4.44	10.22	0.22	21.11	9.00
Caramba 1.2	13.44	11.11	4.11	62.78	22.86
Falc 0.8+ Kolf 1.5	1.78	7.22	0.67	57.78	16.86
Hec 0.75 F250 069	5.11	17.78	1.78	62.22	21.72
Falcon 0.8	3.44	5.00	0.89	51.67	15.25
Sfera 1.0	17.00	18.33	2.67	60.00	24.50
Stratego	10.22	15.89	1.44	46.67	18.56
Kolfugo S. 1.5	38.89	25.56	4.44	69.44	34.58
Fus. check.	62.22	58.89	5.00	63.33	47.36
Mean	11.72	13.72	1.73	44.54	17.93
LSD 5 %					6.17

Table 3. Fungicides against FHB in wheat, 2002. General means across cultivars and isolates.

Fungicides and rates l/ha	FHB%		FDK	Yield loss	DON	Leaf	Plot yield % to	Mean - efficacy
	FHB%	AUDPC	%	%	ppm	rust %	check	%
Prosaro 1.0	0.40	4.51	0.77	3.85	0.79	0.22	106.01	91.74
Fol. Solo 1.0+Kolf. S 1.5	0.43	3.80	1.83	6.07	0.92	0.33	112.96	87.39
Prosaro 0.8	0.94	9.26	1.30	2.41	0.99	0.78	106.40	92.35
Folicur Solo 1.0	1.06	10.33	1.80	5.95	2.17	0.56	103.75	84.18
Caramba 1.5	1.70	17.74	1.53	4.92	1.61	0.44	109.41	86.50
Falcon 0.8	1.72	17.53	0.82	5.31	1.95	1.67	105.74	85.90
Juwel 1.0	2.97	32.22	1.86	5.56	1.92	0.22	111.13	83.37
Sfera 1.0	5.63	61.17	4.07	6.62	3.64	2.89	106.68	73.35
Kolfugo S 1.5	7.15	81.75	3.73	6.76	3.65	31.11	104.29	71.71
Fusarium check	23.88	294.27	25.79	18.10	10.87	52.22	100.00	0.00
Mean	4.17	48.42	3.95	5.96	2.59	8.22	96.94	68.77
LSD 5 %	1.12	12.13	1.41	3.44	0.87	3.91	4.12	5.98
Mean fungicide efficacy	89.7	91.0	92.3	70.8	81.9	91.8	-7.38	

	FHB%	AUDPC	FDK	Yd loss	DON
AUDPC	0.9996				
FDK	0.9795	0.9839			
Yield loss %	0.9620	0.9644	0.9719		
DON ppm	0.9924	0.9912	0.9742	0.9706	

All significant at P = 0.1 %

Table 4. Fungicide control of FHB in wheat, general means across.

	cultivars and isolates, 2003		
Fungicide	Traits		
	FHB %	FDK %	Yield loss %
Prosaro 1.0	1.75	2.42	6.41
Prosaro 0.8	2.31	2.68	8.10
F. Solo 1.0 +Kolf. 1.5	2.49	5.13	12.15
Falcon 460EC 0.8	2.82	5.71	14.17
Folicur Solo 1.0	3.05	5.18	11.80
Caramba 1.2	3.29	11.31	13.21
Caramba 1.5	4.49	7.50	12.55
Juwel 1.0	4.92	10.50	14.31
Tango Star 1.2	4.93	7.00	13.43
Kolfugo 1.5	15.81	20.77	22.78
Fusarium check	20.72	26.52	32.52
Mean	5.55	8.73	13.45
LSD 5 %	2.15	3.13	4.19

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EFFICACY OF FUNGICIDES ON FHB OF SOFT RED WINTER WHEAT IN ARKANSAS, 2003

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INTRODUCTION

Identifying fungicides that reduce incidence and severity of Fusarium head blight (FHB) in the field and levels of damage and mycotoxins in the grain could have widespread benefits to growers and users of all market classes of wheat in the event of FHB epidemics. This test in Arkansas is part of the Uniform Fungicide Trial that is coordinated by the Chemical and Biological Control Committee, and the objective is to hasten the integration of fungicides and biocontrols that are effective against FHB into cost-effective and environmentally-safe wheat disease management strategies.

MATERIALS AND METHODS

The moderately susceptible wheat cultivar 'Agripro Patton' was planted at the University Farm at Fayetteville on 10 October 2002. Seed was treated with Dividend fungicide (1 fl oz / cwt) for loose smut and seedling diseases and Gaucho insecticide (3 fl oz / cwt) for aphids and barley yellow dwarf. Individual plots were 7 rows by 13 ft. Plots were fertilized with a total of 100 lb nitrogen as ammonium nitrate (75 lb applied on 4 March and 25 lb applied on 2 April). Ryegrass and broadleaf weeds were controlled with recommended herbicides. Infested corn kernel inoculum of *Fusarium graminearum* was applied to the plots on 4 and 14 April for a total of 6 kernels / sq ft. Fungicides were applied in a randomized complete block design with six replications on 28 April when 50% of the main stems had begun to flower. The mist system operated for eight 10-minute periods between midnight and 8:00 am for nine nights between 29 April and 13 May. On 27 May, 50 heads per plot were sampled randomly and evaluated for FHB incidence and head severity, and plot severity was calculated. Plots were harvested with a plot combine on 13 June, and grain was passed once through a seed cleaner before test weight and percentage of scabby grain were measured.

RESULTS AND DISCUSSION

As measured by plot severity and incidence of infected heads, all treatments except V-10116 at 6 fl oz significantly reduced FHB (Table 1). Only three treatments significantly reduced infected head severity. There were no differences among treatments for percent scabby grain and test weight. Differences among treatments for yield were complicated by stripe rust and Septoria leaf blotch epidemics late in the season. Folicur and JAU6476 appeared to be more effective against these diseases than V-10116. Rainfall totaled 8.37 inches during April and May, but most of the rain came in late May after irrigation ceased. This high rainfall favored FHB and Septoria tritici blotch.

Table 1. Effects of fungicides on FHB variables, yield, and test weight of wheat in Arkansas.

Product and rate per acre	Plot severity (%)	Incidence of infected heads	Infected head severity (%)	Scabby grain (%)	Yield (bu/ac)	Test wt. (lbs/bu)
JAU6476 480SC 3.6 fl. oz. + Folicur 4 fl. oz. + 0.125% Induce	13.1a	0.74a	17.7a	32a	56.2a	47.9a
Folicur 3.6F 4 fl. oz. + 0.125% Induce	13.8a	0.82ab	16.7a	31a	50.4ab	47.3a
V-10116 (1.67 SC) 8 fl. oz. + 0.125% Induce	15.3ab	0.84b	18.2ab	35a	47.9bc	44.1a
JAU6476 480SC 5.7 fl. oz. + 0.125% Induce	15.4ab	0.76a	20.4abc	29a	51.8ab	46.8a
JAU6476 480SC 5.0 fl. oz. + 0.125% Induce	17.7b	0.83b	21.4bc	28a	51.9ab	48.4a
V-10116 (1.67 SC) 6 fl. oz. + 0.125% Induce	18.8bc	0.89bc	21.0bc	32a	49.0abc	46.1a
Non-treated check	22.3c	0.93c	24.0c	33a	42.0c	48.9a
CV (%)	19.4	8.5	16.2	14.2	13.3	10.3

Values within a column followed by the same letter are not significantly different by LSD at P = 0.05

SPLIT APPLICATION OF FUNGICIDES FOR INCREASED CONTROL OF FHB AND DON ON BARLEY

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ABSTRACT

Fusarium head blight (FHB) has reduced the quality of barley grown in the Midwest for the last decade due to fungus infected kernels, pinched grain and most importantly the presence of the toxin, deoxynivalenol (DON). Individual cultural and chemical control measures have reduced disease, but have been unsuccessful in getting the level of control necessary for the requirements of the malting barley industry. It is likely that the reduced control with fungicides is due to the long period during which the barley plant is susceptible to infection (flowering through to harvest) and the relatively short period of effectiveness of the fungicides used for control. Field experiments were to find a rate of multiple fungicide application that is both effective and economic. The fungicides tested were Folicur (Bayer CropScience), and JAU 6476 (Bayer CropScience experimental). Both 6-rowed Robust and 2-rowed Conlon were used as they are common barley cultivars sown in the Midwest and they differ in the amount of disease and DON they experience under the same environmental and inoculum conditions. In 2003 at Bottineau ND, fungicides were applied at the recommended rate either split between two applications at 50-75% headed and fully headed or in a single application at fully headed. Due to environmental conditions, FHB was too low to be assessed and only one plot had a positive DON reading. Flag leaf disease was 4.0 % on Conlon and 6.0% on Robust. All timings of both fungicides on Robust and Conlon reduced flag leaf disease compared to the untreated control. Test weight in Robust was increased by both timings of both fungicides but yield was unaffected. In 2003 at Langdon ND, fungicides were applied at recommended and 2x recommended rates, either in one application or split between two applications at awns emerged and fully headed. Percentage of kernels infected by FHB was low, with Robust having 1.3% in the untreated and Conlon having 2.6%. In Robust, split application of the recommended rate or single application of 2x recommended rate of JAU 6476 reduced disease. In Conlon, the single application of the recommended rate and the double application of the recommended rate of Folicur reduced disease as did the single application of 2x recommended rate of JAU 6476. DON levels were significantly lower in untreated Conlon (0.95 ug/g) than Robust (2.28 ug/g). None of the treatments reduced DON in Conlon, but in Robust DON was reduced by a single application of 2x recommended rate of Folicur and both rates and timings of JAU 6476. Yield was unaffected by all treatments. The experiment at Bottineau demonstrates that the fungicides do not influence yield when there is very low levels of disease, but they may control leaf disease. At Langdon, disease was higher but still regarded as low, and under those conditions selected treatments reduced both disease and DON, but there was no significant advantage to using a split application of fungicide. These experiments need to be repeated under a range of environments and disease pressures.

USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT 1: *IN VITRO* AND FIELD TESTING OF THE EFFECT OF UV PROTECTANTS ON FHB ANTAGONISTS

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OBJECTIVES

To 1) discover UV-protectants that enhance survival of FHB antagonist *Bacillus subtilis* OH 131.1 after UV exposure *in vitro* and 2) evaluate the influence of a lignin-based UV protectant on the efficacy of OH 131.1 and the yeast *Cryptococcus nodaensis* OH 182.9 in field tests.

INTRODUCTION

In previous research, we have demonstrated the potential of several antagonists including *B. subtilis* OH 131.1 and *C. nodaensis* OH 182.9, 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., 2002ab; Khan et al., 2003). We have developed an effective frozen biomass concentrate that can be commercially produced, and have produced a more commercially feasible, consumer friendly dried product that maintains CFU/ml but is less effective in reducing FHB, possibly due to the cryoprotectant in the dried product stimulating pathogen activity (Schisler et al., 2002b).

Formulation research, in terms of UV protectants (Behle et al., 1996; McGuire et al., 2001), may be crucial for the continued development of these biocontrol strains from bench discovery to biocontrol product. UV light is particularly devastating to the survival of vegetative microbial cells. A water soluble sodium salt of lignin (Westvaco PC1307, experimental product) and the optical brightener Blankophor BBH (Sigma, Detroit, MI) have demonstrated UV protectant activity (McGuire et al., 2001) but their effect on FHB antagonist survival and efficacy was unknown prior to these studies.

MATERIALS AND METHODS

UV protectants (Westvaco PC1307 and Blankophor BBH) were tested, *in vitro*, for their ability to aid survival of dried cells of OH 131.1 when exposed to artificial sunlight supplied from a xenon light source (Suntest Atlas CPS solar simulator, Heraeus DSET Laboratories Inc., Phoenix, AZ). Cells of antagonist OH 131.1 were grown in flasks containing a semidefined liquid medium (SDCL), harvested from 24 h growth cultures, combined or not with UV protectants, added as microliter droplets of formulated cells to 96 well microtiter plates, air-dried for 1 h or not, and exposed or not to 6 h of UV light. Cell counts at the time of introduction to microtiter plates were approximately 2 x 10^8 CFU/ml. Lignin concentrations were 0.2% and 0.3%, and BBH concentrations were 0.5%, 1.0% and 2.0%. Wells were rehydrated with weak

growth medium and the growth of surviving cells determined over time using a plate-reading spectrophotometer at 620 nm (Fig. 1). Predicted absorbance values (proportional to cell biomass concentration) were determined by weighted linear regression analysis of growth curves.

Because UV protectants enhanced OH 131.1 survival *in vitro*, OH 131.1 and yeast antagonist *C. nodaensis* OH 182.9 were tested with and without the lignin UV protectant in field trials at Peoria, IL and Wooster, OH. Inoculum of OH 131.1 and OH 182.9 was produced using SDCL medium with a carbon:nitrogen ratio of 11 and total carbon loading of 14 g carbon/liter (Schisler et al., 2002a). The soft red winter wheat cultivars Pioneer 2545 (susceptible) and Freedom (moderately resistant) were used at both locations. Biomass was harvested from Fernbach shake flasks and applied at the beginning of wheat flowering (Schisler et al., 2002a). Bacterial and yeast suspensions contained 50 % fully colonized broth (~$1x10^8$ CFU/ml and ~$5 x 10^7$ CFU/ml, respectively) and were applied at a rate of 20 gal/acre. The fungicide Folicur 3.6F was applied at the recommended rate. Controls were untreated plants and plants treated with buffer/wetting agent only. Corn kernels colonized by *Gibberella zeae* (Schisler et al., 2002a) were scattered through plots (~25-40 kernels/m^2) two weeks prior to wheat flowering and mist irrigation provided periodically for approximately one week after treatment application. Heads were scored for disease incidence and severity 2 to 3 weeks after treatment using a 0-100% scale. Data for the deoxynivalenol content of grain and 100 kernel weight is being tabulated (ongoing). Randomized complete block designs were used in both trials (n=5 at both locations). Analysis of variance, linear regression and Fisher's protected LSD or Student's t-test (p#0.05)(JMP 4.0 statistical software) were used as appropriate to separate treatment means in these studies.

RESULTS AND DISCUSSION

In vitro, lignin and BBH enhanced the survival of dried cells of OH 131.1 vs. controls at all concentrations tested when cells were exposed to 6 h of UV as demonstrated by higher predicted absorbance (due to higher cell biomass) in wells during active cell growth 30 h after rehydration (Table 1). For 6 h UV treated wells, approximately 2 to 3 times as much cell biomass was present in UV protectant-amended wells as in unamended wells. The effect of UV protection was not necessarily improved as the concentration of protectants increased. UV protectants also enhanced OH 131.1 cell survival in wells that did not receive UV light, possibly due to reducing cell viability losses due to drying and rehydration stress. UV protectant amendment of fresh OH 131.1 cells generally had no influence on cell growth (data not shown).

Wheat treated with OH 131.1 or yeast OH 182.9 had decreased FHB severity and incidence vs. one or both controls in Peoria, IL and Wooster, OH field trials but the lignin UV protectant did not consistently influence antagonist efficacy (Table 2). Lignin (0.3%) amendment did not influence the efficacy of either antagonist in the Peoria, IL trial while lignin increased efficacy (OH 131.1 on Freedom), decreased efficacy (OH 131.1 and OH 182.9 on Pioneer 2545), or had no effect (OH 182.9 on Freedom) in the Wooster, OH trial. Repetition of these studies with washoff-resistant formulations of UV protectants and testing of additional UV protectants would clarify if these compounds can be used to enhance the efficacy of FHB antagonists in the field.

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ACKNOWLEDGMENTS

This project was made possible, in part, by funding provided by the U.S. Wheat and Barley Scab Initiative.

Fig. 1. Droplet Drying Method to Screen Cell Survival Factors

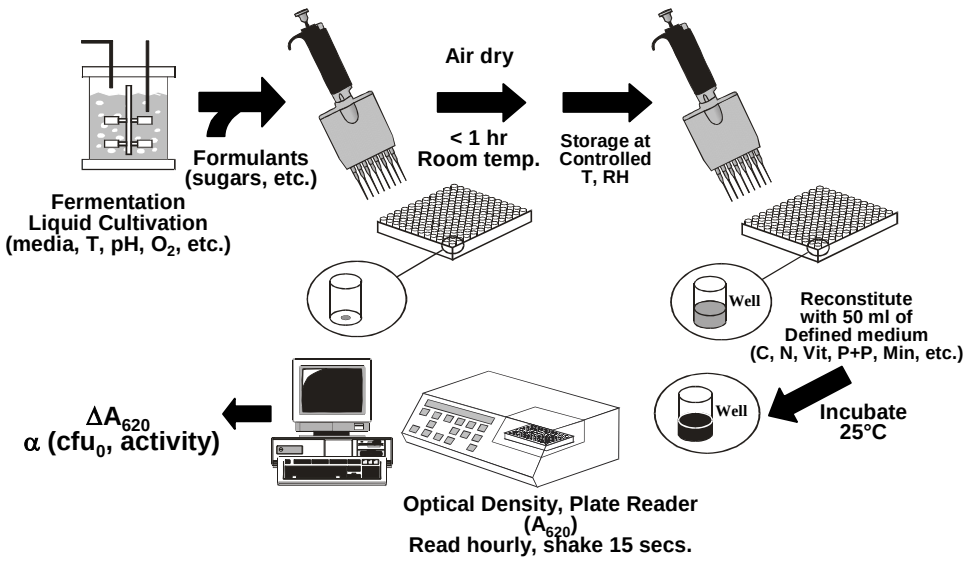

Table 1. Influence of UV Protectants on Dried 24-h Bacillus sp. OH 131.1 Exposed to 0- or 6-h UV Prior to Rehydration of Cells.

Treatment	Predicted A_{620}[1] 30 h after medium addition	
	No UV	6-h UV
OH 131.1 + 0.2% Lignin[2]	0.148 a	0.131 a
OH 131.1 + 0.3% Lignin	0.140 b	0.120 b
OH 131.1 Control	0.082 e	0.040 f
OH 131.1 + 0.5% Blankophor[3]	0.126 c	0.087 c
OH 131.1 + 1.0% Blankophor	0.110 d	0.081 d
OH 131.1 + 2.0% Blankophor	0.156 a	0.114 b

[1]Values followed by the same letter are not significantly different [Duncan's multiple range test ($p<0.05$)].
[2]Experimental product Westvaco PC 1307, water soluble lignin.
[3]Optical brightener.

Table 2. Field results in Peoria, IL and Wooster, OH biocontrol strains OH 131.1 and OH 182.9 with or without 0.3% Westvaco lignin PC 1307 formulation.

Treatment	Peoria, IL[1]			Wooster, OH[1,2]			
	Freedom	Pioneer 2545		Freedom		Pioneer 2545	
	DI[3]	DS	DI	DS	DI	DS	DI
Untreated Check	21.0#	4.0	32.0	25.8#	93.3	47.5	100
Buffer Tween Check	11.0*	3.2	29.3	20.6*	93.9	49.9	99.6
Folicur	12.0*	1.9*#	19.7*#	12.1*#	80.0*#	42.4*#	99.6
OH 182.9	12.7*	3.3	22.7*	17.4*#	92.8	40.9*#	98.9*
OH 182.9 + 0.3% Lignin	17.0*	2.8*	23.0*	16.3*#	89.4	44.3#	99.6
OH 131.1	10.7*	2.1*#	22.0*#	19.6*	95.6	42.5*#	100
OH 131.1 + 0.3% Lignin	9.0*	2.3*	22.0*	16.0*#	87.2#	48.6	100

[1]DS = % Disease severity, DI = % Disease incidence. Within a column, means followed by "*" and "#" are significantly different from the untreated check and buffer/tween check, respectively (student's t-test, P ? 0.05).
[2]Extensive rain and wind caused wheat lodging and permitted excessive disease development at Wooster, OH.
[3]Disease severity values were very low (range = 0.9-1.9), and are therefore not reported.

EVALUATION OF FUNGICIDES FOR THE CONTROL OF FUSARIUM HEAD BLIGHT AND LEAF DISEASES ON 'ELKHART' AND 'PIONEER VARIETY 2540' WINTER WHEAT IN MISSOURI

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OBJECTIVES

To identify fungicides that are effective in minimizing the damage from Fusarium head blight and foliage diseases in winter wheat.

INTRODUCTION

The severity of Fusarium head blight epidemics in the United States has caused enormous yield and quality losses in wheat and barley (McMullen, et al., 1997). The development of this disease is dependent on host genetics, a range of favorable environmental conditions, the prevalence of the causal fungus and the survival and spread of the cause fungus (Sutton, 1982). Control of this disease has been difficult because of the complex nature of the host/pathogen interaction. In addition to the development of varieties with resistance to Fusarium head blight, research focusing on fungicide treatments for the management of Fusarium head blight has been pursued.

In 1998, a Uniform Fungicide Trial was conducted across seven states (McMullen, 1998), which provided data on efficacy of five products or product combinations in reducing Fusarium head blight when applied at heading. This Uniform Fungicide Trial permitted evaluation of the performance of products across numerous states or sites, wheat classes and environments. Across the test sites that had substantial Fusarium head blight in 1998, an average of about fifty percent reduction in Fusarium head blight occurred, as well as a reduction in DON for most products, plus a substantial reduction in wheat leaf diseases. The Uniform Fungicide Trial has been continued since 1998 with additional test sites in more states and changes in products tested as new fungicides and biological control agents have become available. The Uniform Fungicide Trial continues to provide valuable information on efficacy and performance consistency of standard fungicides and new experimental fungicides. Missouri has participated in the Uniform Fungicide Trial since 1998 (Sweets, 2000). Results from the 2003 trial are presented in this report.

MATERIALS AND METHODS

Six fungicide treatments and an untreated control were evaluated on 'Elkhart' and four fungicide treatments and an untreated control were evaluated on 'Pioneer variety 2540' soft red winter wheat at the Bradford Research Center, near Columbia, MO. 'Elkhart' and 'Pioneer variety 2540' were drilled directly into soybean stubble on 15 Oct 02. The soil type at the site was a Putnam silt loam. The planting rate was 100-lbs of seed/A. The experimental design for each variety was a randomized complete block with 6 replications. Individual plots were 4.5 ft (7 rows) by 30 ft in length. The entire plot area was fertilized with 30-lbs/A nitrogen preplant followed by 90-lbs/A nitrogen topdressed in the spring. Treatments were applied with a CO_2 backpack sprayer with nozzles directed towards the heads. Treatments were applied in 400 ml of water. Applications were made at Feeke's Growth Stage (FGS) 10.51 on 11 May 03. Plots were rated for foliage diseases on 24 May 03. Ratings were

done as estimates of the percentage of leaf area covered with Septoria leaf blotch or stripe rust on each of 10 flag leaves randomly collected from each plot. Fusarium head blight incidence and head severity measurements were taken 26 May 03. For harvest the plots were end trimmed and individual plot lengths measured. Plots were harvested on 30 June 03 with a Wintersteiger plot combine. Test weight and moisture were determined with a Dickey-John GAC 2000 Grain Analyzer. Samples were submitted to the Veterinary Diagnostic Services Department at North Dakota State University for DON analysis. Data was statistically analyzed using ANOVA.

RESULTS AND DISCUSSION

Plants emerged well and early stands were uniform. The 2003 season was warm and dry early; cool and wet as the wheat was flowering and heading; and then cool and dry as the crop matured. Septoria leaf spot and leaf rust began to develop during flowering. When foliage disease ratings were made, both stripe rust and Septoria leaf blotch were evident across the trial so rating for these two diseases were made and are reported as percent total foliage disease. Fusarium head blight was widespread throughout the plot at the time Fusarium head blight incidence and severity ratings were made. At harvest most plots had noticeable amounts of shriveled, lightweight kernels or tombstone kernels.

There were no statistically significant differences in yield between the fungicide treatments and the untreated control on either 'Elkhart' or 'Pioneer variety 2540'. Total foliage disease ratings were significantly higher for the untreated control than the fungicide treatments on both 'Pioneer variety 2540' and 'Elkhart'. On 'Elkhart', JAU6476 at the 5.7 fl oz rate + Induce 0.125% v/v had lowest total foliage disease rating.

On 'Elkhart' there were no statistically significant differences between treatments for percent Fusarium head blight incidence, percent average head severity, percent field severity or percent scabby kernels but there was a significant difference between treatments for DON levels. The untreated control had the highest DON level, although it was only 1.10 ppm.

On 'Pioneer variety 2540' there were significant differences between treatments for percent Fusarium head blight incidence. The untreated control had the highest percent FHB incidence and the three JAU6476 480SC treatments had the lowest percent FHB incidence. There were also significant differences in DON levels between the treatments. Again the untreated control had the highest DON level (0.62 ppm) and the three JAU6476 480SC treatments had the lowest DON levels. There were no statistically significant differences between the untreated control and the four fungicide treatments for percent of percent average head severity, percent field severity or percent of scabby kernels on 'Pioneer variety 2540'.

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Table 1. 'Elkhart'

Treatment and Rate/A	Yield[1] bu/A	Test Wt. (lb/bu)	Foliage Disease Rating[2]	%FHB[3]	% Ave. Head Sev.[4]	% Field Sev.[5]	% Scab Kernels[6]	DON ppm
Untreated control	55.4	59.0	15.23	42.00	19.33	7.94	20.8	1.10
Folicur 3.6F 4.0 fl oz + Induce 0.125% v/v	63.8	60.4	0.75	41.67	23.00	9.32	18.5	0.39
JAU6476 480SC 5.7 fl oz + Induce	60.6	60.5	0.57	41.00	19.83	8.04	16.2	0.21
JAU6476 480SC 5.0 fl oz + Induce	61.1	59.9	0.73	35.67	21.67	7.62	11.0	0.01
JAU6476 480SC 3.6 fl oz + Folicur 4.0 fl oz + Induce 0.125% v/v	70.1	60.3	0.70	40.67	24.33	9.61	13.6	0.26
V-10116 1.67SC 6.0 fl oz + Induce	55.8	59.2	0.62	41.33	19.85	8.56	21.0	0.09
V-10116 1.67SC 8.0 fl oz + Induce 0.125% v/v	57.1	59.7	0.74	40.67	25.67	10.48	19.3	0.01
LSD (P=0.05)[7]	N.S.	N.S.	1.38	N.S.	N.S.	N.S.	N.S.	0.32

[1] Yield based on 60-pound bushel weight adjusted to 13% moisture content.

[2] Total Foliage Disease Rating based on the average % of flag leaf showing symptoms of stripe rust and Septoria leaf blotch for 10 flag leaves.

[3]% FHB or percent of Fusarium head blight incidence based on % of heads showing symptoms for 50 heads.

[4]% ave. head sev or percent of average head severity based on % of head showing FHB symptoms for 50 heads.

[5]% field sev or percent field severity calculated using the formula (%FHB x % ave. head sev.)/100.

[6]%scab kernels or percent scabby kernels based on % scabby kernels in a 200 kernel sample.

[7]Data was analyzed by ANOVA with means separated by LSD at P=0.05.

Table 2. 'Pioneer variety 2540'

Treatment and Rate/A	Yield[1] bu/A	Test Wt. (lb/bu)	Foliage Disease Rating[2]	%FHB[3]	% Ave. Head Sev.[4]	% Field Sev.[5]	% Scab Kernels[6]	DON ppm
Untreated control	88.8	59.2	16.92	52.17	23.67	12.93	9.7	0.62
Folicur 3.6F 4.0 fl oz + Induce 0.125% v/v	94.5	59.5	0.80	46.67	22.83	10.57	11.3	0.34
JAU6476 480SC 5.7 fl oz + Induce 0.125% v/v	99.3	57.4	0.76	36.83	25.33	8.83	11.9	0.01
JAU6476 480SC 5.0 fl oz + Induce 0.125% v/v	97.9	59.1	0.72	41.00	19.00	7.48	12.2	0.11
JAU6476 480SC 3.6 fl oz + Folicur 4.0 fl oz + Induce 0.125% v/v	95.4	58.8	0.95	39.67	25.50	10.15	10.8	0.11
LSD (P=0.05)[7]	N.S.	N.S.	1.64	10.54	N.S.	N.S.	N.S.	0.29

[1] Yield based on 60-pound bushel weight adjusted to 13% moisture content.

[2] Total Foliage Disease Rating based on the average % of flag leaf showing symptoms of stripe rust and Septoria leaf blotch for 10 flag leaves.

[3]% FHB or percent of Fusarium head blight incidence based on % of heads showing symptoms for 50 heads.

[4]% ave. head sev or percent of average head severity based on % of head showing FHB symptoms for 50 heads.

[5]% field sev or percent field severity calculated using the formula (%FHB x % ave. head sev.)/100.

[6]%scab kernels or percent scabby kernels based on % scabby kernels in a 200 kernel sample.

[7]Data was analyzed by ANOVA with means separated by LSD at P=0.05.

CONTROL OF FUSARIUM HEAD BLIGHT VIA INDUCED RESISTANCE ELICITED BY *LYSOBACTER ENZYMOGENES* C3 - POTENTIALS AND LIMITATIONS

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ABSTRACT

Lysobacter enzymogenes C3 is unique among biological control agents for Fusarium head blight (FHB) in that induced resistance is involved as a mechanism action. We investigated the spatial distribution of C3-elicited induced resistance by applying the bacterium or water to various parts of wheat plants and assessing the degree to which FHB developed in treated and non-treated spikelets following inoculation with pathogen conidial suspensions. When C3 was applied to flag leaves, there was no effect on FHB severity on heads of treated plants as compared to the water-treated control. When the agent was applied to spikelets on the lower half of flowering wheat heads, inhibition of FHB was observed in the lower half of treated heads but not in the non-treated upper portion. In another set of experiments, C3 was sprayed onto wheat heads and then the treated heads were challenged with pathogen conidia by floret injection. FHD severity in C3-treated heads was found to be lower than in heads treated with water. We conclude that resistance induced by C3 is not systemically expressed, but is localized to the spikelets on which the bacterium is deposited. As a consequence, biocontrol efficacy using C3 will depend in part on the effectiveness of application procedures in depositing the bacterium onto all spikelets. If C3 is well distributed across spikelets in a wheat head, induced resistance can reduce the incidence of infection in the head and inhibit pathogen spread from infected spikelets via the rachis. In studies on induced resistance in other host-pathogen systems, the effectiveness of induced resistance varied depending upon the host genotype to which the inducing agent was applied. Therefore, we also investigated whether or not C3 could be effective in reducing FHB development on different cultivars of spring wheat. In a series of greenhouse experiment in which C3 was applied to 11 cultivars varying in susceptibility to FHB, the bacterium reduced FHB severity, as compared to water-treated controls, in eight of the cultivars. Differential effectiveness of biocontrol by C3 was not associated with relative levels of resistance to FHB reported for the cultivars. These findings suggest that that host genotype may be another determining factor in biological control by C3 and that the interaction of each genotype with C3 must be assessed empirically.

COOPERATIVE MULTISTATE FIELD TESTS OF BIOLOGICAL AGENTS FOR CONTROL OF FUSARIUM HEAD BLIGHT IN WHEAT AND BARLEY

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OBJECTIVES

To evaluate biological control agents for efficacy in control of Fusarium head blight across a range of hosts (species, market class, and cultivars) and environmental conditions.

INTRODUCTION

Microorganism strains are being investigated in several US laboratories as biological control agents against Fusarium head blight (FHB). Strains that have shown potential to reduce scab severity under field conditions include the yeast *Cryptococcus nodaensis* OH182.9 (Schisler et al., 2002), the gram-positive, spore-forming bacterium *Bacillus subtilis* TrigoCor 1448 (Hershman et al., 2002; da Luz et al., 2003), and the gram-negative bacterium *Lysobacter enzymogenes* C3 (Yuen and Jochum, 2002). Additional strains, such as *Bacillus* sp. 1BA (Baye et al., 2002; Luo, 2000), have shown promise in laboratory tests. Crucial to gauging the usefulness of a biocontrol agent is an assessment of its efficacy across the range of field conditions and host genotypes occurring in the different geographic areas in which FHB may be a problem. In 2003, experiments were conducted cooperatively among several biocontrol laboratories to evaluate the same agent strains in three states.

MATERIALS AND METHODS

Six experiments were conducted, two each in Nebraska, Ohio and South Dakota. Nebraska experiments were located at Lincoln and Mead, separated by approx. 40 miles. Cultivar 2137, a FHB-susceptible hard red winter wheat, was planted at both locations. There were four and five replications per treatment at Lincoln and Mead, respectively. Ohio experiments were conducted in Wooster on soft red winter wheat cultivars Pioneer 2545 (susceptible) and Freedom (moderately resistant), with five and four replications per treatment, respectively. South Dakota experiments were conducted at Brookings in 'Oxen' hard red spring wheat and 'Robust' barley, both with four replications per treatment. Randomized complete block designs were used in all locations.

Bacterial strains C3, TrigoCor 1448, and 1BA were propagated in the laboratory at each location. The strains were grown in broth media and the resultant cultures were amended with the surfactant Induce (0.125%) prior to application. The yeast OH182.9 was supplied as fermentation biomass suspended in a

buffer amended with the surfactant Tween 80 (Schisler et al., 2002). Each experiment also included a treatment with tebuconazole (Folicur 3.6F, 4 fl oz./A) amended with Induce. Control plots in South Dakota were nontreated, whereas control plots in Nebraska were treated with water containing Induce; Ohio experiments involved both of these controls in addition to one treated with buffer/Tween solution. At anthesis, a single application of each treatment was made in 70 gal/A at Nebraska and Ohio and 20 gal/A in South Dakota. Pathogen inoculum was provided in the form of conidial suspensions (Nebraska and South Dakota) or pathogen-colonized corn kernels (Ohio and South Dakota). Mist irrigation was applied for over 1 week after treatment to promote FHB development. The incidence and severity of FHB was determined on over 40 heads per plot approx. 3 weeks after treatment. Seed yields, test weight and DON content were determined in Nebraska and South Dakota.

RESULTS AND DISCUSSION

Biological control agents applied in Nebraska and South Dakota experiments did not affect FHB incidence or severity (Table 1). Treatment with Folicur reduced incidence in both Nebraska locations and decreased severity at Mead, but the fungicide but had no effect in South Dakota. The biological control agents and Folicur did not affect DON content or plot yields, and only the fungicide treatment exhibited a positive effect on test weight in one Nebraska location (data not shown). Fusarium head blight severity levels were very low in Nebraska and South Dakota, and therefore, results from these experiments are not conclusive as to the efficacy of the biocontrol agents.

Efficacy was found for biological control treatments in Ohio, but the results varied depending upon the cultivar (Table 2). Very high incidence and severity levels recorded in the controls of susceptible Pioneer 2545 were unchanged by treatment with biological control agents or Folicur. In moderately resistant 'Freedom', FHB severity in plots treated with each of the biocontrol agents was lower than in the non-treated control and were not significantly different from plots treated with Folicur. Differences between the biocontrol treatments and treatment involving only adjuvants, however, were not statistically significant.

In this set of field experiments, no difference could be discerned for disease suppression among the biocontrol agents tested. Further research is needed to ascertain the effectiveness of the agents in different geographic areas and on barley and other wheat market types. Ohio results do lend support to the supposition that biological control agents will be most effective when integrated with host resistance.

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Table 1. Incidence (INC) and severity (SEV) of Fusarium head blight in Nebraska and South Dakota evaluations of biological control agents, 2003[1].

Treatment	NE Lincoln		NE Mead		SD Wheat		SD Barley	
	%INC	%SEV	%INC	%SEV	%INC	%SEV	%INC	%SEV
Control[2]	41 ab	5	85 a	9 a	13	1	69	6
Bacillus sp. 1BA	52 a	7	81 a	8 a	19	2	72	7
Bacillus subtilis Trigocor 1448	51 a	6	74 a	7 a	21	2	76	6
Cryptococcus nodaensis OH182.9	54 a	8	84 a	8 a	11	1	62	4
Lysobacter enzymogenes C3	60 a	8	83 a	8 a	18	2	64	8
Folicur	32 b	3	44 b	4 b	14	1	64	6
P	0.008	0.051	0.001	0.006	0.053	0.125	0.795	0.664

[1]Means in a column with the same letter are not significantly different at P=0.05 according to Student-Newman-Keul's Multiple Range Test. Mean separations shown only when treatment effects in ANOVA were significant at the 95% level.
[2]Control plots in Nebraska were treated with water amended with Induce. Non-treated plots were the controls in South Dakota.

Table 2. Incidence (INC) and severity (SEV) of Fusarium head blight in Ohio, 2003, evaluations of biological control agents on two winter wheat cultivars[1].

	Freedom		Pioneer 2545	
Treatment	% INC	% SEV	% INC	% SEV
Nontreated control	93	26 a	100	47
Induce	93	21 ab	>99	50
Buffer/Tween	94	21 ab	100	43
Bacillus sp. 1BA	88	18 bc	100	49
Bacillus subtilis Trigocor 1448	86	16 bc	100	47
Cryptococcus nodaensis OH182.9	93	18 bc	>99	42
Lysobacter enzymogenes C3	91	18 bc	100	49
Folicur	80	12 c	>99	42
P	0.136	<0.001	0.232	0.294

[1]Means in a column with the same letter are not significantly different at P=0.05 according to Student-Newman-Keul's Multiple Range Test. Mean separations shown only when treatment effects in ANOVA were significant at the 95% level.

USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT 2: EFFECTS OF CARBON-TO-NITROGEN RATIO OF PRODUCTION MEDIA ON THE BIOCONTROL EFFICACY AND THE SURVIVAL OF *CRYPTOCOCCUS NODAENSIS* OH 182.9 AFTER FREEZE-DRYING

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OBJECTIVES

To determine the effect of varying the carbon-to-nitrogen (C/ N) ratio and carbon loading of production media and cultivation duration on the biocontrol efficacy of *C. nodaensis* OH 182.9 against Fusarium head blight and the viability of freeze-dried OH 182.9 cells over time.

INTRODUCTION

Fusarium head blight (FHB), primarily incited by *Gibberella zeae*, is a devastating disease of wheat world-wide (McMullen *et al.*, 1997). The infection of wheat kernels by *G. zeae* reduces grain yield and quality in part due to the pathogen producing a vomitoxin deoxynivalenol (DON) in grain during infection. Extensive research has focused on the use of resistant wheat cultivars for control of FHB. However, no available cultivars are immune to FHB. Chemical management of FHB has proven inconsistent and is hindered by costs and concerns about post-harvest fungicide residues. Cultural practices such as crop rotation and a shift from reduced to conventional-tillage practices are not economically viable. *Cryptococcus nodaensis* OH 182.9 has been evaluated as an effective biocontrol agent for management of FHB (Khan *et al*., 2001; Schisler, *et al*., 2002). Development of a dried product of OH 182.9 is sought because of the potential advantages of ease of handling, favorable economics and acceptance by the users.

MATERIALS AND METHODS

C. nodaensis OH182.9 cells were inoculated in 250-ml Erlenmeyer flasks containing 50 ml of semidefined complete liquid media (SDCL). Flasks were incubated for 48 and 72 h on a shaker at 250 rpm, 25ºC. Two milliliters of samples were distributed in an autoclaved serum vial and then placed in a freeze drier with eutectic temperature – 8ºC operating at – 45ºC for 2 days. Vials were stored at room temperature and over time, cells were resuspended in 2 ml of phosphate buffer, diluted and plated on 1/5 Tryptic soy broth agar (TSBA/5). To determine the efficacy of fresh cells for controlling FHB, wheat heads (cultivar Norm) at anthesis were sprayed with 25% suspensions of fully colonized cultures of OH 182.9 in SDCL (approx. 1×10^8 CFU/ml), followed by *G. zeae* Z-3639 (1×10^4 macroconidia/ml). Inoculated plants were placed in a humid chamber for 3 days before being transferred to greenhouse benches. Disease severity was recorded using a 0-100% scale 14 days after inoculation. Experiments were performed 2 or 3 times, and the treatments were arranged in a completely randomized design.

RESULTS AND DISCUSSION

OH 182.9 was grown in SDCL with C/N ratios of 6.5, 9, 11, 15, 30 and the carbon loading of 14 g/L for 48 and 72 h. Total biomass production was similar for all combinations of cell age by SDCL medium C/N ratio. Survival of freeze-dried cells was greatest for cells grown in SDCL C/N 30 medium for 48 h (Fig. 1). Cells produced in C/N 6.5 medium exhibited the poorest survival (data not shown). In general, cells harvested after 48 h were more tolerant to freeze-drying than those cultured after 72 h; cells grown in higher C/N ratio SDCL survived better than those harvested from lower C/N media when being harvested at 48 h. OH 182.9 produced in C/N 9, 11 and 15 media for 48 h significantly reduced the FHB disease severity compared to the disease control (Table 1).

For the medium C/N 30, research on influence of carbon loading in production media on cell survival after freeze-drying and the efficacy of disease control was conducted. Total carbon loading of 7, 14, 21 and 28 g/L in SDCL C/N 30 medium was tested. In general, cells cultured for 48 h survived better than those harvested after 72 h. OH 182.9 cells from SDCL C/N 30 media with varied carbon loading maintained 6.5-8.5 log CFU/ml for 15 days after freeze-drying (Fig.2). Cells produced in C/N 30 media with 7, 14 and 21g/L carbon and harvested after 48 h had better survival than others, including the standard 48 h C/N 11 culture with 14g/L carbon. OH 182.9 grown in SDCL C/N 30 with carbon loading of 7 or 14 g/L was effective against FHB (Table 2). Our results indicate that C/N ratio, total carbon loading and the stage of microbial growth in production media greatly influence freeze–drying survival of the biomass and the biocontrol efficacy of OH 182.9.

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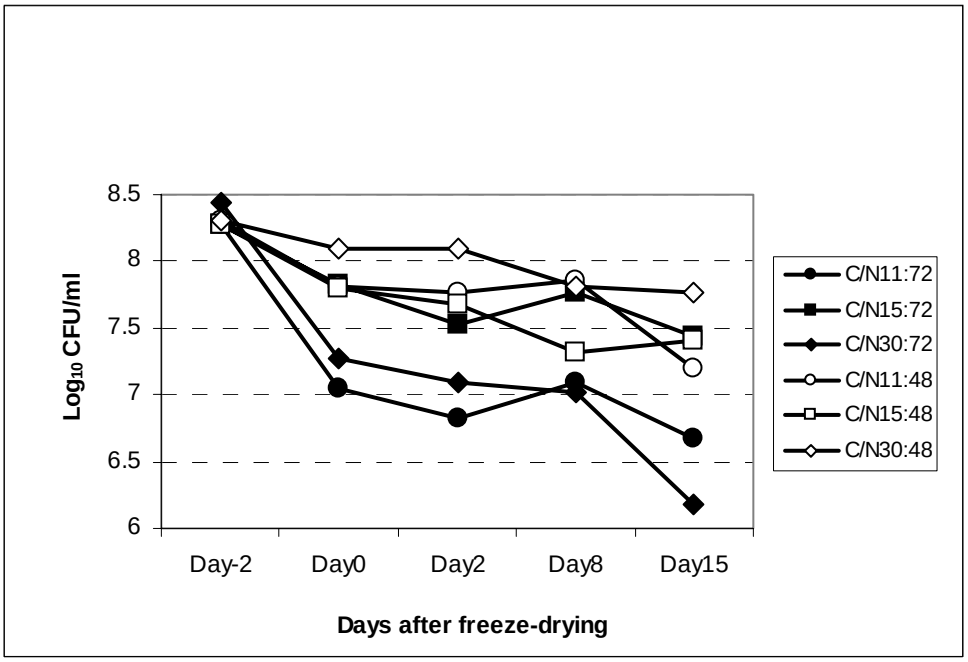

Figure 1. Effect of C/N ratio (11, 15, 30) and cell age (48, 72 h) on OH 182.9 cell survival after freeze-drying (14 g/L carbon).

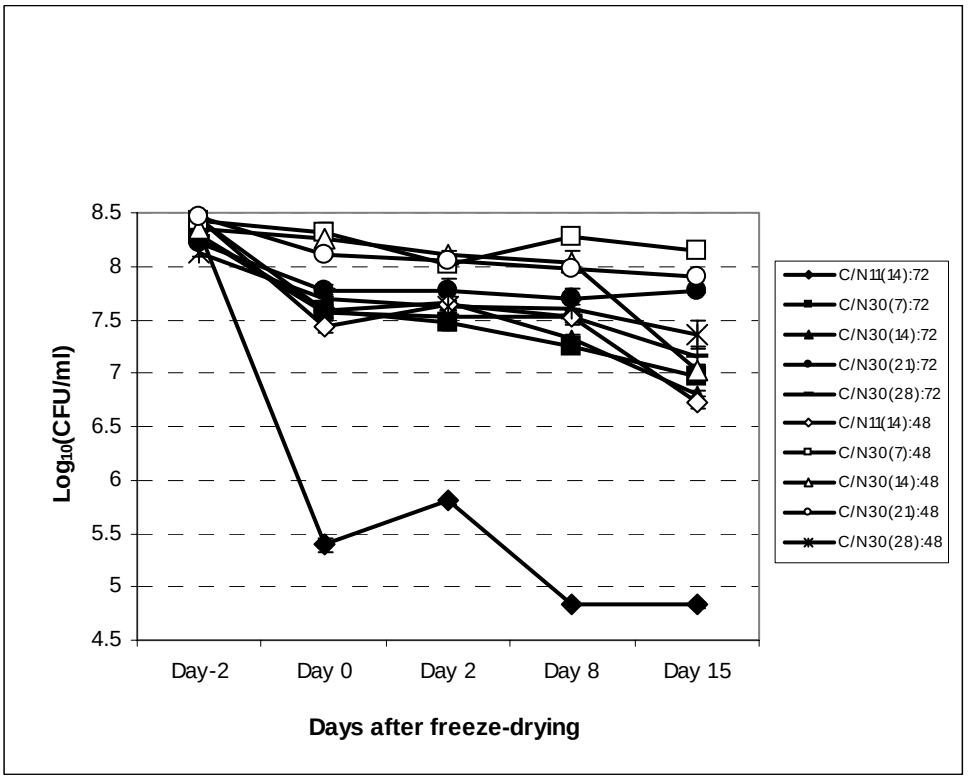

Figure 2. Effect of carbon loading in SDCL and cell age (48, 72 h) on OH 182.9 cell survival after freeze-drying.

Table 1. Biocontrol efficacy of *C. nodaensis* OH 182.9 produced in SDCL C/N 11, 9 and 6.5 media with 14g/L carbon

Treatment [x]	Disease Severity (%) [y]			
	Trial 1	Trial 2	Trial 3	Pooled
C/N 11: 72 [z]	90 a	83 a	83 ab	85 a
C/N 9: 72	90 a	83 a	75 abc	83 ab
C/N 6.5: 72	90 a	77 a	79 abc	82 ab
C/N 11: 48	60 b	80 a	49 bc	63 bc
C/N 9: 48	74 ab	49 a	44 c	56 c
C/N 6.5: 48	90 a	76 a	83 ab	83 ab
Disease CK	86 a	90 a	90 a	89 a
Nontreated CK	0 c	0 b	0 d	0 d
$LSD_{0.05}$	24	47	37	21

[x] 25 % full strength OH 182.9-colonized SDCL

[y] Different letters within a column indicate significant differences among means.

[z] C/N11: 72 indicates OH 182.9 was grown in SDCL C/N 30 medium for 72 h.

Table 2. Effect of carbon loading of 7 and 14 g/L in SDCL C/N 30 media and incubation time (48, 72 h) on biocontrol efficacy of *C. nodaensis* OH 182.9

Treatment [x]	Disease Severity (%) [y]			
	Trial 1	Trial 2	Trial 3	Pooled
C/N 11(14): 72	19 abc	44 abc	46 ab	36 abcd
C/N 30(7): 72 [z]	23 abc	57 ab	49 ab	43 abc
C/N 30(14): 72	48 a	54 ab	53 ab	52 ab
C/N 11(14): 48	7 bc	24 bc	19 bc	16 de
C/N 30(7): 48	24 abc	42 abc	26 bc	31 bcd
C/N 30(14): 48	14 abc	20 bc	28 bc	20 cde
Disease CK	37 ab	69 a	70 a	59 a
Nontreated CK	0 c	0 c	0 c	0 e
$LSD_{0.05}$	36	45	38	23

[x] 25 % full strength OH 182.9-colonized SDCL

[y] Different letters within a column indicate significant differences among means.

[z] C/N 30(7): 72 indicates OH 182.9 was grown in SDCL C/N 30 medium with carbon loading 7 g/L for 72h.

A COMPARISON OF *FUSARIUM PSEUDOGRAMINEARUM* AND *F. GRAMINEARUM* FROM WHEAT IN AUSTRALIA

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ABSTRACT

In Australia both *Fusarium graminearum* and *F. pseudograminearum* cause head blight and crown rot of wheat and this work compares the aetiology and diversity between the two species. A total of 199 isolates of *F. pseudograminearum* and 118 isolates of *F. graminearum* obtained from wheat grown in Queensland and northern New South Wales and were identified using published species-specific PCR assays. The phenotypic measures used include aggressiveness on wheat, corn, sorghum, canola, barley, rye, oat and triticale, mycotoxins produced in autoclaved-wheat culture, growth rate on potato dextrose agar, spore size and the number of macroconidia produced in culture. To measure aggressiveness, the middle spikelet of wheat, oat, barley, rye and triticale was inoculated at flowering with 10-µL suspension of 10^5 macroconidia/mL. Number of spikelets infected was recorded at 14 days after inoculation. Corn, sorghum and canola were inoculated using sterile toothpicks colonised by the pathogen by growing in potato dextrose broth for 7 days and the length of stem rot was recorded after 14 days. Genotypic relationship between the two species was evaluated using Amplified fragment length polymorphism with five primer pair combinations. Both species produced the same mycotoxins Deoxynivalenol (DON), Zearalenone (ZEA) and nivalenol (NIV). NIV concentration in all samples tested was less than 250 ppb, while DON concentration ranged between 2.5 ppm and 0.71 ppm in *F. pseudograminearum* and 4.4 ppm and 2.4 ppm in *F. graminearum*. ZEA concentration was considerably higher in *F. graminearum* (3.0-11.9 ppm) than in *F. pseudograminearum* (0.9-2.2 ppm). The symptoms and severity of diseases produced on the heads of the cereal crops were similar. Both species caused canker and stem rot in canola, and stalk rot in corn and sorghum. A high level of genotypic diversity was observed within each species. All *F. graminearum* isolates produced homothallic perithecia in culture but only 8% of *F. pseudograminearum* isolates produced heterothallic perithecia in culture. Although there were variations in all the phenotypic and genotypic measures for each species, the level of variation in *F. graminearum* was higher than in *F. pseudograminearum.*

RECOVERY OF *FUSARIUM GRAMINEARUM*, CAUSE OF WHEAT HEAD SCAB, AND DEOXYNIVALENOL FROM INOCULATED LEAVES AT ADULT PLANT STAGE IN THE GREENHOUSE

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ABSTRACT

Fusarium graminearum (teleomorph: *Gibberella zeae*) causes Fusarium head blight (FHB) on wheat and other small grains in North Dakota. The fungus has been recovered frequently from wheat leaf samples collected at any growth stage from various fields, and the leaves are thought to serve as an additional source of inoculum for disease development. However, the pathway of the fungal survival on leaves has not yet been explored. It is also not known if the fungus produces deoxynivalenol (DON) in leaves during colonization process. To answer these questions, leaves of two FHB susceptible wheat genotypes, Glenlea and M-3 (W7976), were inoculated at Feekes scale 11.4 with *F. graminearum* conidial suspension (100, 000 conidia/ml) until run off and kept in a humidity chamber twice for 24-hr, with a 24-hr interval between the two cycles. Thereafter, the plants were kept in a growth chamber set at 24°C day and 20°C night for seven days. The plants were examined daily for any symptom development. Inoculated plants were placed a third time in the humidity chamber for 24 hrs 8 days post inoculation and then they were moved back to the growth chamber. Two days later the leaves were clipped, brought to the laboratory, and observed under a dissecting microscope and a compound microscope for the presence of fungal sporodochia and perithecia. Most of the leaves of both cultivars retained their green color with a little chlorosis, which was more conspicuous while holding leaves toward light. Lower leaves of both cultivars turned mostly chlorotic. The green leaves of both cultivars had several sporodochia, while chlorotic leaves had both sporodochia and some immature perithecia. DON was detected at 4 ppm in inoculated green leaves. The results indicate that the fungus can survive on leaves, produce both spore types (depending on the nature of the leaves (green/chlorotic)), and serve as a source of inoculum. Moreover, the fungus can produce DON in the leaves that could make them unacceptable as forage. This is the first report on the occurrence of DON in intact wheat leaves. This study may help in understanding the fungal pathway from seedling to adult plant stage under field conditions and help in the disease management.

DETERMINATION OF WETNESS DURATION USING RADAR-DERIVED PRECIPITATION ESTIMATES

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ABSTRACT

Fusarium head blight (FHB) of small grains tends to be associated with certain environmental conditions, especially rain-induced wetness periods occurring near anthesis. A Geographic Information System-based model simulation which incorporates 4km resolution weather radar (NEXRAD)-derived precipitation estimates into a crop canopy energy balance-based scheme to estimate wetness duration periods for small grains on the 4 km spatial scale has been developed and tested with field observations. During the past year, an analysis of errors and biases in NEXRAD precipitation estimates (the most critical input data involved in the wetness duration simulation) was completed for the May-September time frame, 1999-2002. Comparisons were made across the state of Michigan between gages in: 1) National Weather Service (NWS) and 2) Michigan Automated Weather Network (MAWN) networks. In terms of rainfall frequency, the NEXRAD estimates were correct in identifying precipitation 89.7% and 89.0% of time on a daily basis for NWS and MAWN networks, respectively, and 95.9% (NWS) and 95.6% (MAWN) of the time on an hourly basis. In terms of differences between estimated and observed precipitation totals, mean differences for daily and hourly periods over the 1999-2002 study period were -0.05mm and 0.13mm and -0.10mm and 0.01mm for NWS and MAWN networks respectively. Validation of the simulated leaf wetness duration in six wheat field sites in Lower Michigan at head height was also carried out resulted during June and July of the 2003 growing season. Collectively across all types of wetness events, the simulation understimated the length of wetness duration. Mean differences and mean absolute differences between simulated and observed leaf wetness across all events were approx. -4.4 hours and 4.5 hours, respectively. The mean absolute difference for precipitation events (those of most significance when monitoring for the incedence of Fusarium) alone was 1.0 hour. Overall, while the results suggest satisfactory performance with wetting events associated with precipitation, the simulation also tended to underestimate wetness duration associated with the formation of dew, especially at the onset of the event.

CALCIUM IONS INCREASE TOXICITY OF DEOXYNIVALENOL TO BARLEY LEAF TISSUES

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ABSTRACT

Deoxynivalenol (DON) had a bleaching effect on detached barley leaf segments (Bushnell et al, Phytopathology 92:S11, 2002). Leaf tissues lost pigmentation after incubation with 30-200 ppm DON for 2-4 days in light. The bleaching was accompanied by disorganization of chloroplasts and other cytoplasmic organelles as viewed by transmission electron microscopy (TEM). However, results were inconsistent; some segments were completely bleached, some developed only white spots or streaks, and others remained entirely green, even at high DON concentrations (90-200 ppm). Here we report that toxicity of DON is increased and tissue responses are more consistent when Ca^{2+} is added. The abaxial epidermis was partially stripped from detached primary barley leaf segments (1.2 cm long) and the segments were then floated with exposed mesophyll in contact with DON solutions, with or without added 10 mM Ca^{2+} (applied as $Ca(NO_3)_2$). Segments were incubated at 25°C in light (250 µmol/m^2/sec). With Ca^{2+}, DON at 10-30 ppm gave white spots or streaks by 24 hr and usually turned entire tissues white by 72 hr. Compared to treatments without Ca^{2+}, the loss of pigment with Ca^{2+} occurred 1-2 days earlier, was more complete in individual segments, and was more consistent among segments. In line with this, amounts of chlorophyll and carotenoid pigments, as measured spectrophotometrically, were reduced more with Ca^{2+} than without. As viewed by TEM, chloroplast degeneration was underway after 18 hr of treatment with 30 ppm DON (with Ca^{2+}) and nearly complete by 24 hr. Increased toxicity could be accounted for by greater concentration of DON within leaf segments treated with DON + Ca^{2+}. Without Ca^{2+}, tissues contained 3 ppm DON; with Ca^{2+} they contained 14 ppm DON (after 48 hr incubation in light on 30 ppm DON). Experiments are in progress to evaluate the effect of Ca^{2+} on DON-treated tissues incubated in the dark. The pronounced increase in toxicity of DON in present experiments suggests that Ca^{2+} concentrations within plant tissues may influence the effect of DON in the development of Fusarium head blight.

EPIDEMIOLOGY OF FUSARIUM HEAD BLIGHT AND CROWN ROTOF WHEAT: LESSONS FOR AUSTRALIA

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ABSTRACT

In Australia species of *Fusarium* cause two serious diseases of wheat: Fusarium head blight (FHB) that has emerged as a major problem in recent years, and crown rot (CR), which continues as a chronic problem costing >AUD$56 million every year. Residue-borne *F. pseudograminearum*, *F. graminearum*, F. *culmorum* and *F. avenaceum* have caused recent outbreaks of FHB and many of these cause serious CR. We have adopted a coordinated approach to both diseases because of the linked etiology, biology and epidemiology of *Fusarium spp.* causing CR and FHB. We are studying the interrelationship among *Fusarium* pathogens causing FHB and CR in wheat farming systems using relative abundance; aggressiveness; toxin production; dispersal and effectiveness of inoculum; epidemiology and disease severity. Pathogen isolates collected from field surveys have been identified using species-specific PCR assays and morphology. High throughput bioassays have been developed and/or adopted from other studies to use pathogen aggressiveness as a selection tool to detect small but consistent differences in quantitative resistance for further improvement of host resistance. In Australia, both species and isolates within species differ in aggressiveness and at least 20% of all *F. graminearum* and *F. pseudograminearum* are aggressive to highly aggressive for both FHB and CR, but there are important differences in the form and effectiveness of inoculum. All 17 *Fusarium* species tested caused FHB and all 10 tested caused CR in plant infection assays, with significant ($P < 0.001$) difference in aggressiveness between species and between isolates within species for both diseases. Overall, isolates from stubble and crown were more aggressive for CR whereas isolates from the flag leaf node were more aggressive for FHB. Isolates that were highly aggressive in causing CR were those originating from paddocks with wheat following wheat, while those from fields with wheat following maize or sorghum were highly aggressive for FHB. There is extensive literature on Fusarium affecting wheat and the US wheat and barley scab initiative has helped to collate existing information and to generate new knowledge. Although CR is prevalent in some states of the USA, an overwhelming majority of information on the pathogen and disease epidemiology relates to FHB. A comparison of disease epidemiology and pathogen populations in the two countries is essential to establish if the wealth of research outcomes from the USA is likely to be applicable to the management of CR and FHB in Australia. Through strong and effective collaboration between research teams from the two countries, we hope to share and grow the wealth of knowledge for the management of both diseases.

DEVELOPMENT AND DEPLOYMENT OF THE NEXT GENERATION PREDICTION MODELS FOR FUSARIUM HEAD BLIGHT

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OBJECTIVES

To develop and deliver accurate and timely predictions of Fusarium head blight

INTRODUCTION

Epidemics of Fusarium head blight were common in many states during the 2003 growing season. High levels of disease were reported in throughout the Mid-Atlantic region encompassing NY, PA, MD, VA, and NC. Outbreaks of disease were also reported in OH, IN, and KY. Producers in these areas struggled to find markets for Fusarium damaged grain, and concerns about mycotoxin contamination were common. Providing producers and the wheat industry in the U.S. with accurate and timely forecasts of disease will be important to minimize the impacts of future epidemics. More specifically, disease forecasts can be used to help producers make decisions about the application of a fungicide or biological control, establish harvest priorities, and pursue markets for grain. Grain buyers can use this information to make preparations for mycotoxin testing, grain cleaning, storage, and processing.

Efforts to predict Fusarium head blight is a priority of a cooperative epidemiology effort sponsored by the U.S. Wheat and Barley Scab Initiative. Researchers from this effort have developed two models using 50 cases of hourly weather and disease observations from ND, OH, MO, KS that can be used to provide estimates of the probability of a scab epidemic greater than 10% severity (De Wolf et al. 2003). These models include a pre-flowering model that uses weather variables observed prior to flowering to predict disease, and a post-flowering model that makes predictions using weather variables observed both prior to and during the flowering period. The pre-flowering model has been shown to be 70% accurate and the post-flowering model 83% accurate. One or both these models are currently being used in ND, SD, MI, OH, PA, NY, and IN to provide producers with disease forecasts for Fusarium head blight. However, means of delivery and resources available to provide these disease forecasts vary among these states (Francl 2001; Lipps et al. 2002; Osborne and Jin 2002).

We report here on our recent efforts to improve the accuracy of pre-flowering disease predictions by expanding the amount of weather information considered by these models, and through adjustments in weather variables based on research addressing *Gibberella zeae* perithecial development. We also will introduce a novel source of weather variables that could allow the uniform deployment of Fusarium head blight predictions in 23 states.

MATERIALS AND METHODS

Information used to develop models of this second phase of the disease forecasting effort consisted of observations of disease and crop growth stage within replicated plots. Disease severity was coded as a

binary variable with cases having a disease severity of 10% or greater considered epidemics (1) and cases with lower disease considered to be non-epidemics (0). Each set of observations (case) was associated with hourly observations of weather variables including temperature, relative humidity and rainfall. The cases used to develop these models were collected in seven states representing both spring and winter wheat production systems (Table 1). The total data set consisted in 119 cases, and this information was partitioned into a model development (n=89) and validation (n=30) data sets.

Table 1. Information from multiple states and locations representing both spring and winter wheat production regions were used to develop prediction models for Fusarium head blight.

State	Locations	Cases
North Dakota	6	33
Ohio	3	48
Missouri	3	11
Indiana	1	11
Pennsylvania	2	8
South Dakota	1	6
Kansas	1	2
Total	**17**	**119**

The hourly weather observations were used to construct variables representing potential time periods critical for the reproduction of the fungus or infection of the host. More specifically, variables considered were summarized for 14 or 7 days prior to, or 7, 10 or 14 days after the anthesis date identified for each case. The temperature variables were selected to represent recent research on the reproduction of the fungus, which indicates that perithecial development of *G. zeae* is limited by temperatures less than 9°C as apposed to 15°C as was previously reported (Dufualt et al 2003, Tchsanz et al 1976). A class variable designating each cases as either a winter or spring wheat was also considered in the analysis.

Variables useful in predicting Fusarium head blight epidemics were identified using best- subsets regression procedures. The identified variables were used to construct interaction terms (multiplication of two or three variables), and together these variables and interaction terms were used to develop logistic regression models for classifying cases as epidemics or non-epidemic (low disease years). This modeling effort focused on developing on two groups of models, pre-flowering models and post-flowering models. The pre-flowering models used only variables available prior to flowering and the post-flowering models allowed for combination of variables available prior to and during the flowering period. The prediction accuracy (percentage of correctly classified cases) for each of model was evaluated with the cases of the model development and validation data sets. Models with reasonable prediction accuracy were selected for further evaluation.

RESULTS AND DISCUSSION

Variables identified by the best-subsets procedure included variables that summarized weather conditions 7 or14 days prior and 7 days after flowering. Variables selected also represented the adjusted temperature range for perithecia development and the class variable designating a case as from winter or spring wheat production region (Table 2). Prediction accuracy of models that used only variables available prior to flowering ranged from 80 to 82% for cases used to develop the models, and 80 to 87% for cases used in

model validation. The prediction accuracy of models using variables available prior to and during crop flowering ranged from 78 to 82% for the model development data set, and consistently classified 87% of the validation cases.

Selection of the final model to deploy as part of an updated disease forecasting system will depend on ongoing analysis of model errors and final model validation with cases from the 2003 growing season. The models produced in this analysis improve the accuracy of pre-flowering predictions from 70% to near 80%. This improvement in accuracy should allow for more accurate disease forecasts at the time of flowering when fungicide applications are most effective (McMullen et al. 2000).

Table 2. Prediction accuracy of logistic regression models used to classify Fusarium head blight epidemics.

			Accuracy (%)		
Model	**Type**	**Variables**	**Development**	**Validation**	**Overall**
A	Pre	T9307,T914,TRH93014, (WC*TM7)	80.9	86.7	82.4
B	Pre	WC,RH9014,T914, RH9014*T914),	82.0	80.0	81.5
C	Pre	WC,RH9014,T914,TRH9014	80.9	80.0	80.7
D	Post	WC,RH9014,T914,TRH9014, (RH9014*T914*PRHM7)	82.0	86.7	83.2
E	Post	WC,RH9014,T914,TRH9014, PRHM7	83.1	80.0	82.4
F	Post	T9307,(RH9014*T914), (RH9014*PRHM7), (WC*T914)	77.5	86.7	79.8

Type: Pre-Flowering or Post-Flowering Prediction
Variables: WC = Class variable indicating spring or winter wheat; T914 = Duration (h) that temperature is greater than 9 C for 14 days prior to flowering; T9307 = Duration (h) that temperature is between 9 and 30°C for 7 days prior to flowering; TM7 = Mean temperature for 7 days prior to flowering; RH9014 = Duration (h) that relative humidity is greater than 90% for 14 days prior to flowering; TRH9014 = Duration (h) that both temperature is between 9 and 30°C and relative humidity is greater than 90% for 14 days prior to flowering; PRHM7 = Mean relative humidity for 7 days after the flowering begins.
Accuracy: Percentage of correctly classified epidemics and non-epidemics for cases used to develop (n=89) and validate (n=30) the models. Overall accuracy is the weighted average of development and validations cases.

We are currently working to deploy the models resulting from this analysis during the 2004 growing season in 23 states that have been impacted by scab epidemics. This experimental system will use weather variables collected from hourly reporting stations across the region and from the analysis of the Rapid Update Cycle provided by the National Weather Service. The Rapid Update Cycle (RUC20) provides hourly observations of temperature and dewpoint temperature at a 20 km grid throughout the U.S. using multiple sources of atmospheric observations. Verification for the accuracy of variables generated by the RUC20 is

currently underway. In the near future these sources of weather variables could allow predictions for Fusarium head blight at 20 km spatial resolution for all 23 states.

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FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL ACCUMULATION IN WHEAT INOCULATED AT DEVELOPMENTAL STAGES FROM FLOWERING THROUGH GRAIN MATURATION

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OBJECTIVE

Determine the relative vulnerability of wheat to Fusarium head blight and dexynivalenol contamination when infection occurs at various developmental stages from mid-flowering through kernel maturation.

INTRODUCTION

Fusarium head blight (FHB) is one of the most severe diseases of wheat affecting grain yeld and quality. The most important mycotoxin produced by *Gibberella zeae* is deoxynivalenol (DON), which is harmful to humans and livestock (Parry et al., 1995; McMullen, 1997). Contamination of wheat with DON at levels exceeding 2 ppm results in rejection of sale or severe price dockage by millers and other grain buyers.

It has been stated that, under moist conditions, the fungus can infect wheat spikelets anytime from anthesis to soft dough stages (Parry et al., 1995; McMullen et al, 1997). Yet, controversy still persists over the precise phenological window of vulnerability to infection, and especially that which results in significant accumulation of DON. Some authors demonstrated that infection occurred principally at anthesis and that anthers were a main infection site (Atanasoff, 1920; Pearce et al., 1976). However, others demonstrated an extended window of infection with a peak after flowering (Andersen, 1948; Schroeder and Christensen, 1963). DON accumulation in kernels is influenced by many factors such as strain of the pathogen, aggressiveness, temperature, moisture and host resistance (Hart et al, 1984; Wang and Miller, 1988; Mirocha et al, 1989). We investigated the role that host developmental stage at the time of infection plays in the final level of DON contamination in harvested wheat grains.

MATERIAL AND METHODS

Greenhouse experiments: Seeds of cv. Norm were sown in soil substrate within pots (2.5 L). During early flowering, groups of 6 pots were established according to phenological similarity. In each pot, 7 to 10 spikes (main tillers) were left and late tillers were consistently eliminated until maturity.

Treatments consisted of spraying a macroconidial suspension of *G. zeae* (isolate GZ014 adjusted to 10^5 spores/ml) on the spikes, at different wheat stages, 5 to 6 days apart, as follows: 1) mid-flowering; 2) kernel watery ripe; 3) kernel early milk; 4) kernel late milk; 5) kernel soft dough and 6) kernel early hard dough. The check treatment consisted of a group of spikes inoculated with water. After inoculation, plants were moved into a mist chamber for 48 hours under continuous moisture with temperature in the range of 21-24º C. After the incubation period, plants were moved back to the greenhouse until maturity. FHB incidence, percentage of spikes blighted per pot, and FHB severity, percentage of blighted spikelets per blighted

spike, were recorded when symptoms appeared, generally on the fifth day after inoculation. At maturity, kernels were harvested and evaluated for: *Fusarium* damaged kernels (FDK); 100-kernel weight; kernel infection on selective medium and DON concentration, using an Elisa Kit - DonFast Ridascreen. The experiment was repeated twice.

RESULTS AND DISCUSSION

Norm was susceptible to FHB from flowering through all stages of grain development. At the later stage, i.e., early hard dough, visual incidence and severity could not be evaluated due to natural spike senescence. FHB incidence ranged from 90-100% and typical symptoms were mostly observed 3 to 4 days after inoculation, except for the inoculation at flowering, when symptoms took 7 to 10 days to be noticed. FHB severity was higher in spikes inoculated after flowering, though severity increased until maturity (data not shown). Average percentage of relative severity from flowering to soft dough was 0, 31.3, 40.6, 67 and 84.2, respectively. FDK ranged from 94-100% between flowering and milk stage inoculations, decreasing at the later stages, but still showing 23% of damaged kernels at the early hard dough stage (Fig. 1). *G. zeae* was detected at high incidence on kernels following inoculation at all stages. Peak kernel infection occurred at the kernel late milk and soft dough stages. Average percentage values from flowering to hard dough stage was: 52, 73.6, 93.3, 99, and 70.3, respectively.

DON levels ranged from 1.2 to 98 ppm. The highest levels were detected in kernels inoculated at watery ripe stage, which was two times higher than DON levels resulting from inoculation at flowering or at late milk stages. Considerable (13.3 ppm) toxin levels were detected in grains following inoculation at soft dough, and trace levels were detected in grains inoculated at early hard dough (Fig. 1). Kernel weight was greatly reduced by inoculations from flowering to milk stages. Inoculations at dough stages produced kernels with weight similar to the nontreated check. (Fig. 1). Significant negative correlation was observed between kernel weight and DON (r^2=-0.94) and kernel weight and FDK (r^2= -0.70). Significant positive correlation was observed between DON and FDK (r^2= 0.57). Other correlations were not significant ($P<0.05$).

It was clear that Norm is a highly suscetible variety with a wide window of infection. According to Andersen (1948), wheat heads were also most susceptible to infection at post-flowering stages, contrary to the results of others who have reported that the peak of infection occurs during flowering (Atanasoff, 1920), possibly due to the stimulatory effect of anthers (Strange, 1974). Our results showed that kernels are likely to be infected whenever there is a conducive environment at very late stages of grain development, which is in agreement with a field study carried out by Fernando et al. (1997). Those authors moved plants at different stages from the greenhouse to field conditions and observed that the peak of kernel infection, detected on selective media, occurred from flowering to milk stages and that considerable levels of kernel infection occurred at dough stages. In our study, *G. zeae* was detected at high incidence in kernels inoculated at dough stages. This was not correlated positively with damage in kernels or DON.

Hart et al. (1984) found that production of DON in wheat inoculated in the greenhouse depended on the duration of head wetness, and occurred independently of the stages of kernel development after the kernels were filled. The authors stated that it was clear that DON could be produced in wheat when there was adequate moisture for fungal growth, even at later stages of kernel development. Our findings here are in agreement with those, though we have observed a discernable peak in DON production in kernels resulting from inoculations at milk stages, even in comparison to inoculation at mid-flowering. A steep decrease in

DON accumulation was observed in kernels inoculated later, with only trace amounts of toxin detected in kernels inoculated at dough stages.

In several recent years in New York state, USA (Bergstrom, unpublished), we have observed an apparent uncoupling of DON contamination in soft winter wheat from the occurrence of FHB symptoms and grain weight reduction. That is, plump, high-yielding wheat has been contaminated with DON above acceptable levels. A possible explanation supported by the current study as well as the findings of Hart et al. (1984) is that DON may be produced from infections late in grain development that do not produce dramatic reductions in grain weight or significant FHB symptoms. This complicates the already challenging task of controlling FHB and toxin contamination. Fungicide applications and FHB risk assessments today are focused almost entirely on infection during a narrow window around crop flowering. If in fact that window of vulnerability, especially for DON contamination, extends through much of grain development, then we need to consider integrated strategies that will protect wheat spikes from infection for several weeks rather than several days after flowering.

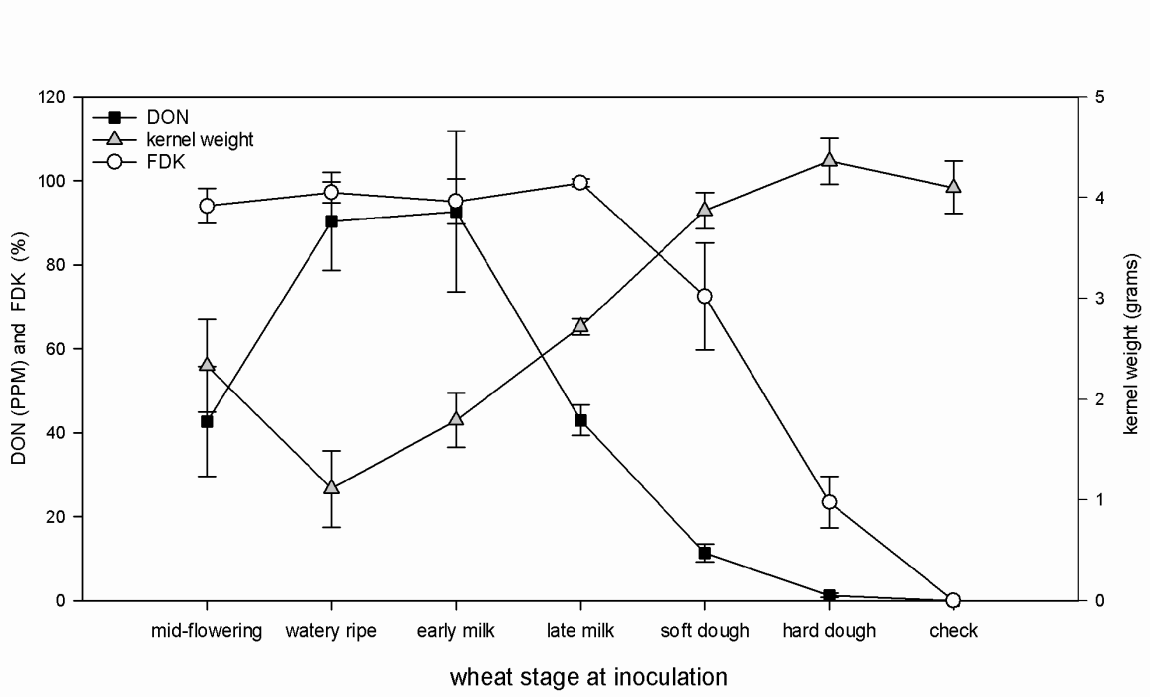

Fig. 1. Effect of wheat growth stage at time of inoculation with *Gibberella zeae* on Fusarium head blight symptoms and quality parameters in mature grain. Vertical bars are the standard deviation. Sample sizes are N=6 (DON) and N=12 (Kernel weight and FDK).

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OXYLIPIN-MEDIATED SIGNALING EVENTS CONNECTING MYCOTOXIN BIOSYNTHESIS AND SPORULATION IN *ASPERGILLUS* AND *FUSARIUM SPP.*

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OBJECTIVE

To identify *Fusarium* dioxygenases and determine their role in spore production.

INTRODUCTION

Fusarium head blight (scab) is one of the most devastating diseases of wheat and barley. It is caused by a number of mycotoxin producing *Fusarium spp.* including *F. graminearum, F. culmorum*, and *F. sporotrichioides.* The latter two *spp.* infect primarily by asexual spores (conidia) whereas *F. graminearum,* the principle scab causing fungus infects host plants with both sexual (ascospores) and asexual spores. Impediments to spore production would be useful in controlling this disease. Biochemical and genetic studies suggest that oxylipins, oxygenated derivatives of unsaturated fatty acids, are conserved signaling and structural molecules modulating fungal asexual and sexual spore development.

In 1987, Champe et al reported the detection of a secreted substance in *A. nidulans*, called psi factor (for precocious sexual inducer), that induced premature cleistothecia formation and sexual sporulation and blocked conidiation. Extensive chemical studies of the psi factor resulted in the identification of linoleic and oleic acid derived oxylipins. We now know that psi factor is a mixture of hydroxylated oleic (18:1), linoleic (18:2) and linolenic acid (18:3) derivatives (termed psiAα,β & γ, psiBα,β & γ and psiCα,β & γ) (2-4) likely produced by all filamentous fungi.

Most recently, we have been able to clone three genes (e.g. *ppoA, ppoB* and *ppoC* for psi producing oxygenase) encoding dioxygeanses that are likely to be responsible for psi production in *Aspergillus nidulans* (7). The amino acid sequence of the encoding proteins shows very high similarity to that of the psi producing protein Lds from the fungus *Gaeumannomyces graminis* (5). A putative ortholog has also been described in *Ustilago maydis* where it is found to be expressed in teliospores (6). Our goal was to identify these genes in *F. graminearum* and start to characterize their role in sporulation. As described below, these genes are not only important for sporulation but also mycotoxin production in both *Aspergillus* and *Fusarium* species.

MATERIALS AND METHODS

Sequence data from a *F. verticilliodes* EST indicated it to be a likely *ppo* gene (e value ca. -75 to *A. nidulans ppoA*). We amplified this DNA sequence (which we call *Fvppo1*) from *F. verticilliodes* genomic DNA, sequenced *Fvppo1* to confirm identity, and then used *Fvppo1* to probe *F. verticilliodes, F. graminearum* and *F. sporotrichiodes* cosmid libraries. Each *Fusarium* species contained several strongly hybridizing cosmids. Subcloning and sequencing of these cosmids yielded putative *ppo* genes. Subsequent

to this, BLAST analysis of the newly released *F. graminearum* genome (http://www-genome.wi.mit.edu/annotation/fungi/) revealed the presence of all three *ppo* genes called *Fgppo1*, *Fgppo2* and *Fgppo3* (Table 1).

A *Fvppo1* disruption vector was created in which the hygromycin resistance gene, *hygB*, was ligated between *Fvppo1* flanking DNA (e.g. ca. 1 kb of flank 5' and 3' to the *Fvppo1* ORF). This vector was then used to transform *F. sporotrichioides* to hygromycin resistance.

RESULTS AND DISCUSSION

An examination of a *ppoA* deletion strain of *A. nidulans* shows it to be defective in both spore and ST production. Deletion of *ppoA* significantly reduced the level of psiB1á and increased the ratio of asexual to sexual spore numbers four-fold (7). In contrast, forced expression of *ppoA* resulted in elevated levels of psiB1á and decreased the ratio of asexual to sexual spore numbers six-fold (7). Additionally, the *ppoA* deletion strain showed reduced ST synthesis (Tsitsigiannis and Keller, data not shown).

To determine if *ppo* genes could be playing a similar role in *Fusarium spp.*, we first identified putative *ppo* genes based on identity to *Asperillus ppo* genes. Three putative genes were found in *F. graminearum.* Transcript analysis of these three genes is shown in Figure

Next we tried to disrupt a *ppo* gene in three *Fusarium* spp. (*F. graminearum, F. verticilliodes* and *F. sporotrichiodes*) using a *Fvppo1* disruption vector. We used the same vector as DNA sequence between the three spp. is conserved and we thought it possible we could disrupt the *ppo* gene in all three spp. using one vector. Examination of 200 transformants of *F. verticillioides* and 60 transformants of *F. graminearum* did not reveal any *ppo* disruptants. Disruption was only successful for *F. sporotrichiodes* where two *ppo* deletion mutants were obtained. Examination of these *Δppo* strains of *F. sporotrichioides* indicate that T-2 toxin gene expression is greatly reduced (Figure 2) and T-2 toxin production has been inhibited (Plattner, Devi and Keller, data not shown). In addition, asexual spore production is severely reduced compared to that of wild type and oxylipin content altered (Table 2).

Taken together, these data suggest that oxylipin signaling affects both sporulation and secondary metabolism. Based on extensive studies of oxylipin signaling in mammals, we predict that oxylipins generated from the *ppo* gene products act as ligands initiating several signal transduction cascades governing global developmental pathways.

We also suggest that the linkage of sporulation and metabolite production is not spurious as other signaling pathways (e.g. a G protein pathway) has also been found to link sporulation and mycotoxin production in *Aspergillus* and *Fusarium* (1 and references therein). The reasons for this co-regulation of sporulation and metabolite production are most likely to be discovered at the ecological and organismal level. It is tempting to speculate that the simultaneous regulation of both processes is associated with protective properties (e.g. allelopathic or anti-herbivoric chemicals, UV damage mitigation) of secondary metabolites in a sporulating colony.

ACKNOWLEDGEMENTS

We thank the Jan G. Jaworski (Donald Danforth Plant Science Center, St Louis, MO) lab and Dr. Robert Zarnowski for assistance in oxylipin analysis. Funding was provided through NRI-USDA 2001-35319-10996 and through the U.S. Department of Agriculture, under Agreement No. 59-0790-3-081. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative.

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

Table 1. Homology of Fusarium dioxygenases to linoleate diol synthase (*lds*) gene of *Gaeumannomyces. graminis*.

		E value	% identity	% similarity
F. graminearum	Ppo1	0.0	42	56
	Ppo2	1×10^{-98}	36	52
	Ppo3	1×10^{-158}	40	54
F. verticillioides	Ppo1	1×10^{-158}	39	53
F. sporotrichioides	Ppo1	1×10^{-157}	40	54

Table 2. Spore production and oxylipin content of wild type and *Δppo1* strains of *F. sporotrichioides.*

	Conidia/µl[1]	8-HOE[2]	8-HODE
Wild type	6.6×10^{4}	8.3	41.0
Δppo1	3.2×10^{4}	1.9	1.9

[1]The number of spores are statistically different ($P < 0.05$) between wild type and *Δppo1* with *Δppo1* consistently producing ca. 1/2 the amount of spores as wild type.

[2]8-HOE = 8 hydroxyoleic acid or psiBß? 8-HODE = 8 hydroxylinoleic acid or psiBα in µg/g mycelium (dry weight). Both 8-HOE and 8-HODE values where significantly reduced in the *Δppo1* strain compared to wild type ($P < 0.05$).

Data was replicated three times.

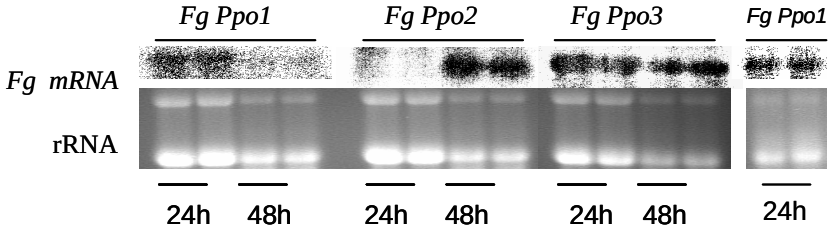

Figure 1. mRNA analysis of *ppo* genes in wild-type strains of *F. graminearum* and *F. sporotrichioides.*

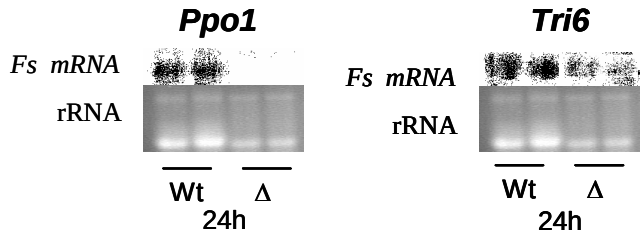

Figure 2. mRNA analysis of *ppo1* and *tri6* in wild-type and *Δppo1* strains of *F. sporotrichioides.* Note that deletion of *ppo1* results in a decrease in *tri6* expression. *tri6* encodes an enzymatic gene required for the production of the trichothecene T2 toxin in *F. sporotrichiodes.*

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RAIN SPLASH DISPERSAL OF *GIBBERELLA ZEAE* SPORES IN A WHEAT CANOPY

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ABSTRACT

To examine the role of rain splash in the dispersal of *Gibberella zeae* (anamorph: *Fusarium graminearum*), the cause of Fusarium head blight of wheat, splashed water was collected during rain events at three heights (0, 30, and 100 cm) above the soil surface over 3 years. Samplers were placed in a reduced tillage wheat field with corn residue and in a breeding nursery. The splash samplers consisted of sheltered funnels and flasks. Splashed rain was collected for three rain events in 2001, seven in 2002, and 10 in 2003. To determine spore deposition levels, for each rain episode, 1 ml of rain splash water was transferred to replicated petri plates with Komada's selective medium, and colony forming units were counted after an incubation period. Based on the flux density of splashed water, and the spores per ml of water, spore flux density (spores per square centimeter per hour) was determined for each rain event. The intensity of splashed rain (mm/h) was highest at 100 cm, indicating that substantial splashing of incident rain occurs from the upper wheat canopy (heads and flag leaves). Spores were detected in every sampled rain event at all heights, with slightly fewer spores at 100 cm compared to the other two heights. There was a strong linear relation, on a log-log scale, between spore flux density and both incident rain intensity and splash rain intensity. Therefore, in addition to aerial dispersal of spores by wind, rain splash dispersal contributes to the movement of inoculum within wheat canopies and may contribute to Fusarium head blight epidemics.

REACTION OF PRIMARY LEAVES OF 26 WHEAT GENOTYPES INOCULATED WITH MACROCONIDIA OF *FUSARIUM GRAMINEARUM* AT THE SEEDLING STAGE AND ASSESSED FOR LESION LENGTH AND DEOXYNIVALENOL ACCUMULATION AT 96 H POST-INOCULATION

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ABSTRACT

Lesion reaction and deoxynivalenol accumulation was investigated in primary leaves of seedlings of 26 wheat genotypes following inoculation with *Fusarium graminearum*. Our objective was to develop a model system to investigate how *F. graminearum* infects and colonizes tissues other than those found in spikes. We developed an inoculum consisting of macroconidia of *F. graminearum* suspended in a weak solution of water-agar (0.3% w/v) containing the surfactant Tween-20 (2 ml/l). The surfactant facilitated adhesion of the inoculum to the leaf cuticle and the weak agar suspension provided an increased viscosity so that the droplets of inoculum would not run or fall off. Water-agar was utilized so that the addition of nutrients for spore germination and fungal growth would be minimal. Primary leaves of seedlings were inoculated 14 days after planting. Seedlings were planted at 4-5 plants per conetainer (4 x 20 cm, dia. x length) containing a soil-less potting mix. The entries were replicated (7 reps, 28 leaves) and the experiment was repeated once. Leaves were inoculated by carefully placing a 10-µl droplet on the center of the leaf, between leaf tip and ligule, of the abaxial leaf surface using a micropipette. Droplets of inoculum on leaf surfaces were allowed to dry for 1-2 hours before moving inoculated plants to a dew chamber providing ca. 100% relative humidity. Plants were maintained in the dew chamber for 72 h then removed to greenhouse benches for another 24 h. Lesions on the inoculated leaves were measured at 96 h post-inoculation. Primary leaves of seedlings of each replicate were first removed then lesions were measured as the length of the longest necrotic lesion dimension in the longitudinal orientation of each leaf. Leaves of seedlings from each conetainer (replicate) of the 26 genotypes were bulked and placed in 1-dram vials and frozen at –20º or -80º C. Leaves were later extracted for analyses and quantification of deoxynivalenol and 15-acetyldeoxynivalenol. In the first experiment we observed significant differences ($P < 0.001$) among the 26 wheat genotypes for both lesion reaction (mean = 3.3 mm, range = 0-27.0 mm) and for accumulation of deoxynivalenol (mean = 0.2 ppm, range = 0-0.92 ppm). We observed large lesions on the primary leaves of Alsen (8.9 mm long), a cultivar known to be resistant to spike and grain colonization by *F. graminearum* in the field, but did not detect any deoxynivalenol accumulation (0 ppm). Frontana was unusual in that it was the only genotype we did not observe any lesion reaction (0 mm) however toxin accumulation (0.53 ppm) was detected in the inoculated but non-symptomatic leaves. The inoculation technique described may be useful to molecular investigations of gene expression and in studies of tissue colonization and Fusarium head blight epidemiology.

DETECTION OF DISTINCT SUBPOPULATIONS OF *FUSARIUM GRAMINEARUM* LINEAGE 7 IN THE U.S.

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ABSTRACT

A collection of the cereal head blight pathogen *Fusarium graminearum* from nine U.S. states, representing 86 fields in 53 counties, was characterized using ten single-copy RFLP probes, a telomeric probe and RFLP probes diagnostic for species and lineage. In addition, isolates were assigned to one of three profiles of trichothecene metabolites (chemotypes) using a PCR-based approach. All 708 isolates determined to be *F. graminearum* were confirmed as lineage 7. The telomeric probe was used for clone determination, leaving 587 isolates for subsequent data analyses. Most lineage 7 isolates (94.6%) from the U.S. were of 15-acetyl deoxynivalenol (15ADON) chemotype. The 3-acetyl deoxynivalenol (3ADON) chemotype was found at 5% and was only identified in samples from North Dakota and Minnesota. The nivalenol chemotype was infrequent at 0.4%. Gene flow analysis demonstrates that the 15ADON population in the U.S. is genetically isolated from the 3ADON population (Nm = 0.5). In comparison, a representative collection consisting of 19 isolates of lineage 7 from Italy was genetically similar to the 3ADON population from the U.S. (Nm > 2), though the Italian collection consisted of all three chemotypes. These results would indicate that lineage 7 consists of at least two distinct subpopulations.

ANALYSIS OF GENE EXPRESSION IN *FUSARIUM GRAMINEARUM* DURING INFECTION ON WHEAT

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ABSTRACT

Fusarium graminearum is a species complex containing at least nine biogeographically structured lineages. With an aim to elucidate gene function related to pathogenicity, we evaluated the abilities of different strains of *F. graminearum* to spread in their hosts and are currently conducting genomic analyses of these interactions. Our previous studies on 31 representative strains from these lineages have shown that they can differ significantly in their aggressiveness on wheat and also in the type and amount of mycotoxin they produce including deoxynivalenol, nivalenol and others. Based on these differences, we selected two strains with high (PH-1; NRRL 31084) and low (NRRL 28303) virulence for genomic studies. Two cDNA libraries were created by suppression subtractive hybridization to compare mRNA populations from wheat heads inoculated with the above mentioned strains and to identify genes specific to each interaction. Upon examination of 1339 EST sequences from these libraries, marked differences in overall gene expression were observed. However, the percentage of fungal sequences found was quite low. Therefore, to further characterize fungal genes expressed during the disease interaction, another subtractive library was constructed using wheat inoculated with NRRL 31084 and mock inoculated wheat heads. Nearly 25% of the 1236 EST sequences examined from this library were of fungal origin as determined by matches to the *F. graminearum* genome sequence. Comparisons of ESTs were also made to databases of other fungi for which whole genome sequences are available including *Magnaporthe grisea* and *Neurospora crassa*. Based on such comparisons and predicted function of genes corresponding to ESTs, candidate sequences potentially involved in pathogenicity have been identified and are being targeted for gene disruption.

THE WHOLE GENOME SEQUENCE OF *FUSARIUM GRAMINEARUM*, LINEAGE 7

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ABSTRACT

We have generated a draft sequence assembly of the *F. graminearum* genome that is available on the web for download and query. The sequence is of high quality with the entire 36 Mb assembly consisting of just 511 contigs (> 2 kb) contained within 43 supercontigs (scaffolds). The second genome release (October 2003) contains automated annotation, preliminary genome analysis and integration with the genetic map. Using organism-specific parameters for gene prediction, 11,640 protein-coding genes have been identified, representing over 1,500 more genes than predicted by the same method for the non-pathogenic filamentous fungi, *Neurospora crassa* and *Aspergillus nidulans*. A genetic map has been constructed that anchors 99.5% of the sequence assembly. Details of the automated annotation, efforts toward manual annotation and coordination of functional analysis of the genome will be discussed.

BIOASSAY VS. CONVENTIONAL CHARACTERIZATION OF FHB RESISTANCE IN NING 7840

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INTRODUCTION

Type II resistance to FHB is the primary focus of many U.S. wheat breeding programs (Rudd *et al.*, 2001). The most common greenhouse method for screening Type II resistance is single floret inoculation followed by counting the number of visually "scabby" spikelets at various days post inoculation (dpi) (Schroeder & Christensen, 1963). Although the number or proportion of scabby spikelets is a convenient method for scoring genotypes for resistance, researchers have questioned the ability of this method to accurately reflect the amount of disease present on a wheat spike (TeKrony *et al.*, 2000; Edge *et al.*, 2001). In this study we examined Type II resistance in FHB resistant line 'Ning 7840' and susceptible 'Norm' in the spikelets and rachis using visual and bioassay techniques.

MATERIALS AND METHODS

Planting: Seeds of the cultivars Ning 7840 and Norm were imbibed at room temperature for 24 hours, and subsequently vernalized (~2°C) in the dark for 5 weeks. After vernalization, germinated seeds were kept at room temperature in the dark for 48 hours before transplanting in the greenhouse at a density of four seedlings per pot. Rows of pots were alternated by genotype to reduce the effects of environmental gradients in the greenhouse.

Inoculation: Inoculum of *Fusarium graminearum* (Schwabe) isolate PH-1 (isolated from MI by Dr. L. Patrick Hart) was produced in Carboxymethyl Cellulose (CMC) liquid medium (Cappellini & Peterson, 1965). The CMC medium was removed through centrifugation and the spore concentration was adjusted to 1.4 × 1066 spores/ml (equivalent to10,000 spores/7µl) by the addition of sterile water. Just prior to anthesis (anthers slightly yellow to very yellow) forty spikes of each genotype were pipette inoculated with 7µl of spore suspension into one basal floret of one central spikelet of each head. Four spikes were randomly selected as non-inoculated controls. Immediately after inoculation, plants were misted for 5 seconds every 5 minutes for 72 hours in a growth chamber (~ 22°C/72°F). Seven and 14 days post inoculation (dpi), 20 inoculated and 2 control heads of each genotype were randomly selected and harvested for disease evaluation. Disease was evaluated using 3 primary methods:

1. <u>Number of Scabby Spikelets (SS)</u>: The number of Scabby Spikelets (SS) was counted based on discolored tissue (due to chlorosis, necrosis, or brownish/reddish discoloration typical of the disease) on one or more glumes, lemma, or palea of any of the florets of the spikelet. Only a portion of a spikelet needed to be symptomatic for the spikelet to be counted as scabby. Both the total SS and the SS up (towards the terminal spikelet) and down (towards the peduncle) from the inoculated spikelet were recorded.

2. Visually Infected Rachis Sections (VIRS): Spikelets were removed from the rachis and the rachis was visually inspected for symptoms of disease. The node subtending the originally inoculated spikelet was termed the 'node inoculated' and was considered a single rachis section. Rachis sections were defined for the remainder of the rachis depending on their location with respect to the 'node inoculated'. *Above* the 'node inoculated' sections were defined as starting just *above* one node and ending just *above* the adjacent node. *Below* the "node inoculated" sections were defined as starting just *below* one node and ending just *below* the adjacent node. In this way, the number of rachis sections infected on a spike was counted, and this count was termed the Visually Infected Rachis Sections (VIRS). Both the total VIRS and the VIRS up (towards terminal spikelet) and down (towards peduncle) from the node inoculated were recorded.

3. Bioassay Infected Rachis Sections (BIRS): The rachis was surface sterilized by soaking in 20% bleach + 0.1% Tween 20 for 2 minutes, then rinsed in sterile water. The rachis was cut into sections (as defined in method 2 above) using a sterile scalpel. Rachis sections from a single spike were plated sequentially in a circular pattern on a large petri dish (150 × 15mm) containing PDA. The number of sections from which *F. graminearum* grew into the media were counted and termed the Bioassay Infected Rachis Sections (BIRS) for that spike. Both the total BIRS and the BIRS up from (towards the terminal spikelet) and down from (towards the peduncle) the node inoculated were recorded.

***Replication*:** The entire experiment was replicated three times over a period of several months.

***Excluded Data*:** BIRS data for replication 1 at 7 and 14dpi and replication 2 at 7dpi was not used because surface sterilization of plant tissue was not effective, resulting in contamination. In addition, twenty-eight spikes (out of 240 inoculated) were not included in SS, VIRS or BIRS data analysis set because of escape, injury, missing data, strange visual symptoms resembling glume blotch (caused by *Septoria nodorum*), or a suspected second infection point.

***Transformation of Data*:** A square-root plus one transformation was used for data analyses. Back-transformation was used to report the estimated means.

RESULTS AND DISCUSSION

Total Spread:

***Within a Genotype* (Fig. 1A):** The mean number of Scabby Spikelets (SS) was significantly less (alpha 0.05) than Visually Infected Rachis Sections (VIRS) and Bioassay Infected Rachis Sections (BIRS) for both Ning 7840 and Norm at 7 and14 days post inoculation (dpi). In contrast, VIRS was not significantly different from BIRS for either genotype at either 7 or 14dpi. These data are consistent with other researchers that have shown that the fungus spread in the rachis is more extensive than in the spikelets (Pugh *et al.*, 1933; Edge *et al.*, 2001; TeKrony *et al.*, 2000). In addition, the greater spread of symptoms in the rachis versus the spikelets suggests that different genetic mechanisms may be responsible for these two measurements, as has been proposed by Yu 1990 (see Bai and Shaner, 1996).

***Between Genotypes* (Fig. 1B):** The mean SS, VIRS and BIRS of Ning 7840 was significantly less than the mean total SS, VIRS and BIRS (respectively) of Norm at both 7 and 14dpi (alpha 0.05).

***Between 7 and 14dpi* (Fig. 1A and 1B):** Comparison between 7 and 14dpi for SS, VIRS and BIRS of Ning 7840 and Norm revealed that Ning7840 SS was not significantly different (p-value = 0.35) between

the two time points, whereas all other measurements for Ning7840 and Norm were significantly different (alpha 0.05). In addition, Ning7840 SS was approximately 53% of the values of VIRS and BIRS at 7dpi, while it was only 37% and 39% of the values of VIRS and BIRS values (respectively) at 14dpi. These data reveal that although Ning7840 shows reduced SS, VIRS and BIRS in comparison to Norm (Fig. 1B), Ning 7840 may be more effective in resisting the spread of scabby spikelets than in resisting the spread of visually and bioassay infected rachis section.

Bimodal Spread of BIRS in Ning7840: Histograms of the percent of the total number of observed spikes at each level of SS, VIRS, and BIRS (Fig. 2) revealed that 34% of Ning 7840 spikes did not show spread of the fungus (BIRS) beyond the initially inoculated node at both 7 and 14dpi. The spread of *F. graminearum* in the rachis of Ning 7840 appeared to have a bimodal trend: either showing minimal or extensive spread of the fungus. For Ning 7840 BIRS at 14dpi approximately 44% of the spikes showed infection in 2 or fewer rachis sections, whereas approximately 56% showed infection in 9 to 14 rachis sections. Norm, in contrast, did not have any spikes in which the fungus was restricted to the initially inoculated node at either 7 or 14dpi. For Norm BIRS at 14dpi 100% of spikes showed at least 6 infected rachis sections, and approximately 84% of spikes showed infection in 9 to 14 rachis sections. Histopathological examinations of Sumai 3 showed that barriers formed in some xylem cells, which were eventually overcome by the fungus (Ribichich *et al.* 2000). In addition, Ning7840 is thought to have the same major QTL for resistance as Sumai 3 (Bai *et al.* 2003). The presence of these structural barriers, which may be overcome, may explain our bimodal results in Ning 7840, where the fungus never moved beyond the initially inoculated node for several spikes, while in others it moved extensively in the rachis but did not appear to invade the adjoining spikelets.

Spread Up and Down from Inoculated Node:

Within a Genotype **(Fig. 1C):** The number of Scabby Spikelets (SS) up (towards the terminal spikelet) from the node inoculated was not significantly different from SS down (towards the peduncle) from the node inoculated except for Norm at 14dpi, in which SS up was significantly less than down. In contrast, visually infected rachis sections (VIRS) and bioassay infected rachis sections (BIRS) up from the node inoculated were significantly less than down for Ning7840 at both 7 and 14dpi, and for Norm at 14dpi. For Norm at 7dpi, none of the measurements (SS, VIRS and BIRS) up were significantly different from the respective measurements down, although all SS, VIRS and BIRS up were significantly less than the respective measurements down at 14dpi. Overall SS, VIRS and BIRS up represented 30-49% of the total spread from the node inoculated, though it was often significantly less (alpha 0.05) than the measurements down. This data is in agreement with other studies that found that the fungus spreads primarily down the rachis from the point of inoculation (TeKrony *et al.*, 2000), although it does reveal that there is also a substantial amount of spread up.

Between Genotypes **(Fig. 1D):** Ning7840 SS, VIRS and BIRS up from the inoculated node were significantly less than Norm SS, VIRS and BIRS (respectively) for 7 and 14dpi. Ning 7840 SS, VIRS and BIRS down from the inoculated node were significantly less than Norm SS, VIRS and BIRS (respectively) for all cases except SS at 7dpi (for which there was no significant difference).

Bimodal Spread of BIRS in Ning7840: The bimodal spread observed in the total BIRS of Ning 7840 (see above) was reflected in BIRS up, but was easily seen in BIRS down (data not shown).

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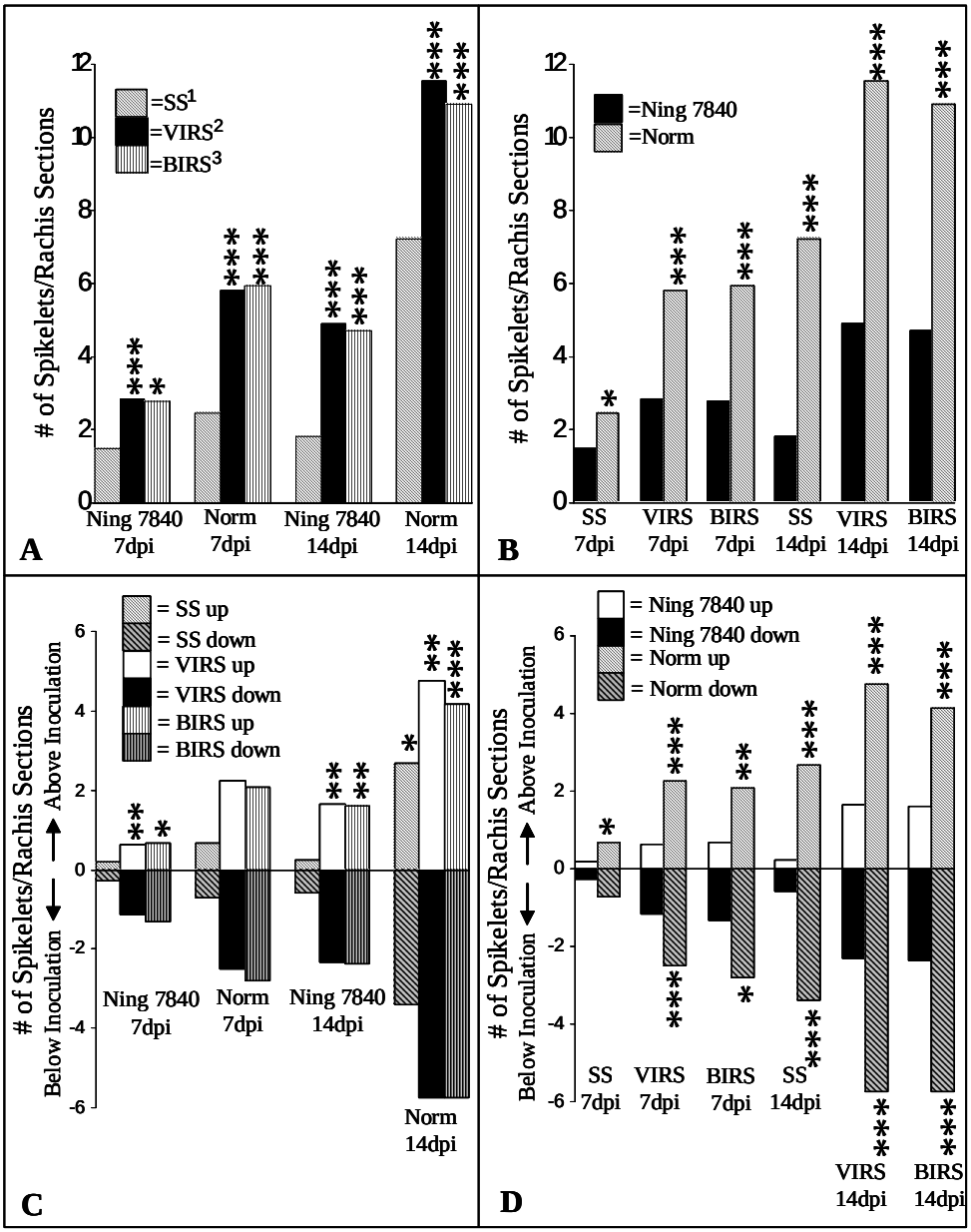

Fig. 1. Effect of Genotype, time of assay, and method of assay on the number of infected spikelets or rachis sections in a resistant (Ning 7840) and susceptible (Norm) wheat cultivar. A. Comparisons between the number of scabby spikelets (SS) and the number of infected rachis sections determined either visually (VIRS) or by bioassay (BIRS) within genotypes at 7 and 14 dpi. Asterisks above a column indicate a significant difference between that column's mean and the # of scabby spikelets for that genotype/dpi combination. **B.** Comparisons between Ning 7840 and Norm for three measures of disease spread at 7 and 14 dpi. Asterisks above a column indicate a significant difference between Ning7840 and Norm for that measurement/dpi combination. **C.** Comparisons between upward and downward spread from the inoculated node for three measures of disease spread in Ning 7840 and Norm. Asterisks above a column indicate a significant difference between the upward and downward spread for that measurement/genotype/dpi combination. The "0" on the Y axis represents the inoculated node. Spread up from the inoculated node is represented by numbers >0, and down from the node inoculated is represented by numbers <0. **D.** Comparisons between Ning 7840 and Norm for upward and downward spread from the inoculated node for three measures of disease. Asterisks indicate a significant difference between Ning7840 and Norm for the upward (asterisks above upper bars), or downward (asterisks below lower bars) spread for that measurement/dpi combination. The "0" on the Y axis represents the inoculated node. Spread up from the inoculated node is represented by numbers >0, and down from the inoculated node is represented by numbers <0. *p< 0.05, ** p < 0.01, ***p< 0.001.
1. SS = Number of Scabby Spikelets. 2. VIRS = Visually Infected Rachis Sections. 3. BIRS = Bioassay Infected Rachis Sections.

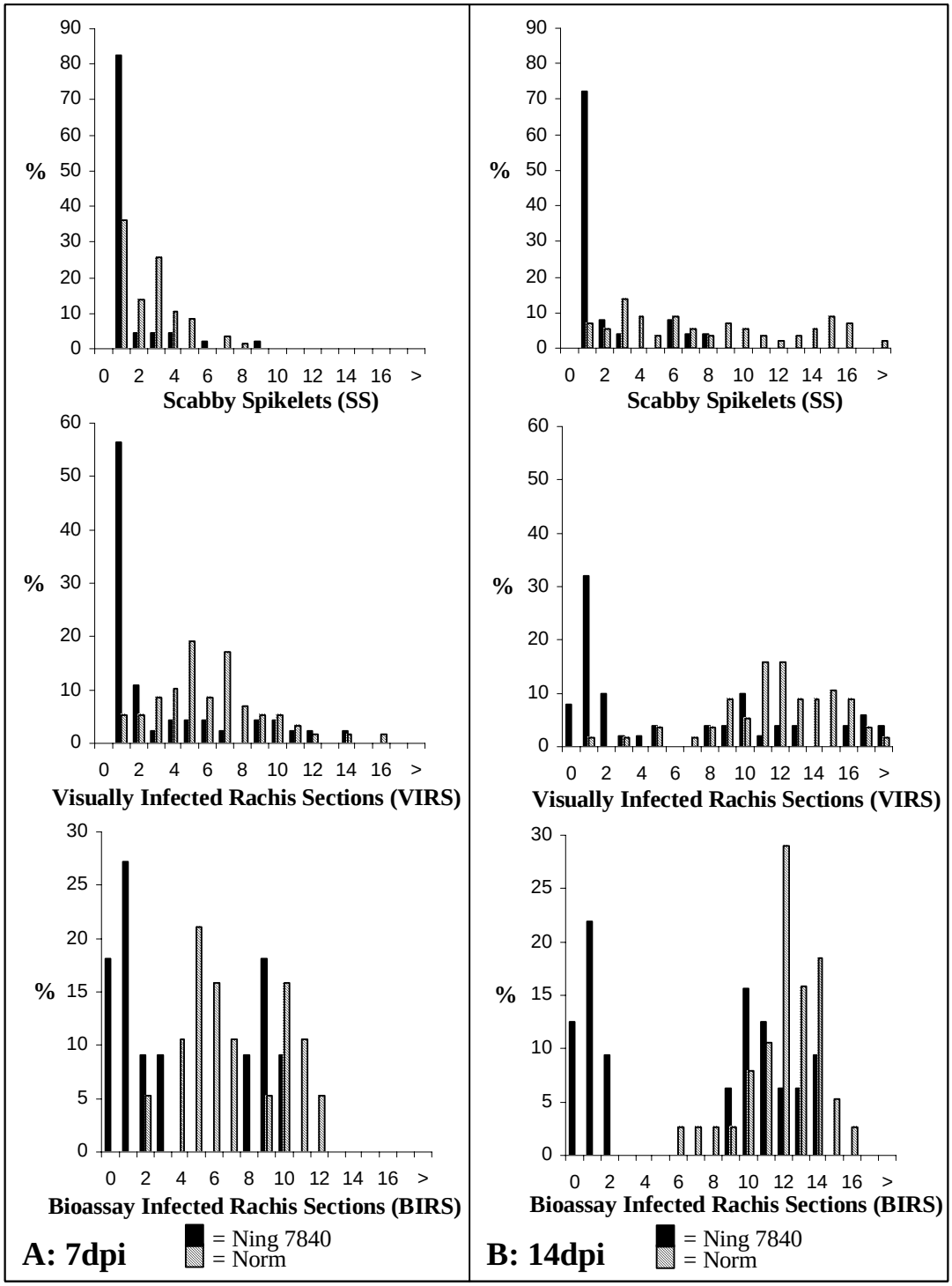

Fig. 2. Relative frequency distributions of disease as determined by three measures in Ning 7840 and Norm at 7 dpi (A), and 14 dpi (B). The Y axis represents the percent of observed spikes with a given X axis category. Note that the scaling of the Y axis values varies with measure of disease.

FUSARIUM HEAD SCAB RISK FORECASTING FOR OHIO, 2002-2003

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ABSTRACT

During the 2002 and 2003 wheat growing season, models were used to predict the risk of Fusarium head scab in Ohio. This was the second and third years for testing these models in the state. Head scab risk assessment probabilities were derived from logistic models previously developed from hourly weather, crop growth and disease observations from 50 location-years representing three wheat production regions in the US. Hourly weather data from weather stations were used to determine duration of weather events for the pre- and post-anthesis time periods examined by the models. Disease risk probabilities were calculated using logistic equations determined by two models representing the critical weather conditions during the time period 7 days prior to anthesis (Model I) and the time period of 10 days post anthesis (Model II). During both years, weather conditions in early April were relatively dry and warm providing conditions for rapid and early development of the crop. Anthesis dates for wheat fields from south to north in the state varied by more than four weeks (10 May to 9 June 2002 and 9 May to 4 June 2003) due to cool weather that slowed plant development in May. Precipitation events became more frequent throughout May across the state with most locations reporting up to 32 and 37 hours of measurable precipitation during the 7 days prior to anthesis for 2002 and 2003, respectively. However, average daily temperatures for most locations in the state were generally below 15 C when most of the wheat was in anthesis. Scab risk probabilities were calculated for early, mid and late anthesis dates for each weather station location. Calculated risk probabilities ranged from 0.00 to 0.81 for Model I and from 0.02 to 0.69 for Model II during 2002, and from 0.00 to 0.82 for Model 1 and from 0.03 to 0.24 for Model II in 2003. Of 42 location-anthesis date scab-risk probabilities calculated during 2002, Model I predicted 31 location-anthesis dates with low to moderately low risk and Model II predicted 40 location-anthesis dates with low or moderately low risk. Of 21 scab risk probabilities calculated during 2003, Model I predicted 17 location-anthesis dates with low to moderately low risk and Model II predicted 21 location-anthesis dates with low to moderately low risk. Based on these results, the head scab risk prediction was reported to be low to moderately low for the majority of locations in the state both years. Head scab risk predictions were posted on the Ohio State University Ohio Field Crop Disease web page (www.oardc.ohio-state.edu/ohiofieldcropdisease/) during the critical time of disease development through harvest. Approximately 14 to 18 days after anthesis, 159 fields in 2002 and 148 fields in 2003 in 30 counties were surveyed for scab incidence by the OSU Extension Agents. From 1 to 10 fields were surveyed per county. Disease surveys indicated the mean incidence of head scab was 4.1% with a range of 0% to 49% in 2002 and 8.9% with a range of 0% to 73% in 2003. Results of the Scab Risk Assessment Models indicated that they generally predicted the risk of scab adequately for the majority of locations in the state both years.

GLOBAL GENETIC DIVERSITY OF *FUSARIUM GRAMINEARUM* CLADE SPECIES AND THEIR MYCOTOXIN POTENTIAL

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ABSTRACT

Although the primary etiological agent of FHB, *Fusarium graminearum,* has been regarded as a single, panmictic species worldwide, phylogenetic analyses of DNA sequences from 11 nuclear genes totaling 13.6 kb [i.e., genealogical concordance phylogenetic species recognition (GCPSR)] have shown that this morphospecies actually consists of 9 phylogenetically distinct and biogeographically structured species (hereafter referred to as the *Fg* clade) [PNAS 97:7905-7910 (2000) and PNAS 99:9278-9283(2002)]. GCPSR is based on the fact that population-splitting events associated with speciation eliminate shared neutral polymorphism over time, resulting in descendant species with reciprocally monophyletic genealogies of orthologs. Given their importance to world agriculture, species rank is formally proposed for the eight unnamed cryptic species within the *Fg* clade using fixed nucleotide characters and conidial characters. In addition to the unexpectedly high level of species diversity within the *Fg* clade, the virulence-associated trichothecene mycotoxin genes are under a novel form of balancing selection resulting in the maintenance of B-trichothecene chemotype polymorphism through multiple speciation events, which may have important consequences for the fitness and aggressiveness of FHB pathogens on particular hosts or in particular environments. Taken together, these studies suggest that the combined species and mycotoxin diversity of FHB pathogens is remarkably high. However, it appears that only a fraction of this diversity is currently represented within North America. Therefore, the introduction of novel FHB pathogens or chemotypes via global trade in agricultural products has the potential to exacerbate the FHB problem in the U.S. We have developed protocols for the multiplex amplification of two sets of chemotype-specific primers, previously designed from genes within the trichothecene gene cluster (TRI3 and TRI12). Using these tests, chemotype diversity has been assessed in a collection of isolates from the U.S., China and Brazil. Chemotype frequencies were more balanced within Brazil (15ADON, 3ADON, NIV) and China (3ADON, NIV) compared with the U.S. [predominantly 15ADON, see Gale at al. poster], although the 15ADON chemotype was completely absent from the four Chinese populations surveyed to date. The development of robust molecular tools for FHB species identification and chemotype determination will significantly improve disease surveillance and global monitoring efforts, and will make available for the first time detailed information on the geographic and host distributions of FHB pathogens and their trichothecene chemotypes, enhancing current knowledge of the ecology, epidemiology and population dynamics of these mycotoxigenic cereal pathogens.

EPIDEMIOLOGICAL STUDIES ON FUSARIUM HEAD BLIGHT OF WHEAT IN SOUTH DAKOTA FOR 2003

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INTRODUCTION AND OBJECTIVES

It has been observed that FHB occurs at epidemic levels when warm, humid conditions and frequent precipitation have occurred at anthesis (Bai and Shaner, 1994; McMullen et al., 1997; Parry et al., 1995). 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 environments, 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 entirely conducive to disease development, inoculum level may be more predictive of final disease than environment on its' own.

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 upper mid-west. The ultimate goal 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. The primary objectives include: 1) establishment of three distinct inoculum (spore) loads through varying the amount of corn stalk residue on the soil surface beneath experimental plots; 2) to determine the effects of high, medium and low inoculum loads and weather on final disease and mycotoxin levels in grain; and 3) to continue to collect and analyze environmental data in conjunction with inoculum and disease monitoring for use in developing and evaluating FHB forecasting models.

MATERIALS AND METHODS

Field plots of spring wheat (*Triticum aestivum* L.) were established near Brookings, SD in a randomized complete block split-plot design. Whole-plots were 6.10m by 15.24m (20ft by 50ft) and consisted of corn stalk residues at levels of: 1) zero (0%), 2) moderate (15%), and 3) heavy (80%) ground cover as measured by the line-transect method. Sub-plots consisted of 2 planting dates (14 Apr and 23 Apr), with a second split for cultivar ("Norm" and "Alsen") resulting in experimental units ('plots') of 3.05m by 6.10m (10ft by 20ft). Whole-plots (residue treatments) were buffered on all sides by 9.14m (30ft) of "Reeder", a tall, late flowering spring wheat variety.

Wheat development was recorded weekly until boot stage, then daily thereafter. Final disease levels were assessing as incidence and severity on 100 heads per 'plot'. Severity was assessed based on percentage of the spike area blighted (Stack and McMullen, 1995). All plots were evaluated at late dough stage (Feekes 11.2) for disease development. Test weights, yield, deoxynivalenol (DON) concentration, and percent scabby kernels were assessed for each 'plot'.

Weather and microenvironment data were continuously collected using a datalogger (Campbell Scientific Inc. model CR10X) and various instruments. Leaf wetness sensors (Campbell Scientific Inc. model 237) were used to estimate the duration of leaf wetness within the canopy. Additional sensors were constructed and deployed to detect moisture at the soil surface (Osborne and Jin, 2000).

Daily airborne inoculum levels were monitored during the sampling period using a Burkhard Cyclone Sampler (Burkhard Manufacturing) placed within the border area of the field. A wash of the cyclone unit was performed daily to ensure uniform sampling. The sample and wash were plated on Komada's medium, selective for *Fusaium* (Komada, 1975). Counts were reported as colony forming units (CFU) per day. Inoculum within 'plots' was enumerated by sampling and washing spikes using protocols described by Francl et al. (1999). On each day, five primary spikes per replicate were collected and placed in a flask with 50ml of sterile deionized water, shaken vigorously for 60 seconds to dislodge spores, then discarded. A 0.5ml aliquot of the wash was then spread-plated onto each of three plates of Komada's medium. Plates were then incubated 5-8 days. Colonies of *F. graminearum* were counted after incubation. Colonies were reported as CFU per spike per day.

Data from the 2003 FHB monitoring plots will be entered into two FHB risk assessment/disease forecast models made available by Ohio State University (Ohio I and Ohio II; De Wolf, et al, 2003). Ohio model I is used to predict risk of a FHB epidemic based on temperature and precipitation variables prior to anthesis. Ohio model II is intended to predict disease risk based on temperature and humidity before and after flowering begins. Model I is intended to predict epidemics before infection, while Model II is intended to estimate disease risk after infection may have occurred.

RESULTS AND DISCUSSION

The 2003 field season was highly favorable for spring wheat yields in much of South Dakota though FHB levels were moderate to low across the region. Temperatures were warm during much of the flowering period, and were considered to be within the FHB-favorable range. Rainfall was limiting during susceptible periods for both planting dates. Only three significant precipitation events (>3mm) fell during the three-week monitoring period corresponding to flowering-to-grain fill periods for both planting dates. Disease levels (incidence and severity) are given in Fig. 1. Values represent low levels of incidence and severity for both varieties. For planting date 2, there is a trend of increased incidence with increased residue cover, which suggests higher levels of spore inoculum present in the higher residue plots during susceptible periods. The severity values show no clear differences in relation to residue cover, as would be expected with low incidence and the dry environment. Figure 2, results of the inoculum bio-assay (head washing), indicate differences in inoculum concentration on spikes corresponding to levels of residue cover for both of the planting dates. Inoculum levels are considered to be favorable for high levels of disease development, therefore it is assumed that some other factor was limiting (presumably precipitation).

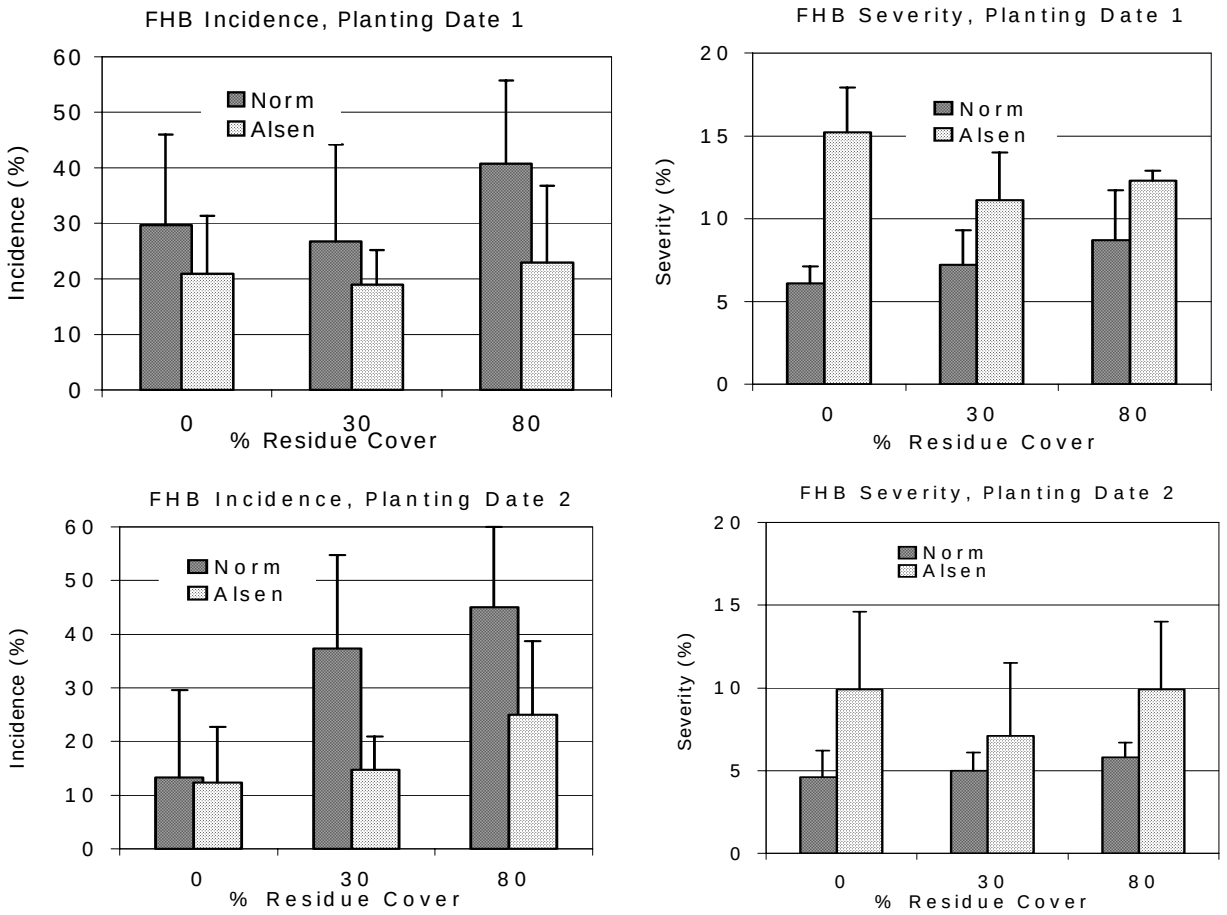

FIGURE 1. Field FHB incidence and severity.

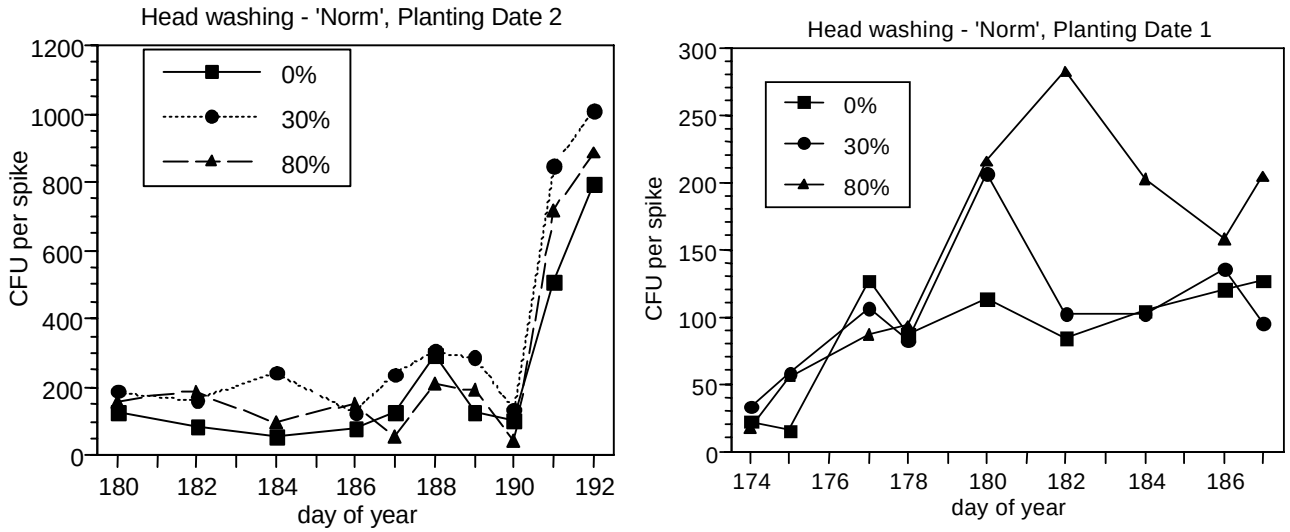

FIGURE 2. Inoculum washed from spikes

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FHB RISK ADVISORY FOR SPRING WHEAT IN SOUTH DAKOTA, 2003

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ABSTRACT

In 2003, the small grains pathology project at South Dakota State University continued to provide a web-delivered, weather-based risk advisory for Fusarium head blight (FHB) in northeastern South Dakota. An twelve-county area comprising the majority of the spring wheat production region in the state was selected for intensive inoculum, disease and environmental monitoring. This area was selected for a FHB risk advisory to be issued on a county by county basis. Advisory information was posted to the internet every one to two days during peak susceptibility periods (flowering) detailing potential risk of disease to wheat crops in each of the 12 counties. Experimental risk assessment models (Ohio I and Ohio II) were utilized to provide risk probability based on a few selected environmental parameters. Model output was considered as part of the overall risk assessment upon which advisories were based. A 'high-risk' advisory was issued for all 12 counties at some point during the three weeks of intensive monitoring (June 16 through July 9). Two counties (Marshall and Roberts) were under 'high-risk' advisory for the entire three weeks. FHB scouting across the region showed disease index levels ranging from low (<less than 8%), to very high (>20%) in parts of Marshall and Roberts counties.

SPATIAL RELATION OF DON CONTAMINATION TO CORN RESIDUES IN SPRING WHEAT

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ABSTRACT

In 2003, as part of a collaborative study investigating the effect of variable inoculum levels on field levels of Fusarium head blight (FHB), plots were established near Brookings, SD to monitor inoculum and disease in spring wheat. Plots were treated by spreading corn stalk residue to achieve three distinct coverage levels (0%, 30% and 80%), thereby attempting to establish low, medium, and high levels of airborne inoculum for FHB. The plots were intentionally isolated with 30' buffer zones surrounding the residue-treated areas. At harvest, these buffers zones and the plots themselves were sampled for *Fusarium* damaged kernels (FDK) and DON contamination. The samples were collected at 5' intervals between and surrounding residue treated plots. The objective of the sampling was to assess the impact of the corn residue levels on DON contamination in grain grown outside of the residue area.

DEVELOPMENT OF FUSARIUM HEAD BLIGHT OF WINTER WHEAT IN OHIO AS INFLUENCED BY PLANTING DATE, CULTIVAR MATURITY, AND INOCULUM LEVEL

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OBJECTIVES

1) Determine the effects of different cultural practices on the development of Fusarium head blight of winter wheat, and 2) determine the relationship among disease intensity, residue level, and inoculum level.

INTRODUCTION

Fusarium head blight (FHB), caused primarily by *Gibberella zeae*, is one of the most devastating diseases of wheat and barley in the North America (McMullen et al., 1997). No single management strategy has been effective against this disease. An integrated approach using cultural practices to reduce inoculum levels and to escape disease-favorable periods, cultivars with partial resistance to FHB, and timely application of fungicides through accurate disease prediction and risk assessment may be the most effective. Weather-driven risk assessment models for FHB have been developed and are currently being validated in several states (De Wolf et al., 2003, Lipps and Mills, 2003). In order to incorporate variables related to cultural practices and inoculum levels into these models, a thorough understanding of the influence of these factors on disease development under different conditions is necessary.

MATERIALS AND METHODS

Three soft red winter wheat cultivars ('Patterson', 'Elkhart', and 'Hopewell') that differ in heading dates were planted on different dates during the 2000/2001, 2001/2002, and 2002/2003 growing seasons at the Ohio Agricultural Research and Development Center, near Wooster. In 2000/2001 and 2001/2002, a split-plot design was used, with planting date, and cultivar being the whole- and sub-plot factors, respectively. In 2002/2003, a split-split-plot design was used. Density of corn residue, planting date, and cultivar were the whole-plot, sub-plot, and sub-sub-plot factors, respectively. Whole plots treatments were established by spreading different densities of corn residue (0, 15, and 80%) over the soil surface in early spring of 2003. Residue levels were determined by the line-transect method. In each growing season, there were three replicate blocks of each treatment combination. Strips of Freedom, a cultivar with moderate scab resistance, were used to separate adjacent blocks and whole plots within each block.

Burkard cyclone spore samplers were used to monitor daily numbers of airborne spores of *F. graminearum* from Feekes growth stage 10 through 11.2. During the same period, wheat heads were collected and assayed directly for spores of *F. graminearum* using head washing.

Beginning at Feekes 10.5.4, incidence and severity of FHB were assessed three times per week in each of the smallest experimental units. Each head within a 1-ft length of row at 10 arbitrarily selected sites within each plot was assessed for percentage of affected spikelets. Diseases incidence was scored as the percentage of diseased

heads, while disease severity was recorded as the average percentage of diseased spikelets per head (= "index").

FHB incidence and severity data were analyzed using Proc Mixed (SAS, Cary, NC) to assess the main and interaction effects of planting date and cultivar on disease development.

RESULTS AND DISCUSSION

In general, late planting of mid-season cultivar, Elkhart, resulted in the highest disease intensity (Table 1). This is probably because the period of greatest crop susceptibility coincided with periods of FHB-favorable weather conditions. In 2001, planting date had no significant effect on FHB development on cultivars Hopewell and Patterson. In both 2001 and 2002, within a given planting date, Elkhart and Patterson, respectively, were the most and least affected by FHB.

In 2003, disease development at 80% residue was comparable with development at 15 and 0% (Figure 1A). This may be due in part to the fact that very similar levels of inoculum were recorded from wheat heads sampled from each residue plot (Figure 1B). In addition, the influence of surface residue on inoculum levels and disease development may be dependent on the weather. Further investigation of this factor over multiple years and locations may provide better insight into its effect of FHB development.

No clear association was observed between levels of FHB inoculum in the air and on wheat heads (Figure 2). Peaks in the level of airborne spores trapped using the Burkard sampler corresponded only to subtle increases in the number of spores recovered from wheat heads. In all three growing seasons, there was a marked association between rainfall amounts and number of CFU recovered from wheat heads via head washes. No overall association was observed between CFU recovered through Burkard spore sampling and rain events. In many cases, peaks in rainfall amounts coincided with reductions in the number of spores sampled from the air and increases in the number of spores sampled from wheat heads. De Wolf et al. (2001) also observed an association between rainfall and *G. zeae* inoculum on wheat heads in North Dakota. In addition to potentially washing spores out of the air, rain may be contributing to increases in inoculum levels on the heads by splashing spores from other sources. Partial results of research on splash dispersal of *G. zeae* support this hypothesis (El-Allaf et al 2003). Further investigation of the relationship among inoculum levels (in the air, on residue and on wheat head), disease intensity, and weather conditions may provide a clearer understanding of the relative importance of airborne and residue-borne inoculum for the development of FHB.

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Table 1. Comparison of the effect of planting date within each cultivar and cultivar within each planting on the final incidence and severity of Fusarium head blight of winter wheat in Wooster, Ohio in 2001 and 2002

Cultivar	Incidence			Severity		
2001						
	18 Sept.	2 Oct.	16 Oct.	18 Sept.	2 Oct.	16 Oct.
Elkhart	72.7 *A b*	77.4 *A a b*	81.6 *A a*	39.6 B b	41.4 A b	52.9 A a
Hopewell	69.1 *A a*	72.3 *A a*	75.5 *A a*	40.7 A a	40.6 A a	44.1 B a
Patterson	50.4 *B b*	56.8 *B a*	58.3 *C a*	30.4 C a	31.0 B a	32.2 C a
2002						
	18 Sept.	1 Oct.	23 Oct.	18 Sept.	1 Oct.	23 Oct.
Elkhart	35.4 *A c*	54.7 *A b*	61.3 *A a*	24.4 A b	27.0 A b	36.5 A a
Hopewell	30.0 *B c*	39.0 *B b*	47.0 *B a*	15.7 B c	19.3 B b	23.2 B a
Patterson	24.0 *C c*	31.2 *C b*	34.9 *C a*	12.6 B c	15.5 C b	18.1 C a

Means followed by the same uppercase letter in each column and by the same lowercase letter within each row are not significantly different at $P \leq 0.05$ by the LSD-test.

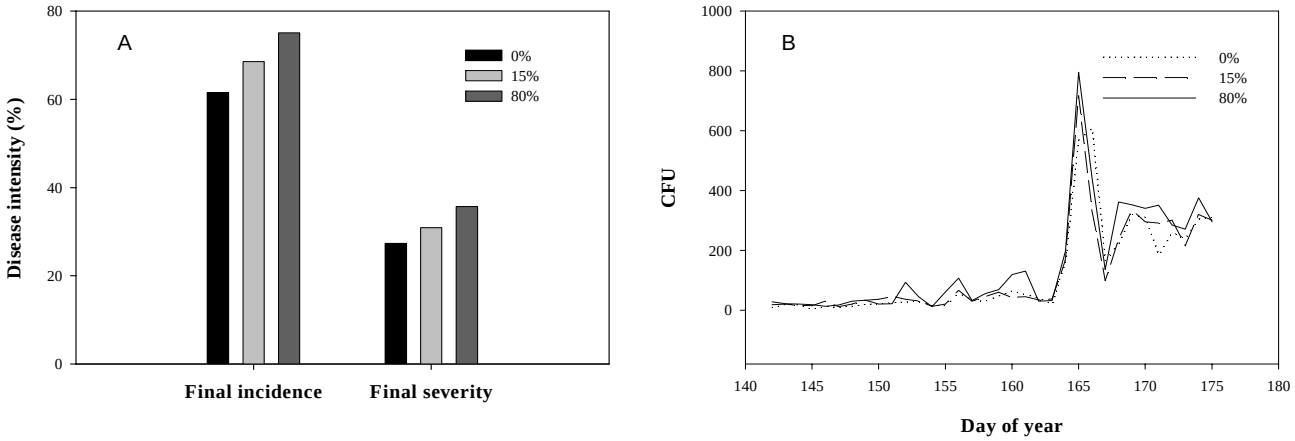

Figure 1 - Effects of surface residue on FHB intensity (A) and inoculum levels on wheat heads (B) in Wooster, Ohio. Final disease assessment was done on 26 June 2003. Head wash assays were used to determine inoculum levels

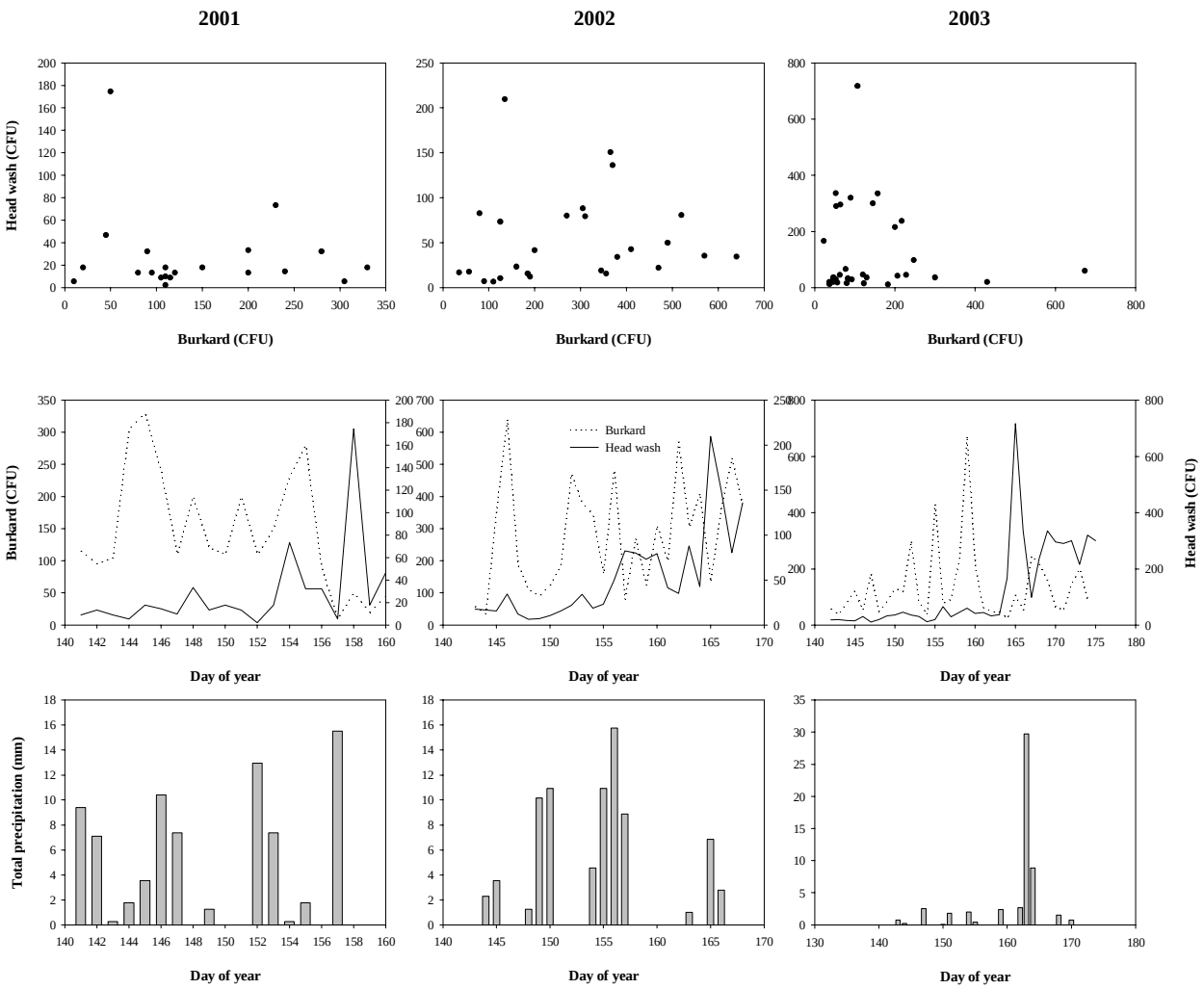

Figure 2 - Relationship among daily inoculum levels of *G. zeae* in the air sampled using Burkard spore traps, on wheat head assayed via head washes, and total daily precipitation during the 2001, 2002, and 2003 winter wheat growing seasons in Wooster, Ohio.

SPLASH DISPERSAL OF SPORES OF *FUSARIUM GRAMINEARUM* USING A SINGLE-DROP GENERATOR

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ABSTRACT

Splash dispersal of *Fusarium graminearum*, causal agent of head blight of wheat, was studied using a single drop generator. Hypodermic needles were used to generate drops 2.7, 3.3, and 3.7 mm in diameter, falling from heights of 50, 75, 100, and 125 cm. A macroconidial suspension of *F. graminearum*, at a concentration of 3×10^4 conidia ml^{-1}, and wheat leaves sprayed with a suspension of 8×10^4 spores ml^{-1} were used as targets. Leaves of wheat cultivar Norm were cut into 3-cm pieces, affixed to microscope slides, and the spore suspension applied in five uniform passes at a rate of 0.23 ml/sec. Three milliliters of the 3×10^4 spores ml^{-1} spore suspension was placed into a small watch glass, forming a 2-mm-deep film. The source materials were placed at the point of drop impact and 50 drops falling at a rate of 130 drops per minute from each fall height/drop diameter combination were allowed to hit the targets. PVC tubes were used to form tunnels along the path of the falling drop to minimize the effects of the wind on the impact position. Five sets of petri plates containing Komada's selective media were placed at 10, 20, and 30 cm from the point of impact to collect splashed droplets. Each set of plates was placed at a different angle from the source. After the splash droplets were collected, 1 ml of sterile distilled water amended with Tween 20 was applied to each plate to enhance the spread and germination of spores in the sampler. Plates were incubated at room temperature under a 14-h photoperiod for 48 h. The number of colony forming units (CFU) was then counted in each plate. There was greater variability in spore dispersal from wheat leaves than from spore suspensions, however, for both targets, the majority of spores were collected between 10 and 20 cm from the point of impact. Drops falling from 125 and 100 cm resulted in greater spore dispersal and more spores collected 30 cm from the source than drops falling from 75 and 50 cm. In general, for a given fall height, more spores were dispersed by 3.7- and 3.3-mm drops than by 2.7-mm drops. For both targets, positive relationships were found between CFUs and functions of the impacting drop velocity and diameter. These relationships were stronger for spore suspensions than wheat leaves. The relationship among the characteristics of different sources of inoculum, physical properties of incident drops, and number of spores dispersed may be used estimate the spread of *F. graminearum* within the wheat canopy and to model rain-splash dissemination of Fusarium head blight.

PATHOGENIC SPECIES, GEOGRAPHIC DISTRIBUTION, AND SEVERITY OF FUSARIUM HEAD BLIGHT ON BARLEY IN THE CENTRAL HIGHLANDS OF MEXICO

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OBJECTIVES

The present study was carried out to: 1) determine the geographical distribution and severity of FHB in the field in the Central Highlands of Mexico; 2) identify *Fusarium* species causing FHB on barley in the field; and 3) confirm the pathogenicity of identified species.

INTRODUCTION

In Mexico, malting barley (*Hordeum vulgare* L.) is grown on approximately 301,000 hectares. The main malting barley producing area of Mexico is the Central Highlands, which accounts for nearly 50% of Mexico's total barley area. Since 1998 there has been an increase of Fusarium head blight (FHB) in barley produced in the Highlands (7,11). Although few studies of FHB on barley have been conducted in Mexico (9,10), field pathogenicity was not confirmed for each species of *Fusarium*. Lack of knowledge of this disease is impeding effective FHB control and putting barley grain produced in Mexico at a competitive disadvantage on the international market.

MATERIALS AND METHODS

Geographical distribution and evaluation of FHB severity on barley heads. Field samples were collected in the 2001 and 2002 growing seasons. Heads showing FHB symptoms were collected at the grain filling stage. The methodology made it possible to collect the greatest possible number of *Fusarium* species present in the field, and to identify other fungal species involved in the symptoms of FHB. Samples taken from the whole area each growing season included a total of 600 heads, which were then evaluated to determine disease severity, isolate species of *Fusarium* and other fungi, and estimate the percent frequency for each isolated species. The percent severity for each sample was the sum of infected grains in the entire sample in relation to the total number of grains in each sample (8). The percent frequency for each species was the number of isolates of the species in relation to the total number of *Fusarium spp.* isolates.

Species identification. The isolates were made in potato dextrose agar and carnation leaf agar (4). Monoconidial cultures were made for each isolate (2,3,8,13). Pathology tests were also conducted in the 2002 season using the Neergard technique (12), as modified by CIMMYT's Seed Health Unit. Monoconidial cultures of different *Fusarium* colonies were obtained. Monoconidial colonies of *Fusarium spp.* obtained from blotters (2002 sampling) and from direct seed isolates (2001 sampling) were identified

according to Booth's classification (2), aided by Burgess et al. (3) and Nelson et al. (13). Identification of *Fusarium* species was confirmed via RFLPs in the Laboratory of Dr. R. De La Torre-Almaraz of the UNAM at Iztacala, State of Mexico (unpublished).

Pathogenicity tests. Pathogenicity tests were conducted in the 2001 growing season at CIMMYT's Experiment Station in Atizapán, State of Mexico (19.10°N, 99.51°W), on 10 species (Table 1) inoculated in the malting barley cultivar Esmeralda harvested in the Central Highlands of Mexico in the 2001 growing season. Isolates were increased in liquid mung bean medium (1), at a concentration of $50x10^3$ conidia per ml (6). Twenty spikelets per experimental plot were inoculated at flowering using the cotton technique (1,5). Sprinkler irrigation was not applied, as daily precipitation favored disease development. Pathogenicity was evaluated 20 days after inoculation.

RESULTS AND DISCUSSION

Species associated with FHB in barley. All symptoms found were related to the presence of *Fusarium spp.*; especially significant was the observation of partial discoloration with dark margins, which indicates the presence of *F. poae*. *Fusarium graminearum* typically causes dark brown coloration in the grain, but this symptom is also strongly associated with *Epicoccum spp.*, *Alternaria spp.*, and *Bipolaris sorokiniana.* Identification of *Fusarium* species was confirmed by RFLPs (unpublished). One outstanding finding is the ubiquitous presence of *Bipolaris sorokiniana*, detected in 100% of the samples in 2002, and of other fungal species, mainly saprophytes or weak parasites such as *Alternaria spp.* and *Epicoccum spp.* (Table 1).

The present study showed that *F. avenaceum* and *F. graminearum* were the main causal pathogens of FHB, given that both were isolated in 96.7% of the sites and showed high frequency levels (Table 1). It is important to note the presence of *F. sambucinum* (although we did not test its pathogenicity), which had not been reported previously (6).

Pathogenicity of individual species. The range of symptoms described in the literature was found in the field, on inoculated heads, and in kernels adjacent to the point of infection. Symptoms at the inoculation points were clearly differentiated, given that moisture and temperature conditions at the test site (Atizapán, State of Mexico) favored disease development. Salmon pink colored mycelial growth on the kernel surface appeared on most of the inoculated heads, in addition to symptoms typical of *F. poae* (14). Re-isolations from the inoculated kernels agree with descriptions of the inoculated species, which confirms their pathogenicity in the field.

Geographic distribution and disease severity. FHB was present throughout the sampled zone, as evidenced by the fact that pathogenic species of *Fusarium* were isolated from 100% of samples showing symptoms. Final disease severity average for both samplings was 6.21%. Disease severity was similar in both years of sampling. Frequency distribution of FHB was higher with *F. avenaceum*, *F. graminearum*, and *F. tricinctum*. Disease severity in general was low; however, the disease causes major economic losses as a result of yield reductions and poor industrial quality due to toxin-contaminated grain (14).

Table 1. Species associated with symptoms of Fusarium head blight (FHB) in barley in the Central Highlands of Mexico, 2001-2002.

Species	Pathogenicity 2001	Sites where FHB was present (%)	Percent frequency	
			2002	2001
Fusarium avenaceum	P	96.7	25.5	30.0
F. graminearum	P	96.7	23.5	20.0
F. tricinctum	P	26.7	6.5	11.0
F. subglutinans	P	43.3	9.0	10.0
F. poae	P	10.0	2.0	5.0
Microdochium nivale	P	13.3	4.5	8.0
F. lateritium	P	13.3	1.0	9.0
F. heterosporum	P	13.3	0.0	5.0
F. equiseti	Nt	33.3	5.0	0.0
F. culmorum	Nt	23.3	7.0	0.0
M. dimerum	Nt	36.7	12.0	0.0
F. sambucinum	Nt	16.7	4.0	0.0
F. merismoides	Np	10.0	0.0	1.0
F. stilboides	Np	10.0	0.0	1.0

Species	Sites where fungi were present (%)
Fusarium spp. and Microdochium spp.	100.0
Bipolaris sorokiniana	100.0
Alternaria spp.	96.6
Epicoccum nigrum	96.6
Trichothecium roseum	66.7
Gonatobotrys spp.	56.6
Penicillium spp.	30.0
Phoma spp.	20.0
Cladosporium spp.	16.6
Cephalosporium acremonium	6.7
Aspergillus flavus	6.7
Acremoniella spp.	3.3

P = Pathogenic, Nt = Not tested; Np = Non pathogenic

CONCLUSIONS

- FHB incidence in commercial barley fields in the Central Highlands is high, as *Fusarium spp.* were detected in 100% of sampling sites in the region.

- Among species isolated in 2001 sample, pathogenicity was confirmed for *F. avenaceum, F. graminearum, F. tricinctum, F. subglutinans, F. poae, M. nivale, F. lateritium,* and *F. heterosporum*.

- Frequency distribution of FHB was higher with *F. avenaceum, F. graminearum*, and *F. tricinctum*. The average disease severity for two years of sampling was 5.7%, within a 4.4 to 8.4% range.

Failure to take measures to control and manage the disease could result in economic losses due to grain yield reductions and the presence of toxins in the grain. This might also have consequences for barley producers and the brewing industry in Mexico.

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TOXINS AND DAMAGE INDUCED BY SEVEN SPECIES OF FUSARIUM HEAD BLIGHT ON BARLEY IN THE CENTRAL HIGHLANDS OF MEXICO

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OBJECTIVES

The present study was carried out to: 1) Evaluate the expression in severity of Fusarium head blight (FHB) caused by *Fusarium avenaceum, F. graminearum, F. tricinctum, F. subglutinans, F. poae, Microdochium nivale, F. lateritium* and *F. heterosporum* 2) Estimate its aggressiveness through its effects on yield components and 3) Determine the production of trichothecenes of each isolate for the different species in the environment of Atizapán, State of Mexico.

INTRODUCTION

Since 1998 FHB has increased, affecting barley (*Hordeum vulgare* L.) in the Highlands of Mexico, where the disease causes losses in both yield and grain quality (8). Among the species causing FHB, *F. avenaceum, F. graminearum* and *F. culmorum* have been reported to be the main toxigenic species, although other less aggressive or opportunistic species are also reported as toxins producers (3). There are diverse reports that indicate the difference in aggressiveness among species as well as among isolates of a single species (10,11).

The mycotoxins most frequently associated with fusariosis caused by *F. graminearum* are deoxynivalenol (DON) and nivalenol (NIV) (3,15) and *F. avenaceum* is reported to be a producer of DON in liquid cultures (1). The capacity of DON production has been related to the aggressiveness of *Fusarium*, which is strongly related to the atmospheric conditions (10) and with the system of cultivation (15).

MATERIALS AND METHODS

Establishment of field test. The evaluation of damages induced by the isolates of the different species of *Fusarium* were carried in the experimental field of Atizapán, State of Mexico (CIMMYT, Int.) (19.10° N, 99.51° W, 2640 masl) during the 2002 growing season. The treatments were established under a random design, with two replications per isolate. The experimental unit consisted of two 1.5 m long rows, which was considered a replication. Planting was done manually using seed of the Esmeralda variety.

Isolates used and method of inoculation. An evaluation was made of 11 monoconidial isolates of the species obtained from the samplings carried out in the Highlands of Mexico during the 2001growing season in barley of the Esmeralda variety. The pathogenicity of the isolates of this species was previously deter-

mined (14). The increase of the inoculum was carried out in liquid mung bean medium (*Vigna radiata*) (2,5). Twenty heads per experimental unit were labeled at the same flowering stage (7,9), and then inoculated by aspersion of conidial solution (50×10^3 per ml) with a manual 1-liter bottle atomizer (7). In order to avoid the spread of conidia, a screen was placed between plots at the moment of inoculation. The application of artificial irrigation was unnecessary, as daily precipitation favored the development of the disease.

Evaluations. Severity evaluation of the 20 labeled and inoculated heads was carried out 7, 14, 13 and 28 days after inoculation (dai), and consisted in counting the infected grains in relation to the total number of grains in each head, thus obtaining a percentage of severity in each reading. Hectolitric weight and thousand kernel weight (TKW) was measured after harvesting.

Confirmation of species. Ten inoculated kernels at each replication were taken to a laboratory to obtain monoconidial isolates and confirmation of the inoculated species (5, 12).

Quantification of toxins. Kernels harvested from each replication were used for the quantification of trichothecenes (DON and NIV). Kernels were ground (commercial Braun mill) and processed with the Romer Labs. Inc. technique (DonFluoroQuantTM method #FQD1NC, version 95.9).

Data analysis. Data obtained of severity, hectolitric weight and TKW was analyzed using the SAS statistical program (version 8.0, SAS Institute Inc., Cary, NC, USA). The PROC GLM procedure was used for the variance analysis, and means were subjected to comparison by Least Significant Difference (LSD) with a= 0.05.

RESULTS AND DISCUSSION

The experiment showed that isolates 1 and 10 (*F. avenaceum* and *F. graminearum*) were the most aggressive. These species came from the same region (Apan, Hidalgo), considered the main zone of barley reception and commercialization, which leads to the supposition that a greater genetic recombination exists in this zone. Same results were found in previous studies (11,18). The sexual and/or asexual recombination and the alternation between the saprophytic and parasitic phases could play an important role in the variations of the populations (11,18), whereas virulence and aggressiveness are influenced by the atmospheric conditions and the cultivation systems, among other factors (10).

Evaluated isolates of *F. avenaceum* from Apan, Hgo. and Calpulalpan, Tlax., showed different values of final severity, although they did not present significant differences, which indicates a high risk (17), taking into consideration that this species is the principal cause of the fusariosis in barley in the Highlands of Mexico (14).

Greater damage was observed from isolate 1 (*F. avenaceum*) than from isolates 10 and 11 (*F. graminearum*), contrary to what was expected. However, the results should be considered with discretion, given that *F. graminearum* is more aggressive in humid areas (16). Strong interactions between the pathogen, the variety and the atmospheric conditions in the expression of aggressiveness were also observed (10).

Table 1. Evaluation of severity for type II resistance in barley heads of the Esmeralda variety inoculated with cotton containing isolates of pathogenic species of *Fusarium* spp. in Atizapán, State of Mexico, 2002.

Isolated	Origen	Severity percentage		
		7 dai**	14dai	21dai
1. *F. Avenaceum*	*Apan, Hgo.*	3.68 a*	5.31 a	9.60 a
2. *F. Avenaceum*	*Calpulalpan, Tlax.*	2.87 a	3.75 a	6.15 a
3. *F. lateritium*	*Zapata,Hgo.*	2.87 a	4.26 a	7.45 a
4. *F. subglutinans*	*Benito Juárez, Tlax.*	2.50 a	4.08 a	6.80 a
5. *F. subglutinans*	*Apan, Hgo.*	3.12 a	3.93 a	6.60 a
6. *F. trincinctum*	*Almoloya, Hgo.*	3.57 a	4.84 a	6.90 a
7. *F. trincinctum*	*Calpulalpan, Tlax.*	2.97 a	4.22 a	7.50 a
8. *F. heterosporum*	*Zapata, Hgo.*	2.85 a	4.43 a	8.05 a
9. *F. Poae*	*Zaragoza, Tlax.*	3.13 a	3.84 a	7.30 a
10. *F. graminearum*	*Apan, Hgo.*	3.06 a	5.93 a	8.50 a
11. *F. graminearum*	*CIMMYT*	2.18 a	3.25 a	5.75 a
Coeficiente de variación		21.98	23.62	16.53

*means with the same letter are not significantly different (DMS, α= 0.05);
** dai, days after inoculation

Table 2. Evaluation of the effect on final severity, hectolitric weight, thousand kernel weight and production of trichothecenes in barley heads of 'Esmeralda' artificially inoculated with isolates of different species of *Fusarium* sp. in Atizapán, State of Mexico. 2002.

Isolate	Origen	Final severity (%)	Hectolitric weight (g/l)	Weight of 1000 grains (g)	Toxins (ppm)**
1. F. avenaceum	*Apan, Hgo.*	9.60 a*	548.25 a	34.49 a	0.00
2. F. avenaceum	*Calpulalpan, Tlax.*	6.15 a	555.07 a	35.51 a	0.00-0.06
3. F. lateritium	*Zapata, Hgo.*	7.45 a	582.83 a	34.79 a	0.00-0.02
4. F. subglutinans	*Benito Juárez, Tlax.*	6.80 a	535.56 a	35.21 a	0.00-0.05
5. F. subglutinans	*Apan, Hgo.*	6.60 a	531.02 a	33.30 a	0.08-0.20
6. F. trincinctum	*Almoloya, Hgo.*	6.90 a	587.68 a	31.64 a	0.00
7. F. trincinctum	*Calpulalpan, Tlax.*	7.50 a	575.28 a	33.18 a	0.00
8. F. heterosporum	*Zapata, Hgo.*	8.05 a	570.12 a	35.70 a	0.03-0.22
9. F. Poae	*Zaragoza, Tlax.*	7.30 a	597.98 a	35.81 a	0.00-0.11
10. F. Graminearum	*Apan, Hgo.*	8.50 a	568.96 a	34.93 a	0.92-2.70
11. F. Graminearum	*CIMMYT*	5.75 a	557.32 a	33.63 a	0.21-0.42
Coeficiente de variación		16.53	4.71	67.56	

*means with the same letter are not significantly different (DMS, α= 0.05)
** deoxynivalenol + nivalenol

F. poae presents high TKW, giving this isolate the highest value in hectolitric weight, for which combined with the data of final severity, it can be concluded that *F. poae* presented the least effects on 'Esmeralda' under the conditions in which the test was developed. On the other hand, *F. subglutinans* presented the greatest effects on hectolitric weight, but not on TKW, where although no statistical differences were shown, *F. tricinctum* presented the greatest reduction in yield. Furthermore, it is important to observe that the isolates of *F. avenaceum* and those of *F. graminearum*, although presenting the greatest final severity, did not reduce yield in relation to the rest of the isolates evaluated.

The production of trichothecenes (DON and NIV), it can be observed that *F. avenaceum* resulted positive to the detection of these compounds, results that have been observed in others studies (1,6). To this respect, studies carried out detected DON in heads inoculated with *F. avenaceum* (15), however, these studies point out that the levels were low, which could be attributed to the fact that some colonies of *F. graminearum* were found in these heads, some contamination could have occurred in the present test, however the results should be considered for confirmation in future investigations.

In the heads inoculated with *F. poae*, the presence of trichothecenes was detected, which coincides with diverse reports (3,4,15), whereas the heads inoculated with *F. lateritium* present low levels of these compounds in the present study, despite the fact that there are not many reports of the production of trichothecenes in this species (4,6). Both isolates of *F. subglutinans* resulted positive to the detection of trichothecenes, although in low levels, thus it is assumed that what was indicated by the case of *F. avenaceum*. Therefore, these results, as well as those obtained with *F. avenaceum*, should be considered as a possibility to be confirmed in future studies.

The results of the present investigation indicate that trichothecenes were not detected in the heads inoculated with the isolates of *F. tricinctum*, although in the case of *F. heterosporum*, low levels of these compounds were present. It is widely known that *F. graminearum* is the principal species that produces trichothecenes (3,4,15), and in the present study the isolates of *F. graminearum* presented the highest levels of trichothecenes of the isolates evaluated.

The literature published on *F. graminearum* suggests that toxins may serve as factors of aggressiveness and virulence of the pathogen (5). It is important to keep this consideration in mind, as the results show that isolates of *F. graminearum*-Apan presented greater severity and a higher concentration of trichothecenes than isolate *F. graminearum*-CIMMYT. However, no differences were observed in the effect on yield, which suggests that the aggressiveness is related to the production of toxins, a situation that has been widely documented (10,13).

The results obtained in the present investigation show that the isolates of the species which cause fusariosis in barley in the Highlands of Mexico are producers of trichothecenes (DON and NIV), demonstrating in a general way that there are no direct relationships between DON concentration with the effect on yield and the aggressiveness. However, it is demonstrated that diverse species produce trichothecenes and cause damage in the yield of the Esmeralda variety, which represents more than 70% of the surface sown with barley in the Highlands of Mexico.

The above, added to the general ignorance of the toxigenic capacity of the Fusarium species in the Esmeralda variety, and under the conditions of the Highlands, needs the programming and execution of research projects that contemplate aspects of ecology, biology, damages and production of toxins of the different species which cause fusariosis in barley.

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INCIDENCE OF *FUSARIUM GRAMINEARUM* IN KERNELS OF WHEAT AND BARLEY CULTIVARS AT FOUR LOCATIONS IN MINNESOTA

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ABSTRACT

Harvested grain of the wheat and barley entries in the 2003 Red River Valley On-Farm Yield Trials grown at Fergus Falls, Humboldt, Oklee and Strathcona MN, were assayed for colonization by Fusarium head blight pathogens. Each trial site consisted of 7.6 m x 1.98 m plots of each of the sixteen wheat and eight barley entries arranged in a randomized complete block design with two replications. Plots were subject to natural infection by Fusarium head blight. Kernels (100 per plot), arbitrarily selected from the harvested grain, were surface sterilized, plated onto Komada's medium (selective for *Fusarium spp.*) and incubated at 20-24ºC under fluorescent lights (12:12, light:dark) for ca. 12 days. *Fusarium* species isolated were identified using standard taxonomic procedures. Regardless of trial location and cultivars, *Fusarium graminearum* was the *Fusarium* species most frequently isolated from kernels (wheat, 9.2%; barley, 11.3%), followed by *F. avenaceum* (wheat, 2%; barley, 4.3%), *F. sporotrichioides* (wheat, 0.8%; barley, 2.4%), and *F. poae* (wheat, 0.7%; barley, 0.9%). In wheat, the highest incidence of *F. graminearum* colonized kernels was found at Strathcona (11.7%) and Humboldt (11.6%). In barley the highest incidence of *F. graminearum* colonized kernels was found at Oklee (14.4%) and Fergus Falls (14.3%). Ranking of wheat cultivars for kernel colonization by *F. graminearum* was significantly (P = 0.01) affected by the interaction of cultivar by location. Overall, the wheat cultivars Alsen (6.3%) and Hanna (3.4%) had lower levels of *F. graminearum* infection than Reeder (12.8%), Oxen (12.5%), Mercury (12.3%), Norpro (10.9%), Parshall (10.7%), MN97803A (9.8%) and Walworth (9.6%) which were significantly more highly colonized ($LSD_{(P = 0.05)}$ = 3.2). Ranking of barley cultivars was not affected by the interaction of cultivar by location (P = 0.11). The six-rowed barley lines MN110 (17.2%) and Drummond (13.2%) had the highest levels of kernel colonization by *F. graminearum*. Robust (9.6%) and the two-rowed barley Conlon (5.8%) had the lowest incidence of *F. graminearum* of the eight barley cultivars examined ($LSD_{(P = 0.05)}$ = 4.1). These data suggest that the kernels of wheat and barley cultivars are colonized differentially by *Fusarium* species depending on their resistance. Other factors such as the environment during the window of infection may also affect kernel colonization.

PREVIOUS CROP AFFECTING SOIL POPULATIONS OF FUSARIUM HEAD BLIGHT PATHOGENS IN MINNESOTA

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OBJECTIVES

To examine the effect of previous crop on soil populations of *F. graminearum* and other cereal pathogenic Fusaria.

INTRODUCTION

Fusarium head blight of wheat and barley, caused primarily by *Fusarium graminearum*, has become a major problem in the Upper Midwest of the United States. In an effort to control FHB, studies have been undertaken to understand the epidemiology of FHB and implement control practices including host resistance, chemical, biological and cultural control practices (Dill-Macky and Jones, 2000; Sutton, 1982). Although FHB epidemics are known to be associated with the inoculum present in host residues (Dill-Macky and Jones, 2000; Sutton, 1982), it is possible that *F. graminearum* in soil is also a source of inoculum. Knowledge of factors affecting soil populations of *F. graminearum* may help in the management of this devastating disease, however an understanding of *F. graminearum* populations in soils is lacking. The objective of this study was to examine soil populations of *F. graminearum*, and other cereal pathogenic Fusaria, in wheat fields as affected by the preceding crop.

MATERIALS AND METHODS

Fifty wheat fields in five counties (Becker, Clay, Mahnomen, Norman and Polk) in Minnesota, and one field in North Dakota (Trail) were surveyed for soil populations of *F. graminearum* and other Fusaria pathogenic to wheat. Information on the preceding crop for each field was obtained from county agents or property owners. In each field, five soil samples (26 g) were collected from the surface (0-2 cm depth) one meter apart along each of two five-meter transects, located at least 30 m from the edge of the field. Soil samples from each transect were air dried for four days at 20-24ºC, and sieved through a battery of sieves (250, 500,1000 and 2000 microns). Based on preliminary experiments, six milligrams of the finest soil particles (<250 microns) from each sample were dispersed onto each of five Petri plates containing Komada's medium (selective for Fusarium species) (Fig. 1A). Plates were incubated at 20-24ºC under cool white and UVA (1:1) fluorescent lights (12 hr photoperiod) for 14 days. *Fusarium* colonies were identified according to Burgess *et al.* (1994). Statistical analysis of soil populations of Fusaria as affected by the previous crop was analyzed using SAS PROC GLM.

RESULTS

Recovery of *F. graminearum* was significantly (P = 0.01) higher from soil particles <250 micron than from soil larger particles (251-499 microns). *F. graminearum* was present in all soil samples tested and was the most frequently isolated *Fusarium* species (78%) among the FHB pathogens recovered (Fig. 1). Other FHB pathogens were isolated at lower frequencies. Populations of *F. graminearum* in soil samples ranged

from 18 CFU/g to 1435 CFU/g dry soil. Population ranges for each species, as affected by rotational crop in 2001, is presented in Table 1. Populations of *F. graminearum* in soil after 2002 wheat crops were significantly (P = 0.01) affected by the immediate previous crop. Although we sampled only one field where wheat followed corn, the highest *F. graminearum* population (1159 CFU/g) (Table 2) was found in this particular field. Populations of *F. graminearum* in the soils of wheat crops following soybeans (356 CFU/g) and sugarbeets (341 CFU/g) were significantly less than in wheat after wheat (642 CFU/g) and wheat after dry beans (497 CFU/g) (Table 2). Populations of *F. culmorum*, *F. sporotrichioides* and *F. avenaceum* were also high in the soil sampled from wheat planted after corn (Table 2).

DISCUSSION

Our data shows that *F. graminearum* can readily be isolated from fine soil particles (<250 microns). It also indicates that soil may be a more important source of inoculum in FHB epidemics than previously thought. Recent epidemics of FHB in wheat and barley crops may have enabled *F. graminearum* populations to build up in the soils. In previous studies, *F. graminearum* was isolated from only 30% of soil samples from corn fields in non-epidemic years (Windels and Kommedahl, 1984) or soil populations found were low (17–24 CFU/g soil) (Salas, 1991). While we only sampled the soil of one field where wheat followed corn, it is not surprising that *F. graminearum* population was high in this particular field as other workers have reported similar results in relation to *F. graminearum* in association with residues of wheat crops following corn (Dill-Macky and Jones, 2000; Sutton, 1982; Windels and Kommedahl, 1984). In contrast, soil populations of *F. graminearum* in wheat after dry beans or soybeans were lower than in wheat after corn or wheat. A lower incidence of FHB has been reported in wheat following soybeans (Dill-Macky and Jones, 2000). Although the relationship between FHB and soil populations of *F. graminearum* has not previously been reported, it appears that monitoring soil populations of *F. graminearum* may be helpful in identifying the most effective crop rotation to help manage FHB epidemics.

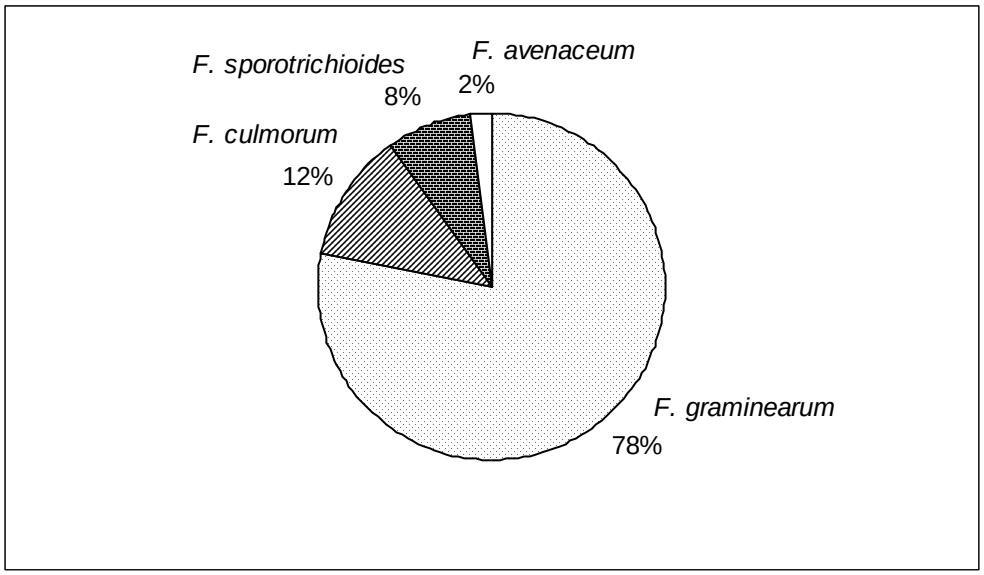

Figure 1. Frequency of recovery of *F. graminearum*, *F. culmorum*, *F. sporotrichioides*, and *F. avenaceum* among pathogenic Fusaria recovered from fifty field soils in Minnesota and one field in North Dakota in 2002.

Table 1. Range of soil populations of *F. graminearum* and other cereal pathogenic Fusaria in soil sampled following the 2002 wheat crop as affected by previous crop.

Previous crop (2001)	Fields sampled (No.)	Colony forming units (CFU)/g dry soil)			
		Fusarium graminearum	*Fusarium sporotrichioides*	*Fusarium culmorum*	*Fusarium avenaceum*
Corn	1	1159	91	168	21
Wheat	5	474 - 1074	38 - 71	63 - 138	10 – 22
Dry bean	3	421 - 628	37 - 84	62 - 103	7 – 20
Soybean	32	49 - 943	6 - 139	7 - 188	1 – 35
Sugarbeet	10	96 - 639	9 - 57	17 - 98	1 – 21

Table 2. Effect of previous crop on soil populations of *F. graminearum*, *F. sporotrichioides*, *F. culmorum*, and *F. avenaceum*. Values given are the mean of the number of field samples examined for each previous crop treatment.

Previous crop (2001)	Fields sampled (No.)	Colony forming units (CFU)/g dry soil)			
		Fusarium graminearum	*Fusarium sporotrichioides*	*Fusarium culmorum*	*Fusarium avenaceum*
Corn	1	1159	91	168	21
Wheat	5	642	52	89	14
Dry bean	3	497	54	79	13
Soybean	32	356	37	56	10
Sugarbeet	10	341	37	55	9
LSD $_{P = 0.05}$		237.3	24.8	33.5	8.2

ACKNOWLEDGMENTS

We thank Abolfazi Kowsari for technical assistance.

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PREDICTING DEOXYNIVALENOL IN WHEAT FOR ONTARIO

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ABSTRACT

Eight years of data were used to develop DONcast– a model to predict deoxynivalenol (DON) in mature wheat grain for fungicide-spray decisions at heading. A website was launched in 2000 for providing DON predictions to agri-business through the Ontario Weather Network (OWN) (http://www.ownweb.ca). The model was adapted for Uruguay, where severe *Fusarium* epidemics have resulted in DON concentrations of up to 5 ppm in baked goods. For predictions in 2004, DONcast will have evolved using an array of weather and agronomic data from over 630 private farms across Ontario and Uruguay. In addition to daily rainfall and temperature data, DONcast for 2004 will include relative humidity (RH) >80% at 11:00 between 3 to 10 d after heading for more accurate decisions of whether or not to apply a fungicide at heading. For the first time, the model will also be extended to include rain and RH between 20 and 36 d after heading (near harvest). Using actual weather and agronomic variables specific to individual farm fields, the overall model explains 75% of the variation of DON using data from 600 farm fields from 1996 to 2003. DON concentrations of less than 1.0 ppm were predicted correctly on 88% of the fields at heading. In other fields where DON concentrations exceeded 1.0 ppm, the model predicted correctly on 72% of the fields at heading.

AIRBORNE PROPAGULES OF *GIBBERELLA ZEAE*: TECHNIQUES FOR MONITORING SPORE RELEASE AND VIABILITY

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ABSTRACT

We report a series of techniques for monitoring spore release and viability of propagules of *Gibberella zeae*. A series of five identical wind tunnels were constructed to monitor the spore release of *G. zeae* under a variety of environmental conditions. The tunnels were operated at temperatures within a range of 10 to 30°C, and at varying degrees of air pressure. In preliminary experiments, ascospore discharge and distant movement in turbulent air currents occurred between 10 and 30°C, with peak discharge and movement at 25°C. To estimate propagule survival in air, viable propagules of *G. zeae* (ascospores and macroconidia) were applied to natural and artificial (plastic) wheat heads and placed in natural environments for varying durations of time. Spores were washed off treated heads, and quantified by plating out washes on selective medium and observing the number of resultant colony forming units. We documented significant ascospore and macroconidia viability on both natural and artificial wheat heads following up to three days of exposure to natural environments.

IDENTIFYING VIRULENCE FACTORS IN *FUSARIUM GRAMINEARUM* USING FORWARD AND REVERSE GENETIC APPROACHES

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ABSTRACT

Fusarium head blight or scab caused by *Fusarium graminearum* is a destructive disease on wheat and barley. Infested cereals are reduced in yield and contaminated with harmful mycotoxins. A molecular approach to the study of *F. graminearum* is critical because there are no effective fungicides and highly resistant plant varieties for controlling scab. Our goal is to determine molecular mechanisms of fungal pathogenesis in *F. graminearum*. In the past few years, we have generated over 9,000 REMI (restriction-enzyme mediated integration) transformants and identified 14 mutants defective in plant infection. One objective of this research is to generate randomly tagged mutant populations and characterize the genes disrupted in these REMI mutants that are reduced in virulence or are nonpathogenic. Genes that have been recovered to date include the HMG-CoA reductase and cystathionine beta-lyase genes. Further characterization of these genes will be discussed. We also have generated over 10,000 ESTs and 10x coverage of *F. graminearum* genome sequence in collaboration with the Whitehead Genome Research Institute at MIT. As a pilot test for large scale functional analyses in *F. graminearum*, over 15 candidate genes have been selected for targeted gene disruption or replacement. These genes are either homologous to known fungal virulence factors or predicted to be involved in various fungal developmental processes or secondary metabolism, such as polyketide synthases and signaling components. Phenotypes of mutants deleted of specific genes will be presented. Overall, both reverse and forward genetic approaches were found to be useful for identifying genes important for *F. graminearum* pathogenesis. The creation of a mutant population and functional analysis approaches developed in these studies will be useful resources for pursuing systematic characterization of *F. graminearum*-wheat interactions at the genome level.

REMI MUTAGENESIS IN *FUSARIUM GRAMINEARUM*

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ABSTRACT

Fusarium graminearum is an important pathogen of small grains and maize in many areas of the world. Infected grains are often contaminated with mycotoxins harmful to humans and animals. To better understand the molecular mechanism of plant infection and virulence of *F. graminearum*, we used the REMI (Restriction-Enzyme Mediated Integration) approach to generate random targeted mutants. Over 9,000 hygromycin-resistant transformants have been generated in the wild-type strain PH-1. Genes disrupted in two of these REMI mutants were recovered by plasmid rescue. In mutant 222, the transforming vector was integrated at the 268 amino acid of the hydroxymethylglutaryl CoA reductase gene (*HMR1*) that is essential for lipid biosynthesis. Disruption of *HMR1* significantly reduced the growth rate and aerial hyphal development in *F. graminearum*. Mutant 222 was non-pathogenic on flowering wheat heads and produced hyperbranching hyphae that are wider in diameter than that of the wild type strain PH-1. In mutant M8, the plasmid was integrated in the promoter region (110 bp upstream) of the cystathionine beta-lyase gene (*CBL1*). Mutant M8 had normal growth rate but produced rare aerial hyphae. Its virulence was significantly reduced. Gene replacement mutants deleted of *CBL1* had phenotypes identical to that of REMI mutant M8. Further characterization of the *HMR1* and *CBL1* genes will be presented.

RELATION BETWEEN HEAD BLIGHT AND GRAIN QUALITY IN THE INDIANA FHB EPIDEMIC OF 2003

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OBJECTIVES

To compare head blight severity, visible damage to grain, rate of infection of sound grain, and contamination by deoxynivalenol in wheat grown under a range of FHB epidemic intensities.

INTRODUCTION

Fusarium head blight (FHB) reduces yield of wheat. Various fungicide trials have demonstrated a negative correlation between FHB index and yield (Kawamoto et al. 2003, Lipps et al. 2003, McMullen et al. 2003). Of concern also is the quality of the grain, particularly the content of dexoynivalenol (DON). High levels of DON further reduce the value of the crop for the farmer, and pose problems for millers and bakers. In 2003, there were reports of high levels of DON in grain from southern Illinois, southern Indiana, and western Kentucky that did not appear to be badly damaged from scab, had good test weight, and came from fields with good yield. We observed that FHB ranged from severe in southern Indiana to almost absent in the north. Cultivar trials in several locations throughout the state allowed us to quantify head blight severity and various expressions of disease in the harvested grain. We used these data to examine relationships among these traits.

MATERIALS AND METHODS

Soft red winter wheat cultivar trials were conducted at research farms in different regions of Indiana. These included the Agronomy Center for Research and Education in west central Indiana (WC), Davis-Purdue Agricultural Center in east-central Indiana (EC), the Southeast Purdue Agricultural Center (SE), and the Southwest Purdue Agricultural Center (SW). Altogether there were 29 cultivars in the tests, but only 17 were common to all tests. At each location cultivars were sown in a randomized complete block design with 4 replications. Sowing dates were at the recommended time for each location and ranged from 27 Sep in the north to 15 Oct in the south.

To assess head blight, we counted the blighted heads in ten 1-ft lengths of row when symptoms were clearly visible and before natural ripening. We also recorded the total number of heads in several 1-ft lengths of row in order to express head blight incidence as a percentage. We rated severity of head blight as the percentage of spikelets blighted on those heads that showed any blight symptom. From this, the FHB index (product of incidence and severity, expressed as percent) could be calculated.

We counted the *Fusarium*-damaged kernels (FDK) in a sample of 100 kernels from each plot. We also plated, on Komada's medium, 25 kernels that showed no evidence of scab from each plot. The kernels were surface-sterilized in 5% bleach (0.2625% sodium hypochlorite) for 2 min and rinsed for 1 min in sterile water before being plated. The plates were incubated at 25°C and 12-h photoperiod for 5 d, after which

kernels contaminated with *Fusarium* were counted. This variable is identified as FCG (*Fusarium*-contaminated grain) and is expressed as a percentage. Finally, a 100-g sample of kernels from each plot was ground in a blender, and the coarse flour was sent to Dr. Pat Hart at Michigan State University for DON analysis.

RESULTS

Head blight was severe in the two southern Indiana sites (SW and SE), moderate at the WC site, present only on one very susceptible cultivar at the EC, and nonexistent at a site in northwest Indiana. Analysis of variance revealed that the effects of location, cultivar, and the location x cultivar interaction were highly significant for FHB index, FDK, FCG, and DON level.

To examine the correlation between head blight and variables associated with grain, we used the FHB index, because this is an overall estimate of head blight intensity that combines incidence and severity. For the three sites where head blight could be rated, there was a low correlation between FHB index and FDK or DON (Fig. 1). When the FHB index was above16%, there were no low values of FDK or DON, but for lower FHB indexes there was a wide range of FDK (Fig. 1A) and DON (Fig. 1B). For samples over the full range of FHB index values some DON levels were greater than 5 ppm. Correlation between FDK and DON was moderately high (Fig. 2A), as was the correlation between FDK and FGC (not shown, r = 0.75).

In every sample, we found infection of apparently sound kernels by *F. graminearum*. Levels of infection ranged from 5 to 69%. The correlation between FCG and FDK was moderately high (r = 0.76). The correlation between DON and FCG was 0.66, lower than that between DON and FDK (Fig 2A). We calculated a total percentage of *Fusarium* contamination (TFCG) by combining FDK and FCC (TFCG = [{100-FDK} × FCG/100] + FDK). The correlation between DON and TFCG was 0.72. Even though all of these correlations were significant, there was a considerable scatter of points, and the use of regression to predict kernel contamination or DON content from FHB index or FDK would not be reliable. Even for the relation between DON and FDK, which had the strongest correlation, there was a considerable range in DON levels over the range of 3% to 15% FDK. Only when FDK was 1% or less was DON below 2 ppm (Fig. 2A).

DISCUSSION

We took the opportunity provided by natural epidemics of various intensities to investigate the relation between head blight intensity in the field and grain quality. We found only modest correlations between FHB index and various grain traits associated with infection by *F. graminearum*. Above an FHB index of 16%, the frequency of FDK or level of DON in the harvested grain was consistently high. We do not know to what extent this threshold would vary from year to year. Unfortunately, many samples below this threshold also had high frequencies of FDK or high levels of DON (see particularly the boxed points in Fig. 1B). Thus, a low FHB index in the field does not necessarily mean a crop of high quality grain. This reflects what millers buying wheat from southern Illinois and Indiana, or western Kentucky, experienced this year.

It might be expected that the frequency of FDK in a grain sample would be a better predictor of DON than head blight symptoms in the field. Of all the correlations between various disease variables and DON, that between DON and FDK was the highest. DON levels below 2 ppm were confined to samples that had less

than 6% FDK. However, among samples of grain with less than 6% FDK, about half had DON levels of 2 ppm or greater.

The data we examined are all from natural epidemics of FHB. The data are from 17 cultivars, some of which have been bred for a degree of resistance to FHB. However, the outliers were not represented by any particular group of cultivars. For example, each of the boxed points in Fig 2B is a different cultivar. Our correlations between DON and field severity or damaged kernels are similar to those reported for field studies in which plants were inoculated and in misted or bagged to promote disease development (Bai et al. 2001, Liu et al. 1997, Mesterhazy et al. 1999).

Weather based risk assessment models have been developed (De Wolf et al. 2003) and are being refined. These models focus on head blight severity in the field. Results of this study indicate that additional effort should also be devoted to prediction of DON (Hooker et al. 2002). In the meantime, when current models used in the U.S. give even a modest chance of severe head blight, tests for DON should be performed on a sufficient sample of fields or grain lots to indicate whether a problem with grain quality exists.

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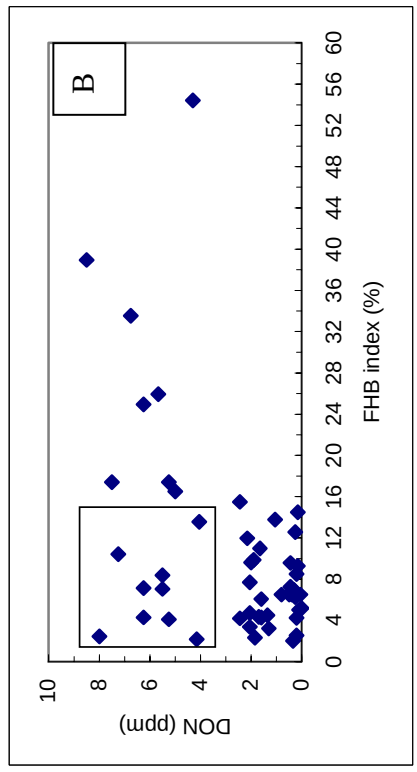

Fig. 1. Relation between frequency of *Fusarium*-damaged kernels (FDK) or DON and FHB index for 17 wheat cultivars grown at 4 locations in Indiana in 2003. For the graph of DON data, one outlier is not depicted because its DON level was much higher than any other entry (DON = 12.5 ppm, FHB index = 15.5%)

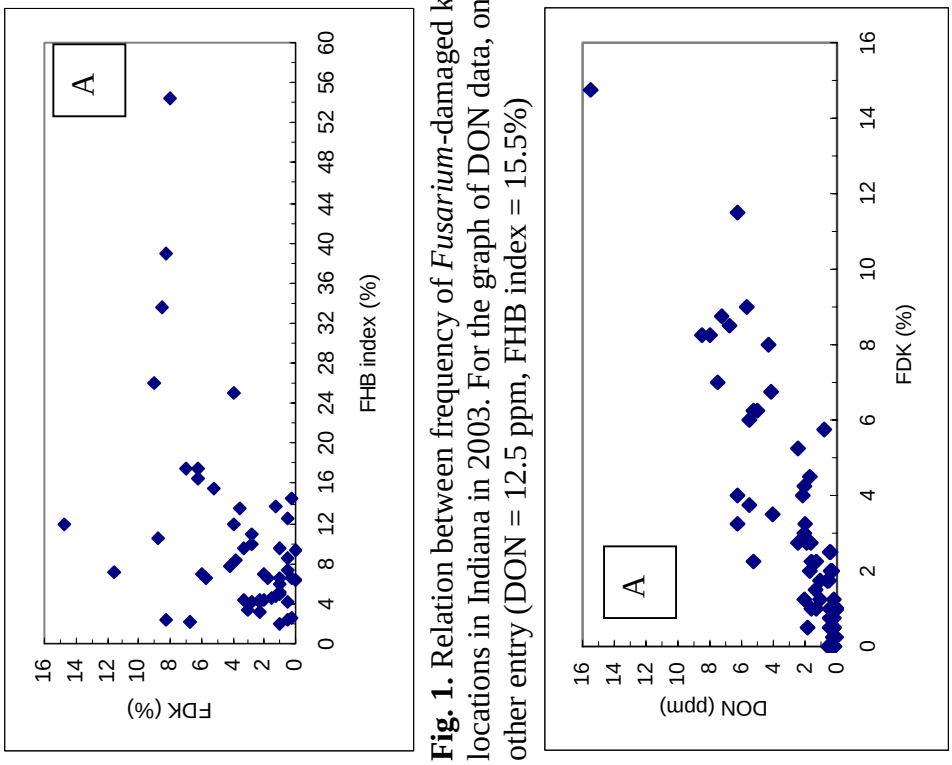

Fig. 2. Relation between DON and frequency of *Fusarium*-damaged kernels (FDK) or Fusarium-contaminated kernels (FCG) for 17 wheat cultivars grown at 4 locations in Indiana in 2003.

SEXUAL DEVELOPMENT AND FUNCTION IN *GIBBERELLA ZEAE*

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ABSTRACT

Gibberella zeae (anamorph *Fusarium graminearum*) produces its sexual spores (ascospores) in sacs called asci. The asci are produced in ephemeral perithecia which are produced on the surface of field debris and which, when mature, fire their spores into the air. We have been investigating formation of perithecia and the mechanism of forcible ascospore discharge through a variety of molecular, histological and physiological techniques. We will present the results of these studies. Among the findings will be results of microarray analyses to identify genes expressed during perithecium maturation, evidence that accumulation of mannitol and potassium ions is important to generation of the turgor pressure for discharge of these spores, and identification of tissue specificity during colonization of wheat. The recent availability of a genomic sequence for *F. graminearum* has greatly facilitated the study of perithecium development and function, and host colonization. Some results facilitated by the availability of the genome sequence will be presented.

EFFECT OF HARVESTING TIME ON INCIDENCE OF SEEDBORNE *FUSARIUM SPP.* IN SPRING WHEAT IN EASTERN ONTARIO

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ABSTRACT

In the last two decades, there has been an increase in the incidence of fusarium head blight (FHB) in spring wheat in eastern Ontario. As a result, seed harvested from the region often contaminated by *Fusarium spp.* and mycotoxins, making wheat unacceptable for milling. This study was undertaken to examine the effect of five harvesting times on incidence of seedborne *Fusarium spp.* using three spring wheat cultivars grown at two locations in Ontario in 1999 and 2000. Twelve *Fusarium spp.* were isolated from 3,831 of the 24,000 seeds which were surface disinfected and plated onto modified potato dextrose agar. *Fusarium sporotrichioides, F. graminearum, F. poae, F. equiseti*, and *F. avenaceum*, were the most frequently detected species and were isolated from 6.8, 3.7, 2.8, 1.8, and 0.6% of the seeds, respectively. The remaining species, *F. acuminatum, F. crookwellense, F. culmorum, F. oxysporum, F. sambucinum, F. solani*, and *F. tricinctum*, collectively infected only 0.3% of the seeds. The incidence of *F. graminearum, F. sporotrichioides,* and total *Fusarium spp.* increased about two fold, from 1.7, 3.9, and 9.5% in seed harvested very early to 5.5, 8.7, and 19.8%, respectively after delayed harvest. Also, *F. poae* had significantly lower incidence at very early and early harvest times compared to normal or later harvest dates. Incidence of the other *Fusarium spp.* was relatively low and not affected by harvesting time. Cultivar, location, and year variation in the incidence of *Fusarium spp.* were observed and likely related to the different levels of varietal resistance to these pathogens, inoculum present, and weather conditions before and during harvesting times.

PATHOGENICITY OF *FUSARIUM* SPECIES CAUSING HEAD BLIGHT ON BARLEY

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ABSTRACT

The pathogenicity of eight *Fusarium spp.* causing fusarium head blight (FHB) in barley was studied under controlled conditions. Six barley lines varying in resistance to FHB were artificially inoculated with six isolates each of *F. acuminatum, F. avenaceum, F. crookwellense, F. culmorum, F. equiseti, F. graminearum F. poae,* and *F. sporotrichioides* at the late-flowering stage. Symptoms of FHB were rated as disease severity on a 0-9 scale, 4, 7, 14, 21, and 28 days after inoculation, and as percentage of infected spikelets (IS) after 21 days. All species caused visible infections in the barley lines, but only *F. crookwellense, F. culmorum,* and *F. graminearum* resulted in severe disease development (>60% IS) and were considered highly pathogenic. *F. avenaceum* had IS of 48.3%, which was significantly lower than those of the three highly pathogenic species, being moderately pathogenic; and, the remaining species had <20% IS, being weakly pathogenic. There were significant differences ($P < 0.05$) in aggressiveness among isolates within species and in susceptibility among barley lines, suggesting that screening for resistance to FHB requires the use of aggressive isolates or a mixture of several isolates. This is also the first report showing that *F. crookwellense* is highly pathogenic and *F. avenaceum* is moderately pathogenic in barley.

POPULATION STRUCTURE OF *GIBBERELLA ZEAE* (*FUSARIUM GRAMINEARUM*) CAUSING FUSARIUM HEAD BLIGHT OF WHEAT IN MEXICO

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ABSTRACT

We have characterized 217 strains of *G. zeae* isolated from wheat in two locations in Mexico collected during growing seasons in 2000 and 2001. AFLP data from 215 isolates is consistent with *G. zeae* phylogenetic lineage 3. Of the remaining two isolates, one clusters closely with phylogenetic lineage 7, and the other does not cluster closely with representatives of the described phylogenetic lineages. We are assessing phylogenetic affiliations of representative lineage 3 isolates, and of the two other isolates relative to the described phylogenetic lineages by comparisons of DNA sequence data from the *benA*, *red*, and *tef* loci. Within these two populations, AFLP diversity is high (>100 AFLP genotypes among 215 lineage 3 isolates), and linkage disequilibrium is low, suggesting that sexual recombination has occurred. The allelic divergence between populations is also low ($G_{ST} < 0.045$), suggesting that there has been extensive genetic exchange between populations. From these data we conclude that populations of *G. zeae* causing Fusarium Head Blight (FHB) of wheat in Mexico differ from those in the United States, which include only isolates of phylogenetic lineage 7.

DETECTION OF SCAB-DAMAGED WHEAT KERNELS BY NEAR-INFRARED REFLECTANCE

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ABSTRACT

Both wheat breeder and wheat inspector must currently deal with the assessment of scab in harvested wheat by manual human inspection. We are currently developing and examining the accuracy of a semi-automated wheat scab inspection system that is based on near-infrared (NIR) reflectance (1000 to 1700 nm) of individual kernels. Our initial work revealed that, for scanning, the kernels could be oriented in just a semi-random basis, in which the rotational angle about a kernel's long axis was arbitrary. Classification analysis has involved the application of various statistical classification techniques, including linear discriminant analysis, soft independent modeling of class analogy (SIMCA), partial least squares regression, and non-parametric (*k*-nearest-neighbor) classification. For the most recent year evaluated (2002), average cross-validation accuracy ranged from 82.1% (a wavelength difference, without kernel mass, model) to 89.6% (a *k*-nearest-neighbor, with kernel mass, model). Although the lower value in this range was indeed lower than that for a model using mass alone (83.8%), the corresponding accuracies of these models on a separate (fully independent) test set indicated that the spectrally based models, with accuracies in excess of 92% were clearly better than the mass alone model. Based on test set accuracy, there were only slight differences between models that were based on principal component scores and those that were based on a simple wavelength difference. Typically, test set accuracies were between 94 and 97 percent. For the *k*-nearest-neighbor model, the number of neighbors needed to achieve stable optimal accuracies was approximately 20. An exhaustive search of the most suitable wavelength pairs for the absorbance [$A = \log(1/R)$] difference, [$A_{\lambda 1} - A_{\lambda 2}$], revealed that the low-wavelength side of a broad carbohydrate absorption band (centered around 1200 nm) was very effective at discriminating between healthy and scab-damaged kernels, with accuracies at about 95%. The best wavelength difference was [$A_{1248\ nm} - A_{1140\ nm}$]. Although the average cross-validation accuracy was lower for this model (82.1%) compared to the *k*-nearest-neighbor model (83.3%), the test set accuracies were nearly identical (94.9% and 95.0%). Many other wavelength differences, [$A_x - A_y$], produced cross-validation accuracies that were within 0.5 percentage units of the optimal difference, with values for *x* favoring the 1150-1300 nm region, and values for *y* favoring the 1000-1150 region. Combined, these regions define the broad absorption band centered near 1200 nm, which is attributed to the second overtone of a carbohydrate CH stretch. The achieved accuracy levels demonstrate the potential for the use of NIR in inspection operations for wheat scab. Therefore, development of an automated, high-speed device utilizing as few as two wavelengths for wheat scab detection appears to be feasible.

UPDATE ON USWBSI DON DIAGNOSTIC LABORATORIES

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OBJECTIVES

To provide DON testing services for collaborators investigating Fusarium head blight (FHB) in barley and wheat.

INTRODUCTION

Deoxynivalenol (DON) is a mycotoxin produced by Fusarium molds. DON analysis is used as an indicator of FHB contamination. Researchers are studying ways to reduce/eliminate the effects of FHB contamination in grain and grain products. In 2003, the U.S. Wheat and Barley Scab Initiative again have provided funding to four regional labs for free DON analysis to researchers. The four regional DON testing labs are located in North Dakota, Minnesota, and Michigan. Contacts for these regional labs are as follows:

Patrick Hart, Ph.D., and Benjamin Munn, Department of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824;
Ph: 517-353-9428, Fax: 517-353-5598, E-mail: hartl@pilot.msu.edu
Method: Neogen Veratox test (ELISA)
Sample type(s): wheat

Paul Schwarz, Ph.D., and James Gillespie, Department of Plant Sciences, North Dakota State University, Fargo, ND 58105;
Ph: 701-231-7732, 701-231-1040, Fax: 701-231-8474, E-mail: James.Gillespie@ndsu.nodak.edu, Paul.Schwarz@ndsu.nodak.edu
Method: Tacke and Casper (1996) by GC/ECD
Sample type(s): barley, malt, and single kernel

Michelle Mostrom, Ph.D., and Beth Tacke, Dept. of Veterinary Diagnostic Services, North Dakota State University, Fargo, ND 58105;
Ph: 701-231-7529, Fax 701-231-7514, E-mail: Michelle.Mostrom@ndsu.nodak.edu
Method: Tacke and Casper (1996) by GC/ECD
Sample type(s): wheat and barley

Yanhong Dong, Ph.D., Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108;
Ph: 612-625-2751, Fax: 612-625-9728; E-mail: dongx001@umn.edu
Method: Tacke and Casper (1996) by GC/MS
Sample type(s): wheat and barley (bulk, single head, single spikelet, single kernel, and small fragment)

MATERIALS AND METHODS

Labs in North Dakota (Mostrom and Schwarz) use the method of Tacke and Casper (1). The lab in Minnesota (Dong) using the extraction procedure from Tacke and Casper but with quantification using GC/MS. The lab in Michigan (Hart) uses the Neogen Veratox system (ELISA).

All labs maintain there own intralab quality control with analysis of check samples. An interlab wheat check program was also maintained by Michigan State University. Each lab received monthly a wheat sample that was divided and analyzed on separate days for repeatability. Also North Dakota State University (Schwarz) sent out monthly check samples of barley and malt for analysis to the collaborative labs as part of a larger collaborative with industry.

RESULTS AND DISCUSSION

Table 1 shows the number of collaborators and number of state sending samples to be analyzed by the four regional labs. The numbers have increased in the past two years and are also shown in table 2 where the number of samples has increased the past four years with 30,000 samples expected to be analyzed in 2003/2004.

Intralab data shown in table 3 shows the coefficient of variances between 6-18% which is the same as previous years. Tables 4 and 5 show the interlab data from North Dakota State University and Michigan State University respectively. Data from both collaboratives with barley, malt, and wheat samples show that there are no significant differences between labs.

REFERENCES

Tacke, B.K., and Casper, H.H. 1996. Determination of deoxynivalenol in wheat, barley, and malt by column and gas chromatography with electron capture detection. J. AOAC Intl. 79:472-475.

Table 1. Number of collaborators and states.

DON Lab	Number of Collaborators				Number of States			
	00/01	01/02	02/03	03/04	00/01	01/02	02/03	03/04
MI-P. Hart	9	17	20	18	8	11	9	12
MN-Y. Dong	11	12	9	11	2	1	3	3
ND-P. Schwarz	4	3	4	8	2	2	3	4
ND- M. Mostrom	23	10	25	21	6	4	7	8
Total	47	42	58	58	18	18	22	27

Table 2. Samples analyzed for DON by labs.

DON Lab	Number of Samples Analyzed			2003/2004	
				Analyzed	Expected
	00/01	01/02	02/03	03/04	03/04
MI-P. Hart	2481	3371	3000	2420	4500
MN-Y. Dong	7533	8500	10000	3764	10500
ND-P. Schwarz	5222	4612	7500	2862	10000
ND- M. Mostrom	4436	4600	4000	3113	5000
Total	19672	21083	24500	12159	30000

Table 3. Intralab quality control date for DON analysis 2003/2004.

DON Lab	2000-2001				2001/2002			
	Sample	Number	Mean (ppm)	CV %	Sample	Number	Mean (ppm)	CV %
MI-P. Hart	Wheat	56	1.6	7	Wheat	94	2.3	5
MN-Y. Dong	Wheat	34	12.8	12	Wheat	38	9.0	14
ND-P. Schwarz	Barley	120	6.3	14	Barley	108	6.3	11
	Barley	112	1.6	16	Barley	104	1.5	16
	Barley	124	5.3	15	Barley	104	5.1	10
ND- M. Mostrom	Wheat	83	1.8	9	Wheat	31	1.7	5
	Barley	83	3.1	9	Barley	31	2.9	5
	Corn	83	5.0	9	Corn	31	4.6	7

DON Lab	2002/2003				2003/2004			
	Sample	Number	Mean (ppm)	CV %	Sample	Number	Mean (ppm)	CV %
MI-P. Hart	Wheat	122	0.9	12	Wheat	84	2.4	8
MN-Y. Dong	Wheat	30	7.2	13	Wheat	102	7.1	12
ND-P. Schwarz	Barley	31	13.8	15	Barley	75	1.9	18
	Barley	18	39.7	12	Barley	54	14.5	13
	Barley	9	2.1	13	Barley	18	40.3	12
ND- M. Mostrom	Wheat	104	1.8	7	Wheat	100	1.8	6
	Barley	104	3.1	6	New Wheat	100	1.1	10
	Corn	104	4.7	11	Barley	100	3.0	8
					Corn	100	4.7	7

Table 4. Interlab quality control data 2003 NDSU.

LAB	Malt Check Samples (ppm DON)									
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct
ND-P. Schwarz	1.70	1.10	0.70	0.40	0.50	0.50	0.07	0.00	0.40	0.43
ND- M. Mostrom	1.90	1.20	0.50	0.40	0.40	0.40	<0.20	<0.20	0.30	0.40
MN-Y. Dong	1.21	0.44	0.33	0.33	0.41	0.34	0.00	0.00	0.30	0.39
MI-P. Hart	3.20	1.20	0.50	<0.50	0.60	0.60	0.00	0.00		
Mean	2.00	0.99	0.51	0.38	0.48	0.46	0.02	0.00	0.33	0.41
SD	0.85	0.37	0.15	0.04	0.09	0.11	0.04	0.00	0.06	0.02
CV	42.41	37.20	29.81	10.73	19.52	24.85	173.21		17.32	5.12

LAB	Barley Check Samples (ppm DON)									
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct
ND-P. Schwarz	1.20	0.00	1.60	0.00	10.20	0.60	6.90	1.60	1.55	0.63
ND- M. Mostrom	1.30	<0.20	1.90	<0.20	10.40	0.80	5.70	2.30	1.60	0.80
MN-Y. Dong	0.77	0.00	1.67	0.00	10.90	0.66	4.96	2.08	1.38	0.72
MI-P. Hart	1.40	0.00	2.10	0.00	6.00	0.60	4.30	2.00		
Mean	1.17	0.00	1.82	0.00	9.38	0.67	5.47	2.00	1.51	0.72
SD	0.28	0.00	0.23	0.00	2.27	0.09	1.11	0.29	0.12	0.09
CV	23.75		12.53		24.20	14.19	20.39	14.65	7.64	11.87

Table 5. Interlab quality control data 2003 Michigan State University.

LAB	Wheat Check Samples (ppm DON)								
	5	6	7	8	9	10	11	12	13
ND-P. Schwarz	0.06	2.30	1.50	0.90	1.20	1.30	1.00	1.22	0.38
ND- M. Mostrom	<0.20	2.20		1.20	1.25	1.65	1.10	1.40	0.50
MN-Y. Dong	0.12	1.24	1.46	1.11	1.17	1.02	1.01	1.36	0.51
MI-P. Hart	0.00	2.30	2.60	1.00	1.60	1.60	1.20	1.64	0.60
Mean	0.06	2.01	1.85	1.05	1.31	1.39	1.08	1.41	0.50
SD	0.06	0.52	0.65	0.13	0.20	0.29	0.09	0.17	0.09
CV	100.00	25.65	34.91	12.40	15.28	21.01	8.65	12.43	18.16

APPLICATION OF REAL TIME POLYMERASE CHAIN REACTION TO THE DETECTION AND QUANTIFICATION OF *FUSARIUM* IN WHEAT

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OBJECTIVES

To examine the relationship between Fusarium head blight symptoms, levels of deoxynivalenol, and the amount of *Fusarium* DNA in individual kernels of wheat.

INTRODUCTION

Fusarium head blight (FHB) of small grains has been problematic for commercial buyers and processors of small grains. To processors, the contamination of the grain with deoxynivalenol (DON) is a food safety issue, and the FDA has specific guidelines for the amounts of DON that can be in food and feed intended for animal and human consumption (1). Generally, small grains are screened for DON at the buying point using one of the several tests commercially available. The amount of testing is dependent on how much disease is observed in fields prior to harvest, which is usually based on visual symptoms on the heads. Jones, et al (2) reported a significant correlation of DON levels with FHB symptoms in spring wheat, but there was wide variation in DON for any one category of symptoms. Since the year 2000 there have been several reports of DON levels in winter wheat that don't always correlate with the amount of FHB symptoms in the field, i.e. low levels of DON when more than ten percent of the heads had FHB symptoms, and high levels of DON when FHB head symptoms were below ten percent. Our objectives in these preliminary experiments were to divide a single sample of grain into categories of kernel symptoms, and then examine individual kernels for DON and *Fusarium* DNA.

METHODS AND MATERIALS

A sample of *Fusarium* infected wheat seed was obtained from Gene Milus, University of Arkansas, with a DON level of >30 ppm. The sample was divided into seven categories based on visual symptoms. The categories were;

1) Normal appearing seed.
2) Seed slightly smaller than sample 1 seed.
3) Seed similar in size to sample 1 but with some whitish discoloration.
4) Shriveled seed but no discoloration.
5) Shriveled seed with some white discoloration.
6) Slightly shriveled seed with a definite white discoloration of the entire seed, and
7) Similar to 6 but seeds also had some red discoloration of the seed coat. An additional 8) Michigan seed from 2003 crop with kernels normal in appearance. Used as a negative control.

Ten individual seeds from each sample were weighed and ground in 500 µl water to extract DON. The homogenates were centrifuged for 5 min and the water transferred to new 1.5 ml eppendorf tubes. The remaining pellet was mixed with 600 µl of Qiagen extraction buffer and total genomic DNA was extracted

with Qiagen DNeasy Plant Mini Kit according to manufacturer instructions. The resulting DNA was diluted 10^2 times (980 µl PCR water + 20 µl DNA eluate) to approximately 1 ng/µl (1µg/ml).

Real time PCR was carried in 25 µl volumes and consisted of 12.5 µl SYBR Green PCR Master mix, 300 nM of each primer, PCR water, and 10 µl of diluted DNA. Forward primer Tri5F (5'-CGACTACAGGCTTCCCTCCAA-3') and reverse primer Tri5R (5'-ATCCGCCATGCACTCTTTG-3') were designed using Primer express software and used to amplify an 85 bp sequence of Tri-5 DNA. Wheat ribosomal DNA 18 S Forward primer (5'-GCCTTCGTGCAAGTGATCCT-3') and reverse primer (5'-CAAGCGGTCAAACCAACCA-3') were used to amplify 18 S ribosomal DNA to normalize the quantities of Tri5 DNA. DNA standard were prepared for both Tri5 gene and 18S gene from the known amounts of DNA. Real-time PCR was performed in Microamp optical 96-well reaction plates using the automated ABI Prism 7000 sequence detector. The Real-time PCR reactions were run as follows: initial denaturation at 95°C for 10 min followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min. PCR results were analyzed using ABI Prism 7000 SDS software. The amounts of DNA in each reaction well were calculated by the software according to standard DNA amounts (Standard Curve Method). Relative amounts of fungal DNA was obtained by normalizing the quantities of fungal DNA to the quantities of Wheat 18S DNA and by calibrating with a calibrator (wheat sample number 8).

RESULTS AND DISCUSSION

The standard curves for estimating concentrations of *Fusarium* and wheat DNA are shown in figures 3 and 4, respectively. There was a good correlation between DON and DNA in individual kernels (r^2=74.2%), and between sample means of DON and DNA (r^2=99.0%) (Table 1, and Figures 1 and 2). Using DON as a comparison with symptom categories, the categories in increasing order of DON were samples 8, 3, 1, 4, 2, 5, 6, and 7 (Table 1). Using increasing levels of DNA to compare with visual symptoms, the categories were samples 8, 3, 1, 4, 5, 2, 6, 7. Samples 5, 6 and 7 had high levels of *Fusarium* DNA and DON. These results suggest that DON and DNA are not necessarily correlated well with kernel symptoms of FHB infection. Schnerr, et al (3) also reported a good correlation between DNA and DON in infected wheat kernels, but they did not correlate either with FHB symptoms of individual kernels. Although these results are from a single sample of wheat divided into subjective categories based on appearance, they do suggest that individual kernels can be variable in relation to levels of infection and DON. More research is needed to support these data indicating that high levels of DON can occur in wheat with minimal symptoms of infection.

Table 1. Sample means and standard errors for DNA concentration (pg), DON (ppm), and kernel weight (mg).

SAMPLE	DNA (pg)		DON (ppm)		SEED WEIGHT (mg)	
	Mean	Std.Dev	Mean	Std.Dev	Mean	Std.Dev
Sample8	0.5	0.3	0.1	0.4	40.0	7.4
Sample3	3.0	2.8	0.9	1.3	23.7	6.1
Sample1	3.9	5.3	1.8	2.8	35.9	3.3
Sample4	5.2	5.7	3.9	8.6	16.0	3.2
Sample2	20.6	26.0	7.7	5.3	35.4	3.6
Sample5	15.9	10.8	31.6	56.4	15.5	4.1
Sample6	94.1	89.5	106.9	77.6	27.8	2.3
Sample7	207.8	132.6	245.8	126.5	15.1	1.9

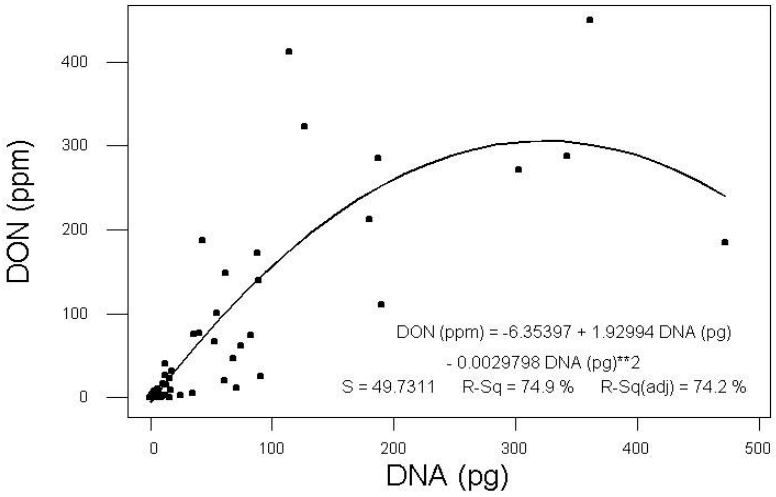

Figure 1. Regression plot for DON (ppm) and DNA (pg) for each of the eighty samples used in the study.

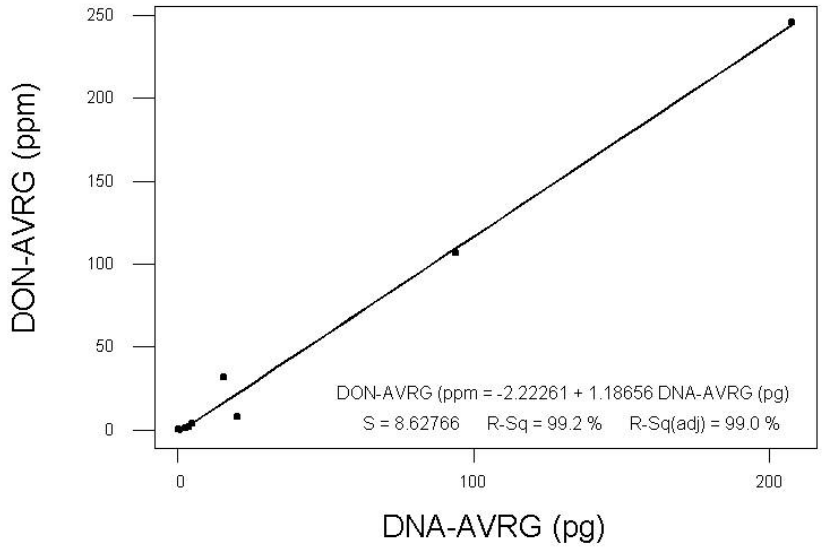

Figure 2. Regression plot of sample means for DON (ppm) versus DNA pg).

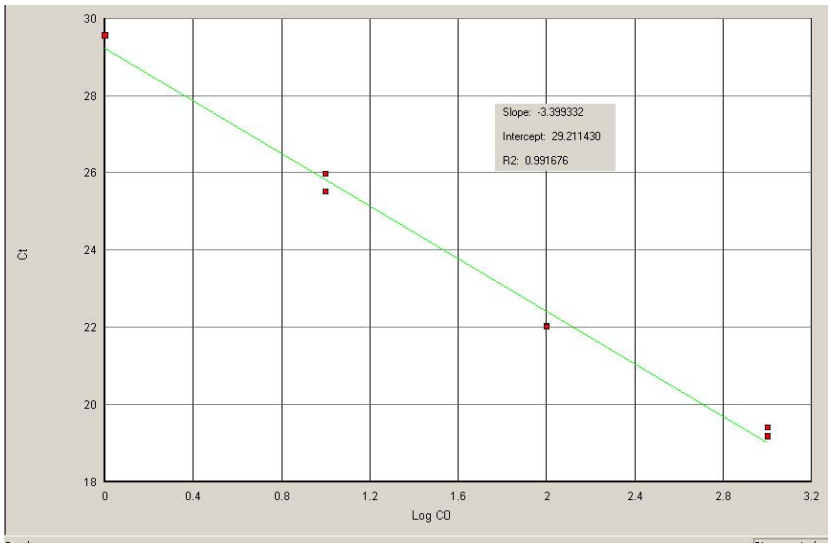

Figure 3. Standard Curve for Tri-5 gene primers-Tri5F-Tri5R.

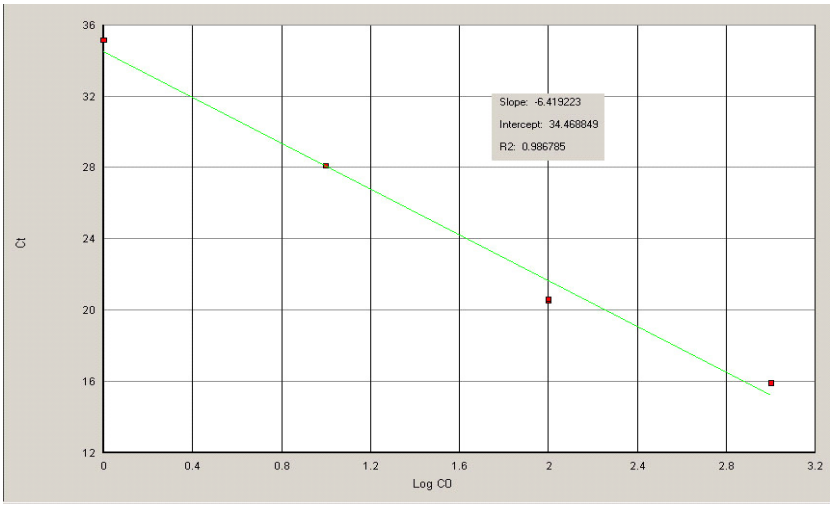

Figure 4. Standard Curve for Wheat rDNA 18S gene primers- W18SF-W18SR.

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FUSARIUM HEADBLIGHT QUALITY ASSURANCE VIA IMMUNOCHEMISTRY

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OBJECTIVE

Determine the relationship between quantitative values of *F. graminearum* and DON in barley

INTRODUCTION

Quality assurance and quality control of any product is predicated upon a **direct, accurate, simple, and cost-effective** method of quantifying the component to quality. Currently FHB quantification is limited to a visual scoring system of seeds or seed heads. Visual scores have limitations to disease quantification and have led to the following conclusion. "… disease ratings in the field with other agronomic characteristics (yield) may reveal the limitations of relying on visual ratings customarily used to quantify disease severity" (Smith et al., 2003). This could also be the reason why no genetic source with complete resistance to FHB is yet known (Miedaner et al., 1995; 2003). Secondary fungal metabolites (DON) have also been used as an indicator of *Fusarium* presence. In the case of barley, however, we can not currently predict malt DON levels from barley DON levels with a high degree of accuracy when DON is <2.0 ppm (Schwarz et al 1995; Beattie et al., 1998). This is because DON production is dependent on environmental conditions (temperature and moisture), physiological development of the fungus (Hooker et al., 2002; Martins and Martins, 2002;) and storage conditions (Beattie et al., 1998). Thus, a need exists to develop a more direct and quantitative analysis for *Fusarium* to replace, or be used in conjunction with, FHB and DON analyses. Immunochemistry is a proven analytical tool that capitalizes on the specificity of antigen/antibody interactions capable of detecting minute quantities of the target antigen. Previously, we capitalized on our immunochemical experiences assessing infection status and toxin production of fungal endophytes in grasses to develop monoclonal antibodies specific to *Fusarium graminearum* during the winter of 2001/2002 (Hill et al., 2002a, 2002b, 2002c). Accordingly, our objective was to assess quantification of *Fusarium graminearum* with species-specific monoclonal antibodies in barley.

MATERIALS AND METHODS

Characterization of Antibody Utility for Barley – Two hundred and fifty grams of three barley samples were used to develop immunochemical methods. The three samples were a non-contaminated sample of 'Drummond' grown in Arizona, a low-contaminated sample of Drummond grown in Langdon, ND, and a highly contaminated sample of ND9712 grown in Langdon, ND. 1) Antigen extraction protocols were tested to determine the optimum conditions for immunoblot analysis by testing: a) temperature and time requirements for antigen extraction, and b) seed aspect when placed on nitrocellulose membranes for antigen extraction. 2) Antigen extraction protocols were tested to determine the optimum conditions for ELISA assays by testing: a) extraction of whole vs. ground seed, b) time necessary to extract the antigens, and c) single vs. a sequential extraction of antigens (antigens extracted after DON extraction performed).

Analysis of Barley Lines - Eighty-five doubled haploid (DH) lines from the cross Zhedar 2/ND9712/ Foster were planted into single row plots at Osnabrok and Langdon, ND in spring 2003. Corn was previously grown on each field, the stubble was chiseled in the fall, and plots planted directly into the corn stubble. The lines had two replicates at each location. The plots were rated for FHB at the soft dough stage of kernel development. After harvest, each line was tested for DON using HPLC-mass spectroscopy at North Dakota State University. Seed from each line was shipped to the University of Georgia, antigens extracted with the best protocol from antibody characterization studies in 2a, 2b, and 2c, and indirect ELISA used to quantify *F. graminearum* in each. Fifty seed from each sample were placed on nitrocellulose membranes and immunoblot analysis conducted using the best protocol from 1a and 1b to determine percent infection.

Analysis of variance was conducted to examine means and coefficients of variability for FHB, DON, and ELISA data within each location. FHB, DON, and ELISA data were correlated with each other within locations. To compare consistency of analysis among locations, FHB from Osnabrock was regressed with FHB from Langdon, DON from Osnabrock was regressed with that from Langdon, and ELISA quantification of *F. graminearum* from Osnabrock was regressed with that from Langdon.

RESULTS AND DISCUSSION

Characterization of Antibody Utility for Barley – We found the best method to extracting antigens for immunoblot was to place seed on top of nitrocellulose membranes saturated with extraction buffer. Seed had to be placed with the **crease side up** and extracted overnight at room temperature (Figure 1). Placing seed with the crease side down resulted in a meniscus of water in the crease that, upon removal of seed, led to spreading of the antigens over the entire nitrocellulose membrane. We found that ground seed decreased the detectable F. graminearum in our ELISA test. Antigen detection was possible with as little as 15 minutes seed incubation on the nitrocellulose membrane but we found that incubating overnight gave greater intensity of color development in seed contaminated with *F. graminearum* while having no effect on the seed that were not.

Extraction of *F. graminearum* antigen from ground seed suppressed the ELISA analysis. Mean of whole seed and ground seed extracts in our experiment was 148 and 92 ug *F. graminearum* per g seed, respectively. Antigen presence in whole seed were greatest when extracted for 1 hour (Figure 2) and ELISA quantification of *F. graminearum* in the three samples gave results consistent with DON even though the two North Dakota samples were 100% infected (Table 1).

Analysis of Barley Lines - The range of ELISA data for *F. graminearum* was greater than either FHB or DON. CV's were greatest for FHB followed by DON and ELISA data (Table 2). FHB scores did not correlate with DON or ELISA in the Langdon samples but were correlated to both in the Osnabrock samples. DON and ELISA data correlated with one another regardless of where the samples originated (Table 3). FHB scores for barley lines grown at Osnabrock did not correlate with those at Langdon, but DON and ELISA values for the lines among locations had low correlations.

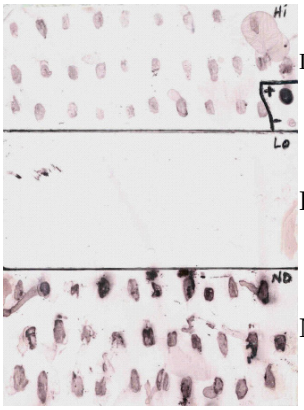

Figure 1. Immunoblot of *F. graminearum* in Drummond from ND, Drummond from AZ, and ND18172 from ND.

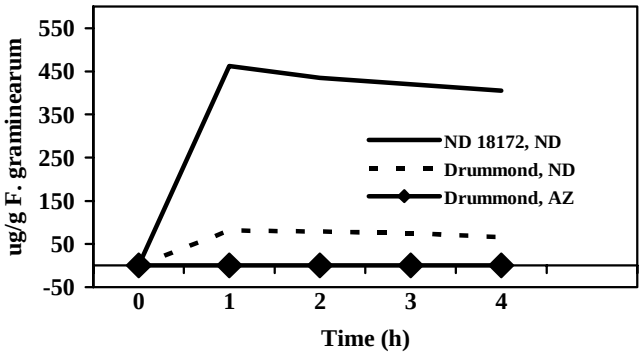

Figure 2. ELISA quantification of *F. graminearum* as affected by extraction time in barley.

Table 1. Infection frequency and mycelial mass of *F. graminearum* in three barley samples. These values correspond to the samples from the immunoblot in Figure 1, to the left of this table.

Line ID	DON	% infected	*F. graminearum*
	ppm	(immunoblot)	ug/g seed
Drummond-AZ	0	0	0.00
Drummond-ND	1.3	100	81
ND18172-ND	24.7	100	462

Table 2. Range, means, and coefficients of variation (CV) for head blight scores, DON, and ELISA quantification of *F. graminearum* in North Dakota field-grown barley.

	Osnabrock			Langdon		
	FHB	DON	ELISA	FHB	DON	ELISA
	%	ppm	ug/g seed	%	ppm	ug/g seed
Range	.06-.43	13.0-71.6	58.9-133.0	0.0-8.7	19.8-106.1	85.9-252.4
Mean	0.18	34.3	97.3	1.36	54.0	147.4
CV (%)	58.1	39.9	14.1	84.5	25.7	28.8

Table 3. Correlation matrix among FHB, DON, and ELISA data for barley grown at Osnabrock and Langdon, ND.

	Osnabrock			Langdon		
	FHB	DON	ELISA	FHB	DON	ELISA
FHB	-	0.42	0.49	-	-0.04	-0.14
DON			0.57	-	-	0.44
Osn vs. Langdon	0.07	0.21	0.23			

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EVALUATION OF ELECTRON-BEAM IRRADIATION FOR REDUCING *FUSARIUM* INFECTION AND MICROBIAL LOADS IN BARLEY MALT

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ABSTRACT

Utilization of *Fusarium* infected barley for malting may lead to mycotoxin production and decreased malt quality. Electron-beam irradiation of *Fusarium* infected barley may prevent these safety and quality defects and allow use of otherwise good quality barley. We evaluated electron-beam irradiation for effectiveness in reducing *Fusarium* infection while maintaining germinative energy in barley samples. Four barley samples with varying deoxynivalenol concentrations (0.10, 0.40, 1.20, and 1.27 µg/g) were evaluated for this study. 200g samples were placed in sterile plastic bags and irradiated at doses of 0, 2, 4, 6, 8, and 10 kGy. Treatments were done at Surebeam Corporation, Chicago and repeated three times. Treated samples were malted in a pilot-scale malting unit at North Dakota State University. The barley samples were analyzed for *Fusarium* infection (FI), germinative energy (GE), aerobic plate counts (APC), and mold and yeast counts (MYC). Malted barley samples were analyzed for FI, APC, and MYC. FI decreased with increase in radiation dosage in both the barley and malted samples. In barley samples exposed to 10 kGy, FI was reduced by 50-98%. APC significantly decreased (1-5 logs) in barley with increase in irradiation dosage. A 5-log reduction in APC was observed at 10 kGy for all barley samples. MYC significantly decreased in barley with increase in irradiation dosage. A 1-2.5 log reduction in MYC was observed for all barley samples exposed to 10 kGy. A 20-70% increase in FI in malt, as compared to 8-10 kGy irradiated barley, was observed. The APC's for malts from barley exposed to 8-10 kGy were significantly higher than in other malted samples. A 5-6 log increase in APC, as compared to 8-10 kGy irradiated barley, was observed. MYC exhibited similar trends as observed with APC in malted barley. A 1-3 log increase in MYC, as compared to 8-10 kGy irradiated barley, was observed. GE in barley samples was significantly decreased (3-15%) at higher irradiation dosages. The largest decrease (14%) in GE was observed for the 1.27 ppm sample treated at 10 kGy. The results suggest that dosages between 6-8 kGy may be effective in reducing the FI significantly while maintaining the GE in barley. Dosages over 8 kGy reduce GE and appear to lead to higher microbial loads in malt. Whether the significant increase in APC and MYC counts after malting of irradiated barley was due to cross-contamination or due to the growth of resistant flora requires further investigation.

INHIBITION OF MOUSE SPLEEN LYMPHOCYTE PROLIFERATION BY DEOXYNIVALENOL

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ABSTRACT

We hypothesized that acute exercise stress combined with feeding deoxynivalenol (DON, 2 ppm) for 14 d would exascerbate immunosuppressive effects of DON. Twenty-four 8-wk-old male BALB/c mice were fed DON for 14 d after 1 wk acclimation to AIN-93G diet. On d 14, mice were placed on a treadmill (10-20 m/min) and exercised to exhaustion (2.5-4.25 h). Mice were then killed by decapitation. Trunk blood and spleens were collected. Single cell suspensions of splenocytes were made by stomacher. Splenocytes were cultured at 5 x 10^5 cells/well for 72 h in the presence of the mitogen concanavalin A (10 µg). Cell proliferation was measured by Cell-Titer™dye uptake. Proliferation of splenocytes from DON-fed mice was 40% of non-exercised controls. Exercised controls and exercised animals fed DON showed proliferation of 68-70% of non-exercised controls. Only the non-exercised DON-fed mice showed significant suppression of lymphocyte proliferation. Thus, our hypothesis was not confirmed, suggesting a protective effect of exercise stress against DON toxicity.

MODERN METHODS FOR DETECTION AND QUANTIFICATION OF TRICHOTHECENE PRODUCERS AND DON LEVELS IN CEREALS AND MALT

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OBJECTIVES

To develop diagnostic tools for detection and quantification of trichothecene producing *Fusarium spp.* and for DON as the major mycotoxin produced in cereals and cereal products, to compare the results with established methods and to apply the newly developed tools in the analysis of contaminated sample materials.

INTRODUCTION

Several *Fusarium spp.* are widespread pathogens on cereals in both temperate and semitropical areas, including the cereal growing regions of Northern America and Europe (Bottalico 1998). Warm and rainy weather conditions during the flowering stage promote infection of cereals with *Fusarium* species, resulting in a disease known as Fusarium Head Blight. Low temperatures following infection may promote production of trichothecene mycotoxins in some species (Jacobsen *et al.* 1993). Contamination of grains with *Fusarium ssp*. and their toxins is of great economic concern to cereal producers and to the grain-processing industry (Demcey Johnson *et al.* 1998; Windels 1999), including maltsters and brewers. Deoxynivalenol (DON) and nivalenol (NIV) have been found to be the most frequently occurring trichothecene contaminants in agricultural crops throughout the world. Fusaria which are capable of producing these key toxins may grow in cereals before harvest but also during storage and food processing under unfavourable conditions (Müller *et al.*, 1998; Homdork *et al.*, 2000). As a result of their high stability during storage and processing (Widestrand and Pettersson 2001) and their occurrence in a wide range of agricultural commodities, trichothecenes are regularly detected in animal feed and in human food (WHO 1990). The U.S. Food and Drug Administration advisory level for DON in cereals and cereal products for human consumption is 1 mg kg^{-1}. With respect to health hazard and consumer protection, uniform regulations of trichothecene limits in several countries and in the European Community are also to be expected within the near future.

Standard methods for the analysis of mould contamination in grain comprise micro-biological investigation of samples on suitable agar media. However, this procedure is time-consuming and only viable mycelia can be detected. Therefore, qualitative and quantitative estimation of fungal biomass is often inaccurate.

The polymerase chain reaction (PCR) offers an alternative to micro-biological procedures. Quantitative PCR methods have previously been developed for fungal pathogens including *Fusarium spp.* (Nicholson *et al.* 1998 ; Niessen *et al.* 1998b ; Doohan *et al.* 1999; Edwards *et al.* 2001). Recently, a group specific quantitative PCR assay for trichothecene producing *Fusarium spp.* was established, which is based on

real-time quantification of the *tri5* gene sequence using the LightCycler™system (Schnerr *et al.* 2001). This assay provides the basis for a rapid quantification of toxigenic *Fusarium* species in cereal samples.

Analytical procedures for trichothecene mycotoxins usually differ in extraction, clean-up, and final analytical steps, depending on the kind of substance is actually analysed. A detailed review of analytical standard methods such as GC, GC/MS, HPLC and immunochemical techniques has been published by Krska et *al.* (2001).

Real-time Biomolecular Interaction Analysis (BIA) from Biacore AB (Uppsala, Sweden) uses the optical phenomenon of surface plasmon resonance (SPR) to monitor biomolecular interactions, without labelling any of the interactants. It detects changes in the concentration of molecules in a surface layer of the solution in contact with the sensor surface. Bound antibody can be removed using chaotropic reagents, which allow the sensor surface to be reused repeatedly. BIAcore has been used in many applications such as kinetic and affinity analysis and investigations of specificity and concentration of ligands. The system has been previously used in the detection of various mycotoxins (van der Gaag *et al.*, 1998), aflatoxin B_1 (Daly *et al.*, 2000), and fumonisin B_1 (Mullett *et al.*, 1998).

The current study describes the use of a real-time PCR based system to detect and quantify the contamination of cereal samples with trichothecene producing *Fusarium spp.* as well as a dip stick based system for specific detection of *F. graminearum* PCR products. Also quantification of DON in an inhibitive indirect immunoassay on a BIAcore SPR device is described. Results obtained with the new analytical tools are compared with results obtained using established analytical protocols.

MATERIALS AND METHODS

Prepartion of DNA - DNA from pure fungal cultures was prepared using the method previously described (Niessen & Vogel, 1997). The method described by Knoll et al. (2002a) was used for rapid preparation of *Fusarium*-DNA from cereal and malt samples.

Real-time PCR analysis - The LightCycler™system was applied for amplification and online quantification of extracted DNA. The PCR primers Tox5-1 (forward) and Tox5-2 (reverse) were used to amplify a 658 bp fragment from the trichodiene synthase gene (*tri5*) of trichothecene producing *Fusarium spp.* (Niessen & Vogel, 1998a). The PCR master mix used was composed as previously described (Schnerr et al., 2001). One microliter of DNA template either extracted from a pure fungal culture of from sample material was used per reaction. Thirty two samples were run in parallel by performing 35 cycles of amplification in LightCycler™capillaries (Roche Diagnostics, Mannheim, Germany) under the following thermal cycling protocol. Samples were preheated at 95°C for 2 min. Subsequently 35 cycles of 0 sec 95°C, 5 sec 63°C, 30 sec 72°C (heating and cooling at 20°C/sec) were run. Detection of fluorescent product was carried out after the last step of each cycle at 83°C. Following the final amplification cycle, a melting curve was acquired by one cycle of heating to 95°C, cooling to 75°C at 20°C/sec and slowly heating to 95°C at 0.1°C/sec with continuous measurement of fluorescence at 520 nm. For quantitative analysis, a serial dilution of purified *F. graminearum* DSM 4527 DNA was used to set up a calibration curve.

PCR Detection Test Strips (Roche) for F. graminearum – PCR using digoxygenin labelled *F. graminearum* specific primers was carried out as described by Niessen and Vogel (1997). Following PCR, 9 µl of the reaction were mixed with 1 µl of product specific oligonucleotide probe biotinylated at both ends (5 µmol l^{-1} in 4 x SSC buffer) (Niessen et al. 1998b). The mix was boiled for 5 minutes and immedi-

ately transferred on ice for hybridisation. Five microliters of the mixture were applied to a DNA Detection Test Strips ™ and the reaction developed for 10 min as recommended by the manufacturer.

BIAcore immunoassay for DON –Purified antibodies from a polyclonal anti-DON antiserum (Niessen et al., 1993) were used to detect and quantify free DON in sample extracts. Unreacted antibody was detected using a BIAcore X device (Biacore AB, Uppsala, Sweden) and a sensor chip SA (research grade) with pre-immobilised streptavidin to which biotinylated DON (produced according to Casale et al., 1988) was coupled. The sensor surface was regenerated with 3 µl 6 M guanidinium-chloride in 10 mM glycine, pH 2.9 after each measuring cycle. DON was extracted from 4 g of ground wheat or barley with 12 ml of 10 % (v/v) methanol in water with 6 % (w/v) polyvinylpyrrolidone by shaking on an orbital shaker at 100 rpm for 30 min at ambient temperature. Extracts were clearyfied by centrifugation (1 min, 15,000 x g) and cleaned up with MycoSep™columns (Romer Labs Inc., Union, USA). For analysis 10 µl of the extract or of a serial dilution of purified DON (Sigma-Alsdirch, Deisenhofen, Germany) were incubated with 30 µl of a 0.5 µg μl^{-1} anti-DON antibody solution for 1 min at ambient temperature and subsequently passed over the surface of the sensor chip. Evaluation of results was done using the BIAevaluation® Software 3.0 softtware package (Biacore AB (Uppsala, Sweden).

RESULTS AND DISCUSSION

Real-time PCR analysis of DON contaminated wheat - Figure 1A shows real-time PCR kinetics obtained with a serial dilution of purified DNA from *F. graminearum* DSM 4527 and the calibration curve calculated from the data (see figure 1B). Real-time PCR on the LightCycler™ (Wittwer et al., 1997) was applied to analyse 300 samples of wheat which had previously been field inoculated with *F. culmorum*. All samples were analysed for DON using a GC/MS method (data kindly provided by BASF, Limburgerhof, Germany). Concentrations of the toxin ranged from not detectable to 34.3 mg/kg. Concentrations of template DNA in the samples were calculated from the calibration curve given in figure 1B and expressed as ppb DNA per gram sample.

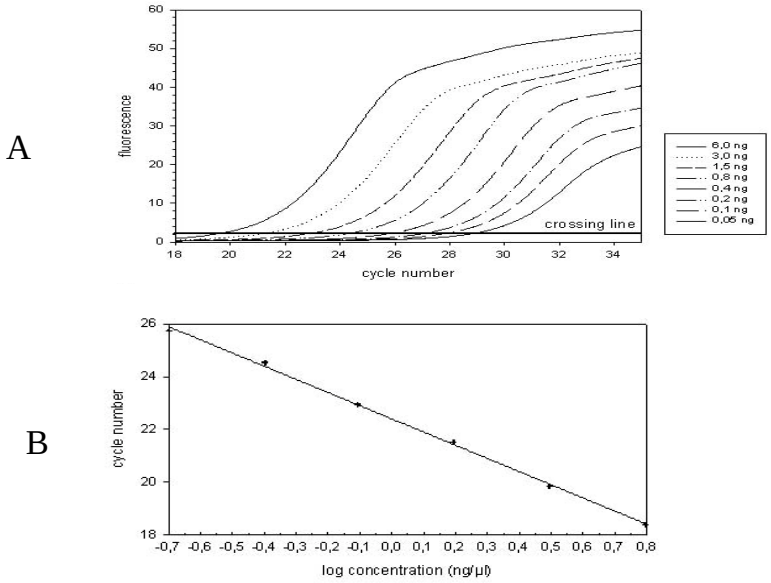

Figure 1: Calibration of the LightCycler™ PCR assay. **A**. Real-time PCR kinetics of a dilution series of DNA isolated from a pure culture of *F. graminearum* DSM 4527 used as template. B. Calibration curve calculated from the data obtained in A, cycle number at crossing line, r = 1.0. Redrawn from Schnerr et al., 2001.

DNA concentrations of trichothecene producing *Fusarium spp.* ranged between not detectable and 16.3 mg/kg in the 300 samples studied. For correlation analysis, DNA concentrations were plotted against DON concentrations and analysed by linear regression (SigmaPlot®4.0 for Windows®, SPSS Inc. Chicago, USA). A coefficient of correlation between both parameters of r=0,9557 was calculated on the basis of all samples. Calculation based only on samples which had DON concentrations between not detectable and 1,5 mg/kg (n = 234) revealed a coefficient of correlation of r = 0,7476 (see figure 2). The correlation was statistically highly significant. Only 12 of the 234 samples had data points which were outside the calculated interval of confidence (95 %). A similarly high correlation was found, when DNA contents and DON concentrations (HPLC) analysed in 100 naturally contaminated wheat samples were plotted against each other (data not shown).

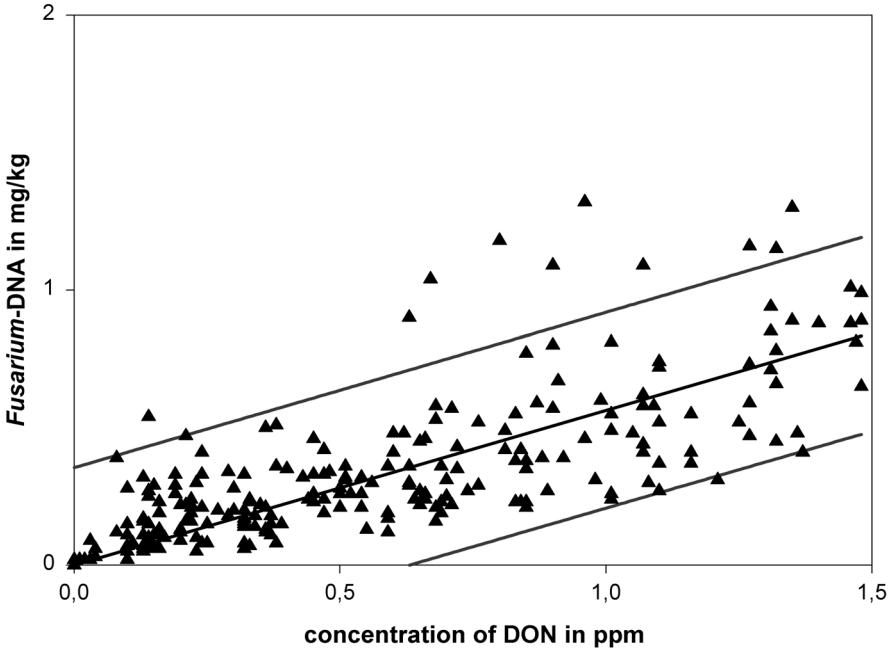

Figure 2: Correlation between concentrations of trichothecene producer DNA and DON concentration in samples of wheat infected with *F. culmorum.* (n = 234; r = 0.7476). Brighter lines indicate 95 % interval of confidence. Redrawn from Schnerr et al., 2002a.

Using the PCR assay described, the minimum detectable quantity of template DNA in sample material was 16 µg/kg corresponding to 290 haploid genomes (Xu *et al.* 1995). The quantitative PCR application analyses the potential for production of trichothecenes in *Fusarium* contaminated cereals. In the current study it was used to analyse relations between this potential, measured as ppm of trichothecene-producer DNA, and the concentrations of DON actually found in a corresponding sample. Data obtained demonstrate that a positive, linear correlation exists between both parameters in the grain samples analysed. This correlation was shown to be statistically highly significant. It was clearly demonstrated that specific diagnosis and quantification of quality relevant *Fusarium spp.* are possible in only a small portion of time and expenditure of labour compared to both microbiological methods and to chemical analysis of mycotoxins. The correlation between DNA and DON concentrations was shown in field inoculated sample material. According to Edwards *et al.* (2001), relationship found here might be weaker in naturally infected samples, because various strains of the different *Fusarium spp.* may be present with different capabilities for production of trichothecenes. This statement was contrasted by results obtained from the analysis of 100 samples of

naturally contaminated wheat in the authors lab, where also a coefficient of correlation exceeding 0.9 was found between DNA concentrations and DON as analysed by GC/MS.

Results reported here clearly show that analysis of samples for the presence of contaminants potentially producing trichothecenes by quantitative PCR may provide a powerful tool for future quality control in cereals. Furthermore, correlation of biomass with other parameters like fungicide treatment, irrigation, regional and climatic differences or even *Fusarium* resistance of cereals might be worthwhile to study with the system developed.

SPR based biosensor for the determination of DON in cereals **-** As an alternative to established analytical procedures for the key mycotoxin DON, a BIAcore-based indirect inhibition immunoassay was developed for the rapid quantification of this mycotoxin in sample material. A calibration curve ranging between 0.07 and 50 ng/µl injection volume (i.v.) of pure DON was used to quantify the toxin in wheat samples (figure 3). The detection limit of the assay for DON was 2.5 pg μl^{-1} i.v., corresponding to 7.5 ppb DON in a contaminated wheat sample. The working range of detection (lower limit + 3x standard deviation) lay between 0.13 and 10.0 ng μl^{-1} i.v. corresponding to 390 and 3000 µg kg^{-1} DON in sample material. For this range of contaminations a R^2 = 0.992 was calculated for the correlation between DON concentration and response signal. The 50 % inhibitive concentration of this assay was calculated to be IC_{50} = 0.72 ng μl^{-1} i.v.

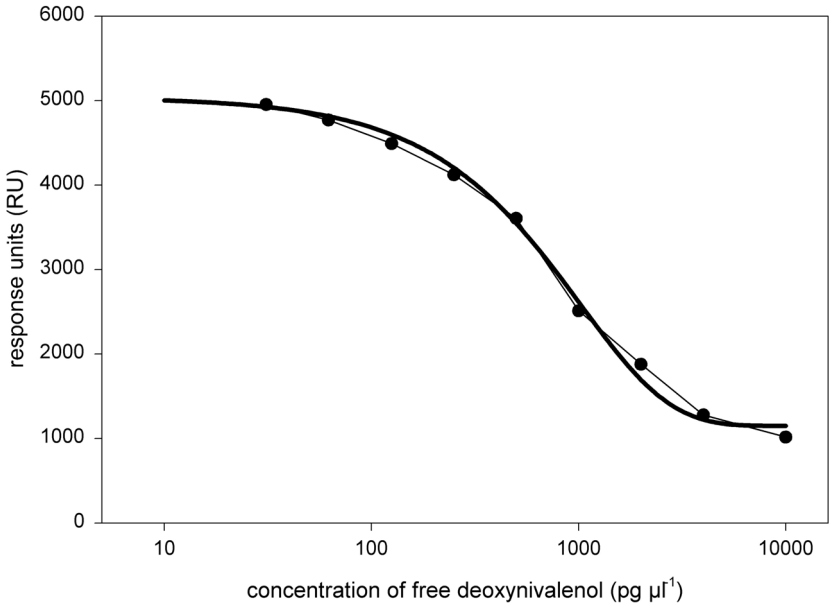

Figure 3: Calibration curve of the inhibition immunoassay for DON. A constant concentration of anti-DON antibodies was mixed with a dilution series of the toxin in binding buffer. The concentration of unreacted antibody was quantified using the BIAcore X SPR device. Redrawn from Schnerr et al., 2002b

Average recovery of DON in wheat samples spiked at levels of 50, 100, 500 µg/kg was 104 ± 15 % with the extraction and cleanup procedure described in materials and methods. Analysis time was 15 min per sample including extraction and quantification. Correlation of DON concentrations analysed with the biochip and with GC/MS (n = 15) or HPLC (n = 50) data showed coefficients of correlation of R^2 = 0.9464 (GC/MS) and R^2 = 0.9066 (HPLC), respectively.

Only few immunoassays have been described using a surface plasmon resonance biosensor for the detection of mycotoxins, such as aflatoxin, ochratoxin A, fumonisin B_1 and zearalenone (van der Gaag *et al.*, 1998, Mullett *et al.*, 1998, Daly *et al.*, 2000). In the present study, simple and rapid sample preparation was combined with real-time measurement applying the optical phenomenon of surface plasmone resonance in a BIA application. For the grain-processing industry the BIAcore assay developed may provide high-speed measurement of DON concentrations for screening of large sample numbers. The significant advantage of this technique is that a biosensor can be used as a rapid, on-line detection system without any further reactions to detect the binding event. In addition, a sensor surface may withstand up to 100 cycles, leading to considerable decrease of costs per analysis. This permits analysis of high sample numbers in the food and feed industry for improved quality control and as a tool for realisation of HACCP concepts. Assays of the BIAcore type are rapid and easy to use even by low trained personnel. It can therefore be anticipated that assays like the one described here might become increasingly important in the cereal industry in the future.

***Test strip for detection of* F. graminearum** – In order to compare sensitivity of the Test Strip™ method with conventional methods of product detection, e.g. ethidium bromide staining, PCR product analyses was performed by gel electrophoresis and the new method in parallel experiments. Several dilutions of purified *F. graminearum* DSM 4527 DNA were amplified by PCR. One aliquot of the reaction mixture was applied to agarose gel electrophoresis following PCR (figure 4B). A second aliquot of the same reaction was used for product detection with the DNA Detection Test Strips™ (figure 4B). The limit of detection was found to be $2.6*10^{-4}$ µg template DNA/reaction with the Test Strip™ system. Comparing results in the agarose gel, a clearly visible signal was still present at a concentration of $6{,}50*10^{-5}$ µg template DNA/ reaction showing a slightly higher sensitivity of product detection in agarose gels.

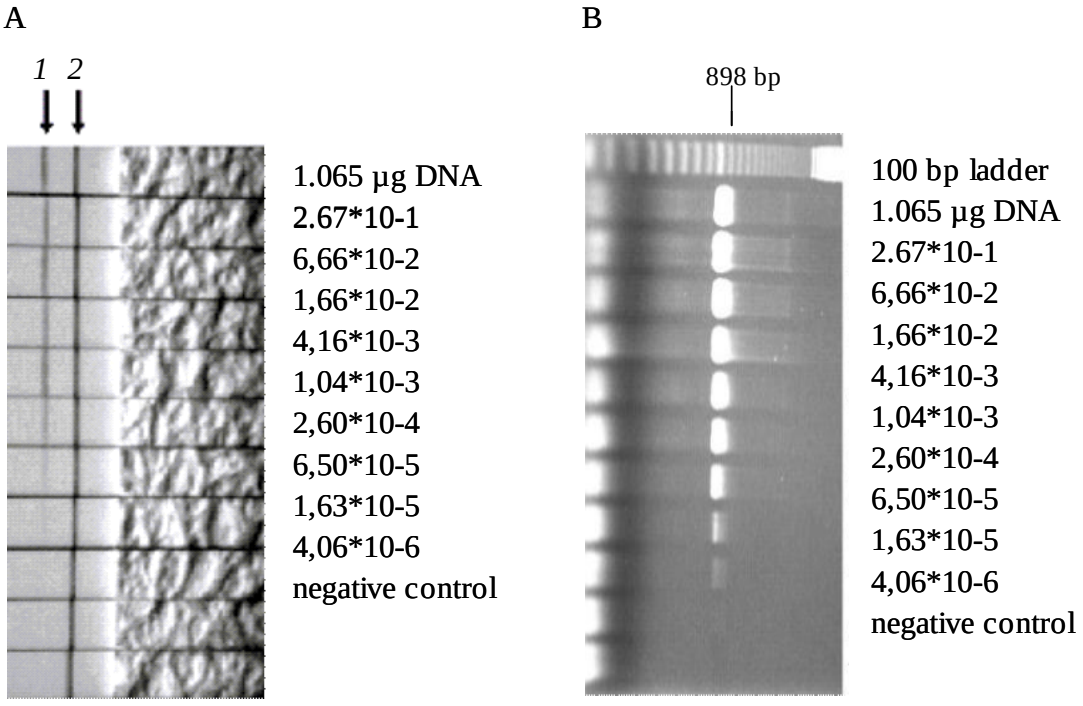

Figure 4: Comparison of two detection methods for PCR products. A serial dilution of DNA from *F. graminearum* DSM 4527 was used as template. Sterile distilled water was used as negative control. **A**: DNA Detection Test Strips™ 1: Signal from PCR product (streptavidin line) 2: Signal from internal control (immuno-gold labelled anti-mouse antibody). **B**: Agarose gel with PCR products stained with ethidium bromide. Redrawn from Knoll et al., 2002.

DNA was extracted from cereal samples contaminated with *F .graminearum* and amplified in a PCR as described under materials and methods. Product was detected using DNA Detection Test Strips™ and results were compared to those obtained with detection in agarose gel electrophoresis with ethidium bromid staining (figure 5). Samples with no *F. graminearum* contamination showed no signal neither with the Test Strips™ nor on agarose gels. In the group of samples with 1-30 % infected grains (microbiological analysis of surface disinfected grains on SNA medium, Nirenberg 1981) the number of samples which were positive in PCR was 10 % higher with detection in agarose gels as compared to detection using the Test Strips™. In the group of samples with more than 40 % of contaminated grains the percentage of PCR positive results in agarose gels and with the Test Strips™ were found to be identical.

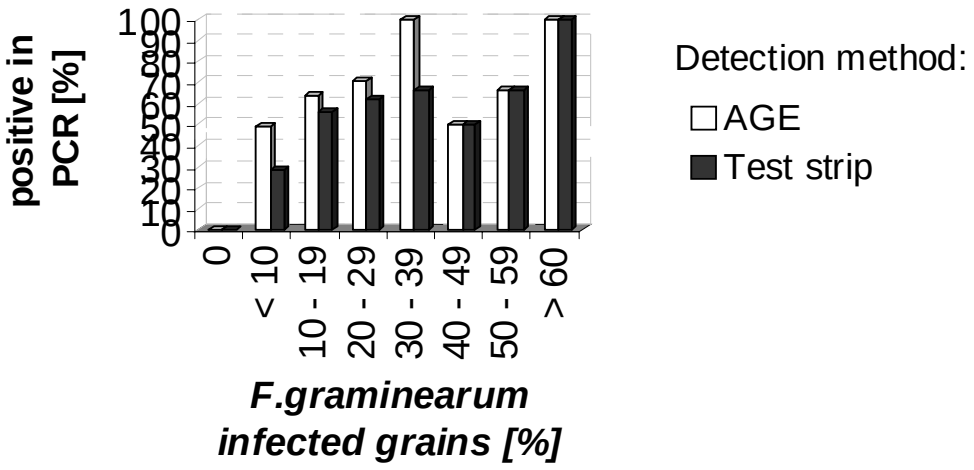

Figure 5: Comparison of detection of *F. graminearum* from cereal samples with agarose gel electrophoresis and DNA Detection Test Strips™. Samples were grouped according to their percentage of grains infected with *F. graminearum*. AGE = agarose gel electrophoresis. Redrawn from Knoll et al., 2002b.

Currently, detection of amplification products in conventional PCR is most frequently performed by staining agarose electrophoresis gels with fluorescent dyes such as ethidium bromide or SYBR Green I, which fluoresce upon intercalation into the DNA double strand. Besides the need for appropriate electrophoresis equipment, analyses are labour intensive and time consuming and mutagenicity of DNA dyes pose a hazard to human health. In contrast, DNA Detection Test Strips™ need no instrumentation, handling is easy and no hazardous reagents are used. Results are obtained within 20 minutes, compared to at least 70 minutes if agarose gel electrophoresis is applied. Detection and verification are achieved with one experiment because the specifically amplified PCR product is detected by a product specific probe. The method described here facilitates detection of *F. graminearum* in routine applications and in screening studies.

Conclusions - Results presented in the current study point to the fact that the modern analytical tools described can act as useful tools in the screening of large numbers of samples in the agro-food industry. Real-time PCR methods like the one described indicate the potential of a sample for the presence of trichothecene mycotoxins within short time omitting time consuming pre-incubation steps and mycological analysis. Antibody based bio-sensorial methods can quantify key mycotoxin like DON within 15 min or

even less in cereals but also in processed samples. Finally, use of DNA Detection Test Strips™ may contribute significantly to a broader acceptance of PCR as a powerful tool in the quality control of the food and feed industry.

ACKNOWLEDGEMENTS

This study was supported by the German Ministry of Economics, Berlin (Arbeitsgemeinschaft industrieller Forschungseinrichtungen „Otto von Guericke“ e.V.), grant AiF 12163/N, and Wissenschaftsförderung der Deutschen Brauwirtschaft, Bonn. We are grateful to Claudine Seeliger and Sabine Faulhammer for technical assistance.

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HUMAN CYTOKINE MRNA RESPONSE TO DEOXYNIVALENOL (VOMITOXIN) USING WHOLE BLOOD CULTURES

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ABSTRACT

Deoxynivalenol (DON or vomitoxin),a tricothecene mycotoxin, is a naturally occurring contaminant frequently found in grain-based foods. This toxin has adverse effects on human and animal health, causing a multisystemic shock-like syndrome including dermal irritation, nausea, vomiting, diarrhea, hemorrhage, leukopenia and anemia. A critical step in DON toxicity is action on leukocytes by activation of cytokines. Previous studies have shown an upregulation of several cytokines, in the U937 cells, a cloned human macrophage model. To study the acute effects of DON on human cytokine production in peripheral mononuclear blood leukocytes, we have developed a culture approach using a 20% dilution of whole blood in RPMI-1640 media and a 6 hour exposure. Cultures were exposed to DON at concentrations of 0, 10, 50, 100, 250, and 500 ng/ml. RNA was then isolated and assayed using real-time PCR primer/probes, for both cytokines (IL-6, IL-8, and TNF-alpha) and 18S rRNA. Cytokine levels were normalized using 18S rRNA levels and relative expression levels determined. IL-6 was significantly induced by DON at 250 ng/ml (~9.5 fold) and 500 ng/ml (~14 fold). IL-8 was significantly induced by DON at 250 ng/ml (~8.5 fold) and 500 ng/ml (~3 fold). TNF-alpha was significantly induced by DON at 10 ng/ml (~1 fold), 250 ng/ml (~1 fold), and 500 ng/ml (~4 fold). Taken together, the capacity of DON to induce IL-8, IL-6, and TNF-alpha gene expression and the threshold doses to achieve these effects were consistent with previous findings in cloned human and mouse macrophage cultures. Interestingly, a high degree of variability was observed in blood cultures from different donors, thus raising the possibility that some individuals may have greater sensitivity to DON than others. Further research is being undertaken to clarify this possibility. (This abstract was presented at the Society of Toxicology Annual Meeting, Salt Lake City, UT, March 10-13, 2003)

DETECTION OF DEOXYNIVALENOL IN BLOOD AND TISSUE BY ELISA

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ABSTRACT

Deoxynivalenol (DON or vomitoxin) is a trichothecene mycotoxin commonly found in cereal grains that adversely affects the gastrointestinal and immune function. In assessing risk of this toxin to humans, it is important to be able to monitor its absorption, disposition and clearance in tissues of exposed animals and compare this to in vitro studies with human and animal. Here we developed approaches to analyze for DON in tissue by immunochemical assay and used these to follow the kinetics of distribution and clearance of this mycotoxin in the mouse. Spiking studies revealed that DON in plasma could be analyzed directly using a competitive direct enzyme-linked immunosorbent assay (ELISA) providing that standard curves were prepared in human plasma as diluent. For analysis of organs, tissues were homogenized in phosphate buffered saline (1:10 ratio). Resultant extracts were then heated in boiling water for 5 min, centrifuged, and supernatants analyzed by ELISA. Tissue disposition and clearance of DON were measured in B6C3F1 male mice (8 wk-old) that were orally administered 25 mg/kg BW of the toxin. Blood was collected from retro orbital plexus and organs removed after 0.08 (5 min), 0.25 (15 min), 0.5 (30 min), 1, 2, 4, 8 and 24 hr intervals. Maximal DON was detected at 5 and 15 min in all tissues tested with a rapid clearance over a 24 hr period. At 5 min, DON concentrations in ng/g of tissue were 19552 ± 1910 in liver, 12140 ± 461 in plasma, 7568 ± 515 in kidney, 7294 ± 839 in spleen, 6755 ± 854 in heart, 5486 ± 175 in thymus and 723 ± 79.4 in the brain. DON concentrations were significantly higher in all the organs tested from 5 min to 8 hr compared to untreated mice. At 24 hr, DON concentrations were also significantly higher except in heart and kidney. Taken together, the results showed that, in the mouse, DON rapidly distributed in all organs within a short time after exposure according to the rank order liver> plasma> kidney> spleen> heart> thymus> brain. The approach presented here will enable predictive studies on human toxicity by correlating in vivo animal studies to ongoing in vitro animal and human investigations.

FIRST REPORT OF TRICHOTECENES PRESENCE IN COMMERCIAL BARLEY GRAINS IN THE HIGHLANDS OF CENTRAL MEXICO

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OBJECTIVES

The present study was carried out to detect and quantify trichothecene levels in commercial barley grain in the Highlands of Central Mexico.

INTRODUCTION

In México, malting barley (*Hordeum vulgare* L.) is grown on approximately 301,000 hectares under both irrigated and non-irrigated conditions. The main malting barley producing area of México is the Central Highlands (encompassing the states of Hidalgo, Tlaxcala, México, and Puebla) (6). Within this region, the area between the valleys and hills of Hidalgo (Apan and Cd. Sahagún) and Tlaxcala (Calpulalpan, Tlaxco, Españita, and Benito Juárez) accounts for nearly 50% of México's total barley area.

Since 1998, Fusarium Head Blight of cereals (*Fusarium spp.*) has increased, affecting barley (*Hordeum vulgare* L.) in the Highlands of Mexico. This region includes the states of Mexico, Tlaxcala, Puebla and Hidalgo (2,3) where the disease causes losses in both yield and grain quality (3).

MATERIALS AND METHODS

At harvest in the 2001 and 2002 growing seasons, samples (1.5 kg) of grain produced by the growers in the Central Highlands of México were taken. Sampling was carried out in warehouses in the Calpulalpan and Apan areas. Samples were taken directly from grain shipments from each area; the objective was to obtain a significant number of samples representative of the Highlands.

Samples were analyzed in CIMMYT's Toxins Laboratory in El Batán, México. The grain samples were ground (Braun commercial mill) and processed using the technique of Romer Laboratories, Inc. (Don FluoroQuant™ method #FQD1NC, version 95.9).

RESULTS

For the 2001 growing season, 87.1% of samples tested positive for trichothecenes (0.05-2.10 ppm), whereas in the 2002 season, 67.7% of samples were positive (0.03-7.10 ppm). In general, toxins were found in 96.8% of the grain from all sampling sites (Table 1) in one of the two years, which indicates that pathogens are present throughout the sampled area.

Results of this study show that 67.7% of the samples from the 2001 growing season and 32.3% of those from the 2002 growing season have a toxin content above 0.5 ppm, the maximum tolerated level used in this study. This finding suggests that most commercial barley grain in México could be contaminated in the future a condition that might be dangerous for the known harmful effect of toxin in human health (4).

To control toxin contamination, the European Community recommends a maximum tolerance level of 0.5 ppm in cereal grains for direct human consumption and 0.75 ppm in flour used as a raw material in food products. Germany restricts the levels to 0.35 ppm in bread and pasta, and 0.10 ppm in edible cereals for children and babies (Notification 2002/138/D).

In countries such as Russia, the USA, and China, the maximum level tolerated in wheat for human consumption is 1.0 ppm (1). Furthermore, the FAO-Codex Alimentarius Commission has proposed a maximum of 0.5 ppm for all cereal-derived products for human consumption, except those for babies, which should contain no more than 0.1 ppm (1). In this study the tolerance level applied was 0.5 ppm, the same recommendation followed by Anheuser-Bush, Inc., the most important beer manufacturer in the USA (5).

CONCLUSIONS

Toxins were found in 96.8% of the sampling sites in one of the two test years. About 68% of the samples from the 2001 growing season and 32% of those from the 2002 growing season have a toxin content above 0.5 ppm.

Failure to take measures to control and manage the disease could result in economic losses due to grain yield reductions and the presence of toxins in the grains. This might also have consequences for barley producers and the brewing industry in the Central Highlands of México.

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Table 1. Detection and quantification of trichothecenes (deoxynivalenol + nivalenol) on the commercial barley variety Esmeralda in the Central Highlands of México (Tlaxcala-Hidalgo), 2001-2002.

	Trichothecene concentration (ppm)	
Sampling site	2001	2002
Almoloya	2.10	2.50
Apan	0.90	0.00
Calpulalpan	1.10	0.00
Chimalpa y Tlalayote	0.10	0.00
Coatlaco	0.83	0.00
Emiliano Zapata	0.05	0.00
La Estancia	2.00	1.00
La Laguna	0.52	0.44
La Soledad	1.70	0.52
La Unión	1.10	0.39
Lagunillas	0.40	0.31
Lázaro Cárdenas	1.70	0.11
Lomorriel	0.80	0.69
Matamoros	1.30	1.00
Ocotepec	0.00	0.03
Paredón	2.10	0.28
Rancho Nuevo	1.50	0.00
San Andrés Buenavista	2.00	1.80
San Diego	1.40	0.00
San Felipe Sultepec	0.88	0.00
San José Jiquilpan	0.00	0.46
San Juan Ixtilmaco	1.80	0.14
San Mateo Activan	0.17	0.18
Santa Bárbara	0.00	0.00
Santa Clara	0.48	0.82
Santiago Tletlapayac	0.00	0.13
Santiaguito	0.33	0.00
Teapan	1.40	0.68
Tepetlayuca	1.10	0.49
Tierra y Libertad	0.40	0.97
Zotoluca	2.10	7.10
Ranges	0.05-2.10	0.03-7.10
Sites where toxins found (%)	87.10	67.7

COMPARATIVE GENETIC ANALYSIS OF FHB-RESISTANT GERMPLASM FOR WHEAT IMPROVEMENT

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OBJECTIVES

To examine the difference of genetic constitution for resistance among FHB-resistant wheat germplasms by using the molecular markers.

INTRODUCTION

Previous studies indicate that resistance to FHB varies not only among wheat cultivars but also among some of their wild relatives. No accession, however, has yet been identified to be completely immune to FHB among the Gramineae. It was also demonstrated that resistant wheat germplasm could be divided into three gene pools: winter wheats from Eastern Europe, spring wheats from China and Japan, and spring wheats from Brazil and Italy (Snijders, 1990). Repeated screening of the genetic resources led to the identification of several resistant cultivars of spring wheat, such as Sumai 3 and Ning 7840 from the Chinese gene pool in the early 1990's. On another front, Shinchunaga, Nobeokabouzu-komugi and Nyubai were also identified as resistant cultivars to FHB from the Japanese gene pool (Nishikado, 1958; Gocho, 1985; Feadak *et al.*, 1997). Among them, Nobeokabouzu-komugi is highly resistant to FHB (Gocho, 1985; Miedaner, 1997). The genetic constitutions of sources for resistance to FHB originating from different gene pools, however, have not yet been elucidated. It is essential to study the genetics of the resistance to FHB, including the identification of the genes for different types of the resistance to FHB in several gene pools, so that different genes can be combined to improve the overall resistance of wheat. The objectives of our studies were to examine the difference of genetic constitution of resistance to FHB among the resistant wheat germplasms.

MATERIALS AND METHODS

Comparative QTL analysis for different types of resistance - This study was conducted to identify the number, the position, and the magnitude of QTLs for the resistance to initial infection of FHB (Type I) and fungal spread within plant tissues (Type II), and tolerance to Fusarium mycotoxins in wheat with DNA markers. Two populations of double haploid lines (DHLs) derived from the F_1 crosses of Sumai 3 (VR)/ Gamenya (VS) and Nobeokabouzu-komugi (VR)/ Sumai 3 were evaluated on the genetic constitution for the component of FHB resistance.

Screening of chromosome regions associated with FHB resistance in Sumai 3 with its susceptible NILs by using SSR and AFLP markers - The plant materials used in this study were Sumai 3 and its four susceptible NILs. The NILs were derived from a cross between Sumai 3 and Chuan980, a susceptible cultivar, followed by seven backcrosses with Sumai 3 and screening for FHB susceptibility in each generation by artificial inoculation with F. graminearum. SSR and AFLP analyses were applied to screen the DNA polymorphism between Sumai 3 and its four NILs.

Genetic variation of accessions within FHB resistance wheat cultivars revealed by SSR markers - We also revealed the genetic variation within accessions of Frontana (Brazil, USA, Canada and Japan) and Sumai 3 (China, USA, Canada, Iran, Austria and Japan) that had been sent to CIMMYT by using 242 SSR markers that encompass the whole of the wheat genome.

RESULTS AND DISCUSSION

Comparative QTL analysis for different types of resistance – Three and two genomic regions were significantly associated with Type I and Type II resistance, respectively. One of the QTLs on chromosome 2DS showed negative effect on both Type I and Type II resistance, which meaning Sumai 3 not only contain resistance genes but also have susceptible gene. The results suggested that the genetic constitutions for Type I and II resistance are not identical. We also examined the difference of the genetic constitution for resistance to FHB between Sumai 3 and Nobeokabouzu-komugi to find unique resistance genes in Japanese germplasm. From the cross combination of both highly resistant cultivars, transgressive segregants with the reaction of moderately resistant to FHB were detected. It is suggested that the unique configurations of resistance genes are causing the skewed distribution of the resistance in this cross. The genetic mode of the resistance indicated that three resistance genes to FHB were different between Nobeoabouzu-komugi and Sumai 3. Consequently, it is suggested that Nobeokabouzu-komugi harbours three dominant genes for the resistance, of which two are unique and another gene is identical with the one of Sumai 3 (Ban and Inagaki, 2001).

Screening of chromosome regions associated with FHB resistance in Sumai 3 with its susceptible NILs by using SSR and AFLP markers – The detected polymorphic markers were mapped using a mapping population of 118 DHLs of Sumai 3 and Gamenya. Eighty-eight SSRs markers and 107 AFLP primer combinations that produced approximately 900 AFLP markers were analyzed. Of these markers, nine (four SSR and five AFLP markers) showed polymorphism between Sumai 3 and its four NILs. The band patterns of these markers were not identical for the four NILs, indicating that the genetic constitutions of the four NILs were different with regard to susceptibility to FHB. Seven of the nine polymorphic markers were mapped on a region of chromosome 3BS where the resistance QTLs have been consistently detected in the populations including Sumai 3 or its derivatives. The remaining two markers were located on chromosome 2AL or 2DL. By using the NILs for FHB resistance, it is revealed that a critical region for Type II resistance to FBH located on chromosome 3BS in Sumai 3, and Sumai 3 may have other genes that affect the FHB resistance. The success for development of the susceptible NILs to FHB and the results obtained in this study demonstrated that some quantitative traits could be genetically analyzed like qualitative traits.

Genetic variation of accessions within FHB resistance wheat cultivars revealed by SSR markers - In the case of Sumai 3, there was no difference in band pattern in any SSR markers examined among the Chinese (CHN), US (USA), Canadian (CAN) and Iranian (IRN) accessions; they were an identical genotype. Sumai 3-AUT showed polymorphism for 32 markers (13.2%) on 11 chromosomes. Some of them were linked on chromosomal regions. It is suggested that Sumai 3-AUT also derived from an original Chinese accession following outcross and selection. All Sumai 3 accessions had a high level resistance to FHB, while Sumai 3 AUT had higher levels of resistance. We can conclude that additional resistance genes to FHB should exist on the chromosome regions identified by aberrant types of SSR markers in Sumai 3-AUT. It was conclude that we need to pay attention to the source and genotype of such accessions when discussing the results of QTL analysis and using them in breeding programs with marker assisted selection. (see more detail in our poster presented in 2003 NFHBF)

Inheritance of resistance to FHB in wheat has been studied extensively over the last five decades. Various studies have determined the mode of inheritance and numbers of genes involved in resistance depending on the materials and methods used. However, lacking of knowledge about the genetic constitutions of different sources for resistance to FHB has hampered significant progress in the breeding of FHB-resistant wheat cultivars for a long time. Different types of genetic resistance to FHB in common wheat have been described. We can find many reports of QTL analyses for Type II resistance derived from Chinese germplasm (mainly Sumai 3). Further studies on the genetic basis of relationship between the characteristics of FHB and resistance mechanisms in wheat should be required to develop FHB-resistant wheat. We are tracing the genetic constitution for different types of resistance to FHB in Japanese wheat breeding systems combining the information of DNA markers and pedigree analysis (Fig. 1). The information obtained in these studies might provide a better understanding of the genetic resistance to FHB, and thereby enhance the resistance levels in wheat through introgression or pyramiding of several resistance genes.

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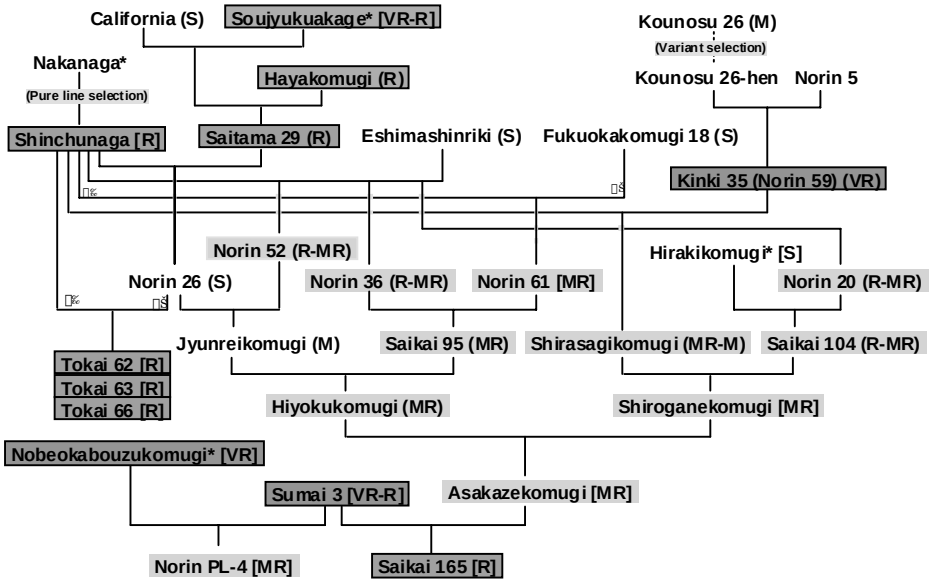

Fig. 1. Pedigrees of FHB-resistance cultivars in improved Japanease wheat

Each resistance level to FHB examined is following with parentheses, and previously reported ones (Yoshida *et al.* 1993) are with bracket. Cultivars in meshed boxes are selected as FHB-resistance cultivars. * Local variety.

GENETIC VARIATION OF ACCESSIONS WITHIN FUSARIUM HEAD BLIGHT RESISTANCE WHEAT CULTIVARS REVEALED BY SSR MARKERS

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ABSTRACT

Fusarium head blight (FHB) is one of the most destructive diseases of wheat. Repeated screening of genetic resources has led to the identification of several resistant cultivars of spring wheat, such as Frontana from Brazil, Sumai 3 from China and Nobeokabouzu-komugi from Japan. It is known, however, that several morphological and ecological variations with different responses to FHB exist within global accessions of Frontana and Sumai 3 (Nishio et al. 2002), and different sources of Sumai 3 have been identified by DNA markers (Bai et al. 2003). In this study, we revealed the genetic variation within accessions of Frontana (Brazil, USA, Canada and Japan) and Sumai 3 (China, USA, Canada, Iran, Austria and Japan) that had been sent to CIMMYT by using 242 SSR markers that encompass the whole wheat genome. Frontana-USA and -CAN were identical with, or variants of, the original Brazilian accession. In the case of Sumai 3, the US, Canadian and Iranian accessions were the same genotype as the Chinese one. Sumai 3-JIR might be a derivative from them. The Austrian accession was considered a derivative of an original Chinese accession following outcrossing and selection with additional resistance genes for FHB. The results of genetic variation within the accessions of Frontana and Sumai 3 reveal that we need to pay attention to the source and genotype of such accessions when discussing the results of QTL analysis and using them in breeding programs with marker assisted selection. (This poster was presented at the 10th International Wheat Genetic Symposium, Paestum, Italy, 1-6 September 2003.)

IMPORTANCE OF FUSARIUM HEAD BLIGHT IN RUSSIA AND THE SEARCH FOR NEW SOURCES OF GENETIC RESISTANCE IN WHEAT AND BARLEY

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Russia is large country occupying over 17 million km^2 and very diverse climatic conditions. Cereal crops are cultivated in the European (North Caucasus, the Central and Volga regions, North-Western region) and Asian regions (Siberia and the Far East) of the country. Wheat and barley are significant components of the agricultural economy of Russia. Winter and spring wheat are grown on over 23-25 million hectares with an average annual production of 30.9-50.6 million tons (average for 1999-2002). Winter and spring barley are grown on 9.8-10.3 million hectares with an average yield of 10.9-19.5 million tons.

The first mention of Fusarium head blight (FHB) within the territory of Russia was from the Far East. The Far East region of cereal production is typically very damp and warm during the summer due to the influences of Sea of Japan and Pacific Ocean. As early as the 18th century, FHB was known as a problem of cereals in the Far East (Palchevsky, 1891; Voronin, 1890). From 1882 until about 1914, FHB epidemics occurred almost every year in the region. The use of seeds and straw contaminated with mycotoxins produced by various *Fusarium* species caused numerous cases of food poisoning in people and animals. Initially, the symptoms observed in people ingesting this tainted grain resembled alcohol intoxication and was often referred to as "intoxicating bread" syndrome. The research of several Russian mycologists revealed that the fungus *Gibberella saubinetii* Sacc. (now *G. zeae* or *F. graminearum)* was the principal causal organism of FHB (Jaczewski, 1904; Naumov, 1913, 1916). FHB was a persistent problem in the Far East during the first half of the 20th century (Abramov, 1938; Naumov, 1940) and continues to be today. High severities of FHB are reported nearly every year in the region. Mycological analyses of seed samples from 1998-2002 have shown a high level of FHB-infected wheat and barley seed (from 23-32%). The most frequently isolated pathogens were *F. graminearum, F. avenaceum,* and *F. poae.*

Another major FHB outbreak within the territory of Russia occurred in the Altai region and Bashkirij (south of Siberia) in 1932-1945. Food shortages during this time forced rural people to consume inferior grain that was left out in the field under snow during the winter. After ingesting this grain, many people suffered the serious disease of alimentary toxic aleukia. Extensive research led by A. Sarkisov revealed that the disease was due to the ingestion of cereals contaminated with T-2 toxin produced by fungus *F. sporotrichioides* (Sarkisov, 1954). In recent years, the level of Fusarium infected grains has been relatively low (0-10%). The principal pathogens found on grain in this region are *F. poae* and *F. sporotrichioides* (Levitin et al., 2000).

Nearly one hundred years after the first major epidemic was reported in the Russian Far East, FHB became one of the most important fungal diseases in the North Caucasus region. There, FHB epidemics occurred in 1985, 1987, 1988, 1992, 1993, 1997, 1998, 2000, and 2002. In the North Caucasus, maize and winter wheat and winter barley are the main crops and are often cultivated in continuous rotation with each other. Favorable weather conditions, coupled intensive agricultural practices have led to a dramatic increase in

FHB severity. Since cereals in the North Caucasus comprise nearly one third of the total cereal production in Russia, the severity of FHB epidemics was of great concern and research efforts to combat the disease were initiated. *F. graminearum (G. zeae*) is the most important FHB pathogen in this region and was responsible for widespread mycotoxin contamination problems. For example, in 1992, analysis of wheat grain from the Krasnodar district showed that DON was present in 100% of the examined samples with concentrations ranging from 0.15-10.5 ppm. In 57% of samples, the level of DON exceeded the permissible level for human consumption (1 ppm). Zearalenone was observed in 68% of the samples in concentrations ranging from 0.01-1.4 ppm (Lvova et al., 1997).

The NorthWest region (near Baltik Sea) is characterized by a damp climate and moderate summer daytime temperatures. In past years, FHB was reported on cereal crops in the region, but was never considered a significant problem (Naumov, 1940). A study of seed infection from recent years revealed significant levels of FHB. In 2000, FHB seed infection averaged 16% (maxiumum 23%) and 22% (maxiumum 24%) for spring barley and spring wheat, respectively. In 2002, the average FHB infection level for both barley and wheat seed was about 6% with a maximum of 15%. The 2003 season was very wet and all harvested grain was infected by FHB. The average level of grain infection by *Fusarium* species was 16% for barley (maximum 32%) and 12% for wheat (maximum 20%). The dominant species isolated from seed were *F. poae, F. sporotrichioides, F. avenaceum.* In this area, symptomatic kernels are rarely observed, except in warm and moist seasons like in 2003. More often asymptomatic kernels are found to carry infections of various *Fusarium* species. Analyses of trichothecene toxins conducted with Finnish colleagues in 2002 revealed principal toxin in this region is nivalenol (0,2-3.7ppm), most likely produced by *F. poae* (Yli-Mattila et al. 2002).

Disease resistance is one of the best means of combating FHB in wheat and barley. In the past, the breeding programs for cereals in the Central, North-West regions, and Siberia never focused on FHB resistance. This led to the release of many susceptible cultivars, which were planted over a large area. In Russia, the most active breeding program focusing on FHB resistance is at the Krasnodar Research Institute of Agriculture (North-Caucasus). This is an area of winter cereal production and recently released winter wheat cultivars such as Krsnodarskaya 6, Yuna, Delta, Demetra, Kolos, Leda, Rufa, Eho, Basianka appear to have tolerance to toxin accumulation (Anpilogova et al., 1996; Ablova, Gritcai, 2001; Kolesnikov et al., 2001; Ribalkin et al., 2000). To breed for FHB resistance, breeders are making crosses among ecogeographically diverse lines and making early generation selections of individuals. Successive crosses are complex involving highly resistant in Krasnodar region Nung Ta 173 (China), Lee (USA), Frontana (Brazil), WSP96.6, Livius (Austria), Kincso, Ringo Sztar (Hungary) and local varieties (Ribalkin et al., 2000).

To broaden resistance to FHB, efforts are being made to identify new resistance sources. The N. I. Vavilov Institute of Plant Industry (VIR) in Russia houses one of the largest and most diverse collections of cereal genetic resources in the world. The wheat collection contains more than 44,000 accessions. Approximately two-thirds of the collected accessions (27,832) include bread wheat germplasm originating from 85 countries. Wild and primitive wheat accessions number 2,867 and goat grass (*Aegilops* L.) accessions number 3,847. A considerable part of the wheat collection was obtained from 1907 to 1940 and are mostly landraces or old varieties. The barley collection comprises 20,197 accessions of cultivated barley: 39% are landraces, 46% are cultivars, 8% are breeding lines, 6% are mutants and genetic stocks and 1% is wild species. In 1947, the number of accessions in the collection amounted to 9000, representing 38 countries besides Western Europe (Kovaleva, 1999; Mitrofanova, 2003; Terenteva, 2001). Estimation of the degree of biodiversity for disease resistance in the genetic resources of cultivated plants and their wild relatives is being documented in joint projects between VIR and VIZR.

Evaluation of wheat and barley accessions from VIR collection identified a group of landraces and old local cultivars of wheat and barley with relatively high levels of FHB resistance (Gagkaeva et al., 2002). All these samples were collected from 1915-1936 in the Far East territory where environmental conditions are favorable for FHB infection.

Two hundred fifty-two accessions comprising 26 species of *Triticum* L. with different ploidy levels were evaluated for FHB resistance in the field. No correlation was found between ploidy level and FHB resistance. However, some feature of plants may be associated with resistance. For example *T. durum, T. aethiopicum,* and *T. turanicum* have a high frequency of florets that undergo open flowering and are susceptible to FHB. Likewise, *T. urartu* has a prolonged flowering period and is susceptible to FHB. *T. timopheevii, T. persicum, T. ispahanicum,* and *T. karamyschevii* originated from regions with high moisture and were generally resistance to FHB. *T. vavilovii, T.turanicum, T. dicoccoides, T. sphaerococcum* originated from dry regions of Middle Asia and were highly susceptible to FHB. The most resistant wild accessions were *T. timopheevii, T. karamyschevii,* and *T .militinae* (from Georgia), *T. persicum* (from Dagestan), *T. dicoccum* (from Germany), *T. spelta* (from Switzerland). A wide diversity for resistance was detected in *T. aestivum* accessions (Gagkaeva et al., 1993)

Aegilops species are of particular interest because they carry genes for resistance to many fungal pathogens that may be introgressed into common wheat. *Aegilops* accessions belonging to 9 different species and several ploidy levels (*Ae.tauschii, Ae.triuncialis, Ae.cylindrica, Ae.juvenalis, Ae.vavilovii, Ae.ovata, Ae.crassa, Ae.kotchui, Ae.bicornis*) were evaluated for reaction to FHB. *Ae. tauschii* was most resistant to *F. graminearum*. Of 56 samples belonging to this species 19.6% were highly resistant. All tested samples of *Ae. triuncialis* and *Ae. ovata* were highly susceptible (Gagkaeva, Navruzbekov, 1991). The most of resistant *Ae. tauschii* germplasn originated from Afghanistan. One of them is used in crossing with wheat at the Crop Breeding Institute (Harbin, China) (Lianfa et al., 2000). The wild accessions (*T. militinae, Ae. squarosa, Ae. sharonensis, Ae. umbellulata, Ae. speltoides, Ae. glaucum, S. cereale*) are potential sources of resistance to the disease and toxin accumulation and are being utilized for breeding purposes at the Krasnodar Research Institute of Agriculture (Kolesnikov et al., 2001). The combining of different resistance genes from diverse sources will broaden the effectiveness of FHB resistance in released cultivars.

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HAPLOTYPE DIVERSITY AT FUSARIUM HEAD BLIGHT RESISTANCE QTLS IN WHEAT

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ABSTRACT

Fusarium head blight (FHB) reduces grain yield and quality in common and durum wheat. Host FHB resistance is an effective control measure that is achieved by stacking multiple FHB resistance genes. Resistance gene stacking would be facilitated if breeders knew which FHB resistance sources carry different resistance genes. A diverse collection of FHB resistant and susceptible wheat lines was characterized with microsatellite markers linked to known FHB resistance quantitative trait loci (QTLs) on chromosomes 2DL, 3BS (distal to the centromere), 3BSc (proximal to the centromere), 4B, 5AS, and 6BS identified in Maringa, Sumai 3, and Wuhan 1. Putative Sumai 3 QTLs were commonly observed in advanced breeding lines, whereas putative Maringa and Wuhan 1 QTLs were relatively rare. The microsatellite data suggested that the 3BS, 3BSc, and 5AS QTLs in the Brazilian cv. Maringa were derived not from Frontana, as previously thought. Maringa appeared to be closely related to Asian germplasm at the 3BS, 3BSc, and 5AS QTL regions. Other Brazilian wheat lines did not appear closely related to other FHB resistance sources. These Brazilian wheats may have novel FHB resistance that will be useful for stacking with FHB resistance derived from Asian germplasm.

MOLECULAR GENETIC DIVERSITY OF GEOGRAPHICALLY DIVERSE SCAB RESISTANT WHEAT LINES

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ABSTRACT

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), also known as scab, is an increasingly important problem in wheat and barley globally because of the emphasis on conservation tillage, the lack of effective cultural and/or fungicide control, and the lack of effective sources of genetic resistance. Yield losses in spring and soft red winter wheat regions of the US alone have exceeded $4 billion in the last decade. Host plant resistance is considered the most practical and effective means of control but breeding has been hindered by a lack of effective resistance genes and by the complexity of the resistance in identified sources. No source of complete resistance is known, and current sources provide only partial resistance, therefore, the identification of different sources of resistance and their incorporation into adapted wheat varieties is critical to the continued improvement of Fusarium head blight resistance in winter wheat. Research funded by the National Wheat and Barley Scab Initiative (USWBSI) has led to the systematic evaluation of scab resistance of accessions contained in the National Small Grains Collection at Aberdeen, Idaho, and, through a collaborative effort with CIMMYT, has led to the introduction of germplasm containing potentially different sources of resistance from scab programs globally. A group of 191 lines, with varying levels of resistance, have been assembled from globally diverse geographic regions. They likely contain genes for scab resistances that differ from those derived from the Chinese line "Sumai 3", which is currently, the most widely used source of resistance. The material includes lines from CIMMYT (38), Romania (7), China (17), Argentina (40), Brazil (12), Japan (12), Hungary (6), Italy (3), South Korea (1) and the United States (55). These lines have been evaluated with 10 different AFLP primer pair combinations resulting in over 50 bands per primer pair. Cluster analysis was performed using Phylip Version 3.6 to assess genetic diversity among the material and to identify genetically different sources of scab resistance that can be used for further studies. Relationships among germplasm will be discussed as will their potential for carrying novel genes for scab resistance.

IDENTIFICATION OF NOVEL SOURCES OF FUSARIUM HEAD BLIGHT RESISTANCE FROM WHEAT-ALIEN SPECIES DERIVATIVES

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ABSTRACT

Sources of resistance to Fusarium head blight (FHB) are limited in wheat. Identification of novel sources is vital for enhancing resistance of wheat to this disease. We have evaluated 284 wheat lines derived from crosses between wheat and its relatives for FHB resistance. Materials were evaluated in the greenhouse using the point inoculation method and the percent of infected spikelets was measured. Approximately 20 spikes were inoculated for each of the wheat lines. Of the 284 lines evaluated, 100 were resistant (<15% infected spikelets), 108 were moderately resistant (15-50% infected spikelets), and 76 were susceptible (>50% infected spikelets). Resistant lines include synthetic common wheat lines, wheat-alien species amphiploids, addition lines, and other wheat-alien species derivatives. Further evaluation is being conducted to confirm these results. Cytogenetic characterization is in progress to understand chromosome constitutions of wheat lines with high levels of resistance. Following an initial chromosome count, fluorescence *in situ* hybridization (FISH) is performed to determine the amount of alien chromatin present in each resistant line. FISH patterns of mitotic chromosomes indicate that 4 of the most resistant lines carry 14 *Thinopyrum ponticum* chromosomes and 42 wheat chromosomes. Additional crosses are being made to localize resistance genes, to minimize alien chromatin, and to pyramid FHB resistance genes.

REACTION OF *AEGILOPS SHARONENSIS* TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* is a serious disease of wheat in many production areas. The deployment of resistant cultivars is one of the best means for controlling this disease. To obtain broad-based resistance in wheat, it is important to combine into cultivars diverse alleles for FHB resistance. Wild relatives are rich sources of resistance alleles for wheat. The diploid wheat relative *Aegilops sharonensis* (2n=2x=14, genome S^1S^1) is native to the coastal areas of Israel and Lebanon and is known to be a rich source of resistance to diseases such as leaf rust, stripe rust, powdery mildew, and Karnal bunt. However, few data are available on the reaction of this species to FHB. Thus, the objective of this study was to evaluate the reaction of a large collection of *Ae. sharonensis* accessions to FHB. Eighty-two accessions originating from nine sites within the coastal plain of Israel were tested. The spray inoculation method was used to assess resistance to initial infection, and the single floret inoculation method was used to assess resistance to spread. A high level of diversity was observed in *Ae. sharonensis* for reaction to FHB as infection levels ranged from 0-100% for both inoculation methods. Eleven accessions exhibited a very high level of resistance (0% infection) with both inoculation methods. These accessions were from two different sites (Ashdod and Ben Zakai) in Israel. Accessions with putative resistance will be evaluated again in the greenhouse in 2003-04 and also in the field. The results suggest that potentially useful sources of FHB resistance may be present in *Ae. sharonensis*.

RELATION BETWEEN TYPE II AND TYPE I RESISTANCE TO *FUSARIUM GRAMINEARUM* IN WHEAT

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OBJECTIVES

The purpose of these experiments was to evaluate germplasm for both type I and type II resistance to *Fusarium graminearum* and to explore the relation between the two types of resistance.

INTRODUCTION

Effective control of Fusarium head blight (FHB) in the field with genetic resistance may require cultivars that have at least resistance to both primary infection (type I resistance) and resistance to spread of the fungus through the spike after primary infection (type II resistance). Although these two types of resistance are conceptually distinguishable, it is not known to what extent the same genes influence both.

Greenhouse screening of material in breeding programs or for genetic studies usually employs the point inoculation method, with subsequent measurement of the spread of blight symptoms in the spike. This measures type II resistance. Type I resistance is assessed by spraying the entire head with a spore suspension, with the goal of exposing all florets to inoculum. Evaluation of wheat lines in the field probably reveals information about both type I and type II resistance, although the relative contribution of each type to the phenotype cannot be distinguished

In previous work, we selected lines for stable expression of type II resistance from several wheat accessions (Shaner and Buechley 1999). Here, we report the results of both spray and point inoculation of these selections. We also evaluated recombinant inbred lines from a cross between wheat cultivars Clark (susceptible) and Chowkang (resistant) for type II resistance (Buechley and Shaner 1999). We evaluated these lines for type I resistance. Results from these experiments allow us to compare the correlation between these two types of resistance.

MATERIALS AND METHODS

We evaluated 32 selections and cultivars (hereafter referred to as selections) simultaneously for type II and type I resistance. Most of the selections were from accessions that showed some degree of type II resistance in initial screenings. We tested selections in the fall of 2002 and again in the spring of 2003. Seedlings were vernalized and then transplanted to the greenhouse. Selections were evaluated for type II resistance by point inoculation.When flowering had progressed to the top of the spike (GS 65), we injected 10µL of a suspension of macroconidia (10^4 spores/ml) of *F. graminearum* into a well-developed floret near the top of the spike. We used spray inoculation to test for type I resistance. When anthers were extruded on all florets of a spike (GS 67), we sprayed the spike with the same suspension of spores used for point inoculation. Plants to be inoculated were set out in a row, with their heads oriented such that spikelets on both sides of the spike would receive direct spray. The sprayer was passed down the line of plants at a speed such that each plant received about a 1-s burst of spray. After inoculation by either method, inoculated head was enclosed in a clear polyethylene bag. For several years we had used 5

cm × 10 cm, clear polyethylene bags, which could be stapled closed around the peduncle. The bags were left in place for 48 h. Within 3 h after placing a bag over a head, its interior would become clouded with condensed water, indicating a saturated atmosphere surrounding the inoculated head. In the fall of 2002 we used self-closing "Ziploc type" bags. However, these could not be tightly closed around the peduncle without risking severing the head. As a result, moisture did not condense on the interior of the bag, suggesting that the atmosphere surrounding the head was not as humid as when the other bags were used. For the experiment conducted in the spring of 2003, we reverted to the bags that could be stapled shut around the peduncle. Severity of head blight was measured by counting the blighted spikelets on each head (Shaner and Buechley 2001). This was done at 5-d intervals beginning 5 d after inoculation.

We conducted similar experiments with recombinant inbred lines from a cross between Chokwang and Clark. Chokwang is moderately resistant to FHB and Clark is susceptible. This population was tested for type II resistance by point inoculation in 1999 and 2000. In 2003, the same population was evaluated for type I resistance after spray inoculation.

RESULTS

Germplasm selections. Most lines showed some resistance and a few had a high degree of resistance to head blight. In each experiment there was a moderate but significant correlation between type I and type II resistance ($r = 0.39$, $P = 0.03$ for the fall; $r = 0.68$, $P = 0.0000$ for the spring). For data averaged over the two experiments, the correlation was even greater ($r = 0.75$, $P = 0.0000$; see Fig. 1). The line in Fig. 1 is not a regression, but represents a perfect agreement between the two types of resistance. The preponderance of points below this line indicates that most selections had a greater degree of type II resistance than type I resistance. Between the two experiments, expression of type II resistance was reasonably consistent ($r = 0.63$, $P = 0.0002$), but, the expression of type I resistance was inconsistent between experiments ($r = -0.04$, $P = 0.83$). The poor correlation between expression of type I resistance between the two experiments was largely the result of several selections that had a low severity in the fall experiment, but a high severity in the spring experiment.

In each experiment, a few selections had some degree of type II resistance, but very little type I resistance. A few selections showed the opposite trend: some degree of type I resistance, but not much type II resistance. No line showed either of these traits consistently in both experiments, probably a consequence of the inconsistency in expression of type I resistance.

RILs. We evaluated type II resistance in a population of recombinant inbred lines (RILs), derived from a cross between wheat cultivars Chokwang and Clark. The correlation between experiments for number of blighted spikelets 22 d after inoculation was 0.505. There was transgressive segregation for resistance. We evaluated this same population for type I resistance. Family mean severities at 20 days after inoculation were normally distributed and ranged from 2 to 17 blighted spikelets (Fig. 2). Chokwang averaged 10 blighted spikelets; Clark averaged 15. Of 77 RILs, 52% had a lower severity than Chokwang. There was no significant correlation ($r = 0.17$) between type I and type II resistance in this population.

DISCUSSION

For the germplasm selections, the correlation between head blight ratings for the two methods of inoculation was significant, but low, suggesting that type I and type II resistance are not entirely under control of the same genes. The correlation was even lower for the RILs derived from Clark × Chokwang. The germplasm selections were

from cultivars that were identified elsewhere as having resistance, or from areas where FHB has long been a problem. It is possible that these cultivars were subjected to selection in the field, where both type I and type II resistance would contribute to a low overall severity of FHB. Even though we performed our reselection work using point inoculation, if these lines possessed genes that conferred type I resistance, they would likely have been retained in the selected plants. The situation was different for the RILs derived from Clark × Chokwang. If genes that condition type I and type II resistance were mostly different, then one would not expect a correlation between the two types of resistance in the RILs. This is what we found. Among the RILs there was considerable transgressive segregation for type I resistance. Chokwang had only a moderate degree of type I resistance (Fig. 2). Although Clark was susceptible when sprayed, not all heads were completely blighted, and possibly it contributes genes that enhance type I resistance when combined with genes from Chokwang. Tamburic-Ilincic et al. (2002) also found transgressive segregation for greater resistance when plants were spray-inoculated.

Part of the variation between experiments with the germplasm selections may have been the result of different methods of bagging heads to provide high humidity after inoculation. The self-closing bag we used in the fall did not seem to retain moisture as well as the bags we had used in earlier tests. In the spring experiment, we resumed use of the original bags and severity of blight was higher than in the fall.

Our findings of a poor correlation between type I and II resistance agree with those of McKendry et al. (2002) and Tamburic-Ilincic et al. (2002). McKendry et al. evaluated severity 21 d after point inoculation and at both 10 and 21 d after spray inoculation. Although not explicitly discussed by them, the earlier evaluation (10 d) of symptoms after spray inoculation would be expected to give a clearer indication of type I resistance than the later evaluation (21 d). Type II resistance could confound the assessment of type I resistance when ratings are deferred until 21 d after infection. If a line had type I resistance, but little type II resistance, it would have a low severity early (10 d), but subsequent spread of the fungus from only one or a few primary infections would result in severe head blight by 21 d, so by that time it would appear to be susceptible. A line with both type I and II resistance would still have a low severity by 21 d, but it would be difficult to determine the relative contribution of each type of resistance to the final severity. We considered this possibility in our analyses and did compare severity at 10 d after spray inoculation with severity at 20 d after point inoculation. There seemed to be little difference compared to what we saw in the comparison of severities at 20 d after either type of inoculation (the data presented in Figs. 1 and 2) We plan to further explore the effect of time of severity rating on the perceived degree of type I resistance.

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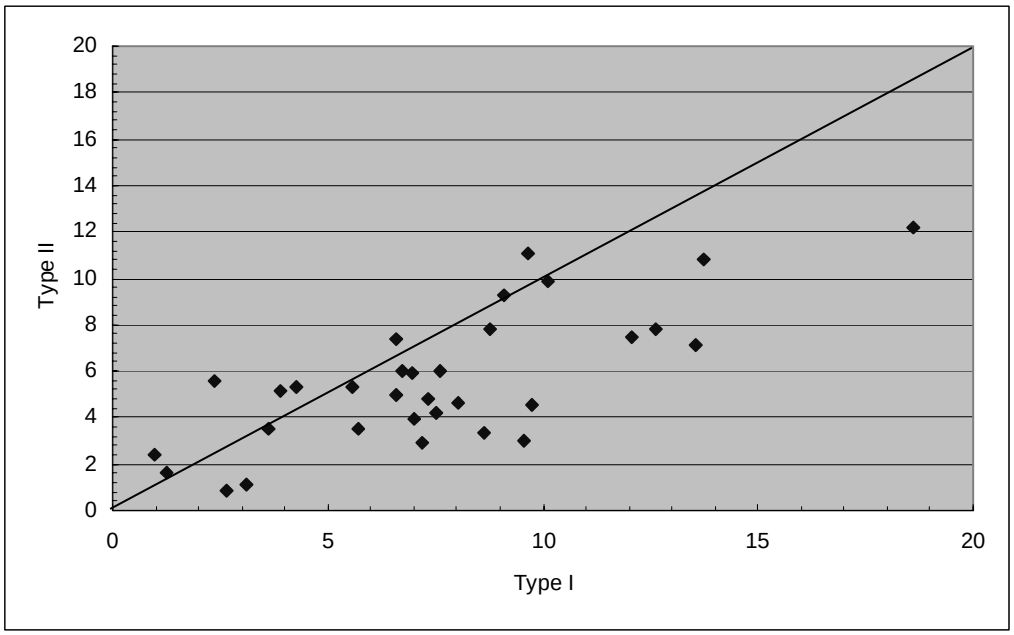

Fig. 1. Relation between type I and type II resistance to *Fusarium graminearum* in a group of wheat selections originally selected for consistent expression of type II resistance. Data are summarized over 2 experiments. The line depicts a perfect association between the 2 types of resistance, not the regression.

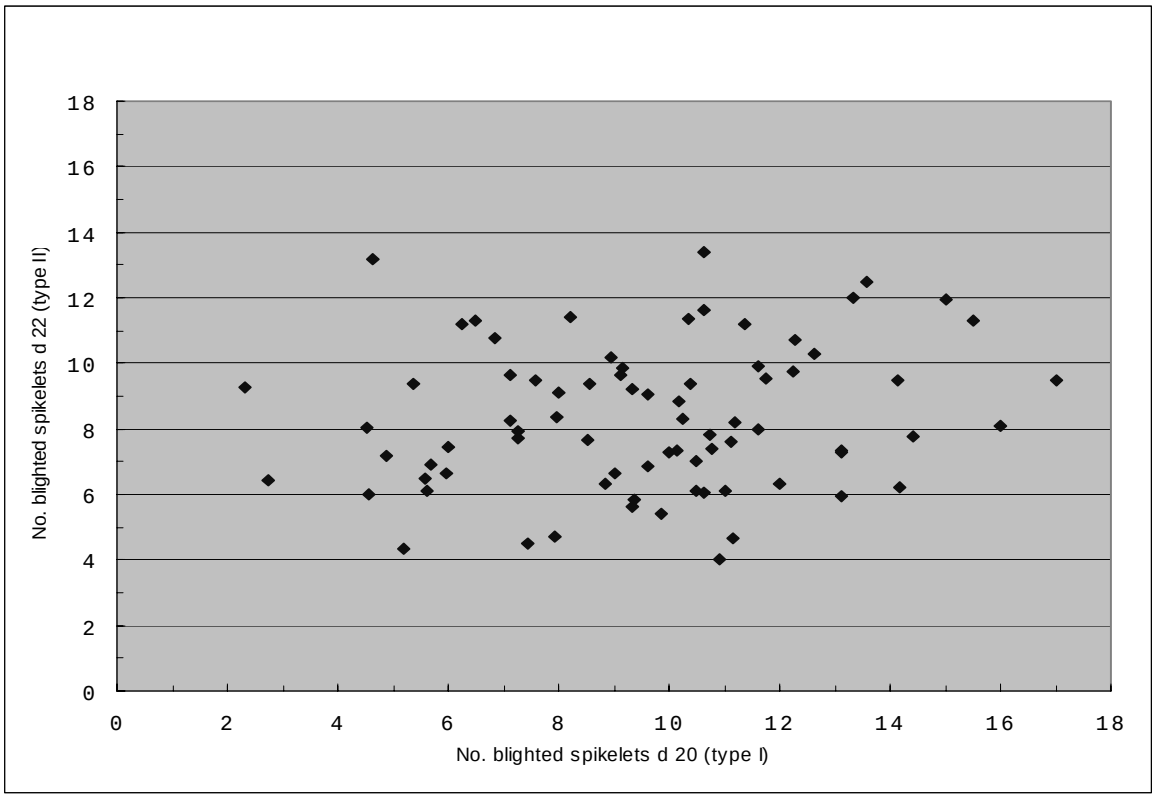

Fig. 2. Relation between type I and type II resistance to *Fusarium graminearum* in a group of recombinant inbred lines derived from a cross between cultivars Clark and Chokwang. Data for type II resistance are averages from 2 experiments; data for type I resistance are means from a single experiment. Clark averaged 11.4 blighted spikelets for point inoculation and 15.2 for spray inoculation; Chokwang averaged 5.0 blighted spikelets for point inoculation and 10.1 blighted spikelets for spray inoculation.

GENETIC CHARACTERIZATION OF FHB RESISTANCE SUPPRESSION CONDITIONED BY CHROMOSOME 2A OF *TRITICUM DICOCCOIDES*

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ABSTRACT

Disomic substitution lines, LDN(DIC), prepared by replacing chromosomes of Langdon durum with those from wild emmer (*Triticum dicoccoides* (TDIC)) showed highly significant differences in response to Fusarium head blight (FHB) when challenge inoculated with *Fusarium graminearum* in the greenhouse (Stack et al. 2002. Crop Science 42:637-642). Several of the LDN(DIC) lines showed increased resistance to FHB. One line, LDN(DIC2A), was highly susceptible to FHB, just as is the TDIC accession that contributed the chromosomes to the substitution lines. In F-1 hybrids with other substitution lines the gene(s) on 2A behaves as a "susceptibility gene" that acts in an additive manner. In most F-1 hybrids involving LDN(DIC-2A), the FHB score was intermediate between the parents. The F1 hybrid between the resistant line LDN(DIC-3A) and LDN had a FHB score similar to the parent, suggesting that the 3A resistance is dominant. The F1 hybrid between Langdon and the LDN(DIC-2A) however, was intermediate, suggesting a quantitative inheritance. In populations derived from crosses of the LDN(DIC-2A) to an FHB resistant line, such as LDN(DIC-3A), the FHB resistance frequency distribution in the F-2 suggests that the gene(s) on chromosome 2A is epistatic to any resistance genes. The distribution of FHB severity values in the F-2 exhibited a trimodal distribution suggestive of a 1:2:1 segregation ratio that might be expected due to segregation of a single gene that acts additively. This supports the hypothesis that a single gene is present on chromosome 2A that not only increases FHB susceptibility, but also suppresses the action of the FHB resistance on chromosome 3A. (This poster was presented at the annual meeting of the American Phytopathological Society, Charlotte, NC, August 2003).

TRANSFER AND EXPRESSION OF RESISTANCE TO FUSARIUM HEAD BLIGHT FROM WILD EMMER CHROMOSOME 3A TO BREAD WHEAT

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ABSTRACT

One of the main goals in breeding for resistance to Fusarium head blight (FHB) is to diversify the resistance gene pool available. To date most FHB resistant spring wheat lines trace back to the Chinese line "Sumai-3" or its derivatives, most of which share the same QTL's. Stack et al. found resistance located on three chomosomes (1A, 3A, 6B) of *T. dicoccoides* when these were substituted into a durum background (Crop Sci. 42:637-642. 2002). The major FHB resistance was present on the wild emmer chromosome 3A and was expressed in the durum disomic substitution line "LDN(DIC-3A)". This line was the resistant parent in a cross with the hexaploid spring wheat "Reeder." Reeder is a good quality, well adapted North Dakota hard red spring wheat cultivar which is quite susceptible to FHB. Lines were advanced by single seed descent. In 2002, F4 seed of this population was planted in an inoculated and mist-irrigated FHB screening nursery. FHB severity was scored at 3 weeks postanthesis; plots were harvested when mature and proportion of scabby kernels determined. In 2003, the F5's of this population were planted in this nursery. In addition the parent line Reeder and two check lines, Alsen, a moderately resistant cultivar of Sumai3 parentage, and 2398, a highly susceptible cultivar, were included. In several previous trials, the FHB severity of LDN(DIC-3A) was similar to that of Alsen. FHB severity was scored at 3 weeks after anthesis. Distribution of FHB scores of lines in this population showed a mean similar to the mean of the parents and some lines showed moderately resistant FHB scores, similar to Alsen the surrogate for the resistant parent. The results indicate that the 3A resistance was successfully transferred to bread wheat. Preliminary results indicate that a sample of the most resistant of the lines in this population carry the 3A QTL marker while a sample of the susceptible lines do not. (This poster was presented at the ASA/CSSA Annual meeting, Denver, Colo. November 2003.)

A POPULATION APPROACH FOR IDENTIFYING FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY

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ABSTRACT

Several composite cross populations of barley have been developed for breeding and basic genetic research. One of these populations (composite cross XXX or CC XXX) originated from natural crosses between the USDA world barley collection and a male sterile line (Ramage et al. 1976, Crop Sci. 16:314). Each accession in the world collection had an opportunity to contribute pollen for seed set on the male sterile line in the original population grown in the field. Thus, CC XXX represents an extremely diverse assemblage of gametes segregating in a single population. As an alternative means for identifying Fusarium head blight (FHB) resistance in barley, bulked seed (~1 kg or 25,000-32,000 seeds) of CC XXX-G (an $F_1Sib_4F_2$ derivative of CC XXX) was evaluated in the 1997 FHB nursery at Hangzhou, China. Uniform and heavy disease pressure allowed for easy differentiation of lines with low and high disease levels and reduced the chance of selecting escapes. From this population, over 350 early maturing, six-rowed lines with low (<5%) infection were selected for further evaluation in the Midwest. From this second round of screening, only 20 lines were found to possess an adequate level of resistance (i.e. less than 15% infection) under Midwest conditions. Two (COMP 351 and COMP 355) of these 20 selections exhibited consistently low levels of FHB and deoxynivalenol (DON) after several successive years of field testing (1998-2003). Overall average FHB and DON levels were 1.9% and 4.5 ppm for COMP 351, 1.8% and 3.5 ppm for COMP 355, 2.0% and 4.0 ppm for Chevron (six-rowed resistant check), 3.9% and 4.2 ppm for CIho 4196 (two-rowed resistant check), 8.4% and 22.9 ppm for Stander (widely grown six-rowed cultivar), and 42.1% and 36.9 ppm for PI 383933 (susceptible six-rowed control). If COMP 351 and COMP 355 carry resistance alleles that are different from those found in other sources, they will be useful in barley programs breeding for FHB resistance.

EVALUATION OF SWISS BARLEY LANDRACES FOR RESISTANCETO FUSARIUM HEAD BLIGHT

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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 increase the diversity of FHB resistance in breeding programs, we have evaluated thousands of accessions of *Hordeum* from the USDA National Small Grains Collection (NSGC) from 1995-2003. The germplasm screened included the entire six-rowed spring barley collection (>8,100 accessions), over a third of the winter six-rowed collection (900 accessions), and over half the wild barley (*Hordeum vulgare* subsp. *spontaneum*) collection (585 accessions). The six-rowed cultivar Chevron is one of the best sources of FHB resistance and was discovered over 70 years ago. Chevron originated from Switzerland. Several of the most resistant accessions from our recent screening effort of the NSGC also originated from Switzerland. To further characterize germplasm from this region, we obtained 74 Swiss barley landraces from Geert Kleijer (Nyon, Switzerland) and evaluated them for their reaction to FHB at St. Paul and Crookston, Minnesota. The foliar spray (using macro-conidia) and grain spawn (ascospores) methods of inoculation were used at the St. Paul and Crookston nurseries, respectively. In general, two-rowed accessions exhibited lower levels of FHB and deoxynivalenol (DON) than six-rowed accessions. Thirteen accessions (11 two-rowed and 2 six-rowed) exhibited FHB severities less than 3%, which was comparable to the range observed on the Chevron control (0-3.6%). Three of these 13 accessions had very low DON levels of <2 ppm (Chevron average=5.6). Additional screening tests will be made in both the greenhouse and field to confirm the resistance of these landraces. We will also genotype these accessions with molecular markers to determine whether they possess the same alleles as Chevron.

MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS ASSOCIATED WITH FUSARIUM HEAD BLIGHT RESISTANCE IN BARLEY

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ABSTRACT

We have investigated barley traits in relation with FHB infection to see if it was possible to establish a breeding strategy for the development of FHB-resistant cultivars. Recent mapping studies indicate that many of the quantitative trait loci (QTL) for FHB resistance coincide with the QTL for plant height, heading date, and spike characteristics. Therefore, this study was conducted to investigate the relationship of morphological and physiological traits to FHB infection and deoxynivalenol (DON) accumulation in a doubled-haploid (DH) population derived from a Léger/CI9831 cross of barley. During two years, 190 DH lines were grown at Ottawa (Ontario) and Hangzhou (China) and also in Charlottetown (Prince Edward Island) for one year. The field plots were inoculated with *Fusarium graminearum* at each location. FHB incidence was positively correlated with DON content. Resistance to FHB was associated with two-row spike, purple lemma, long glume awn, tall stature and/or resistance to lodging, but it was not associated with long rachilla hairs, rough lemma awn, or heading date. Two-row was associated with tall stature and resistance to lodging. These associations in two-row lines combined with spike characteristics helped reduce FHB infection and DON accumulation to a greater extent compared to six-row lines. Purple lemma contains a high level of phenolic compounds, which in turn could inhibit FHB development. The association between long glume awn and FHB resistance could be due to genetic linkages. Therefore, trait associations should be taken into consideration when breeding for FHB resistance in barley.

RESULTS OF SSR FINGERPRINTING OF 94 NEWLY IDENTIFIED FUSARIUM HEAD BLIGHT RESISTANCE SOURCES

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ABSTRACT

Molecular analysis of the parents is the first step for a marker-assisted breeding. This poster reports a molecular fingerprinting of 96 bread wheat accessions with 32 simple sequence repeat (SSR) markers. 93 of the 96 accessions came from Europe, Asia and South America and were selected from the entries of the Spring Wheat Germplasm Survey of the US Wheat Barley Scab Initiative for their excellent performance for fusarium head blight (FHB) resistance in the multi-year examination. The objectives of this study were: 1) study the novelty of these newly identified FHB resistance sources by comparing them with 'Sumai 3', 2) analyzing the genetic background of these lines and 3) provide breeders a molecular fingerprint for each of the accessions assayed. A total of 254 alleles at 105 loci were uncovered. Most of these SSR alleles were not evenly distributed worldwide. A total of 49 alleles were not observed in any Asian accessions assayed, while 34 and 32 alleles were respectively absent from the European and the South American accessions. Forty-four alleles were found to be continent-specific. Genome-wide cluster analysis clearly divided the 96 accessions into two groups with 48 accessions per group. Accessions in the group where 'Sumai 3" is not in should be a good source for novel FHB resistance QTLs if they are also different from 'Sumai 3' for the 3BS chromosomal region defined by SSR markers *Xgwm389, Xgwm493* and *Xgwm533*. Examples of such FHB resistance sources include 'Tokai 66' and 'Nobeoka Bozu' from Japan 'Laureano Alvarez Laah' and 'Tezanos Pintos Precoz' from Argentina, 'Chudoskaja' and 'Ostka Wierzbienska' from Poland, and 'Abura' from Brazil.

EFFECTS OF ROW TYPE, FLOWERING BEHAVIOR AND SEVERAL OTHER SPIKE CHARACTERS ON RESISTANCE TO FUSARIUM HEAD BLIGHT IN BARLEY

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ABSTRACT

Barley varieties show a wide range of resistance to Fusarium head blight (FHB), however, the expression of resistance to FHB is complex and various spike characters such as row type, kernel density, etc. are thought to possibly influence the FHB resistance in barley. The possible effect of row type have been particularly noted, because two-rowed types generally are more resistant than six-rowed types, and the most resistant barleys recognized so far are all two-rowed types. Our test also showed obvious difference in resistance level between two-rowed and six-rowed Japanese varieties. Two-rowed and cleistogamous (closed-flowering) varieties in Japan belonged to the highest resistant group, while six-rowed and chasmogamous (opened-flowering) varieties were mostly susceptible. In order to assess effects of several spike characters including row type and flowering behavior to FHB resistance, we investigated the resistance level of near-isogenic lines (NILs) with genetic background of Japanese two-rowed varieties differing for the traits. The evaluation of FHB resistance was performed using "pot-plant" and "cut-spike" method reported previously. In both cases, spikes exactly at anthesis were spray-inoculated with macroconidia suspension of *F. graminearum*. The chasmogamous and six-rowed NILs were tend to be more diseased than cleistogamous and two-rowed NILs, respectively, and the difference in FHB severity was greater and more consistent in chasmogamous/cleistogamous NIL pairs than in two-/six-rowed pairs. No or little differences were observed in lax/dense spike, normal/uzu type (semi-dwarf and have dense spike), and wax-coated/wax-less spike NIL pairs. Our results indicate that cleistogamy and genetic background of the resistant two-rowed varieties contribute to FHB resistance more greatly than row type and spike density, and therefore, that trait and the germplasms are useful for the resistance breeding of barley.

EVALUATION OF SPRING WHEAT GERMPLASM FOR FUSARIUM HEAD BLIGHT RESISTANCE

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OBJECTIVE

To identify sources of resistance to Fusarium head blight in spring wheat germplasm.

INTRODUCTION

The use of host resistance will be one of major components in managing Fusarium head blight (FHB) in wheat. Success of breeding for resistance relies on the availability of a diversified and well-characterized resistant germplasm pool. Evaluation of the spring wheat germplasm collections deposited in the USDA National Small Grain Collection has enabled us to identify diverse sources of putative resistance (Zhang and Jin 2003). This report summarizes the progress made in 2003 in the spring wheat germplasm screening.

MATERIALS AND METHODS

In the 2003 field season, a multi-nursery system (Zhang et al. 2000) was used. This system composed of several inter-related nurseries and environments (field and greenhouse), each of which handles germplasm at different stages of evaluation/characterization. Below is a brief description of the experiments.

Field nurseries: Newly introduced materials were planted in non-replicated single-row plots and evaluated for FHB reaction in the Preliminary Screening Nursery (PSN). ND 2710 and BacUp were used as resistant checks and Sonalika and Wheaton as susceptible checks. The nursery was inoculated with corn grain (colonized by *Fusarium graminearum*) and conidial suspension (a mixture of ten isolates). Details in nursery management, inoculation, and data collection were as described previously (Zhang et al. 2000; Zhang and Jin 2002). Accessions or plants within an accession with a relatively low FHB index (incidence*severity) and/or low percentage of Fusarium damaged kernels (FDK) were selected. Selections were further evaluated in subsequent years in Elite Germplasm Nurseries (EGN). Entries of EGN were planted in row-plots with three replicates. The materials were blocked based on three maturity groups. For data analysis purpose, each maturity group was considered as an individual experiment. The EGN plots were hand-harvested. Yield, volume-weight, FDK, and DON of each plot were recorded.

Greenhouse characterization: Field selections were evaluated in the greenhouse by both point and spray inoculations in the fall and spring greenhouse seasons. When the plant was at full heading to the beginning of anthesis stages, the 11th floret (counting upward from the base of the spike) was inoculated with a conidial suspension (ca. 70,000 – 80,000 conidia/ml) using a single *F. graminearum* isolate. The inoculated spike was covered using a zip-loc specimen bag for 48 hours. The number of infected spikelets (counting downward from the inoculation site, i.e. the 11th spikelet) was recorded 21 days after inoculation. Spray inoculation was applied at the beginning to half anthesis stage. Inoculated plants were incubated for 72 hours in a mist chamber. Disease severity was collected seven days after inoculation. For each greenhouse season,

eight replicates per test-entry were planted for point inoculation or spray inoculation. Approximately 20-40 heads/entry/season were tested by either of the methods.

RESULTS AND DISCUSSION

The 2003 field experiment was conducted in Brookings, South Dakota. The PSN consisted of 466 accessions originated from India, China, Nepal and several other countries in Southeast Asia. Based on visual disease reading and FDK, eighty-five lines were selected for further evaluations. The EGN nursery included 326 accessions that were selected from the preceding three years. Lines in the second and third year of re-evaluation (i.e. selections from the 2001 and 2000 PSN) were subjected to DON analysis (by Dr. Y. Dong at the University of Minnesota). Table 1 presents the 2001-2003 field evaluation data of selections made in 2000. Disease severity and FDK of most lines in Table 1 varied considerably over the years. However, several accessions exhibited consistently low FHB index and low FDK (within the range of resistant checks of ND 2710 and BacUp). Evaluation from greenhouse experiments (data not presented) indicated that some of the selections were highly susceptible to point inoculation. For example, the average disease severity of PI 372137 by point inoculation (based on 40 inoculated spikes) was 99.0%. Greenhouse experiments are in progress to further characterize these selections.

ACKNOWLEDGEMENT

This material is based upon work supported by the U.S. Department of Agriculture, under Agreement No. 59-0790-9-045. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative.

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Zhang X., and Y. Jin. 2003. Diversity of resistance to Fusarium head blight in the US spring wheat germplasm collection. Page 204 *In:* 8th International Congress Plant Pathology Vol. 2. Feb. 2-7, 2003, Christchurch, New Zealand.

Table 1. Three-year (2001-2003) field data of spring wheat germplasm selections with low Fusarium head blight index and percentage Fusarium damaged kernel.

Accession	FHB index (%)				FDK (%)			Country
/ID	2001	2002	2003	Mean	2001	2002	Mean	of origin
ND2710-ck	12.3	11.5	8.2	10.7	7.7	40.0	23.8	USA
BacUp-ck	24.0	28.8	20.5	24.4	20.0	33.3	26.7	USA
Wheaton-ck	88.7	87.0	76.0	83.9	90.0	83.3	86.7	USA
Sonalika-ck	83.0	87.8	85.5	85.4	95.0	85.3	90.2	India
PI 67392	15.1	11.0	7.2	11.1	21.7	40.0	30.8	Russia
PI 69251	22.5	22.0	3.9	16.1	8.3	53.3	30.8	China
PI 225448	18.7	22.1	14.8	18.5	8.3	53.3	30.8	Uruguay
PI 225382	10.9	31.9	17.3	20.1	12.5	53.3	32.9	Uruguay
PI 225449	16.8	26.3	17.5	20.2	11.7	46.7	29.2	Uruguay
PI 155266	19.0	29.7	17.1	21.9	13.3	66.7	40.0	Japan
PI 225396	19.0	34.3	15.8	23.0	11.7	53.3	32.5	Uruguay
PI 197664	21.3	39.7	11.3	23.9	6.7	40.0	23.3	Argentina
PI 225516	18.7	43.3	10.5	24.2	6.0	53.3	29.7	Uruguay
PI 372137	34.0	25.5	15.5	25.0	20.0	43.3	31.7	Ukraine
PI 351500	23.1	37.7	16.1	25.6	6.0	60.0	33.0	Russia
PI 225504	22.7	39.0	15.9	25.9	13.3	53.3	33.3	Uruguay
PI 225384	14.1	46.3	18.1	26.2	21.7	63.3	42.5	Uruguay
PI 225467	15.4	47.1	17.3	26.6	8.3	66.7	37.5	Uruguay
PI 233203	14.4	45.3	20.9	26.9	3.7	60.0	31.8	Russia
PI 285973	23.5	32.5	25.3	27.1	11.7	56.7	34.2	Russia
PI 225378	21.7	45.2	16.1	27.6	17.5	56.7	37.1	Uruguay
PI 285945	44.5	27.6	15.3	29.1	3.0	56.7	29.8	Poland
PI 572636	24.0	50.0	13.8	29.3	6.7	70.0	38.3	Ukraine
PI 584914	23.3	47.8	17.8	29.7	8.3	66.7	37.5	Brazil
PI 69260	29.8	21.8	38.2	29.9	3.0	26.7	14.8	China
PI 337149	37.8	35.2	20.9	31.3	36.7	56.7	46.7	Argentina
PI 285972	40.3	35.9	19.0	31.7	21.7	73.3	47.5	Poland
PI 74494	44.8	33.9	16.5	31.8	13.3	56.7	35.0	Russia
PI 69321	63.8	18.9	13.3	32.0	6.7	23.3	15.0	China
PI 225519	21.4	57.2	19.3	32.6	10.0	46.7	28.3	Uruguay
PI 225376	25.1	52.8	20.2	32.7	15.0	60.0	37.5	Uruguay
PI 372136	29.3	48.9	21.2	33.2	5.0	50.0	27.5	Russia
PI 520540	20.6	64.0	15.3	33.3	6.7	46.7	26.7	Brazil
PI 189816	29.8	56.8	15.7	34.1	23.3	63.3	43.3	Argentina
PI 225446	47.0	25.6	30.6	34.4	56.7	53.3	55.0	Uruguay
PI 225398	23.9	51.8	28.3	34.7	16.7	56.7	36.7	Uruguay
PI 69335	25.7	57.3	22.5	35.2	10.0	33.3	21.7	China
PI 352009	59.1	17.5	28.9	35.2	21.7	40.0	30.8	Russia
PI 70656	61.8	26.6	19.1	35.8	16.7	26.7	21.7	China
PI 74493	54.2	35.5	20.9	36.9	8.3	50.0	29.2	Russia
PI 225375	40.2	37.7	32.9	36.9	33.3	66.7	50.0	Uruguay
PI 281842	66.3	25.9	25.9	39.4	25.0	43.3	34.2	Ukraine

PI 225424	36.7	51.7	30.3	39.6	18.6	53.3	36.0	Uruguay
PI 225372	40.7	48.5	32.5	40.6	10.0	53.3	31.7	Uruguay
PI 225525	64.3	43.3	25.0	44.2	26.7	46.7	36.7	Uruguay
PI 168722	74.0	41.3	20.8	45.4	15.0	36.7	25.8	Argentina
PI 283806	42.8	70.4	29.8	47.7	5.0	53.3	29.2	Argentina
PI 74085	56.0	55.2	32.2	47.8	5.0	56.7	30.8	Russia
PI 69240	51.0	58.7	39.0	49.5	6.7	43.3	25.0	China
PI 62083	68.5	64.8	19.0	50.8	30.0	40.0	35.0	Argentina
PI 559686	53.2	42.8	57.8	51.3	11.7	56.7	34.2	Russia
PI 69261	69.8	54.4	30.2	51.5	23.3	23.3	23.3	China
PI 69747	62.0	32.7	62.0	52.2	26.7	30.0	28.3	China
PI 233204	51.8	49.1	55.8	52.3	10.0	50.0	30.0	Russia
PI 69243	49.2	68.7	47.2	55.0	8.3	23.3	15.8	China
PI 69270	67.8	57.3	46.7	57.3	41.7	33.3	37.5	China
PI 69265	56.8	45.7	75.5	59.3	13.3	33.3	23.3	China

ROLE OF THE USDA REGIONAL GENOTYPING CENTERS

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ABSTRACT

The concept of USDA Regional Genotyping Center was original proposed by Van Sanford et al. (2001) to meet growing need of marker-assisted selection for scab resistant cultivars. With strong support from cereal crop researchers, three USDA regional genotyping centers have been recently established in Manhattan, KS, Fargo, ND and Raleigh, NC, respectively. Their missions include developing high throughput molecular markers for Fusarium head blight and other agronomically important traits of cereal crops, screening breeding materials and germplasm with molecular markers for breeding programs to perform marker-assisted breeding, and providing training and technical consultations on marker analysis to breeders and other researchers. Screening of breeding materials for the major FHB resistance QTL on 3BS will be the first service provided by the Centers. Protocols for sample handling, marker analysis, data analysis and data delivery will be proposed. Scopes of research and service in the Genotyping Centers will be discussed.

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PERCENTAGE SCABBY KERNELS IS CORRELATED WITH FUSARIUM HEAD BLIGHT INDEX FOR KANSAS WINTER WHEAT CULTIVARS

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ABSTRACT

Fusarium head blight (FHB) is a serious disease of wheat and barley that is best controlled by host resistance. Resistance to FHB can be expressed in several ways including a reduction in the percentage florets that are affected and a reduction in the percentage of scabby kernels in harvested grain. This research sought to determine if there is a correlation between the observed reaction of commercial Kansas winter wheat cultivars in the field and the percentage scabby kernels in harvested grain. Twenty (2000 and 2001) or 24 (2002 and 2003) common commercial wheat cultivars were screened over a 4-yr period in the field for reaction to FHB. Experimental design for each year was a randomized complete block with four replications. Corn grains colonized by *Fusarium graminearum* were applied to the soil surface in three applications about 2 wk apart beginning 5 wk prior to heading (93 g/m2 total applied). During heading and flowering, plots were sprinkler irrigated (3 min/hr) from 9:00 p.m. until 6:00 a.m. FHB index (percentage diseased spikelets) was determined for each plot of each cultivar between four and six times and averaged. At maturity, plots were harvested with a small-plot combine and the percentage scabby kernels visually estimated for the harvested grain. Analyses of variance (ANOVA) followed by LSD (P=0.05) were conducted for all cultivars for FHB index, grain yields, and percentage scabby kernels. Correlation coefficients were calculated for percentage scabby kernels in harvested grain with average FHB index and with grain yields (N=80 or 96 depending upon the year). There were 10 cultivars that were common to all four years. Significant differences (P=0.05) occurred among the cultivars for FHB index, percentage scabby kernels, and grain yields. Across all years, Hondo showed the lowest FHB index (8.0%) and Tomahawk the highest (52.7%). Within each year, significant correlations occurred between percentage scabby kernels and either FHB index or grain yields. For common Kansas commercial winter wheat cultivars, FHB index values are a significant predictor of the amount of scabby kernels in harvested grain; however, their predictive value is from 9% to 60%, depending upon the year. Similarly, grain yields of cultivars in a high-scab environment are a significant predictor of the amount of scabby kernels and their predictive value is 26-47%.

SCAB SCREENING OF SOFT RED WINTER WHEAT GENOTYPES IN MARYLAND

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ABSTRACT

The 2002/2003 wheat growing season presented very favorable environmental conditions for the development of a scab (*Fusarium graminearum*) epidemic in Maryland. Rainy, drizzly conditions predominated during the spring of 2003. These conditions led to a high level of scab incidence on Maryland's Eastern shore, the largest wheat growing area in Maryland. The official winter wheat state variety test was grown under field conditions in Queenstown (MD) and the level of scab severity, percentage of tombstones, and DON (Deoxynivalenol) were assessed. Forty genotypes were tested and the incidence of the disease was fairly uniform across the nursery. There were significant genotypic differences. The genotypes Vigoro Tribute, USG3350, Catoctin, McCormick, Coyote, USG3430, MV5-46, Neuse, 25R37, and Patton showed moderate levels of resistance to scab with low percentage of tombstones and low DON levels. On the other hand, the genotypes Southern States 522, Century II, GA931470E62, Coker 9835 and Florida 304 had very high levels of tombstones and DON. This ranking was consistent with other evaluations of resistance of currently grown soft red winter wheat cultivars. It is important to continue to screen currently grown cultivars of soft red winter wheat for even moderate scab resistance because this can be useful for future breeding as well as for immediate use by wheat growers.

COMPARATIVE EVALUATION OF THE UNIFORM REGIONAL SCAB NURSERY FOR SPRING WHEAT PARENTS UNDER DRYLAND AND MIST-IRRIGATED CONDITIONS

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OBJECTIVES

This study sought to compare the disease reactions of wheat entries in the 2003 Uniform Regional Scab Nursery for Spring Wheat Parents (URSN) at nursery locations that are mist-irrigated with locations where mist-irrigation was not used ("dryland screening"). Traditionally the URSN is grown at locations where mist-irrigation is utilized to facilitate the infection of plants by *Fusarium graminearum*. Disease severity in mist-irrigated nurseries is frequently very high, making identification of moderate variation for scab resistance among genotypes difficult due the narrow phenotypic range of disease reactions generally observed. Dryland screening thus was evaluated in this study to see if useful information on the performance of wheat genotypes under lower disease pressure could augment the information obtained from mist-irrigated nurseries, while also demonstrating the utility of conducting Fusarium head blight (FHB) screening under dryland conditions.

INTRODUCTION

Fusarium head blight (FHB or scab) has occurred frequently since the early 1990's primarily in the upper Midwest and Northeastern U.S. The reemergence of FHB has resulted in severe economic losses to wheat producers, the wheat processing industry, and the associated rural communities in the affected regions (Windels, 2000). The severity and continued occurrence of the disease impelled wheat breeding programs to undertake a concerted effort to develop scab resistant varieties. The URSN was established in 1995 as a means of evaluating promising FHB-resistant germplasm, and to encourage open exchange of this germplasm among spring wheat breeding programs in the US. The URSN has traditionally been planted at multiple locations where mist-irrigation facilitates the development of FHB. The nursery provides a useful comparison of germplasm for reaction to scab over multiple locations (Campbell and Lipps, 1999; Groth et al., 1999) as well as a comparison among the locations for quality of data that is obtained. The development of methods which enable scab screening without the cost and time associated with maintaining a mist-irrigated location is now being examined, through the addition of two dryland locations in 2003 to the URSN, to augment the data obtained annually from the six mist-irrigated nursery locations.

MATERIALS AND METHODS

The 2003 URSN was planted at the standard set of misted and irrigated locations in Minnesota, North Dakota, South Dakota, and Canada. In addition, two dryland locations in Minnesota (St. Paul, Barnesville) were also planted. In 2003, the URSN consisted of 41 entries, of which 5 were checks, and 5 were durum wheats.

Dryland plots at St. Paul and Barnesville, MN were spray-inoculated with a suspension of macroconidia of *F. graminearum* when anthers were first observed on spikes within the plot. Plots were inoculated a second time three days after the first application. Inoculum was applied at a concentration of 1×10^5 macroconidia ml^{-1} sprayed at a rate of about 33 ml m^{-1} of row. Rows were sprayed at a rate of 3.3 sec m^{-1}. The CO_2-powered sprayer, fitted with an ss80015 TeeJet flat-fan orifice, was operated at 2.76×10^5 pascals dispensing inoculum at about 10 ml sec^{-1}. Inoculum was directed toward the spikes by keeping the tip of the orifice within 20-30 cm of the spikes. Plots were assessed for FHB incidence (percentage symptomatic spikes) and FHB severity (percentage symptomatic spikelets/symptomatic spike) about 14 days post-inoculation and at about 20 days post-inoculation at the St. Paul and Barnesville dryland sites, respectively.

The mist-irrigated nursery at St. Paul was inoculated with the same spray-inoculation methodology as the dryland plots about three days post-heading. Heading occurred when about 50% of the heads emerged from the primary tillers. Plots were mist-irrigated each night following the first application of inoculum until disease assessment. The mist-irrigation system delivered 14 irrigation events per night each event being about 5 min long. The combined volume of the misting events was about 4 mm per unit land area per night. Plots at Crookston were inoculated with sterilized corn kernels colonized with *F. graminearum*. Colonized grain was spread on the soil surface within the plots at a rate of 18.4 kg/ha at the five to six-leaf growth stage. Plots were mist-irrigated each night following inoculum dispersal to provide adequate moisture for perithecial development. At heading the mist-irrigation system was operated in a fashion to provide supplemental moisture on spikes each night, although excessive moisture was avoided by monitoring rainfall accumulations and duration of leaf wetness, using remote sensor instrumentation. Plots at Brookings, SD were inoculated with colonized corn and mist-irrigation was utilized.

For this study, disease data from three of the mist-irrigated URSN locations in Minnesota (St. Paul and Crookston) and South Dakota (Brookings) was used for analysis. Similarly, disease data was obtained for the two dryland locations (St. Paul and Barnesville). Data obtained at all locations included FHB incidence, FHB severity, and FHB index (product of FHB incidence and FHB severity), and visually scabby kernels (VSK or tombstones). Means, coefficients of variation, and data ranges were calculated for each location's dataset. Additionally, Pearson's correlation coefficients were calculated in a pairwise fashion for FHB index and VSK data from the locations included in the analysis.

RESULTS

A summary of the phenotypic data obtained for the URSN entries at three mist-irrigated locations and the two dryland locations is shown in Table 1. It is clear that mist irrigation following inoculation of wheat spikes with the pathogen increases both FHB incidence and severity relative to the values obtained at the dryland locations. Nonetheless, it is important to note that the range of values for FHB incidence is far greater in the dryland nurseries. The range of VSK ratings at the dryland locations is comparable to those obtained at two of the mist-irrigated locations. The results suggest that scab disease at both dryland locations was significant enough to result in a wide phenotypic range of measurements for the traits measured.

Table 1. Summary statistics (% values) for wheat entries in the 2003 URSN.

Mist-irrigated	FHB Incidence		FHB Severity		FHB Index		VSK	
Locations	Mean (cv)	Range	Mean (cv)	Range	Mean (cv)	Range	Mean (cv)	Range
St. Paul, MN	88.9 (12.7)	66.7-100	29.9 (36.8)	13.7-55.3	28.0 (42.2)	9.5-55.3	14.8 (41.0)	3.3-51.0
Brookings, SD	96.2 (6.8)	54.0-100	37.3 (25.2)	5.7-78.8	36.8 (26.6)	3.1-78.8	16.7 (39.6)	1.0-83.3
Crookston, MN	94.2 (8.3)	65.0-100	36.0 (34.4)	8.8-79.1	35.3 (28.3)	5.7-53.7	19.4 (28.3)	3.5-53.7
Dryland Locations								
St. Paul, MN, MN	52.2 (26.0)	15.0-93.8	12.5 (32.8)	5.2-27.5	7.8 (53.4)	1.1-24.5	7.9 (63.7)	1.0-58.8
Barnesville	74.6 (14.4)	27.5-100	20.1 (23.0)	8.5-58.6	16.6 (29.1)	2.4-58.6	8.7 (52.7)	1.0-57.5

It is of value to our study to determine if entries in the URSN were ranked similarly in both mist-irrigated and dryland locations. We compared the rankings of the five check varieties in the URSN across the locations to determine how similar they ranked in the dryland vs. mist-irrigated locations. These results are shown in Table 2. These results indicate that both the resistant and susceptible checks exhibit similar rankings between locations.

Table 2. Ranks of wheat check cultivars for FHB disease index (DX) and visually scabby kernels (VSK) at three mist-irrigated and two dryland sites.

Check	Brookings Irrig.		Crookston Irrig.		St. Paul Irrig.		St Paul Dryland		Barnesville Dryland	
cultivars	DX[1]	VSK	DX	VSK	DX	VSK	DX	VSK	DX	VSK
ND2710	1	2	1	1	2	2	5	18	2	1
Bacup	3	1	20	7	23	22	7	3	7	3
2375	23	18	27	28	18	9	2	11	14	20
Oslo	36	40	41	36	38	39	41	40	40	37
Wheaton	41	41	40	41	41	41	40	41	41	41

[1] Product of FHB incidence and FHB severity.

Further, to evaluate the relative similarity between data obtained at dryland vs. mist-irrigated locations, correlations were calculated in a pairwise fashion for FHB index and VSK. The results are shown in Tables 3 and 4. The correlation between the two dryland locations for disease index was positive and highly significant, and was the highest among all locations. The correlations between dryland locations and mist-irrigated locations were also highly significant in all instances.

Table 3. Pearson's correlation coefficients for means of FHB index of 41 wheat lines among selected locations participating in the Uniform Regional Scab Nursery (URSN) during the 2003 field season.

	Barnesville Dryland	St. Paul Dryland	St. Paul Irrig	Crookston Irrig
St. Paul, Dry	0.77***			
St. Paul, Irrig	0.70***	0.54***		
Crookston, Irrig	0.77***	0.70***	0.72***	
Brookings, Irrig	0.73***	0.54***	0.39**	0.62***

Asterisks represent significance of coefficients at the *P*=0.05 (*), *P*=0.01 (**), and *P*=0.001 (***).

Similarly, correlations for VSK between the two dryland locations was positive and highly significant (Table 4). In fact, all of the correlation coefficients between locations for VSK were positive and highly significant. Thus, it appears that even though the FHB index at the dryland locations was lower than the mist-irrigated locations, rankings for both FHB incidence and VSK were similar.

Table 4. Pearson's correlation coefficients for means of visually scabby kernels (VSK) of 41 wheat lines among selected locations participating in the Uniform Regional Scab Nursery (URSN) during the 2003 field season.

	Barnesville Dryland	St. Paul Dryland	St. Paul Irrig	Crookston Irrig
St. Paul, Dry	0.77***			
St. Paul, Irrig	0.75***	0.70***		
Crookston, Irrig	0.77***	0.52***	0.69***	
Brookings, Irrig	0.81***	0.76***	0.79***	0.72***

Asterisks represent significance of coefficients at the *P*=0.05 (*), *P*=0.01 (**), and *P*≤ 0.001 (***).

DISCUSSION

The URSN provides breeders with a mechanism to exchange and evaluate promising accessions with putative resistance to FHB. Traditionally the URSN was conducted only at locations with mist-irrigation and usually the intensity of disease limited the ability of cooperators to identify intermediate levels of scab resistance. Intermediate levels of scab resistance often are not detectable under severe scab intensity such as that often encountered in the misted URSN locations. This is unfortunate because often those genotypes with intermediate levels of resistance possess more desirable agronomic characteristics. Our results suggest that dryland screening provides a less severe disease intensity while still differentiating among resistant and susceptible genotypes. This provides an opportunity to identify those genotypes with improved agronomic characteristics with intermediate levels of resistance. Thus, the use of "dryland" nurseries may augment breeders' ability to identify germplasm to utilize in crosses aimed at improving FHB resistance while maintaining agronomic quality. The dryland screening also has the advantage of requiring fewer resources than traditional screening nurseries.

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ANDROGENIC ABILITY OF EIGHT FHB RESISTANT BARLEY ACCESSIONS

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ABSTRACT

Most Fusarium head blight (FHB) resistant barley (*Hordeum vulgare*) accessions are relatively poor from an agronomic point of view. Due to the complex inheritance of FHB resistance, introgression of this trait into well adapted local germplasm will likely require multiple generations of crossing and selection in order to combine resistance and agronomic performance, even with the use of doubled haploids. Unfortunately, little is know concerning the androgenic ability of genotypes providing FHB resistance and so it is not known which of these could prove interesting in the production of doubled haploid populations. The objective of a first experiment was to compare the androgenic ability of eight barley accessions, known to offer some resistance (Chevron, Gobernadora, Seijo II, Shyri, Svanhals, Zhedar I, F104-250-9 and C97-21-38-3), with three cultivars (ACCA and Léger and Cadette) whose androgenic response was already well characterized. In a second experiment, the androgenic ability of F_1 hybrids, involving some of these genotypes used as parents, was measured and compared to that of the parental genotypes. Very large and significant differences were observed in the number of green plants produced by the different accessions and F1s. In some cases, the androgenic potential proved so low that only a conventional approach, based on selfing to reach homozygosity, would seem justified.

RESOURCE ALLOCATION AND CULTIVAR STABILITY IN BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT

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OBJECTIVES

To characterize the stability of spring wheat cultivars for their Fusarium head blight (FHB) reaction and to obtain estimates of screening nursery requirements for an optimum resource allocation for FHB evaluation.

INTRODUCTION

Fusarium head blight, caused primarily by *Fusarium graminearum* Schwabe, is an important disease for spring wheat (*Triticum aestivum* L.) production. The use of disease-resistant cultivars represents the best method of control (Mesterházy, 1995; Parry et al., 1995; Campbell and Lipps, 1998; Groth et al., 1999). Resistance expression often differs among environments (Parry et al., 1995; Campbell and Lipps, 1998); consequently, developing cultivars with FHB resistance requires experimental designs and strategies that consistently discriminate among genotypes.

Fusarium head blight is incited by a highly variable pathogen, and development and evaluation of the response of wheat to FHB are complex and readily altered by environment. Infection incidence and disease severity measure the frequency and degree of colonization of the spike, respectively, and are common measures of disease (Parry et al., 1995). Disease-affected grain is often quantified as percent visually scabby kernels (VSK) and deoxynivalenol (DON) content (Tacke and Casper, 1996; Jones and Mirocha, 1999).

There is a lack of information on the stability of commercial cultivars and on the allocation of environments, replicates, and within plot sub-sampling for FHB evaluation. The objectives of this study were to characterize the stability of spring wheat cultivars for their FHB reaction and obtain estimates of screening nursery requirements for an optimum resource allocation for FHB evaluation.

MATERIALS AND METHODS

Plant Materials - Fourteen commercial wheat cultivars (Table 1), encompassing a broad range of cultivar response to FHB ranging from resistant to susceptible, were used in this study. These cultivars were grown in FHB nurseries at St. Paul and Crookston, MN from 1999 to 2002 in a RCBD with three replicates location^{-1} year^{-1}. Plots consisted of a single 2.4 m row seeded at a rate of 110 kg ha^{-1} with 0.3 m row spacing.

Fusarium Inoculation- At St. Paul, macroconidial inoculum was applied to the spikes at plant heading with a CO_2 powered backpack sprayer employing a single flat-fan nozzle. Plots were sprayed evenly at a rate

of 30 ml m^{-1} of row, ca. 7×10^6 macroconidia $plot^{-1}$. At Crookston, the nursery was inoculated with Fusarium colonized wheat kernels spread uniformly on the soil surface at 100 kg ha^{-1} at about 25 d prior to average plant heading. The nurseries were misted about eight times during a daily cycle with an automatically controlled irrigation system providing about 12 mm of water per day.

Disease Evaluation - Visual disease scores (0 = no symptomatic spikelets to 5 = all spikelets symptomatic) were assigned to dominant spikes from 20 randomly selected plants $plot^{-1}$. The scores were used to calculate the following three variables for each plot: 1) disease incidence (INC) in % — frequency of symptomatic spikes (scores 1 to 5); 2) disease severity (SEV) in % — average score of spikes with scores from 1 to 5; and 3) disease index (DIS) — average score of spikes with scores from 0 to 5.

Spikes were assessed at late grain filling, when healthy spikes were still green and not senescent. Percent visually scabby kernels (VSK) was assessed according to Jones and Mirocha (1999). Samples from the three replications of each cultivar were bulked following VSK determination and DON analyses were conducted according to the methods of Tacke and Casper (1996) with some modifications.

Data Analysis - Analyses of variance and cultivars' means comparisons were conducted on INC, SEV, DIS, VSK, and DON. Pearson correlation and Spearman rank correlation coefficients were calculated to assess the relationships among heading date, INC, SEV, DIS, VSK, and DON. Two stability parameters of each cultivar: regression coefficient b_i and deviation from regression parameter δ_i^2 (Eberhart and Russell, 1966) were estimated for the different FHB parameters. Predicted $LSD_{0.05}$ for differing levels of sub-sampling, replications, and environments were calculated from estimates of cultivar × environment mean squares, $\sigma_\varepsilon^2 + r\sigma_{CE}^2$, where, σ_ε^2 is the plot error variance, r equals replicate number, and σ_{CE}^2 is the estimated cultivar × environment variance.

RESULTS AND DISCUSSION

Significant differences (at 0.05 probability level) among cultivars were found for the FHB parameters. Table 1 summarizes the mean cultivar values across eight environments for INC, SEV, DIS, VSK, and DON. Correlations between heading date and each of the FHB parameters were very low and non-significant, except for INC (r = 0.17**). Correlations among all FHB parameters were very high and significant (Table 2). SEV and DIS had the highest correlation coefficient among all parameters (r = 0.99***) and DON content of the grain had its highest correlation with VSK (Table 2). Yearly rankings of cultivars for their FHB response using SEV, DIS, VSK, and DON were highly repetitive. Cultivar rankings using VSK were the most repetitive, with overall mean values for Spearman rank correlation coefficients of 0.73***.

For DIS, VSK, INC, and SEV, results from stability analyses over eight environments revealed stability to FHB response in some resistant and some susceptible cultivars. For DIS, 'Forge', 'Roblin', and 'Verde' had low stability. Those varieties are intermediate for their FHB reactions. Thus, depending upon conditions, they will vary more than either varieties with better resistance or varieties with poorer resistance.

We predicted $LSD_{0.05}$ for DIS using spike numbers of 10 and 20 $plot^{-1}$, and replicate numbers of 2, 3, 4, 6, and 8 at 1, 2, 3, 4, 6, 8, and 10 environments (Table 3). We suggest that $LSD_{0.05}$ magnitudes ca. 33% or less of the observed range of values is sufficient for finding important differences. The difference between extreme cultivars for DIS, calculated from across environment means, was 2.4 (Table 1). Accordingly, a $LSD_{0.05}$ less than 0.8 is suggested. Two replicates with 20 spikes achieve this goal in three environments.

For our study, the greatest reduction in genotype standard error was obtained by going from 1 to 2 environments and from 1 to 3 environments, representing a 29 and a 42% reduction in genotype standard error, respectively.

CONCLUSIONS

Both colonized grain and conidial spray inoculation methods provide disease levels appropriate to differentiate resistant and susceptible cultivars. When breeding for FHB resistance, it is imperative to evaluate the material using resistant and susceptible check cultivars known to be stable in their FHB response. Stability of FHB reactions was not associated with levels of resistance in the cultivars tested. Increasing the number of environments has the greatest effect in reducing the genotype standard error and therefore increasing the probability of finding significant differences among genotypes evaluated. We recommend that wheat breeding programs testing a large number near homozygous-early generation lines (e.g. $F_4 - F_6$ derived), use one or two environments to identify and discard highly susceptible lines. The selected lines should continue to be evaluated in subsequent FHB trials to more accurately assess their response over more environments. A good assessment of cultivar FHB reaction can be obtained in three or four environments.

Table 1. Cultivar means for INC, SEV, DIS, VSK, and DON over eight environments.

Cultivar	INC	SEV	DIS	VSK	DON
BacUp	76	18	1.4	9.6	5.3
Ingot	84	24	1.9	11.7	6.6
Forge	81	25	1.9	18.9	7.2
P2375	89	25	2.0	16.5	8.6
Gunner	92	24	2.1	13.3	8.1
McVey	94	25	2.1	19.8	12.6
Russ	90	29	2.2	24.5	10.6
Verde	98	33	2.5	23.5	15.0
HJ98	96	38	2.7	25.4	9.8
Marshall	99	36	2.7	27.6	18.9
Oxen	98	41	2.9	28.8	10.9
Norm	99	47	3.1	44.1	36.5
Roblin	99	56	3.4	30.4	12.7
Wheaton	99	68	3.8	49.8	35.9
Mean	92	35	2.5	24.6	14.2
LSD	5	6	0.3	4.2	8.0

Table 2. Pearson correlation coefficients (above diagonal) and Spearman's rank correlation coefficients (below diagonal) among five parameters over cultivars, reps, and environ. (n=330).

	INC	**SEV**	**DIS**	**VSK**	**DON**
INC	-	0.43***	0.52***	0.43***	0.32***
SEV	0.58***	-	0.99***	0.56*	0.21*
DIS	0.65***	0.98***	-	0.58***	0.37***
VSK	0.52***	0.70***	0.72***	-	0.45***
DON	0.61***	0.63***	0.64***	0.73***	-

*, **, *** = significant at 0.05, 0.01, and 0.001, respectively.

Table 3. Predicted LSD's (P = 0.05) for disease index among 14 wheat cultivars under differing levels of sub-sampling plot^{-1} (spike number), replication within environments, and environments.

	No of reps with 10 spikelets plot^{-1}					No of reps with 20 spikelets plot^{-1}				
Env.	2	3	4	6	8	2	3	4	6	8
1	1.46	1.34	1.27	1.21	1.17	1.40	1.30	1.24	1.19	1.16
2	1.03	0.95	0.90	0.85	0.83	0.99	0.92	0.88	0.84	0.82
3	0.84	0.77	0.74	0.70	0.68	0.81	0.75	0.72	0.68	0.67
4	0.73	0.67	0.64	0.60	0.59	0.70	0.65	0.62	0.59	0.58
6	0.59	0.55	0.52	0.49	0.48	0.57	0.53	0.51	0.48	0.47
8	0.51	0.47	0.45	0.43	0.41	0.50	0.46	0.44	0.42	0.41
10	0.46	0.42	0.40	0.38	0.37	0.44	0.41	0.39	0.38	0.37

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FLOWERING CHARACTERISTICS AND INCIDENCE OF *FUSARIUM* INFECTION IN A RI POPULATION OF WHEAT

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ABSTRACT

The wheat cultivar Patterson develops a higher incidence of Fusarium head blight (FHB), caused by *Fusarium graminearum*, under field conditions than cv. Goldfield. The cleistogamous nature of Goldfield is likely responsible for its low incidence of FHB. To study the inheritance of flower opening width and duration in relation to incidence of FHB, Patterson and Goldfield were crossed to generate a recombinant inbred (RI) population consisting of 100 F_2-derived lines. The population was characterized for FHB incidence and heading date in eight field environments. Flower opening characteristics of the RI lines and the parents were characterized in three tests over time in a greenhouse and one test in the field, in 2003. An ANOVA revealed significant variation for both incidence and flower opening characteristics, with the ranking of lines typically consistent across environments. A scatter plot of FHB incidence vs. flower opening width suggests that the smaller the flower opening width, the lower the incidence. Research is in progress to identify DNA markers that are associated with cleistogamous flowering/low FHB incidence, which would be valuable in future breeding research to reduce crop production and grain quality losses in wheat due to FHB.

COMPARISON OF FHB DEVELOPMENT ON HARD WINTER WHEAT USING DIFFERENT PLANTING SCHEMES

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ABSTRACT

Fusarium head blight (FHB) is a destructive disease of wheat causing yield loss and poor grain quality. Winter wheat producers in South Dakota have adopted a reduced tillage cropping system and have increased production of winter wheat in traditional corn-soybean rotations. These practices could lead to an increase in FHB severity. The winter wheat breeding program at South Dakota State University has established a proactive effort to develop FHB-resistant hard winter wheat varieties. Transplanted hill nurseries have been screened since 1999 utilizing an established mist-irrigated field screening nursery designed to test cultivars, elite lines, and preliminary lines for FHB resistance. However, transplanting winter wheat is time consuming, involving vernalizing seedlings in cold chambers followed by hand planting. Also, the poorly established root system in transplanted wheat often leads to poor plant development. Furthermore, the laborious transplanting process does not follow the conventional direct seeding method followed by wheat producers. Therefore, we investigated planting schemes to determine if direct seeded row materials are affected differently than transplanted hill plots when they are inoculated with FHB. In October 2000, several multi-location field trials, including the South Dakota Crop Performance Trials (CPT), were directly seeded into the FHB nursery. The CPT trials were also vernalized and transplanted in May 2001. Significant correlations between the two types of planting techniques were observed for FHB severity and disease indices. However, the FHB incidence for the direct seeded rows was low and was not significantly correlated with the incidence levels in the transplanted hills. This was perhaps due to the early flowering of the direct seeded materials. Also, the cooler temperatures at anthesis may have inhibited FHB development. In 2002 and 2003, we investigated transplanted seedling performance in comparison to delayed seeded CPT lines. The CPT and several other trials were directly seeded on November 26, 2001 and October 25, 2002. The planting scheme helped delay flowering by approximately two to three weeks compared to conventional timely seeding. In May 2002 and 2003, the CPT trial was transplanted into the mist-irrigated field nursery. Significant correlations ($P < 0.01$) between the two types of planting techniques in 2002 and 2003 were observed for FHB disease index. Correlations between the direct planting techniques across years were also significant ($P < 0.05$). Correlation coefficients among transplanted hill nurseries across years were not significant, however. These results suggest that delayed direct seeding should replace transplanting for efficient scab screening in hard winter wheat. However, transplanted hills should be used if improper weather conditions prevent a successful direct seeded nursery.

USE OF ROMANIAN WINTER BREAD WHEAT LINE *FUNDULEA 201R* IN BREEDING FHB RESISTANCE

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ABSTRACT

Fusarium head blight (FHB, scab) is one of the main wheat diseases in Romania, because wheat-maize is a common rotation and because high humidity during flowering time is frequent in some areas and years. A winter breadwheat breeding program for resistance to FHB was started at Fundulea more than 20 years ago, in order to reduce yield losses produced by this disease.

Complex crosses between previously identified sources of moderate resistance to FHB and selection under artificial inoculation have produced several lines, that combine better FHB resistance with improved agronomic type and resistance to other diseases. Among them, resistance to FHB has been more documented in the advanced line *Fundulea 201R*.

The winter wheat line *F201R*, is a transgressive derivative of crosses between several sources of moderate resistance, not related to the Chinese resistant germplasm. It has shown high levels of resistance to FHB in several environments in Romania and in other countries, when various inoculation and assessment methods were used. Recently, investigation of quantitative trait loci (QTL) performed at Purdue University, U.S.A. revealed the presence of four interval regions located on chromosomes 1B, 3A, 3D and 5A, that together accounted for 43.0% of the genotypic variation in FHB resistance of *F201R*. In contrast to the Chinese resistant cultivar Sumai 3, *F 201R* is resistant to the main foliar diseases (powdery mildew, leaf and stripe rusts and septoriosis) and has good winterhardiness. However, like Sumai 3, this line has poor bread making quality. As a consequence, improving bread-making quality in FHB resistant germplasm became a major breeding objective. This proved to be a difficult task, because of the complex genetic control of FHB resistance and because one of the major QTLs for FHB resistance is located on the translocated chromosome 1B/1R of *F201R*.

However, after several cycles of breeding, using crosses between *F201R* and several donors for high breadmaking quality (Dropia, Delabrad, Boema from ARDI-Fundulea, Romania and Karl from KS, U.S.A.) we can report some progress.

Data are presented for 17 advanced lines derived from such crosses, in which the level of resistance was assessed under field artificial inoculation by point inoculation technique and breadmaking quality was estimated using the sedimentation value (sv).

Four of the 17 lines combined good levels of FHB resistance (AUDPC -% damaged spikelets- ranging between 180 and 271, when in *F201R* AUDPC was 122) with acceptable sedimentation values (sv=55-63, as compared to sv=42 in *F 201R*).

The investigation of response to FHB was also performed in doubled haploid (DH) lines derived from a cross between *F201R* (FHB resistant) and Boema (high bread making quality). Based on resistance defined as AUDPC and relative weight of grains (RW, as % of control), lines with reaction to FHB, similar to the resistant parent and bread making quality were identified.

Results indicate that combination of resistance to FHB with desired agronomic traits is a difficult but feasible breeding objective, in order to obtain new winter wheat cultivars more adapted for cultivation in conventional and organic farms.

AN ALTERNATIVE TO THE FHB INDEX: INCIDENCE, SEVERITY, KERNEL RATING (ISK) INDEX

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ABSTRACT

The FHB index is widely used by researchers working on Fusarium head blight (FHB) to express the resistance of breeding lines and cultivars. The FHB index is calculated by multiplying the incidence in percentage by the severity in percentage and dividing the product by 100 to express the FHB index on a scale of 0-100. We propose a new index that incorporates a third factor, the evaluation of harvested grain. We believe it is important to include the evaluation of the grain in an index to be used for selection of scab resistant breeding lines. The incidence, severity, kernel rating index (ISK index) is calculated as follows: 0.3 x (incidence in %) + 0.3 x (severity in %) + 0.4 x (FDK in %) or 4 x (kernel rating on 0-9 scale). The limits for the ISK index are 0-100 (or 0-96 if the kernel rating scale is used). Because the FHB index is calculated by multiplying the incidence and the severity, the variability of the FHB index is often greater than either the incidence or severity separately. Due to the variability associated with the FHB index, in many cases, FHB index means are significantly different only when they differ by large amounts. We have found that mean separation is better with the ISK index than with the FHB index. Susceptible and resistant breeding lines can be separated with either the FHB index or the ISK index; however, the ISK index is more effective for the separation of moderately resistant and very resistant breeding lines. Thus, the advantages of the ISK index over the FHB index are: 1) the ISK index incorporates a harvested grain evaluation, and 2) the ISK index provides better separation of means than the FHB index.

IDENTIFICATION OF QTLS IN THE HARRINGTON/MOREX BARLEY POPULATION FOR FHB REACTION, MATURITY, AND PLANT HEIGHT

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OBJECTIVES

To evaluate the Harrington/Morex (HM) doubled-haploid population for FHB reaction, heading date, and plant height in both long- and short-day environments and to estimate the number, genome location, and importance of quantitative trait loci (QTLs) associated with these traits.

INTRODUCTION

Development of spring barley, *Hordeum vulgare*, cultivars with resistance to Fusarium head blight (FHB), incited by *Fusarium graminearum*, is difficult because the inheritance of FHB resistance and its interactions with morphological traits are complex. These traits map near the six-rowed spike 1 (*vrs1*) locus in the proximal region of chromosome 2HL. Testing of doubled-haploid lines from the HM mapping population may reveal more information about the 2H associations. Harrington, a Canadian two-rowed cultivar, and Morex, a Midwest six-rowed cultivar, are recommended for malting in the US. Testing HM population in both long- and short-day environments could aid evaluation of plant height and maturity genes.

MATERIALS AND METHODS

The HM lines, their parents (Harrington and Morex), and the checks (CIho 4196, Bowman, and Conlon) were arranged in a randomized complete block design with two replicates, and grown in two environments in 2002: Langdon, ND (LA02) and Osnabrock, ND (OS02) and three environments in 2003: Hangzhou, China (CH03), Langdon, ND (LA03), and Osnabrock, ND (OS03). Plant height was measured in the CH03, LA03, and OS03 nurseries as centimeters from the soil surface to the tip of inflorescence excluding awns. Heading date was estimated in the CH03 and OS03 nurseries as the number of days from January 1 to when approximately 50% of the heads were half emerged from the boot. Fusarium inoculum was prepared according to Prom et al. (1996). Disease readings were taken at the soft dough stage. Counting the number of infected kernels and dividing by the total number of kernels per spike multiplied by 100 determined the severity of FHB. Assessments were made on 10 randomly selected spikes per plot. Analyses of variance for FHB severity, heading date, and plant height were conducted for each environment by means of GLM procedures of SAS (1990). Error mean squares across all the environments were not homogeneous as determined by Bartlett's chi-square test; thus, a combined ANOVA across environments was not conducted. Phenotypic data sets on HM lines from the five environments, and the published 107-marker linkage map for the HM population (Marquez-Cedillo et al., 2001) were used to perform QTL analyses with the software package NQTL. Both simple interval mapping (SIM) and simplified composite interval mapping (sCIM) techniques were used for QTL detection (Tinker and Mather, 1995). Each data set was analyzed with 1000 permutations, a 5-cM walking speed, and a Type-I error rate of 5%. Coinci-

dent peaks with both SIM and sCIM analysis above the significance threshold were used to declare the presence of QTL.

RESULTS AND DISCUSSION

FHB results - Only the results at specific sites are reported. Significant differences among HM lines were observed for all traits that were evaluated. The differentiation among lines for FHB reactions appeared best for the OS02, CN03, and LA03 nurseries (Table 1). Phenotypic correlation coefficient values for FHB severity among all environments were significant with a range of 0.40 to 0.69. The moderately resistant parent (Harrington) exhibited lower FHB severity than the moderately susceptible parent (Morex). Few HM lines had lower FHB scores than Harrington and several had higher FHB scores than Morex. With the exception of Chinese nursery, most lines were within the bounds of LSD (P=0.05) and were not transgressive segregates for increased FHB resistance. When averaged over locations, some HM lines appeared as resistant as the resistant check CIho 4196.

Table 1. Mean FHB severity, heading date, and plant height for Harrington/Morex lines, parents, and checks.

Line	Spike type	FHB %				Heading date days from Jan 1		Plant Height cm	
		2002	2003		Five Locations	2003		2003	
		Osnabrock	China	Langdon	Mean	China	Osnabrock	China	Langdon
Checks									
CIho 4196	2	8.7	0.1	22.1	12.0	115.4	201.5	109.1	114.0
Harrington	2	**10.3**	**20.3**	**30.5**	**16.7**	**108.0**	**206.5**	**93.1**	**92.5**
Bowman	2	11.0	32.5	32.9	27.3	102.2	198.0	91.8	87.0
Conlon	2		29.8	37.9		101.4	193.0	93.2	88.5
Morex	6	**25.2**	**37.1**	**40.9**	**35.4**	**97.2**	**195.0**	**110.2**	**96.0**
HM μ		21.1	26.4	39.7	29.1	105.3	200.2	104.2	96.7
σ		3.9	4.5	6.6		1.2	1.8	6.7	2.9
Less susceptible									
HM 124	2	6.6	8.8	26.0	11.4	107.3	206.0	116.7	110.5
HM 1	2	12.1	2.0	19.1	13.1	111.8	204.5	115.2	125.0
HM 244	2	4.4	6.6	22.5	15.0	107.1	199.5	114.7	108.0
HM 49	2	6.3	10.1	25.9	15.7	103.8	199.5	125.3	107.0
More susceptible									
HM 72	6	41.1	69.3	45.6	47.4	101.1	201.0	91.7	76.5
HM 33	6	35.2	66.3	60.1	47.5	103.9	196.0	88.4	81.5
HM 145	6	35.9	50.4	53.8	47.9	100.9	198.0	93.8	74.5
HM 73	6	46.3	70.1	78.7	52.9	106.3	202.0	85.1	77.5
LSD 0.05		10.9	12.5	18.5		3.2	5.1	18.5	8.0
CV		26.1	23.9	23.6		1.5	1.3	9.1	4.2

Heading date and plant height results - Harrington was later and shorter than Morex in all tests (Table 1). The frequency distributions in the HM population for all traits, except spike type, were continuous. A few transgressive segregates for plant height were noted, but not for heading date. Maturity was significantly and negatively correlated with FHB severity in two environments where days to heading were recorded. Maturity in both long- and short-day conditions was significantly and positively correlated. Plant height was significantly and negatively correlated with FHB severity. These data support previous findings about the general tendency of tall, late plants to be more FHB resistant.

Identification of QTLs -The largest QTL for FHB resistance was present in chromosome 2H and was detected in all five environments (Fig. 1A and 1B). A second, but smaller, QTL for FHB was found in 4H in all tests. The largest QTL for heading date was detected in 2H. At the short-day site, peaks in 4H and 7H were significant and peaks in chromosomes 1H and 5H approached significance (Fig. 1C). Two QTLs for plant height detected in 2H and 4H according to the coincident peaks of SIM and sCIM analyses (Fig. 1D). An additional plant height QTL in 7H was found in only two environments. Marquez-Cedillo et al. (2001) previously reported plant height and maturity QTLs in 2H in the HM population.

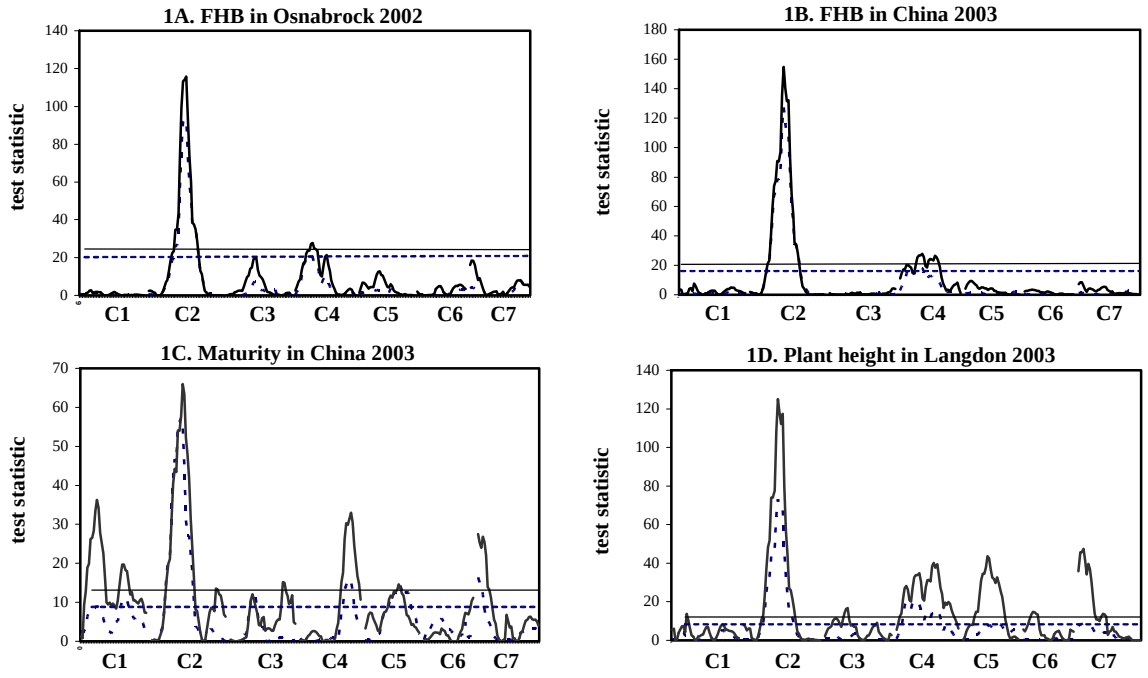

Figure 1. Scans of test statistics (Y-axis) for simple interval mapping (SIM, broken lines), simple composite interval mapping (sCIM, solid lines) for Harrington/Morex DH lines. Chromosomes 1 (7H), 2 (2H), 3 (3H), 4 (4H), 5 (1H), 6 (6H), and 7 (5H) are shown on the X-axis. Horizontal lines indicate corresponding thresholds for testing SIM and sCIM.

Trait relationships - Harrington contributed QTLs for FHB resistance, increased plant height, and late heading. The HM lines with lowest FHB readings were tall and two-rowed, while the lines with highest FHB readings were short and six-rowed (Table 1). Other studies placed at least two plant height genes (*hcm1* and *lin1*) and one heading date gene (*Eam6*) near the *vrs1* locus in 2H (Franckowiak and Lundqvist, 2002). These genes are present in Morex and can partially explain the observed trait associations in 2H. One previous suggested that the *Eam6* gene is expressed in short-day environments. Tohnooka et al. (2000) reported that the QTL for short-day response in the Steptoe/Morex doubled-haploid population is located in 2H with minor factors in 1H, 4H, and 7H. None of the HM lines headed earlier than Morex in China. These results suggest that accumulating desirable factors for FHB resistance and associated morphological traits in cultivars adapted to the Upper Midwest will be difficult.

CONCLUSIONS

1. Lines with FHB reaction levels similar to those of CIho 4196, the resistant check, were observed in the Harrington/Morex DH population.

2. QTLs for FHB resistance were located primarily in chromosome 2H near major genes that control plant height and maturity.

3. Testing of the HM lines under short-day conditions revealed the presence of several QTLs that contribute to earliness in Morex.

4. Both adverse linkages and a number of minor genes for early heading have made development of early, FHB resistant barley cultivars difficult.

ACKNOWLEDGEMENTS

The authors would like to thank Bingxin Zhang, Jason Faller, and Yongliang Sun for their assistance with FHB nursery management and inoculation of nurseries at Hangzhou, Osnabrock, and Langdon, respectively. This paper is based upon work supported by the American Malting Barley Association, Inc. and the U.S. Department of Agriculture, under Agreement Nos. 59-0790-9-034 and 59-0790-9-043. This is a cooperative project with the U.S. Wheat & Barley Scab Initiative.

DISCLAIMER

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

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SCREENING ELITE SOUTH DAKOTA WINTER WHEAT FOR SSR MARKERS LINKED TO FUSARIUM HEAD BLIGHT RESISTANCE

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ABSTRACT

Resistance to wheat (*Triticum aestivum* L.) Fusarium head blight (FHB) -caused by *Fusarium graminearum* Schwabe - is a very complicated quantitative trait. Marker assisted selection (MAS) could be a useful tool to enhance the efficiency of FHB resistance breeding. A major FHB resistance QTL, *Qfhs.ndsu-3BS*, has been identified in Spring wheat cultivar Sumai 3, and SSR markers *Xgwm389-135*, *Xgwm493-190*, *Xgwm533-98* and *Xgwm533-145* were found to be linked to this QTL. In this study, we screened 61 elite winter wheat lines for these four markers. The results indicated that 22 wheat lines had either *Xgwm533-98* or *Xgwm389-135* marker. However, none of the 61 winter lines had either *Xgwm493-190* or *Xgwm533-145*, the two SSR markers that are tightly linked to the *Qfhs.ndsu-3BS* FHB-resistance QTL. Of the four markers, *Xgwm389-135* was the most polymorphic. An *Xgwm533-120* allele, which was a diagnostic marker for stem rust resistance gene *Sr2*, was observed in 35 wheat lines.

RESISTANCE OF GENOTYPES OF THE UNIFORM SOFT RED WINTER WHEAT FHB NURSERY AND INTERNATIONAL GENOTYPES TO FHB

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OBJECTIVES

The main task of the experiment was to test of the uniform soft red winter wheat FHB nursery and its comparison with several advanced Hungarian and international genotypes.

MATERIALS AND METHODS

Each genotype was sown on a 5 m^2 plot at 19 October 2002. Germination was normal, in the second decade of December – 17-19 ^oC frost came without snow. The spring wheats were all killed, therefore Sumey-3 and Nobeoka Bozu could not have been tested. Several winter wheat genotypes suffered also, but mostly survived without problem the long winter. Inoculation was made at full flowering with 2 *F. graminearum* and two *F. culmorum* isolates (see Table 1) separately having different aggressiveness. Inoculation, evaluation of data followed Mesterhazy (1995, 2001) and Mesterhazy et al. (1999). Four evaluations of FHB were made rating the percentage of diseased spikelets. As the weather was hot and dry a fifth evaluation could not have been done, harvest was 14 days earlier than normal. At full ripening the infected groups of heads were harvested, ten heads were randomly separated, threshed carefully at low wind speed, weighted and rated for FDK. Two FDK ratings were made, in the first case the medium to severe FDK was rated, in the second only the severe. The genotypes B 980582 and B 011066 had white color grains, here the evaluation needed more care the contrast between normal a scabby grains was smaller than usual.

RESULTS AND DISCUSSION

Table 1 shows the data across the isolates, ranked by the severe FDK values. Mean of the FHB values is 33 %, for all FDK it is 59 and for severe 45 % and mean yield loss is 48 %. The variation width is very high, from very low to very high values all possibilities occur. Even the correlations between traits are close and highly significant, important genotypic deviations were recorded. There were several genotypes like B 011066 that had a susceptible head reaction, high FDK value for medium to severe infected grains, but rated much lower for severe FDK ratio. The opposite was Huba with relatively low FHB value, but its FDK was very high, 80 and 34 % for the severely infected grains. Normally the difference was much lower; in the most susceptible genotypes the difference was minor. Fundulea 201R had a better than medium FHB rating, but surprisingly very high FDK values above 70 %. Arina, the famous Swiss medium resistant cultivar made the same performance, relatively low FHB with very high FDK values. The most resistant genotypes had lower than 10 % FHB infection, up to 30 % medium and severe FDK, up to 23 % severe FDK and up to 30 % yield loss. Cooker 9835 was the most susceptible with nearly 80 % FHB, more than 80 % FDK and 80 % yield loss. Among Hungarian materials, not presented here, many such lines occur.

Even the resistance differences were considerable, full resistant material has not been found. However, the first four genotypes carry resistance from Nobeoka Bozu, the Japanese spring wheat resistance source. The

Sgv/NB//MM/Sumey3 line performed also well, presenting the lowest FHB value, but for FDK it was less strong. They are much more adapted than their spring wheat ancestors, but they are not yet suitable for a cultivar. However, crosses with these lines promise good lines for variety production.

Table 1. Resistance of American and several international wheat genotypes to FHB, 2003. Data: Means or four isolates.

Genotypes	FHB %	FDK(1) %	FDK (2) %	Yield loss %
81.60/NB//Ko	9.00	3.72	3.50	33.43
Zu//Ré/NB	4.98	28.75	9.63	44.97
RSt/NB	19.29	34.17	9.83	33.60
RSt/NB	7.90	29.58	12.83	35.24
Sgv/NB//MM/Sum3	1.25	20.00	13.00	20.00
ARGE 97-1033-3-5	3.15	25.83	16.00	17.47
Zu//Ré/NB	8.27	25.00	16.25	26.63
B 980582	14.71	25.83	18.33	44.81
ARGE 97 1047-4-2	8.19	29.17	22.25	30.39
ARGE 97 1038-3-5	15.33	31.67	22.33	41.02
NC 99-13296	28.10	35.00	27.08	41.58
B 011066	46.79	74.17	27.08	77.08
NC 98-26192	17.29	35.83	28.42	45.82
Huba	28.83	80.00	34.58	68.66
VA 00 W 566	46.88	40.83	37.08	66.40
B 980416	31.81	53.33	37.92	59.73
ERNIE	17.98	47.50	38.75	23.82
Saman	37.06	44.38	38.75	50.97
ARGE 97-1042-4-5	11.21	53.33	41.25	29.86
Mérő	29.54	71.25	41.25	58.84
AR 857-1-1	18.85	70.00	45.00	47.48
92.111466/Sum3/81.61//Ko	21.88	57.50	45.00	48.48
VA 02 W 713	50.52	46.25	46.25	56.75
ARGE 97 1048-3-6	33.50	62.50	46.67	61.34
VA 01 W 461	54.52	71.25	50.00	80.40
VAN 98 W 342	30.79	77.08	50.42	44.62
92.1117///RSt//MM/NB	30.10	60.00	51.88	60.91
NC 9913308	34.06	68.33	53.33	33.25
VA 00 W 562	35.98	63.33	55.42	47.63
AR 857-1-2	26.63	80.00	55.83	29.08
AR 93019-2-1	41.92	77.92	55.83	60.46
MV 15-42	36.52	65.83	56.25	54.02
B 011117	34.52	70.42	59.58	59.94
GA 931587 E 53	55.15	76.25	62.50	68.86
MV 5-46	39.33	75.00	64.58	53.38
MV 27-28	37.88	81.67	65.83	58.13
GA 931630 E 48	38.23	80.83	65.83	49.71
Ar 93035-4-1	68.10	89.17	68.33	79.46
VA 02 W 732	47.19	79.58	68.75	75.19
Fund.201R	37.65	77.50	71.25	61.61
GA 94261 E 7	62.13	92.92	72.92	83.02
Arina	33.90	95.25	78.75	52.90
GA 941208 E 35	64.73	91.67	79.67	65.48
GA 931233 A 24	78.33	87.92	82.00	82.49
COKER 9835	79.73	91.33	82.50	80.54
Mean	32.88	59.53	45.12	47.88
	2.14	6.82	7.98	2.36

Trait	FHB	FDK(1)	FDK (2)
FDK(1)	0.765212		
FDK (2)	0.806421	0.911796	
Yield loss	0.861387	0.693261	0.643818

All are significant at P = 0.1 %

Saman and Huba are new candidates for cultivars with good medium resistance and Saman has better parameters, especially for yield loss. Both are high yielding and have good baking quality.

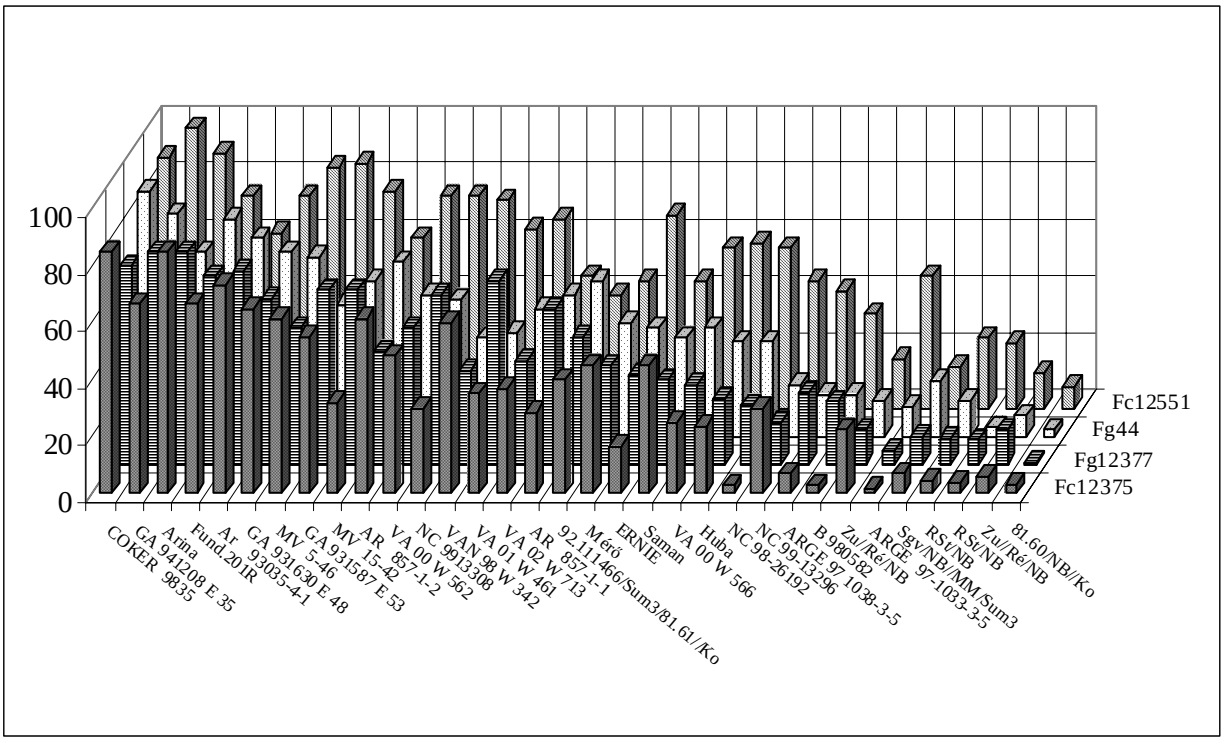

Figure 1. Resistance tests for FHB in wheat genotypes, severe FDK data of some entries according to the isolates used.

Evaluating the resistance, yield response is also important. We need all four parameters in the positive range to have a stability of resistance.

Figure 1 shows the isolate specific data for FDK. It presents clearly that the response of the cultivars is not the same. In most cases as here also, the cultivar x isolate interaction is significant. When we use five inocula of the same isolate, we have the same picture. Therefore these data are not arguments for races. However it shows that a possible reliable evaluation of resistance needs the response as a mean of several aggressiveness level. So the data will not be only more reliable, but the resistance level can also be determined with a higher preciosity. Here we cannot have the case that by using a less aggressive isolate most of the material will be "resistant" and next year full of FHB at a severe epidemic.

We have now the problem, which level of resistance secures excellent field resistance. From these data it is not possible to set e limit value. When a natural epidemic occurs, such a set of cultivars will show according to their natural infection severities where is about the limit value. When we would not have more susceptible cultivars than the limit represented by Ernie it should be an acceptable value. In Hungary possibly less is enough like Saman. For practical reasons it is reasonable to select from the better half of the population tested.

We should stress that this is one year result. Comparing with the other location it seems that a good agreement is between data, even they were produced by different methods. Large differences can be identified well.

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DEVELOPING FHB-RESISTANT SOFT RED WINTER WHEAT VARIETIES FOR THE MID-SOUTH

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INTRODUCTION

Wheat varieties resistant to Fusarium head blight (FHB) are likely to be an important component of any integrated management strategy for FHB. In the Mid-South (Arkansas, Louisiana, Mississippi, and Tennessee), FHB resistance has not been as high of a priority for growers because FHB epidemics have occurred less frequently than diseases such as stripe rust, leaf rust, leaf blotch, and soilborne mosaic. Consequently, before FHB-resistant varieties will be accepted by growers, FHB resistance will need to be incorporated into agronomically suitable, high-yielding varieties with adequate resistance to other diseases. To achieve this goal, the Arkansas Breeding Program is developing resistant varieties as part of its on-going program, and the Arkansas Germplasm Enhancement Program evaluates all FHB winter wheat nurseries for resistance and is developing adapted breeding lines by transferring genes for resistance to FHB and other diseases into soft red winter wheat backgrounds.

MATERIALS AND METHODS

The Arkansas Breeding and Germplasm Enhancement Programs began making crosses specifically for FHB resistance in 1993 and 1997, respectively. Lines developed from these crosses have been selected each season for adaptation, yield, and resistance to FHB and other diseases. Evaluations of the most advanced lines are reported here as a progress report.

RESULTS AND DISCUSSION

Of the 32 entries evaluated in the Southern Winter Wheat FHB Nursery, several AR lines developed by the breeding program and ARGE lines developed by the germplasm enhancement program ranked among the top five entries for FHB incidence, severity, index, scabby seed, DON, and type 2 resistance in the greenhouse (Table 1). Several advanced lines have resistance to FHB as well as stripe rust and leaf blotch which are important diseases in the region (Table 2). ARGE lines were similar to checks for milling score, test weight, and flour protein, but most lines ranked below the checks for baking score, softness equivalent, and flour yield (Table 3). Fifteen advanced lines had yields that were not significantly different from the recently released variety, Pat (Table 4).

Arkansas began developing FHB-resistant soft red winter wheats before the US Wheat and Barley Scab Initiative existed. Funding from the Initiative has allowed the expansion of FHB activities, and the Arkansas program has made valuable contributions toward developing resistant varieties.

ACKNOWLEDGMENTS

We thank participants in the Southern Winter Wheat FHB Nursery for data across locations, Charles Gaines and the staff at the USDA, ARS Soft Wheat Quality Laboratory for the milling and baking data, and Steve Harrison for evaluating lines during the selection process.

Table 1. Performance of advanced lines from Arkansas averaged across all locations in the 2003 Southern Winter Wheat FHB Nursery.

Line[1]	FHB						Scabby		Vomitoxin		Heading		Plant		Greenhouse	
	Incidence		Severity		Index		seed		DON		date		height		severity	
	0-100	Rank	0-100	Rank	0-100	Rank	%	Rank	ppm	Rank	Julian	Rank	in.	Rank	0-100	Rank
ERNIE	49.6	5	23.5	13	13.9	9	27.6	8	6.5	17	129	2	31	8	15	2
COKER 9835	77.8	32	57.3	32	48.9	32	58	32	11.4	31	133	29	29	3	46	24
AR 857-1-1	58.2	11	16.9	3	11	5	32.1	14	4.4	7	132	25	38	31	31	14
AR 857-1-2	48.3	3	15.7	2	10	3	23.4	3	4.1	5	132	26	37	30	51	27
AR 93019-2-1	59.6	15	25	16	19.4	16	46.7	30	7.7	24	134	30	40	32	53	30
AR93035-4-1	65.7	21	26.7	18	23.3	21	49.6	31	7.2	22	132	27	34	20	33	18
ARGE 97-1042-4-5	44.4	2	19.2	9	10.2	4	30.2	10	3.3	2	130	9	35	22	23	3
ARGE 97-1033-3-5	44.3	1	10.7	1	4.5	1	25.2	4	4.1	6	132	23	37	28	14	1
ARGE 97-1048-3-6	51.5	7	17.7	7	14.6	11	27	6	4.5	8	132	22	37	29	23	5
ARGE 97-1038-3-5	54.7	10	20.4	11	12.2	7	27.3	7	3.3	3	130	8	35	24	35	22
ARGE 97-1047-4-2	50.7	6	17	5	9.8	2	31.9	12	3.1	1	129	1	36	26	44	23

[1]Ernie = resistant check, Coker 9835 = susceptible check, Ranks based on 32 total lines in the nursery.

Table 2. Disease ratings for advanced Arkansas lines in 2003.

Line	FHB % diseased florets			% Scabby grain	% Stripe Rust	% Leaf Blotch	% Green leaves
	Fayetteville[1]	Kibler[2]	Greenhouse[3]	Kibler	Fayetteville[4]		Kibler[5]
AR 93095-4-1	50	32	23	85	4	40	69
AR 93035-4-1	36	40	35	88	0	12	60
AR 93035-4-3	39	35	44	83	0	11	60
AR 93035-4-4	40	39	40	85	2	17	50
AR 93035-4-2	41	36	30	83	4	36	55
AR 93035-7-1	44	38	52	85	1	20	50
AR 93108-1-3	46	48	30	93	2	31	45
AR 93091-4-2	44	32	57	68	25	56	60
AR 93108-9-1	46	46	43	98	4	66	31
AR 93108-3-2	48	40	16	90	0	76	65
AR 93069-5-1	36	40	58	70	2	76	32
AR 93019-2-1	64	71	69	100	5	69	44
AR 93001-3-2	29	26	18	60	0	31	78
AR 878-2-1	28	28	19	68	4	59	45
AR 857-1-2	19	16	28	45	2	40	85
AR 857-1-1	22	21	62	64	2	45	78
AR 880-5-1	25	25	32	76	0	65	64
AR 922-5-1	36	19	13	48	2	81	69
ARGE 97-1022-5-1	34	39	40	88	0	20	81
ARGE 97-1042-4-5	30	38	9	88	0	18	50
ARGE 97-1043-6a-5	22	31	12	75	0	14	78
ARGE 97-1033-3-5	20	16	6	59	0	10	60
ARGE 97-1033-10-2	28	28	6	85	15	31	64
ARGE 97-1048-3-6	19	29	12	83	5	20	78
ARGE 97-1064-11-5	35	35	21	85	0	19	78
ARGE 97-1064-13-5	24	24	20	85	2	18	85
ARGE 97-1038-3-5	36	34	32	83	0	28	76
ARGE 97-1008-3-3	26	30	20	80	4	31	78

Table 2. Continued

Line	FHB % diseased florets			% Scabby grain	% Stripe Rust	% Leaf Blotch	% Green leaves
	Fayetteville[1]	Kibler[2]	Greenhouse[3]	Kibler	Fayetteville[4]		Kibler[5]
ARGE 97-1010-3-5	32	40	32	85	4	50	64
STANDARD[1]	41	34	38	65	0	17	76
ARGE 97-1047-4-2	28	24	35	68	0	35	83
Ernie	38	18	9	75	18	44	32
Agripro Patton	29	24	55	94	66	20	23
Agripro Mason	46	33	53	93	0	36	58
Pioneer 2684	58	46	13	90	5	83	42

[1]Mean of four replications rated on May 27 at soft dough stage

[2]Mean of four replications rated on May 19 at soft dough stage

[3]Center floret inoculation, mean of three replications with four to eight heads per replication

[4]Percentage of flag leaf diseased, mean of four replications rated on May 28

[5]Mean of four replications rated on May 12; leaf blotch and stripe rust were the principal diseases

Table 3. Milling and baking scores from the USDA, ARS Soft Wheat Quality Lab for ARGE FHB-resistant lines.

LINE	MILLING QUALITY SCORE		BAKING QUALITY SCORE		TEST WT. SCORE		SOFT. EQUIV. SCORE		MICRO T.W. LB/BU	SOFT. EQUIV. %		FLOUR YIELD %		FLOUR PROT. %	LACTIC ACID RET'N	COOKIE DIAM. CM.		TOP GR.
STANDARD[1]	64.0	C	65.3	C	72.3	B	60.5	C	61.7	54.6		71.1		9.59	113.1	18.11		2
97-1022-5-1	60.2	C	36.3	F	67.6	C	56.3	D	61.1	52.7		70.2	Q	10.42	113.5	16.95	Q	1
97-1042-4-5	65.9	C	-1.2	F	91.3	A	26.1	F	63.9	39.3	Q	71.6		10.38	116.6	15.45	Q	0
97-1043-6a-5	61.2	C	28.8	F	79.7	B	54.8	D	62.5	52.1		70.4		10.93	104.1	16.65	Q	1
97-1033-3-5	63.9	C	-3.0	F	84.4	A	21.2	F	63.1	37.1	Q	71.1		9.93	113.2	15.38	Q	0
97-1033-10-2	67.4	C	-6.2	F	84.6	A	22.4	F	63.1	37.6	Q	72.0		10.09	117.8	15.25	Q	0
97-1048-3-6	54.5	D	23.3	F	89.0	A	36.9	F	63.7	44.1	Q	68.7	Q	10.05	101.8	16.43	Q	1
97-1064-11-5	55.3	D	27.6	F	73.9	B	61.9	C	61.9	55.2		68.9	Q	9.64	139.0	16.60	Q	0
97-1064-13-5	58.7	D	36.3	F	68.0	C	66.5	C	61.2	57.3		69.8	*	10.72	114.3	16.95	Q	1
97-1038-3-5	57.0	D	37.6	F	63.7	C	46.4	E	60.6	48.3	Q	69.3	Q	9.38	109.6	17.00	Q	1
97-1008-3-3	56.1	D	52.0	D	83.2	A	51.4	D	63.0	50.5	*	69.1	Q	9.77	102.2	17.58	Q	2
97-1010-3-5	50.1	D	18.5	F	77.8	B	55.4	D	62.3	52.3		67.6	Q	10.74	105.1	16.24	Q	0
97-1060-5-5	61.6	C	29.8	F	82.5	A	57.5	D	62.9	53.3		70.5		10.03	108.5	16.69	Q	1
97-1047-4-2	58.0	D	38.6	F	75.0	B	54.5	D	62.0	51.9		69.6	*	9.58	110.6	17.04	Q	2
Agripro Mason	63.4	C	46.1	E	76.4	B	69.9	C	62.2	58.8		71.0		9.45	119.9	17.34	Q	1
Pioneer 2684	64.0	C	65.3	C	72.3	B	60.5	C	61.7	54.6		71.1		9.59	113.1	18.11		2
NK Coker9663	59.1	D	56.3	D	70.3	B	54.9	D	61.4	52.1		69.9	*	8.13	111.6	17.75	*	3

[1]Standard was Pioneer 2684

* = values was one LSD below the standard

Q = Values was two or more LSDs below the standard

Table 4. Performance of breeding lines and checks in the Scab Yield Nursery in 2003 across two locations in Arkansas (Stuttgart and Marianna).

Entry	Yield	Test weight	Lodging	Plt ht	Heading date	Maturity date	Leaf rust
	bu/A	lb/bu	%	in.			%
PAT	73.9	54.6	1	38	4/22	5/24	2
AR93108-3-2	71.1	54.2	3	35	4/16	5/17	0.7
AR93035-4-2	71.0	54.6	1	33	4/21	5/21	3.3
ARGE971043-6a-5	70.7	55.5	3	37	4/21	5/20	0.3
AR93035-4-1	70.7	55.4	0	35	4/23	5/22	2.7
AR93035-4-3	69.7	56.2	0	36	4/22	5/22	3
AR93035-4-4	69.2	54.4	0	35	4/21	5/23	3
ARGE97-1033-10-	69.0	54.9	13	40	4/22	5/23	1
AR93095-4-1	68.9	52.1	13	39	4/22	5/17	2
AR93108-1-3	68.0	53.1	12	32	4/18	5/20	2
ARGE97-1022-5-1	67.3	54.5	8	36	4/21	5/21	1.3
AR93001-3-2	66.9	56.3	16	37	4/16	5/19	2
AR93069-5-1	66.7	57.3	7	37	4/18	5/20	2
AR93035-7-1	66.6	54.7	0	34	4/20	5/21	3.3
AR93108-9-1	66.4	52.7	5	33	4/16	5/16	3.7
AR922-5-1	66.4	56.8	19	37	4/16	5/18	1.7
AR93019-2-1	66.1	52.1	0	33	4/21	5/21	2.3
AR857-1-2	64.4	55.3	8	41	4/15	5/16	.
AR857-1-1	63.7	55.7	6	40	4/15	5/16	.
ARGE97-1064-13-	62.9	52.6	13	41	4/20	5/23	0.7
ARGE97-1008-3-3	62.4	56.2	15	39	4/17	5/25	1.7
ARGE971064-11-5	62.2	52.8	16	39	4/20	5/23	2.7
AR880-5-1	62.1	53.9	13	37	4/18	5/19	2
ARGE971010-3-5	60.7	55.7	28	38	4/17	5/20	0
ERNIE	60.7	54.0	11	37	4/21	5/23	1.3
AR878-2-1	60.3	55.2	4	39	4/15	5/18	4
AR93091-4-2	59.7	54.9	6	37	4/21	5/24	0.3
ARGE97-1060-5-5	56.6	55.2	41	40	4/20	5/19	1
Mean	65.9	54.7	9.3	36.9	4/19	5/20	1.9
LSD05	7.5	1.4	13.3	4.2	3.2	4.1	1.6
CV%	10.0	2.3	125.6	5.6	0.4	0.4	52

THE DEVELOPMENT OF FUSARIUM HEAD BLIGHT TOLERANT VARIETIES OF WHEAT IN NEBRASKA FROM 2001 TO 2003

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ABSTRACT

Wheat germplasm that is tolerant to Fusarium head blight (FHB, scab) will be the basis for cultivar development in high rainfall and irrigated acreage, which is at high risk to FHB infection, in the Central Great Plains. The primary objective is to identify and develop elite winter wheat varieties that are tolerant to Fusarium head blight. The second objective was to field screen the elite hard winter wheat lines including those in the Regional Germplasm Observation Nursery (RGON). Eight hundred winter wheat experimental lines, which included the RGON were screened in the field against appropriate controls in 2003. Seventy two % of the lines have been screened in at least three mutually exclusive trials including an independent determination by South Dakota State University (SDSU). Inoculation was done, by spraying the heads at a rate of (70 000 *Fusarium graminearum* conidia of strain SD-4) / (1 ml distilled water), followed by misting in the field or bagging in the greenhouse. Six % of the RGON lines had significantly less than 35 % severity. Twenty one percent of the lines that were extensively screened, had significantly less than 46% severity. Thirteen percent of these extensively screened lines are derived from experimental breeding lines, and are very likely new sources of background FHB resistance. These very promising lines will be crossed with known major genes to enhance the tolerance their tolerance to FHB infection.

INTRODUCTION

Nebraska is second only to California for irrigated crop production. Hence FHB, though a periodic disease, can be an important disease greatly affecting approximately 35% of Nebraska's wheat acreage. As humans consume virtually all of this wheat and over one half is exported, safe, healthy grain is critical for maintaining the reputation of hard winter wheat in the domestic and export markets. All winter wheat lines to be released by the University of Nebraska shall be screened for FHB tolerance, and this information will be shared with producers.

The primary objective is to identify and develop elite winter wheat varieties that are tolerant to Fusarium head blight (FHB, scab), using conventional breeding methods. The second objective was to screen elite hard winter wheat lines including the Regional Germplasm Observation Nursery (RGON).

MATERIALS AND METHODS

Sources of FHB tolerant germplasm originating from our biotechnology efforts, spring and soft wheat germplasm, and exotic germplasm, were collected for crossing into our elite lines. In the field, seventy two transgenics and eight hundred winter wheat breeding lines, which included the RGON nursery were planted and screened, against appropriate controls, for tolerance to FHB, using a system similar to that of Campbell and Lipps (1998). Each variety was planted in a 10 ft^2 plot.

All lines screened in the field and greenhouse in Nebraska in 2003, were inoculated by spraying 70000 conidia of *F. graminearum* strain SD-4 / 1 mL distilled water, onto heads. Inoculum was applied in the field by spraying 50

mL of inoculum/plot using an electrical back pack sprayer, and in the greenhouse 5 mL of inoculum/head was applied using a hand held sprayer. After inoculation in the field the plants were misted for 5 minutes at 30 minute intervals, using a modified system similar to that employed by Zhang et al. (1999). Misting began when the plants were inoculated and continued until the first readings were taken. In the greenhouse inoculated heads were, tagged and then sealed in a 16 x 9.5 cm^2 snack size Ziploc bag for 72 hrs. Bordering the scab nursery with forage triticale provided an excellent buffer and greatly reduced wind in the misting nursery.

FHB was rated by estimating the % head severity on 20 individual heads (Shaner and Buechley, 2001). Plot severity or the FHB index was calculated by averaging these 20 FHB ratings. Intensity was calculated by taking the count of the infected heads and dividing it by 20 (the total # of heads scored). It is not practical to record FHB index and incidence in the greenhouse, however % severity was recorded, by counting the # of infected spikelets relative to the total # of spikelets. The grain, from one of our three most advanced nurseries was analyzed for Deoxynivalenol (DON) by the Veterinary Diagnostic Service at North Dakota State University.

RESULTS AND DISCUSSION

The Nebraska breeding project has extensive field and greenhouse FHB tolerance data, from 9 independent trials conducted since 2001 in Nebraska and South Dakota. Seventy two % (i.e. 574 lines) of the eight hundred lines screened in 2003 have been screened in at least 3 mutually exclusive trials. However we were careful not to indiscriminately combine all the trials, because one should not base important decisions regarding line selection, upon experiments which may have unusual variability. Therefore we were careful to only compare lines, from experiments in which overall experimental variability was similar. Trials were considered similar if their variability differed by less than 5 fold in magnitude.

FHB severity was combined for 4 of the 9 greenhouse and field trails and incidence data was combined for 2 of the 4 field trials. Table 1 shows select lines including "Wesley" that were more tolerant to the spread of FHB infection than Millenium or NE99495. Wesley is a recently released widely grown line from our program. It appears to have a better than average level of FHB tolerance and a low DON level in our field tests which confirms our earlier greenhouse tests. Wesley is adapted to the primary regions where FHB is most common. Over half of these lines were derived from experimental breeding lines, and are very likely new sources of background FHB resistance. These very promising lines will be crossed with known major genes to enhance the their tolerance to FHB infection.

FHB tolerance in winter wheat breeding nurseries was generally high. The most FHB tolerant transgenics will be crossed to varieties having some FHB tolerance, and to Wesley, The best lines for FHB tolerance and agronomic performance will be retested in 2004.

We have initiated a collaboration with Dr. Ismail Dweikat to test our lines with microsatellite markers to determine if they have the expected genes from the parents and to identify the marker diversity in known regions containing FHB quantitative trait loci.

In the 2003-2004 cycle, we will plant 80 out of 819 F_2 bulks and 33 out of 750 F_3 bulks that were deliberately made for FHB tolerance. Our most advanced lines from the FHB crosses are in the F6 generation. We will screen 420 lines from our elite germplasm (our three most advanced nurseries), 46 lines from the FHB screening nursery, 20 - 50 transgenic spring wheat lines (initially) from our biotechnology efforts, and 277 lines coming from the RGON in the field

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.

Table 1: Pedigree of Extensively Tested Select FHB Lines Tolerance to FHB Severity Compared to More Susceptible Lines.

Line	Pedigree of the most FHB Tolerant Lines	LsMean Severity	Standard Error
NE99579	**TOMAHAWK/NE88584//NE89657**	22[A]	14
CULVER	NE82419 X ARAPAHOE	26[AB]	14
ARAPAHOE	BRULE/3/PKR4*/AGENT//BELOT198/LCR	28[AB]	14
NE97465 (Goodstreak)	**SD3055/KS88H164//NE89646 (=COLT*2/PATRIZANKA)**	31[AB]	14
ALLIANCE	ARKAN/COLT//CHISHOLMsib	31[AB]	14
NIOBRARA	TAM105*4/AMIGO (TX80GH2679)//BRULE Fsel	31[A]	14
N97V121	**N87V106/OK88767**	31[A]	14
W91040		32	16
WESLEY	**KS831936-3//COLT/CODY**	31[A]	14
CHEYENNE	CI8885	32[B]	14
NE00544	**SD89180/KARL 92**	32[B]	14
NE00403	**PRONGHORN/ARLIN//ABILENE**	33[B]	14
NE96737	N95L421 N87V106/NE88582	33	14
PRONGHORN	CENTURA/DAWN//COLTsib	35	15
NE00679	NE93462 (=ARAPAHOE/NA HW-81-170)/NE90616 (=ARAPAHOE/COLT)	35	14
NE97689 (Harry)	NE90614 (=BRL/4/PKR*4/AGT//BEL.198/LCR/3/NWT/BRL) /NE87612 (=NWT//WRR*5/AGT/3/NE69441)	35	14
NE00479	IL85-3132-1/ARAPAHOE	35	14
NUPLAINS	ABILENE/KS831872	36	14
NE99656	TX89V4138/NE89657//KARL 92	36	14
NE00507	KS831936 - 3 / NE86501 / / TX86V1405 - 1	37	14
NE99464	NE86606/RAWHIDE//ABILENE	37	14
WAHOO	ARAPAHOE/Abilene//ARAPAHOE	37	14
NE99489	NE90625/NE91525//KARL 92	37	14
NE98632	NIOBRARA/NE91525	37	14
NE00658	NE93462 (=ARAPAHOE/NA HW-81-170)/NE92608 (=NE82413/COLT)	38	14
NI98439	NE90476/(10Ax88-1643)X10927 592-1-5	39	14
NE99543	ALLIANCE/KARL 92	40	14
NE98471	NE90461/NIOBRARA	40	14
NE98466	KS89H50-4/NE90518	40	14
NE00633	NE92614 (=CENTURA/RL8200003)/IKE	41	14
SCOUT66	CI73996	41	14
NE99445	RAWHIDE/TOMAHAWK//KARL 92	45	14
MILLENNIUM		46	14
NE00481	NE93462 (=ARAPAHOE/NA HW-81-170)/NE92608 (=NE82413/COLT)	46	14
NE99495	ALLIANCE/KARL 92	47	14
NE00435	WI87-018/2*ARAPAHOE	49	14
NE00429	WI87-018/2*ARAPAHOE	51	14
NE00564	T81/NE91635 (=NE82761/NE82599)	57	14

Lines with severity values followed by [A] or [B] respectively were significantly more tolerant than Millenium and NE99495 respectively.

ACKNOWLEDGEMENTS

This material is based upon work supported by the Scab Initiative and the U.S. Department of Agriculture, under Agreement No.59-0790-9-026. We would like to thank J.Yue, and X. Zhang for their advice about inoculation procedures and their *Fusarium graminearum* sample and Dr. Boosalis for his assistance making field inoculum, and T.J. McAndrew and his team at the Mead Experimental station for help with the irrigation system.

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OBSERVATIONS FROM SRWW VARIETY DEVELOPMENT NURSERIES WITH SEVERE FHB PRESSURE

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OBJECTIVE

Screen soft red winter wheat (SRWW) germplasm for FHB resistance.

INTRODUCTION

Our 2002-03 soft red winter wheat nurseries in Ohio experienced severe disease pressure. The moderately resistant check Freedom had an average FHB index of 48 over 11 tests while the susceptible check Pioneer 2545 had an average FHB index of 79. This report summarizes results from screening breeding lines in our variety development program.

MATERIALS AND METHODS

We evaluated > 800 SRWW lines in a mist-irrigated field nursery inoculated with infested corn kernels. Spring was cool and wet which delayed anthesis and the onset of visual symptoms. Early heading lines were rated up to 37 days after anthesis, while later lines were rated about 20 days after anthesis. Some nurseries (YR1, YR2) were visually rated for % infected spikelets, for others we assessed disease incidence and severity on 20 heads per line per replication to calculate FHB index. We used three reps for all trials.

RESULTS AND DISCUSSION

First Year (YR1) of Testing: We tested 483 F4 derived lines from 69 crosses in the first year of replicated evaluation for eventual variety release. The lines were selected from the 2001-02 head row nursery based on agronomic value. The average FHB index of all lines was 53, slightly higher than Freedom (48) in the YR1 trial, and much lower than Pioneer 2545 (83). Heading was spread over 19 days with FHB rated 20 to 37 days after anthesis. FHB index was not correlated to heading date ($r = 0.01$) or days between anthesis and rating ($r = -0.02$).

Of the 483 lines, 141 had FHB index less than or equal to Freedom, 49 were significantly ($P<0.1$) more resistant than Freedom, and 454 were more resistant than Pioneer 2545. Eight lines were significantly more resistant than Freedom at the 0.05 probability level (Table 1). These eight lines were derived from eight different crosses and 15 different parents.

Comparing the range of family means within each cross to an $LSD_{0.05}$ of 17.4 indicated that significant segregation occurred in 72% of the populations with n is greater than or equal to 5 (Fig. 1). There was no association between population mean and range of FHB index. Even the population with the highest mean had one line that had an FHB index equal to that of Freedom (Fig. 1). The minimum family mean within a population was negatively associated with the range of FHB index values in the population (Fig. 2): families

with the lowest FHB index came from populations with the greatest range of FHB values. All the lines in Figure 2 with means significantly (P<0.1) lower than Freedom came from crosses with significant segregation. The nine populations that produced lines with low means (Table 2) were derived from 17 parents.

Table 1. FHB index of eight breeding lines with FHB index significantly less (P<0.05) than Freedom (47.9) in the 2002-03 YR1 test of 483 F4 derived lines.

OSU Line	Heading Date	FHB Index (%)	Pedigree
OH02-3059	147	23.3	FREEDOM/OH618
OH02-15652	142	24	PATTERSON/HOPEWELL
OH02-8771	144	26.7	IN881064A12-3-3-1/OH552
OH02-5103	142	28.3	OH536/OH615
OH02-8183	146	30	GOLDFIELD/P89204A8-1-59
OH02-14111	141	30	IL87-1917-1/IN83127E1-24-5-2-1-31//FOSTER/P. 2548
OH02-10578	143	30	OH552/OH599
OH02-11675	144	30	OH618/OH552

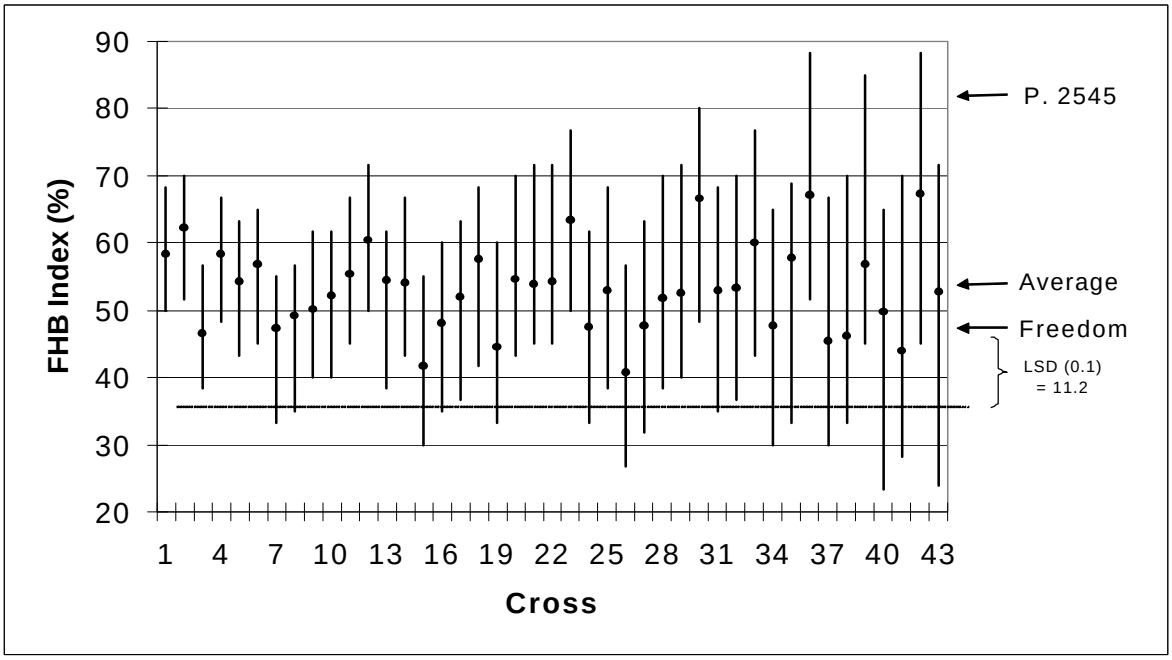

Figure 2. Association of the FHB index range of a population, and the minimum family mean of a population, for 43 populations (n is greater than or equal to 5) evaluated in the 2002-03 YR1 nursery.

Interpretation of YR1 results

- Many SRWW parent combinations generate genetic variation for FHB resistance
- Useful resistance (FHB index less than or equal to Freedom) is possible in many populations
- Segregation and recombination are needed to produce low FHB index
- Recurrent selection may be useful to create high levels of FHB resistance from the different FHB resistance alleles in SRWW.

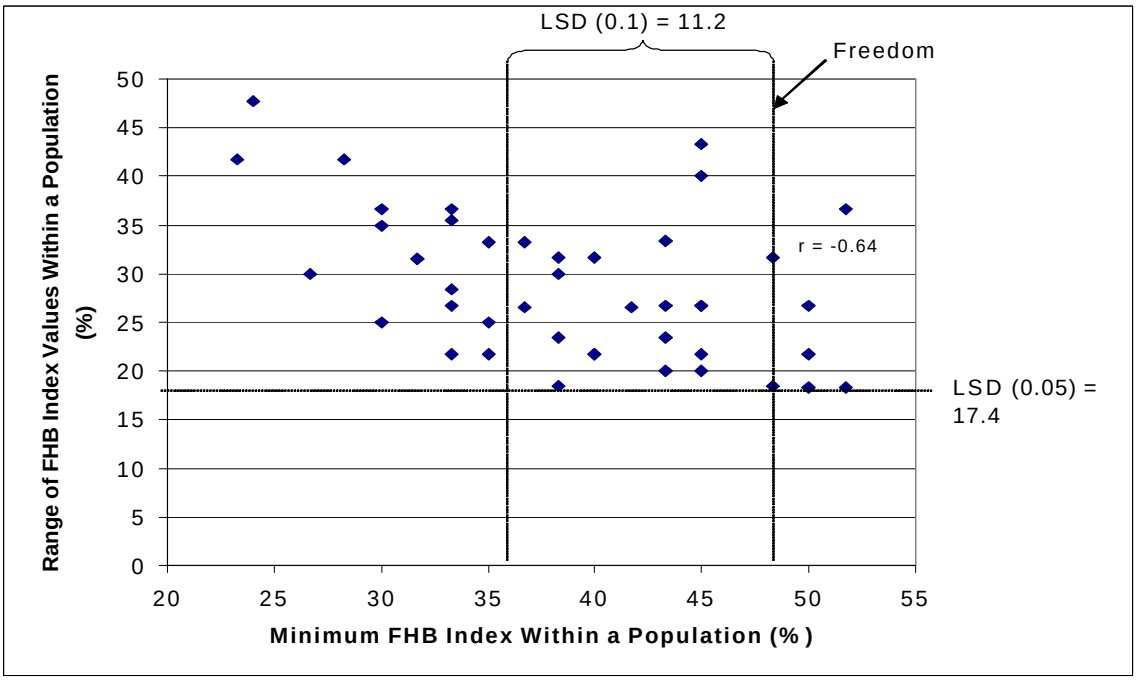

Figure 2. Association of the FHB index range of a population, and the minimum family mean of a population, for 43 populations (n is greater than or equal to 5) evaluated in the 2002-03 YR1 nursery.

Table 2. Mean, minimum, maximum, and range of populations (n>4) that produced lines with low FHB index values in the 2002-03 YR1 nursery.

Population	n	Average	Minimum	Maximum	Range
FREEDOM/OH618	10	49.8	23.3	65	41.7
PATTERSON/HOPEWELL	43	52.7	24	71.7	47.7
IN881064A12-3-3-1/OH552	15	40.8	26.7	56.7	30
OH536/OH615	11	43.9	28.3	70	41.7
IL87-1917-1/IN83127E1-24-5-2-1-31//FOSTER/P.	7	47.6	30	65	35
GOLDFIELD/OH546	8	47.7	31.7	63.3	31.6
PIONEER 2548/FREEDOM	7	46.2	33.3	70	36.7
OH581/IN83127E1-24-5-2-1-31//5088B-D-32-1/OH601	7	47.4	33.3	55	21.7
FREEDOM/PATTERSON	10	57.8	33.3	68.8	35.5

Second Year (YR2) of Testing: Disease pressure was light in the 2001-02 YR1 test of 316 breeding lines with checks averaging 76.5% less disease than in the 2003 YR1 test. We selected 117 lines and evaluated them in 2003 YR2 trials. The average FHB index of the selected lines (10.6) in the 2002 YR1 was 71% of the mean of all lines (15.1) and 34% of the mean of Pioneer 2545 (31.0). In the 2003 YR2 trial, the 117 lines had a mean FHB index of 58, which was 103% of the mean of Freedom (56.4) and 68% of the mean of Pioneer 2545 (85.0). The correlation of the YR1 and YR2 scores was 0.52. Several lines had low scores in both years (Table 3).

Interpretation of YR2 Results

- Resistance under light and severe disease pressure was associated.
- As in YR1 tests, useful resistance can be derived from many different crosses

Table 3. FHB index of the best lines selected from the 2002 (YR1) and 2003 (YR2) nurseries.

Line		Average	2003 (YR2)	2002 (YR1)
OH01-6467	OH534/12485-23-2//FREEDOM/R28785	19.3	37.5	1
OH01-1212	OH526/HOPEWELL	20.9	40	1.7
OH01-2642	OH534/12485-23-2//FREEDOM/OH572	21.3	36.7	6
OH01-1949	OH581/OH569	22.3	43.3	1.3
OH01-7658	HOPEWELL/OH601	23.6	43.3	4.3
OH01-3162	OH534/OH513//14884/VA91-54-219	23.7	41.7	5.7
OH01-2683	OH534/12485-23-2//FREEDOM/OH572	24.4	45	3.7
Freedom		30.5	56.4	4.6
Pioneer 2545		58	85	31

Advanced Testing: We evaluated 140 lines for incidence, severity and index in 2002-03 in five tests (FHB, YR3, YR4+, NUWWS, USFHB). Lines more resistant than the check were found in each trial. Lines with low index values (e.g .< 20) were derived from crosses with both exotic (ZM10782, Ning 7840) germplasm as well as adapted-only crosses.

Table 4. FHB incidence (INC), severity (SEV), and index (IND, also given as % resistant check [I%C]) for best lines and resistant checks from the 2003 advanced tests.

Test	Name	Pedigree	INC	SEV	IND	I%C
FHB	OH904	ZM10782/Free.//305/VA91-54-219	90	7.9	7.1	33%
FHB	OH903	Ning 7840/Glory//OH526	97	7.6	7.4	35%
FHB	Freedom		92	21.8	21.4	
YR4+	OH740	L89060/OH529	100	29.1	29.1	53%
YR4+	OH753	Catoctin/OH536	100	31.2	31.2	57%
YR4+	OH736	OH462/Ckr 9663	100	34.5	34.5	63%
YR4+	Freedom		100	55.2	55.2	
YR3	OH806	Freedom/OH530	100	14.8	14.8	49%
YR3	OH886	Freedom/OH530	100	22.6	22.6	75%
YR3	Freedom		100	30.2	30.2	
NUWWS	RCATL33	R/FR#1/ACRon//25R18/ACRon	100	16.2	16.2	53%
NUWWS	P.981233A1-10	Freedom//Goldfield/X117	100	17.6	17.6	58%
NUWWS	IL97-6755	IL90-4813//IL85-3132-1/Ning 7840	100	17.9	17.9	59%
NUWWS	Freedom		100	30.5	30.5	
USFHB	ARGE97-1033-3-5	Freedom/Catbird	100	14.1	14.1	24%
USFHB	AR857-1-1	Madison/YMI6	93	18.9	17.6	30%
USFHB	B011117	YMI6/Ckr 9877	92	19.7	18.8	32%
USFHB	Ernie		100	58.1	58.1	

REPORT ON THE 2002-03 NORTHERN UNIFORM WINTER WHEAT SCAB NURSERY (NUWWSN)

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OBJECTIVE

Evaluate soft winter wheat lines for resistance to FHB in a multi-site uniform nursery.

INTRODUCTION

Each year the USWBSI funds coordination of a uniform nursery to assess FHB resistance in soft winter what lines adapted to the northern US.

MATERIALS AND METHODS

The 2002-03 NUWWSN evaluated 51 breeding lines from 10 breeding programs (Table 2). Data was collected from 10 field trials and five greenhouse tests. The field trials used various methods of inoculation and rating, though most use mist irrigation and Fusarium infected corn kernels as inoculum. Most cooperators reported incidence (INC) and severity (SEV) collected on 20 heads per replication. INC and SEV were used to calculate index (IND=INC*SEV/100). Some also collected data on percent scabby seed (%SS) based on seed weight and DON. The greenhouse tests were conducted using single floret inoculation and all reported severity (GHSEV). For this report, data is averaged over all locations and the genotype x location interaction was used as the error to calculate an LSD (0.05). For each trait, each entry was compared to the entry with the lowest mean, and the entry with the highest mean using the $LSD_{0.05}$.

RESULTS AND DISCUSSION

A summary of the full NUWWSN report is presented here. The full version of the 2002-03 NUWWSN report will be available at the 2003 USWBSI forum and at the USWBSI web site (http://www.scabusa.org/). Based on proportion of total sum of squares (TSS) accounted for by genotype by environment interaction (GEI) effects, GEI was an important source of variation for SEV (40% of TSS), INC (23%), GHSEV (22%), and DON (21%). The data summarized over all locations for the best and worst lines is summarized in Table 1 while the data for all lines is presented in Table 3.

Table 1. Mean over all locations for all traits for the best and worst entries in the 2002-03 NUWWSN. Entries with a mean that is not significantly different than the highest mean in that column are designated with an "h", while the entries with a mean that is not significantly different than the lowest mean in that column are designated with an "l"

	SEV n=7	INC 9	IND 9	%SS 4	DON 2	GHSEV 5	# LOW TRAITS	# HIGH TRAITS
P.981359C1-4	16.5 l	49.3 l	10.7 l	23 l	13.9	34.7 l	**5**	0
MO980829	21.6 l	41.9 l	11.4 l	35.7 l	12.4	24.9 l	**5**	0
MO011174	16 l	43 l	11.8 l	32.3 l	13.2	24.3 l	**5**	0
VA02W708	15 l	44.3 l	11.9 l	25.7 l	16.3 h	31.5 l	**5**	1
IL96-24851-1	16.3 l	47.7 l	12.8 l	43	9.5 l	27.1 l	**5**	0
IL97-6755	15.7 l	43.3 l	13.1 l	30.7 l	16 h	30.4 l	**5**	1
IL99-27048	14.4 l	42.8 l	13.1 l	28.2 l	13	37.2 l	**5**	0
MO981020	17.9 l	45.1 l	15.5 l	34.3 l	11.9	26.1 l	**5**	0
MO011175	21.4 l	46.1 l	15.8 l	32.2 l	8.3 l	41.6	**5**	0
VA02W709	16.5 l	49.3 l	16.6 l	52.9 h	11.4 l	29.8 l	**5**	1
P.981238A1-1	17.4 l	49 l	18 l	49.5	8.7 l	35 l	**5**	0
E0009	20.9 l	52.7 l	18 l	36.2 l	11.7	36.6 l	**5**	0
ERNIE	17 l	45.7 l	19 l	36.3 l	8.6 l	27.6 l	**6**	0
P.97397E1-11	21.1 l	49.7 l	20.5	45.5	9.4 l	23.4 l	**4**	0
MO010708	20.7 l	55.7	18 l	33.4 l	12.7	34.6 l	**4**	0
RCATL33	15.3 l	39.9 l	14.6 l	39.3	6.9 l	46.2	**4**	0
PIONEER 2545	30.7 h	71.4 h	34.4 h	58.6 h	10.7 l	43.5	1	**4**
KY93C-1238-34-1	36.2 h	64.7 h	34.4 h	52 h	13.2	46.8	0	**4**
F0008	27.5 h	66.3 h	34.6 h	55.3 h	5.7 l	50.5	1	**4**
VA01W448	30.8 h	57.3	35.1 h	55.3 h	19.1 h	47.9	0	**4**
RCATL13	31.1 h	65.3 h	36 h	51.5 h	15.5 h	65.9 h	0	**6**
OH738	31.8 h	66.9 h	37.3 h	57.2 h	15.4 h	45.3	0	**5**
RCATTF17/34	36.6 h	60.8 h	39.3 h	53.8 h	11.9	61.2 h	0	**5**
VA02W734	36.1 h	64.7 h	41.3 h	49	7 l	55 h	1	**4**
AVERAGE	24.3	54.6	23.4	44.6	11.3	43.9		
R2	0.6	0.84	0.77	0.95	0.79	0.78		
CV	45.1	25.4	41.5	22	30.2	34.3		
LSD	11.5	12.8	9	13.7	6.8	18.7		

Table 2. List of entries in the 2002-03 Northern Uniform Winter Wheat Scab Nursery (NUWWSN)

Source	Entry	Name	Pedigree
Ohm	1	P.97397E1-11	96204//Gfd/INW9824
Ohm	2	P.981227A1-1	Gfd/9824//96204/3/x117/4/9853
Ohm	3	P.981233A1-10	Fdm//Gfd/X117
Ohm	4	P.981238A1-1	Ernie//91193D1/X117
Ohm	5	P.981359C1-4	Acc3130/Patterson
Ohm	6	P.99646C2-7	961331/9811//283-1/Fdm
Sorrels	7	NY89064SP-7139*	88029(84061(6120-15/F29-76)/Augusta)/Harus
Sorrels	8	NY89052SP-9232	88119(Geneva/84004/6-1MR)/Geneva
Sorrels	9	CALEDONIARESEL-VT	Caledonia offtype
Sorrels	10	NY88046-8138	Susquehanna/Harus
Sorrels	11	NY89052-7142	88119(Geneva/84004/6-1MR)/Geneva

Table 2 (Continued)

Source	Entry	Name	Pedigree
Ohm	1	P.97397E1-11	96204//Gfd/INW9824
Ohm	2	P.981227A1-1	Gfd/9824//96204/3/x117/4/9853
Ohm	3	P.981233A1-10	Fdm//Gfd/X117
Ohm	4	P.981238A1-1	Ernie//91193D1/X117
Ohm	5	P.981359C1-4	Acc3130/Patterson
Ohm	6	P.99646C2-7	961331/9811//283-1/Fdm
Sorrels	7	NY89064SP-7139*	88029(84061(6120-15/F29-76)/Augusta)/Harus
Sorrels	8	NY89052SP-9232	88119(Geneva/84004/6-1MR)/Geneva
Sorrels	9	CALEDONIARESEL-VT	Caledonia offtype
Sorrels	10	NY88046-8138	Susquehanna/Harus
Sorrels	11	NY89052-7142	88119(Geneva/84004/6-1MR)/Geneva
Kolb	12	IL97-6755	IL90-4813//IL85-3132-1/Ning 7840
Kolb	13	IL99-13436	IL91-14163/IL93-1517
Kolb	14	IL99-27048	IL90-6364/Pioneer brand 2571
Kolb	15	IL97-4915	IL87-2834-1/OH470//MO9965-52/IL90-6364
Kolb	16	IL96-24851-1	IL90-6364//IL90-9464/Ning 7840
Baenziger	17	ARAPAHOE	BRULE/3/PKR4*/AGENT//BELOT198/LCR
Baenziger	18	NE97V121	N87V106/OK88767
Baenziger	19	NE99445	RAWHIDE/TOMAHAWK//KARL 92
Baenziger	20	NE98466	KS89H50-4/NE90518
Vansanford	21	KY94C-0094-11-2	L880119/2684//2510
Vansanford	22	KY93C-0403-23-1	VA88-52-69/KY83C-004//2510
Vansanford	23	KY93C-1238-34-1	VA87-54-558/ KY83C-004//2510
McKendry	24	MO980829	MO 11769/Madison
McKendry	25	MO981020	MO 11769/Madison
McKendry	26	MO011175	MO 91-19/Pioneer 2552
McKendry	27	MO011174	MO 980521 reselection. (MO 11769/Madison)
McKendry	28	MO010708	MO 94-182/VA 91-54-219
McKendry	29	MO010719	MO 12278/Pioneer 2552
Sneller	30	OH738	L890690/T814
Sneller	31	OH743	OH529/OH506
Sneller	32	OH750	OH536/OH506
Sneller	33	OH751	10584-0801/COKER 9663
Sneller	34	OH753	CATOCTIN/OH536
Sneller	35	OH736	OH462/COKER 9663
Schafsmaa	36	RCATL33	R/FR#1/AC RON//25R18/ACRON
Schafsmaa	37	RCATL10	BALKAN//AC RON/SUP72017-17-5-10-1
Schafsmaa	38	RCATL13	2737W/25R57//R/FR#1/2737W
Schafsmaa	39	RCATTF19/26	EX9806/AC RON
Schafsmaa	40	RCATTF2/4	2737W/EX9806
Schafsmaa	41	RCATTF17/34	2737W/25R57
Griffey	42	VA01W448	PC-11(SHANGHAI4/CHILL"S":SCAB-RES) /3/92-51-39(IN71761A4-31-5-48//71-54-147 /MCN1813)//FFR555W/RCT/4/COKER9803, F8
Griffey	43	VA02W694	96-54-250(CK9803/FREEDOM)/P92823A1-1-2-3-5(CLARK*4/NING 7840), F6
Griffey	44	VA02W708	NING 7840/PION2684//96-54-244 (CK9803/FREEDOM), F6
Griffey	45	VA02W709	NING 7840/PION2684//96-54-244 (CK9803/FREEDOM), F6

Table 2. (Continued)

Source	Entry	Name	Pedigree
Griffey	46	VA02W729	PC-11(SHANGHAI4/CHILL"S":SCAB-RES) /3/92-51-39(IN71761A4-31-5-48//71-54-147 /MCN1813) //FFR555W/RCT/4/93-52-23&24 (MSY//HUNTER/WLR), F8
Griffey	47	VA02W734	PC-7(CHILL"S"/YM16:SCAB-RES)/3/92-51-39(IN71761A4-31-4-48//71-54-147 /MCN1813)//CK9803/RCT/4/93-52-55 (MSY*3/BALKAN//SAL), F8
Ward	48	D6234	(X1291,I3118/FRANKENMUTH//FRANKENMUTH)/3/(C5107,B2218/B2142//B5250)
Ward	49	E0009	NY82-105-2 / NY262-37-422
Ward	50	E0010	NY82-105-2 / NY262-37-422
Ward	51	F0008	(D2217,C4680/AUG//AUG)/3/(PIONEER_2555,PNR_W3017/PNR_W521)
CHECKS	52	ERNIE	
CHECKS	53	PIONEER 2545	
CHECKS	54	FREEDOM	

Table 3. Mean over all locations for all traits for all entries in the 2002-03 NUWWSN. Entries with a mean that is not significantly different than the highest mean in that column are designated with an "h", while the entries with a mean that is not significantly different than the lowest mean in that column are designated with an "l"

	SEV n=7	INC 9	IND 9	%SS 4	DON 2	GHSEV 5	# LOW TRAITS	# HIGH TRAITS
P.97397E1-11	21.1 l	49.7 l	20.5	45.5	9.4 l	23.4 l	4	0
P.981227A1-1	27.5 h	56.5	27.6	47.1	14.5	43.9	0	1
P.981233A1-10	22.9 l	57.8	20.4	50.5 h	14.2	21.9 l	2	1
P.981238A1-1	17.4 l	49 l	18 l	49.5	8.7 l	35 l	5	0
P.981359C1-4	16.5 l	49.3 l	10.7 l	23 l	13.9	34.7 l	5	0
P.99646C2-7	29.5 h	62.7 h	28.1	46	10.8 l	64.1 h	1	3
NY89064SP-7139*	22.1 l	55.6	18.7 l	49.8	21.5 h	39.2 l	3	1
NY89052SP-9232	22.2 l	53.5	20.6	39.6	11 l	59.1 h	2	1
CALEDONIARESEL-VT	23.4 l	47.1 l	18.9 l	52.3 h	6.5 l	58.8 h	4	2
NY88046-8138	26.6	54.9	29.7	43.1	8 l	43	1	0
NY89052-7142	29.8 h	57	25.5	39.7	4.7 l	57.9 h	1	2
IL97-6755	15.7 l	43.3 l	13.1 l	30.7 l	16 h	30.4 l	5	1
IL99-13436	27.8 h	58.2	25.5	48.5	8.8 l	49.8	1	1
IL99-27048	14.4 l	42.8 l	13.1 l	28.2 l	13	37.2 l	5	0
IL97-4915	28.5 h	52.5 l	20.7	39.3	8.2 l	47	2	1
IL96-24851-1	16.3 l	47.7 l	12.8 l	43	9.5 l	27.1 l	5	0
ARAPAHOE	19.9 l	55.1	18.7 l	42.9	9.7 l	53.4 h	3	1
NE97V121	33.1 h	66 h	32	59.1 h	13.9	49.7	0	3
NE99445	38.4 h	57.8	36.3 h	49.7	8.5 l	59.7 h	1	3
NE98466	29.9 h	54.5	22.8	41.9	10.5 l	47.7	1	1

Table 3 (Continued)

	SEV n=7		INC 9		IND 9		%SS 4		DON 2		GHSEV 5		# LOW TRAITS	# HIGH TRAITS
KY94C-0094-11-2	21.1	l	57.3		23.5		43.7		6.7	l	43		2	0
KY93C-0403-23-1	26.5		59.4	h	31.3		63.6	h	8	l	71.4	h	1	3
KY93C-1238-34-1	36.2	h	64.7	h	34.4	h	52	h	13.2		46.8		0	4
MO980829	21.6	l	41.9	l	11.4	l	35.7	l	12.4		24.9	l	5	0
MO981020	17.9	l	45.1	l	15.5	l	34.3	l	11.9		26.1	l	5	0
MO011175	21.4	l	46.1	l	15.8	l	32.2	l	8.3	l	41.6		5	0
MO011174	16	l	43	l	11.8	l	32.3	l	13.2		24.3	l	5	0
MO010708	20.7	l	55.7		18	l	33.4	l	12.7		34.6	l	4	0
MO010719	22.9	l	48.1	l	18	l	37.5		15.3	h	41.1		3	1
OH738	31.8	h	66.9	h	37.3	h	57.2	h	15.4	h	45.3		0	5
OH743	22.2	l	57.2		24.8		48		15.7	h	33.8	l	2	1
OH750	23.5	l	56.8		21.2		50.7	h	12.2		51.2		1	1
OH751	21.9	l	55.7		22.5		41.2		9	l	37	l	3	0
OH753	23.1	l	56		24.7		48.6		10.1	l	48.6		2	0
OH736	26		57.2		22		43.4		7.7	l	50.4		1	0
RCATL33	15.3	l	39.9	l	14.6	l	39.3		6.9	l	46.2		4	0
RCATL10	29.8	h	61.5	h	27.6		48.9		7.8	l	34.7	l	2	2
RCATL13	31.1	h	65.3	h	36	h	51.5	h	15.5	h	65.9	h	0	6
RCATTF19/26	27.2	h	56.2		25.4		50.4	h	6.4	l	51.9		1	2
RCATTF2/4	22.3	l	48.9	l	19.3	l	49.1		7.3	l	56.2	h	4	1
RCATTF17/34	36.6	h	60.8	h	39.3	h	53.8	h	11.9		61.2	h	0	5
VA01W448	30.8	h	57.3		35.1	h	55.3	h	19.1	h	47.9		0	4
VA02W694	22.5	l	45.6	l	25.2		40		16.3	h	61.5	h	2	2
VA02W708	15	l	44.3	l	11.9	l	25.7	l	16.3	h	31.5	l	5	1
VA02W709	16.5	l	49.3	l	16.6	l	52.9	h	11.4	l	29.8	l	5	1
VA02W729	22.2	l	58		22		43.8		20.1	h	44.9		1	1
VA02W734	36.1	h	64.7	h	41.3	h	49		7	l	55	h	1	4
D6234	24.8	l	57.2		27.1		41.2		15	h	45.8		1	1
E0009	20.9	l	52.7	l	18	l	36.2	l	11.7		36.6	l	5	0
E0010	26		61.1	h	26.2		48.4		8.1	l	36.8	l	2	1
F0008	27.5	h	66.3	h	34.6	h	55.3	h	5.7	l	50.5		1	4
ERNIE	17	l	45.7	l	19	l	36.3	l	8.6	l	27.6	l	6	0
PIONEER 2545	30.7	h	71.4	h	34.4	h	58.6	h	10.7	l	43.5		1	4
FREEDOM	21.5	l	58		21.6		49.9	h	11.1	l	37.8	l	3	1
AVERAGE	24.3		54.6		23.4		44.6		11.3		43.9			
R^2	0.6		0.84		0.77		0.95		0.79		0.78			
CV %	45.1		25.4		41.5		22		30.2		34.3			
LSD(0.05)	11.5		12.8		9		13.7		6.8		18.7			

BREEDING FOR SCAB RESISTANCE IN SOFT RED WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) caused significant damage to the Kentucky wheat crop in 2003. Many producers had grain rejected from wheat millers due to high levels of dexoynivalenol (DON). This problem is not new. Each year FHB affects producers in the state by causing economic damage. The yearly impact from FHB infection and resulting DON accumulation demonstrates the need of screening wheat for FHB resistance. In 2001 approximately 80 breeding lines were selected from the breeding program to be tested in the scab nursery. The lines were dividing into two tests, the Magnum and Mondo. The breeding lines were developed from parents that had some level of scab resistance. For the past two years the Magnum and Mondo have been evaluated in the greenhouse and two locations, Lexington and Princeton, KY. Evaluations of the Magnum and Mondo have resulted in several lines with good resistance. The selected breeding lines are being evaluated for the third year in the scab nursery. Plots outside of the scab nursery are being used to increase seed for the selected lines. We anticipate including several of the lines in the 2005 Northern Uniform Winter Wheat Scab Nursery. The selected lines are also being evaluated in elite tests for agronomic traits. Several of the breeding lines with low severity also were above average in yield. Dependent on further testing, we would like to release the breeding lines as scab tolerant culitvars or germplasm. The scab nursery in Lexington 2003 was evaluated for FHB severity over time using area under disease progress curve (AUDPC). The goal of collecting this data is to determine the amount of disease pressure each year and to pinpoint when symptom development begins. By using this data, disease pressure can be quantified to compare the scab nursery over years and locations. A 7-day interval (post-anthesis) was used for severity evaluation. Fifteen rows within the scab nursery and fifteen rows in the border of the nursery were evaluated for severity. The data collected from the AUDPC along with the data from culitvar checks will be a useful tool in determining the uniformity of FHB in the scab nursery.

BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE: AN INTERNATIONAL APPROACH

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INTRODUCTION

The International Maize and Wheat Improvement Center (CIMMYT) begun research on Fusarium head blight (FHB), initially denoted as 'scab', in the mid 1980's. The center's roots trace back to 1943/44, when a bilateral program between Mexico and the Rockefeller Foundation was established to develop modern wheat varieties for irrigated production in Mexico, with barley added in 1970 (later under ICARDA/CIMMYT). When these high-yielding rust resistant lines were sown globally, it became obvious that in rainfed areas additional traits needed to be introgressed: first resistance to *Septoria tritici* resistance in the 70's and later *Fusarium spp.* in the 80's. Another motivating factor emphasizing FHB was CIMMYT's increasing relationship with China, the largest producer, consumer and importer of wheat, from the mid 1980's onwards. FHB, stripe rust, powdery mildew and BYDV were the most important diseases in China. Adapted germplasm from China was used to combine high yield, slow rusting, and FHB resistance, with spin-offs expected to the FHB-prone regions of Africa and Latin America. In barley just 23 lines out of 5000 tested formed the basis for FHB resistance breeding, resulting in the widely grown Chinese variety Gobernadora (Zhenmai-1) in which resistance QTLs were mapped in collaboration with Oregon State University.

MATERIALS AND METHODS

After several years of experimentation in Toluca (800 mm rainfall per crop cycle) begun by Girma Bekele in the mid 80's, the methodology settled on sprinkler-enhanced screening of a 0.5-1 ha field. Resistance was characterized in terms of reduced fungal spread throughout the spike (Type II) and good grainfill (Type IV and V), later augmented with resistance to infection (Type I) and recently with low toxin levels (Type III) in both wheat and barley (Miller and Arnison, 1986; Mezterhazy, 1995; Schroeder and Christensen, 1963; Vivar, 1996). On barley 14 *Fusarium* species were found; *F. avenaceum* was the most frequent (25.5 – 32.0 %) in field conditions, followed by *F. graminearum* (20.0 – 23.5 %). In barley Type I resistance appears most important, particularly under Midwest (US) conditions (Capettini, 1999). Lucy Gilchrist was the main researcher for the past 10-plus years on this topic in collaboration with the breeders. An international Scab Resistance Screening Nursery (SRSN) was started in the late 1980's and distributed on an *ad hoc* basis (data was received from: Argentina, Brazil, Canada, China, France, Germany, Guatemala, India, Iran, Korea, Pakistan, Paraguay, Peru, Poland, Tanzania, Ukraine, and Uruguay). After CIMMYT joined the USWBSI in 2000, the SRSN was assembled annually and all entries shared with USWBSI. Toxin levels of seed sent from FHB hotspots in China were also tested: these include Wuchang, Hubei Province, Nanjing, Jiangsu Province, Putian and Jianyang, and Fujian Province). The breeding/selection methodology aims to combine resistance mechanisms by accumulating distinct alleles (Singh and van Ginkel, 1997; van Ginkel and Gilchrist, 2002; See table 1 for two examples)

Table 1. Complementary parental combinations 1x2 and 3x4 to accumulate FHB resistance Types in wheat. (bold font = FHB resistant; normal font = FHB susceptible)

		RESISTANCE TYPE				
		I	**II**	**III**	**IV**	**V**
Entry	**Cross**	**Damage (%)**	**Damage (%)**	**Toxin (ppm)**	**Grain losses (%)**	**Grain (1-5)**
1	Milan/Sha7	**0.00**	6.07	**0.14**	13.29	2
2	Bcn*2//Croc_1/Ae. squarrosa (886)	11.56	**4.82**	0.38	**1.68**	**1***
3	Mayoor//Tk Sn1081/Ae. squarrosa (222)	**0.86**	7.26	0.49	**1.3**	**1***
4	Gov/Az//Mus/3/Dodo/4/Bow (= Gondo)	2.51	**2.66**	**0**	21.16	2

The inheritance of resistance in key FHB resistant wheat parents (e.g. Sumai#3 and derivatives, Frontana), later also gaining favor with USWBSI, was studied and published (Singh and Rajaram, 1995; Van Ginkel *et al*. 1996). Table 2 lists key resistance sources in barley, based on data from cooperation between Mexico, the US, Canada, China, Ecuador, Brazil and Uruguay, shared with USWBSI. Molecular studies in progress will look at genetic diversity, aiming to combine resistance with yield and malting quality.

Table 2. Effective barley sources of Type I and Type II resistance (ICARDA/CIMMYT).

Cross	**Head Type**		**Cross**	**Head Type**
Atahualpa	**2**		Gobernadora	**2**
Azafrán (Misc. Cal. 21)	**2**		Humai 10	**2**
Chamico	**6**		PFC 88209	**6**
Chevron	**2**		Shyri	**2**
CIho 4196	**2**		Svanhals	**2**
Fredrickson	**2**		Zhedar 1	**2**

When the molecular position of some FHB resistance genes and associated markers was published, also the genetic diversity in CIMMYT wheats began to be studied (Anonymous, 2003; Sixin Liu and Anderson, 2003; this paper). Three doubled haploid populations involving three resistant parents (Gondo; Bcn*2// Croc_1/Ae. squarrosa (886); Sha3/Cbrd) crossed to a susceptible line (Flycatcher) are being studied using BSA to identify linked markers (Table 6). Resistance from 11 CIMMYT synthetic hexaploid spring wheat derivatives was back-crossed into five wheats nominated by the USWBSI: Ivan, Reeder, Russell, Verde, and Wheaton.

RESULTS

In the past three years, following the association with USWBSI 50,000 – 150,000 spikes of 5,000-10,000 of wheat and barley lines are inoculated annually. These materials include global introductions (from e.g. Argentina, Austria, Brazil, Bulgaria, Canada, Chile, China, France, Germany, Japan, Hungary, Romania, South Africa, Turkey, UK, Uruguay, and USA), and breeding products, and were inoculated and evaluated/ characterized for the five types of resistance. The most resistant 50-200 spring and winter entries are sent to the USWBSI annually (Table 3).

Table 3. Types of germplasm materials sent to USWBSI since inception of agreement.

Type of material	2000	2001	2002	2003
Bread wheat, spring, advanced lines	15	55	6	117
Bread wheat, spring, alien derivatives	21	1	2	20
Bread wheat, spring, introductions	19	116	29	186
Bread wheat, winter, advanced lines	-	-	-	2
Bread wheat, winter, alien derivatives	-	-	15	2
Bread wheat, winter, introductions	-	-	18	34
Durum wheat	5	-	-	14
Barley	9	27	-	166
Triticale	-	-	-	7

Table 4 lists the globally most resistant SRSN spring bread wheat entries.

Table 4. Top Type II resistant wheat entries in global testing of Scab Resistance Screening Nursery (equal or better than Sumai#3 and Frontana; most resistant first)

1st SRSN	2nd SRSN	3rd SRSN	4th SRSN	5th/6th SRSN	7th SRSN
Shanghai #3	Ng82149/Kauz	Wuhan #3	Ng8675/Ng8645	Sha5/Weaver	Catbird
CMH78A.544	Ng8201/Kauz	China #7	Mayoor	Catbird	Gondo
Fan #1	Sha#3/Kauz	Ning7840	Ng8675/Cbrd	Chum18//Jup/Bjy	Shanghai
Ning7840		Shanghai #3	Lu 95	Gondo	Ng8675/Cbrd
Yangmai #6		F3.71/Trm//3383.20			Sha3/Cbrd
		Suzhoe #6			
		Ng82149			

The following SRSN lines expressed the lowest toxin content after heavy FHB epidemics in China (including Jianyang and Fujian Provinces, where levels reached 17-21 ppm in some entries): Sha#3/Cbrd, Ng8675/Cbrd, Milan/Sha7, Shanghai, and Mayoor//Tk Sn1081/Ae. squarrosa (222), toxin levels ranging from 0.35 to 1.65 ppm.

When barley genotypes from different programs from the US, Mexico and Latin America were evaluated in the Mexican highlands for Type I and Type II resistance to artificially inoculated *F. graminearum* and *F. avenaceum*, genotype x *Fusarium* species interaction was found (Marchand, 2003). The most resistant barleys were also tested in China and Canada (see Table 5), and will enter into the national nursery NABSEN.

Table 5. Top ICARDA/CIMMYT barley germplasm evaluated in Canada and China.

	Brandon, Canada 2001				Hangzhou, China, 2001-02		
Cross	Row	Rating (1-5)	DON		Diseased spike	Diseased index	Diseased seed
Canela/Zhedar#2	2	2	21.4		45.2	13.7	5.2
Zhedar#1/4/Shyri//Gloria-Bar/Copal/3/Shyri 2000/5/Arupo/K8755//Mora	2	2	46.3		45.2	11.3	6.0
Canela/Zhedar#2	2	2	28.7		35.5	8.9	7.3
Guayaba	2	3	44.0		53.3	20.8	8.8
Atah92/Gob	2	3	29.7		35.3	13.2	13.3
Atah92/Gob	2	2	21.6		45.8	15.6	16.4
Chevron, Robust, Stander (susceptible checks)	*6*	*5*	*92.0*		*77.4-96.8*	*29.0-55.6*	*25.4-45-7*
Zhedar 1, CI 4196 (resistant checks)	*2*	-	-		*6.6-10.0*	*1.9-2.8*	*0.6-3.1*

Some synthetic wheats show great promise, with 15 expressing multiple resistance: these are also being studied for marker identification. Wheat/*Thinopyrum bessarabicum* addition lines are being converted into translocation lines using the ph-system, FISH, C-banding, DHs and markers. To improve durum wheats *T. monococcum, T. urartu, T. boeoticum* and *Ae. speltoides* are being crossed, but success is not guaranteed. Some of the newly identified resistant parents were intercrossed allowing determination of four possibly new genes (Gilchrist *et al*., 2002; Table 6). The resistant and susceptible tails of the three distributions are now being studied for marker identification.

Table 6. Gene postulations of three crosses among three FHB resistant lines.

#	Parent 1	Parent 2	#	Postulation P1	Postulation
1	Bau/Milan	Gondo	2	AABBCCDD	aabbCCDD
2	Bau/Milan	Catbird	2	AABBCCDD	AABBccdd
3	Catbird	Gondo	4	AABBccdd	aabbCCDD

A total of 186 F7, F6, and BC1F4 populations, in which synthetic resistance Type II was back-crossed into five US commercial varieties, most confirmed twice to be resistant, were sent to USWBSI. They are also resistant to the most virulent Mexican races of stem, leaf and stripe rust. In collaboration with Busch Agricultural Resources Inc., FHB resistance is being crossed into US commercial barley varieties. Multiple disease resistant lines are now available (Table 5), also to the USWBSI.

In recent molecular studies at CIMMYT about one third of an elite group of CIMMYT wheats were shown to carry resistance distinct from the full Sumai#3 haplotype. See Figure 1.

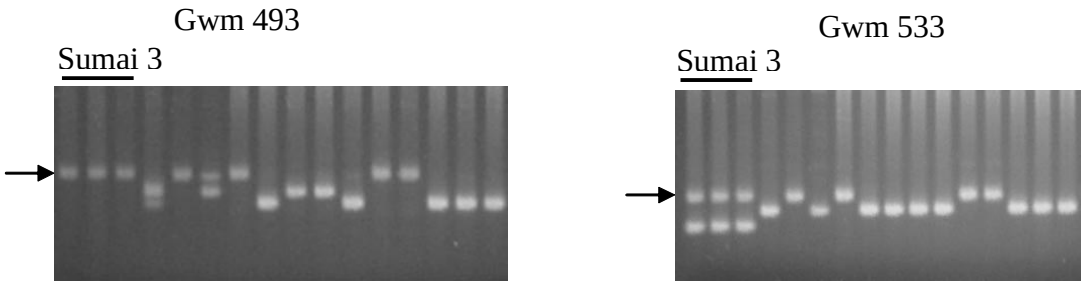

Figure 1. Allelic variations among a subset of CIMMYT bread wheat germplasm for SSR markers *Xgwm 493* and *Xgwm 533* associated with chromosome 3BS region. Sumai 3 alleles are indicated by an arrow.

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BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE: PHENOTYPIC VS. MARKED-BASED SCREENING IN EARLY GENERATIONS

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ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* (Schwabe) causes significant losses in the soft red winter wheat crop in Kentucky and in small grain crops in many regions of North America. FHB epidemics result in significant yield losses, and can cause serious reductions in grain quality. Phenotypic screening for FHB resistance is expensive and time consuming. Use of markers linked to FHB resistance genes may increase efficiency of the breeding process. For this purpose, three $F_{2:4}$ populations of 40 lines each were evaluated during 2001 in one location (Lexington, KY) and 2003 in two locations (Lexington and Princeton, KY). Screening for the Type II resistance QTL located on the chromosome 3BS was done using the SSR markers GWM493 and GWM533. Pedigree information led us to expect that all three populations would be segregating for these alleles. However, marked-based screening showed that only 19 lines of population 2 had the Sumai 3 alleles associated with markers GWM493 and GWM533. Broad sense heritabilities were calculated for severity in the three populations. Estimates were (0.83, 0.63 and 0.66) based on one location and (0.31, 0.60 and 0.59) based on two locations, suggesting that considerable progress could be made through selection. Some non-Sumai 3 resistance is evidently present in populations 1 and 3. Field results showed that severities ranged from 30 to 32 % and incidence from 43 to 50% in the three populations. Respective differences between lines in population 2 with marker and without were : severity - 28 vs 32%; incidence - 47 vs 53%; scabby kernels - 9.41 to 11.27%; and DON - 9.7 vs 10.64 ppm. These lines will continue to be analyzed for their FHB resistance phenotype and agronomic performance in FHB nurseries at Lexington and Princeton.

SUCCESS OF ALTERNATIVE BREEDING METHODS IN TRANSFERRING FUSARIUM HEAD BLIGHT RESISTANCE TO SOFT RED WINTER WHEAT

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OBJECTIVE

Accelerate development of FHB-resistant soft red winter wheat varieties using breeding methods other than traditional topcrossing.

INTRODUCTION

The Mid-Atlantic region has suffered significant economic losses in recent years, including 2003, from Fusarium head blight epidemics (Griffey et. al., 1999). This has reinforced the need to accelerate development of FHB-resistant varieties suited to our area. The Virginia Tech Small Grains Program has been involved in developing scab-resistant soft red winter wheat varieties using both traditional and alternative breeding methods. Traditional methods include topcrossing elite parents to obtain an improved variety with disease resistance. This method has proven to be successful in developing the scab-resistant Virginia Tech varieties Roane, McCormick, and Tribute. Alternative breeding methods include the transfer of FHB resistance from unadapted germplasm to adapted elite varieties via backcrossing and the acceleration of breeding progress using the wheat ´This paper evaluates progress made to date using such alternative breeding methods.

MATERIALS AND METHODS

Backcrossing. The first crosses between unadapted, scab-resistant lines and adapted elite varieties were initiated in 1998. Following these initial crosses, a series of one to five backcrosses were made to elite (recurrent) parents. During each of these cycles, the backcrosses were evaluated for scab resistance in a mist-irrigated greenhouse using the single floret inoculation technique. At various stages of the backcrossing process, populations were developed from selected individuals and planted at Mt. Holly, VA under mist-irrigated, scab-inoculated conditions. FHB-infected maize seed was the primary inoculum applied to these populations each year. Scab-resistant populations were bulk-selected in early generations and advanced; head-selections were made in later generations and evaluated in headrows.

Doubled Haploid. Use of the wheat × maize doubled haploid (DH) system was initiated in 2000 with nine 3-way crosses comprised of diverse scab-resistant parents. Emasculated wheat heads were hand-pollinated with maize pollen and immersed in 100 mg/L 2,4-D solution 1 to 2 days later. Embryos were excised 12-16 days post-pollination and cultured in test tubes containing nutrient agar medium. The resulting seedlings were then immersed in 0.1% colchicine solution, rinsed, transplanted into soil, and vernalized for up to 8 weeks. This process was repeated in 2001 with ten additional 3-way crosses.

RESULTS AND DISCUSSION

Backcrosses. In 2002, 29 BC_1F_4 and 3 BC_2F_4 headrows were selected and subsequently evaluated in observation yield tests at two locations and in a FHB nursery in 2003. Of these, 12 lines were selected and advanced for testing in replicated preliminary yield tests at three locations in 2004 (Table 1). Five of these lines are also being tested in the 2004 Uniform Scab Nurseries. Most of these lines had higher yield and lower FHB incidence than Roane, and 4 lines had FHB incidence levels lower than Ernie. FHB severity and index values followed a similar pattern.

In 2003, 124 backcross lines (28 BC_1F_5, 15 BC_2F_5, 59 BC_2F_4, 3 BC_3F_4, 18 BC_4F_3 and 1 BC_5F_3) were selected as headrows and advanced for evaluation in observation yield tests at two locations and in a FHB screening nursery in 2004. Field and greenhouse FHB screening data for the most advanced backcross lines are presented in Table 2. Most of the backcross lines were more resistant to scab than their respective recurrent parents in the field test. In the 2002 greenhouse screening, all of the backcross lines were more resistant than their recurrent parent. Other scab-resistant parents used in developing backcross lines being evaluated in 2004 observation yield tests but not included in Tables 1 and 2 include Er-Mai 9 and Yan-Ahi 9. The recurrent parent Jackson was also used in developing backcross lines.

Doubled Haploids. In 2003, 135 H_3 doubled haploid lines were evaluated in inoculated, mist-irrigated greenhouse and field tests. Of these lines, 30 were selected for further evaluation in observation yield tests at two locations and in a FHB screening nursery during the 2003-04 season. Twelve of the19 original 3-way crosses are represented among these selections. Field and greenhouse FHB screening data on the DH lines are presented in Table 3. For most of the lines, scab incidence and severity percentages are 30 or less, and Type II resistance ratings are within the resistant to moderately resistant range. A few DH lines with higher disease severities were selected due to outstanding agronomic characteristics, but in general, most lines performed very well in the presence of FHB.

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Table 1. Performance of Backcross Lines Selected for Advancement to the 2003-04 Virginia Tech Preliminary Yield Test.

		2003 Observation Data			
Line	**Pedigree**	**Yield (bu/a)**	**INC %**	**SEV %**	**IND**
ERNIE	RESISTANT CHECK	52.7	9.7	11.9	0.3
ROANE	RESISTANT CHECK	52.1	26.7	13.7	0.4
PION 2545	SUSCEPTIBLE CHECK	n/a	40.0	24.3	1.2
VA03W-633	VR95B717 / 2*Sisson"S"	61.5	15.0	12.6	0.3
VA03W-635	VR95B717 / 2*Sisson"S"	65.6	21.3	15.5	0.7
VA03W-646	Ning9016 / 2*Sisson"S"	73.2	18.3	13.0	0.5
VA03W-643	W14 / 2*Roane	53.9	4.3	7.4	0.1
VA03W-644	W14 / 2*Roane	55.9	16.7	11.3	0.3
VA03W-647	Shaan85-15 / 2*GA891283LE18	60.2	11.0	6.4	0.1
VA03W-651	Yumai 7 / 2*Pioneer2684	65.7	20.0	16.7	0.6
VA03W-652	Ning9016 / 3*Pioneer2684	64.0	28.3	17.0	0.8
VA03W-655	VR95B717 / 2*Ernie	61.1	16.7	9.3	0.2
VA03W-661	Ning7840 / 2*Ernie	46.3	4.3	5.4	0.1
VA03W-662	Ning9016 / 2*Ernie	51.4	3.7	4.0	0.1
VA03W-664	Ning9016 / 2*Ernie	50.8	7.0	8.6	0.2

Table 2. Reaction of FHB-Resistant Backcross Lines Versus Recurrent Parents in the 2003-04 Virginia Tech Scab Observation Test.

		Field Screening (2003)			Greenhouse Screening (2002)	
Entry	**Pedigree**	**INC %**	**SEV %**	**IND**	**SEV %**	**Type II Reading (1-5)**
Recurrent Parent	**Renwood 3260**	**55**	**60**	**33**	**47.8**	**3.9**
VA04W-163	W14 / 5*Renwood 3260, BC4F3	10	18	1.8	17.2	3.3
VA04W-164	W14 / 5*Renwood 3260, BC4F3	15	25	3.8	27.6	3.6
VA04W-165	W14 / 5*Renwood 3260, BC4F3	25	29	7.3	20.7	3.4
VA04W-166	W14 / 5*Renwood 3260, BC4F3	15	23	3.5	21.4	3.4
VA04W-167	W14 / 5*Renwood 3260, BC4F3	10	21	2.1	17.9	3.3
Recurrent Parent	**Roane**	**30**	**19**	**5.7**	**39.7**	**3.9**
VA04W-218	W14 / 5*Roane, BC4F3	30	27	8.1	11.1	2.7
Recurrent Parent	**Madison**	**75**	**61**	**46**	**31.9**	**3.7**
VA04W-231	Futai8944 / 5*Madison,BC4F3	55	69	38	30.0	3.7
VA04W-232	Futai8944 / 5*Madison,BC4F3	50	34	17	30.0	3.7
VA04W-234	Futai8944 / 5*Madison,BC4F3	55	52	29	17.1	3.4
Recurrent Parent	**Agripro Mason**	**35**	**36**	**13**	**48.2**	**4.0**
VA04W-239	Shaan85-15 / 6*Agripro Mason,BC5F3	50	45	23	29.4	3.7
Recurrent Parent	**Ernie**	**5**	**23**	**1.2**	**44.5**	**4.0**
VA04W-257	Shaan85-2 / 5*Ernie, BC4F3	4	13.3	0.5	22.2	2.9
VA04W-260	VR95B717 / 5*Ernie, BC4F3	20	39	7.8	20.7	3.4
VA04W-265	W14 / 5*Ernie,BC4F3	5	20	1	28.1	3.7
VA04W-266	W14 / 5*Ernie,BC4F3	5	18	0.9	20.8	3.3
VA04W-274	Futai8944 / 5*Ernie,BC4F3	4	18	0.7	21.7	2.8
VA04W-275	Futai8944 / 5*Ernie,BC4F3	10	21	2.1	22.9	3.0
VA04W-276	Futai8944 / 5*Ernie,BC4F3	4	15.7	0.6	20.7	3.4
VA04W-277	Futai8944 / 5*Ernie,BC4F3	20	34	6.8	28.0	3.5

Table 3. Field and Greenhouse Reaction of FHB Doubled Haploid Lines in the 2003-04 Virginia Tech Scab Observation Test.

		Field Screening (2003)			Greenhouse Screening (2002)	
Line	**Pedigree**	**INC %**	**SEV %**	**IND**	**SEV %**	**Type II Score: 1-5**
Ernie	**Resistant Check**	5	23	1.2	44.5	4.0
Roane	**Resistant Check**	30	19	5.7	39.7	3.9
Renwood 3260	**Moderately Susceptible Check**	55	60	33	47.8	3.9
Madison	**Susceptible Check**	75	61	46	31.9	3.7
VA04W-118	Ning7840/Ernie//Tribute,H3	40	52.0	21.0	23.3	3.2
VA04W-119	Roane/Freedom//Ernie,H3	25	24.0	6.0	6.3	2.0
VA04W-120	Roane/Freedom//Ernie,H3	5	17.8	0.9	5.2	1.5
VA04W-121	Roane/Freedom//Ernie,H3	20	13.0	2.6	9.1	2.5
VA04W-122	Roane//W14/Coker9134,H3	20	19.0	3.8	6.8	2.0
VA04W-123	Roane//W14/Coker9134,H3	5	11.7	0.6	7.2	2.4
VA04W-124	Roane//W14/Coker9134,H3	4	0.0	0.0	4.7	2.1
VA04W-125	Roane//W14/Coker9134,H3	4	14.3	0.6	8.9	2.4
VA04W-127	Renwood 3260//Freedom/Ernie,H3	20	33.0	3.3	11.1	2.0
VA04W-128	Renwood 3260//Freedom/Ernie,H3	4	21.7	0.9	27.5	3.6
VA04W-129	Renwood 3260//Freedom/Ernie,H3	15	28.0	4.2	8.0	2.7
VA04W-130	Renwood 3260//Freedom/Ernie,H3	4	13.0	0.5	26.5	3.3
VA04W-131	Renwood 3260//Shaan85-2/Ernie,H3	4	10.0	0.4	6.9	2.8
VA04W-132	Renwood 3260//Shaan85-2/Ernie,H3	40	30.0	12.0	8.6	2.7
VA04W-133	Renwood 3260//Shaan85-2/Ernie,H3	4	16.7	0.7	4.7	2.0
VA04W-134	VR95B717/Roane//Pioneer26R24,H3	4	15.0	0.6	14.4	2.9
VA04W-135	VR95B717/Roane//Pioneer26R24,H3	4	11.7	0.5	12.3	3.3
VA04W-136	Ernie//INW 9824/McCormick,H3	4	10.0	0.4	7.0	2.7
VA04W-137	IL4162//INW 9824/McCormick,H3	5	22.0	1.1	14.1	2.9
VA04W-138	INW 9824/VA375WS//Pioneer25W33,H3	5	17.0	0.9	7.5	2.4
VA04W-139	INW 9824/VA375WS//Pioneer25W33,H3	20	22.0	4.4	25.3	3.6
VA04W-140	INW 9824/VA375WS//Pioneer25W33,H3	35	31.0	11.0	13.6	2.7
VA04W-141	Roane//IL89-6489/AGS 2000,H3	30	27.0	8.1	29.5	3.8
VA04W-142	Roane//IL89-6489/AGS 2000,H3	20	24.0	4.8	27.5	3.0
VA04W-143	Roane//IL89-6489/AGS 2000,H3	15	15.0	2.3	11.7	2.5
VA04W-144	Roane//IL89-6489/AGS 2000,H3	15	15.0	2.3	13.0	3.2
VA04W-145	Roane//INW 9824/AGS 2000,H3	15	18.0	2.7	11.0	2.3
VA04W-146	Roane/AGS 2000//Agripro Gibson,H3	10	15.0	1.5	14.3	3.0
VA04W-148	Roane/AGS 2000//Agripro Gibson,H3	35	38.0	13.0	18.5	2.7
VA04W-149	Roane/AGS 2000//Agripro Gibson,H3	20	15.0	3.0	5.9	1.8

MARKER-ASSISTED BACKCROSSING SELECTION OF NEAR-ISOGENIC LINES FOR A 3BS FUSARIUM HEAD BLIGHT RESISTANCE QTL IN *TRITICUM AESTIVUM*

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OBJECTIVE

Our objective was to develop near-isogenic lines for the 3BS Fusarium head blight resistance QTL using marker-assisted backcrossing.

INTRODUCTION

Near-isogenic lines (NILs) differing in disease resistance quantitative trait loci (QTL) are valuable materials for the study of the genetic basis of quantitative resistance. A major QTL on the distal part of chromosome 3BS known to have an effect on Type II Fusarium head blight (FHB) resistance in hexplaoid wheat was used in a marker-assisted backcross program. This QTL explained up to 60% of the phenotypic variation in several mapping populations (Bai et al., 1999; Zhou et al., 2000, 2002; Buerstmayr et al., 2002). A number of research groups have reported that this QTL has the largest effect on FHB resistance among all FHB resistance QTL identified to date (Bai et al., 1999; Anderson et al., 2001; Ban and Watanabe 2001; Zhou et al., 2000, 2002; Sneller et al., 2001; Buerstmayr et al., 2002; Shen et al., 2003). This 3BS QTL was also verified in two breeding populations and used successfully in marker-assisted selection for FHB resistance (Zhou et al., 2003).

MATERIALS AND METHODS

A marker-assisted backcrossing selection program was begun in 1999 to develop NILs for the 3BS Fusarium head blight resistance QTL. A recombinant inbred line, RIL90, was selected from among 133 RILs derived from Ning7840×Clark as the donor parent of the 3BS QTL. RIL90 was selected based on AFLP mapping results and FHB screening tests. Based on 617 AFLP markers mapped on 133 RILs, the genetic similarity between Clark and RIL90 was 65%. RIL90 was backcrossed with Clark and the F_1 was further backcrossed with Clark. Five BC_1F_1 plants were selfed, and fifty BC_2F_2 plants were genotyped with SSR markers Xgwm389, Xgwm533 and Xbarc147 during the seedling vernalization period and evaluated for Type II scab resistance after flowering in the greenhouse. Two plants which were homozygous for marker alleles from Ning7840 for Xgwm533 and Xbarc147 with moderate resistance were selected to backcross with Clark for two additional generations. Five BC_4F_1 plants derived from the two BC_2F_2 plants were selfed, and 56 and 64 BC_4F_2 plants derived from these two BC_2F_2 plants were genotyped and Type II scab resistance was evaluated. NILs for the major scab resistance QTL were identified, and 15 $BC_4F_{2:3}$ plants derived each line were evaluated for Type II scab resistance conferred by the 3BS QTL.

Genetic similarity between Clark and NILs was calculated by means of simple matching coefficients (SMC), $S_{ij} = (a + d)/(a + b + c + d)$, where a = number of fragments in common between genotypes; d = number of fragments

absent in both genotypes, and *b* and *c* = number of fragments not in common between two genotypes (Sokal and Michener, 1958).

RESULTS AND DISCUSSION

The average percentage of scabbed spikelets (PSS) values of RIL90 over four FHB screening tests was 47%. Based on AFLP markers linked to the 3BS major QTL, RIL90 carries the resistant allele from Ning7840. With the development and application of simple sequence repeat (SSR) markers in wheat genome mapping, 121 polymorphic SSR markers (out of 728 SSR markers analyzed) were mapped on the 133 RILs. All of these SSR markers were integrated with 617 AFLP markers mapped on the same population. Six SSR markers and one STS marker (Guo et al., 2003) linked to the 3BS QTL were identified (Figure 1.). During the process of developing NILs, the genome region containing the scab resistance QTL in backcross plants was retained through SSR marker analysis. NILs differing in Type II FHB resistance and alleles from Ning7840 and Clark were identified in BC_4F_2 populations. Greenhouse evaluation of FHB resistance of single BC_4F_2 plants and a progeny test and SSR analysis confirmed the identification of RILs with the 3BS QTL. Frequency distribution of PSS values for BC_4F_2 plants is shown in Figure 2.

NILs were selected from 120 BC_4F_2 plants based on SSR and STS marker genotypes and FHB phenotypic data. Genetic similarity between NILs and Clark was tested based on all SSR markers polymorphic between Ning7840 and Clark. Plants obtained after the fourth generation of backcrossing resembled the recurrent susceptible parent based on phenotypic and genotypic evaluation. NILs had genetic similarity with Clark of more than 98%, but retained the major FHB resistance QTL from Ning 7840 and a 3BS region from Ning 7840 of less than 8 cM in length. These NILs will be useful for further molecular characterization of the major QTL on 3BS.

ACKNOWLEDGMENTS

We thank Amy Wilson for excellent technical assistance. We thank Dr. P. B. Cregan and Dr. Q. J. Song from USDA-ARS, Beltsville, MD, for providing sequence information of newly developed microsatellites (Xbarc). We thank Dr. Marion Röder from the Institute for Plant Genetics and Crop Research, Gatersleben, Germany, for providing sequence and mapping information of fifty pairs of unpublished Xwms SSR primers and Dr. M. D. Gale, John Innes Center, UK for providing forty-two Xpsp SSR markers. Funding for this research was provided by the University of Illinois Agricultural Experiment Station, the Illinois Council for Food and Agricultural Research (C-FAR), and the U. S. National Wheat and Barley Scab Initiative.

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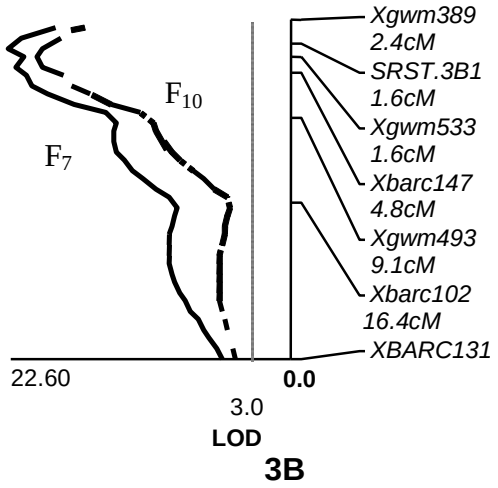

Figure 1. Linkage and interval analysis of SSR and STS markers linked to the major Type II FHB resistance QTL on chromosome 3BS in a set of 133 recombinant inbred lines from a cross of Ning7840 ×Clark. Phenotypic data was collected in F7 and F10 RILs, respectively.

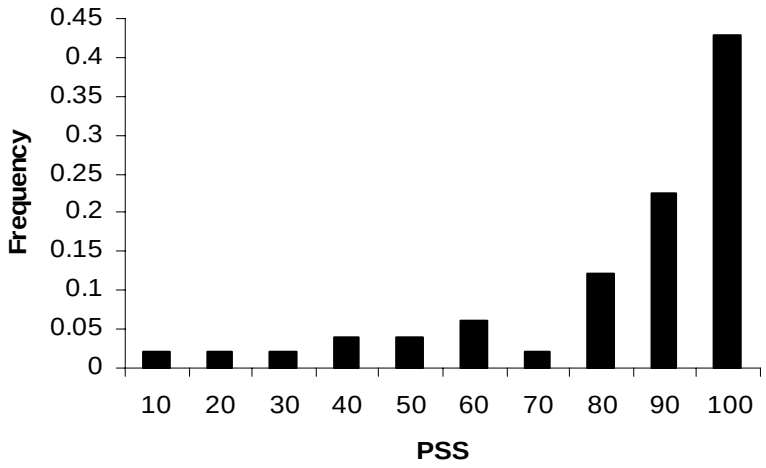

Figure 2. Frequency distribution of PSS values of 120 BC_4F_2 plants derived from backcrossing RIL90 with recurrent susceptible line, Clark. RIL90 is a F_{11} recombinant inbred line derived from a cross between Ning7840 and Clark.

THE US WHEAT AND BARLEY SCAB INITIATIVE WEB SITE

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ABSTRACT

The US Wheat and Barley Scab Initiative (USWBSI) web site(http://www.scabusa.org) is an integral part of the USWBSI. The site is an important information resource for all aspects of the Initiative, IncludingResearch, News, Forums, and Literature. The site includes an online searchable database containing detailed information on all Projects, Grants, Institutions, Documents, Committees, and Contacts associated with the Initiative. Maintenenance of the web site involves database development, web programming, and system administration. Each section of the web site is constantly being improved and expanded. Some key goals for improvement of the site are to enhance the Research section (results, methods, online informatics resources, etc.), and to facilitate communication among Initiative members, Scab researchers, wheat/barley breeders and growers, and others affected by Scab. Another key goal is to integrate the USWBSI web site more closely with GrainGenes (http://wheat.pw.usda.gov), an online database of molecular and phenotypic information for the Triticeae. This poster provides an overview of the USWBSI web site, solicits ideas and suggestions for future improvements to the site, and addresses the use of the GrainGenes web site as a resource for Scab research.

GRDC STRATEGIC INITIATIVE ON CROWN ROT, COMMON ROOT ROT AND FHB IN AUSTRALIA

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ABSTRACT

Crown rot caused by the fungus *Fusarium pseudograminearum* is a major constraint to winter cereal production in Australia. Although it is generally more common in the northern cropping belt, it can occur throughout all mainland cereal growing areas and is estimated to cost the Australian grains industry $56 million per annum. Common root rot, caused by the fungus *Bipolaris sorokiniana*, is often found in association with crown rot and has been estimated alone to cost growers $22 million per annum. Losses from Fusarium head blight (FHB) caused predominantly by *Fusarium graminearum* have not been estimated in Australia. However, severe FHB on the Liverpool Plains in northern NSW in 1999 and 2000 inflicted yield losses of around 20-100% with associated downgrading in quality. Outbreaks of FHB have occurred sporadically in Australia and have also been associated with the rainsplash of *F. pseudograminearum* macroconidia formed on lower nodes into heads. A strategic initiative on crown rot, common root rot and FHB with funding from the Grains Research and Development Corporation (GRDC) was formed in 2002 to address these disease problems in the Australian grains industry. The initiative encompasses seven projects across four states with the aims of: i) providing an integrated and coordinated approach to the management of these diseases, ii) facilitating communication and collaboration between research groups in Australia and internationally, and iii) extending research outcomes to growers. Research projects are:

1. Epidemiology and pathology of Fusarium in relation to crown rot and FHB - Dr Sukumar Chakraborty, CSIRO-Plant Industry, St. Lucia, Brisbane.
2. Management of Fusarium diseases and common root rot of cereals in the northern cropping zone – Dr Steven Simpfendorfer, NSW Agriculture, Tamworth.
3. Genetic approaches to resistance to Fusarium and Bipolaris in wheat and barley - Dr Graham Wildermuth, Queensland Department of Primary Industries, Toowoomba.
4. Crown rot management in durum and bread wheats for the southern region - Dr Hugh Wallwork, South Australian Research and Development Institute, Adelaide.
5. Development of a molecular diagnositic for crown rot - Dr Alan McKay, South Australian Research and Development Institute, Adelaide.
6. Epidemiological principles, inoculum dynamics and nitrogen effects on crown rot - Dr David Backhouse, University of New England, Armidale.
7. Intermediate hosts and the management of crown rot and head blight - Professor Lester W. Burgess, University of Sydney.

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