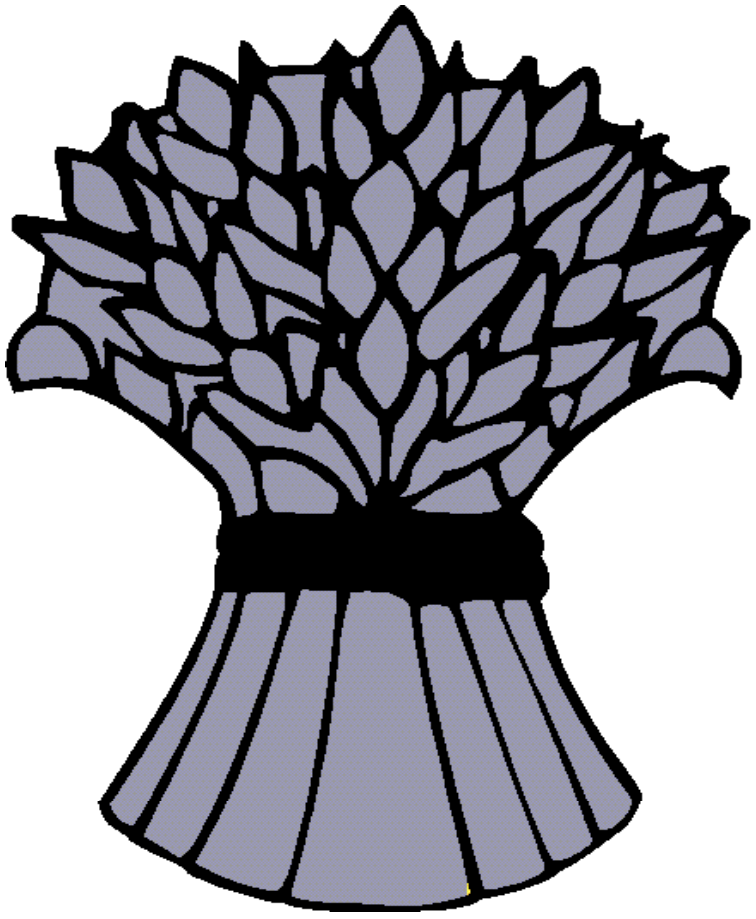
2002 National Fusarium Head Blight Forum Proceedings



Holiday Inn Cincinnati-Airport
Erlanger, KY
December 7-9, 2002

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Table of Contents

BIOTECHNOLOGY

CHEMICAL AND BIOLOGICAL CONTROL

EPIDEMIOLOGY AND DISEASE MANAGEMENT

FOOD SAFETY, TOXICOLOGY AND UTILIZATION

GERMPLASM INTRODUCTION AND ENHANCEMENT

VARIETY DEVELOPMENT AND UNIFORM NURSERIES

OTHER REPORTS

FUSARIUM VIRULENCE AND PLANT RESISTANCE MECHANISMS: A PROJECT WITHIN THE AUSTRIAN GENOME PROGRAMME GEN-AU

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ABSTRACT

In 2002 the Austrian Federal Ministry for Education, Science and Culture has established the national genome programme GEN-AU (GENome Research in AUstria: http://www.gen-au.at/). The first call has brought together a broad spectrum of Austrian scientists (coordinator GA) focussing on the *Fusarium* problem. We proposed (as one part) that Austria should contribute 25% of the costs of sequencing the *F. graminearum* genome, but the panel felt that our proposal was too much dependent on the (in April 2002) not yet submitted proposal by US partners (Birren, Kistler, Xu, Trail). In the meantime a dramatically downscaled pilot-project (with only 5 of the initially 12 partner institutions remaining) has been funded (about 795.000 US dollar).

In the next two years researchers from the Center of Applied Genetics (CAG) of the University of Agricultural Sciences, Vienna, the Technical University (TU) of Vienna, the Institute for Agrobiotechnology in Tulln (IFA), the Austrian Research Center Seibersdorf (ARCS), and from the wheat breeding company Saatzucht Donau will collaborate on several aspects. The following principal investigators are involved: Josef Straub, Gerhard Adam (CAG) and Robert Mach (TU) will collaborate on the development of efficient gene disruption methods for *F. graminearum*. Mutants will be tested for altered virulence at the IFA Tulln (Marc Lemmens) and for altered metabolite production by LC-MS-MS (Rudolf Krska, IFA Center for Analytical Chemistry). Also analytical techniques and reference materials for “masked mycotoxins” will be developed. In the group of Gerhard Adam *Arabidopsis thaliana* genes encoding mycotoxin inactivating enzymes will be characterized, and Marie-Theres Hauser will explore the role of zearalenone in plants (CAG). The group from ARCS will establish wheat suspension cultures and work on the identification of differentially expressed genes in wheat and the development of DNA arrays. The genetic basis of so far uncharacterized highly *Fusarium* resistant wheat genetic resources will be elucidated by Hermann Büerstmayr (IFA), the knowledge gained will be utilized by the commercial partner (Julia Lafferty, Saatzucht Donau) in a marker assisted backcross breeding program (QTL pryamiding).

The aim of the GEN-AU program is “to secure and expand Austria’s competitiveness and ability to cooperate on an international level”. It may be of interest for US researchers, that the EU 6th framework programme is open to the participation of entities from nonmember countries on the project level on the basis of mutual benefit.

QTL ANALYSIS OF FUSARIUM HEAD BLIGHT IN BARLEY USING THE CHINESE LINE ZHEDAR 2

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ABSTRACT

Fusarium head blight (FHB) in barley and wheat caused by *Fusarium graminearum* is a continual problem worldwide. Primarily, FHB reduces yield and quality and produces the toxin deoxynivalenol (DON), which can affect food safety. Locating QTLs for FHB severity, DON level and related traits heading date (HD) and plant height (HT) with consistent effects across a set of environments would increase the efficiency of selection for resistance. A population of seventy-five double haploid lines, developed from the three-way cross Zhedar 2/ND9712//Foster, was used for genome mapping and FHB evaluation. Phenotypic data were collected in replicated field trails from five environments in two growing seasons. A linkage map of 214 RFLP, SSR and AFLP markers was constructed. The data were analyzed using MQTL software to detect QTL x environment interaction. Because of the presence of QTL x E, the MQM in MAPQTL was applied to identify QTLs in single environments. MQM mapping identified nine QTLs for FHB severity and five for low DON. Only three of these QTLs were consistent across environments. Five QTLs were associated with HD and two with HT. Regions that appear to be promising candidates for MAS and further genetic analysis including the two FHB QTLs on chromosome 2H and one on 6H which also were associated with low DON and later heading date in multiple environments. This study provides a starting point for manipulating Zhedar 2-derived resistance by MAS in barley to develop varieties that will show effective resistance under disease pressure.

ISOLATION AND CHARACTERIZATION OF *TRI16* FROM *FUSARIUM SPOROTRICHIOIDES*

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ABSTRACT

Many of the genes involved in the trichothecene biosynthetic pathway in *Fusarium* have now been identified within a 29 kb section of DNA. Within this cluster are 3 genes (*Tri4, Tri11, and Tri13*) encoding P450 oxygenases, a gene (*Tri5*) encoding sesquiterpene cyclase, a gene encoding an esterase (*Tri8*), two acetyltransferase genes (*Tri3 and Tri7*), a transport pump gene (*Tri12*), and two regulatory genes (*Tri6* and *Tri10*). One gene encoding an acetyltransferase, *Tri101*, is not located within the cluster. However, not all of these genes are functional in every *Fusarium* species. The *Fusarium* toxins can be divided into two groups based on the substitution of the A ring. *Fusarium sporotrichioides* produces A-type trichothecenes, such as T-2 toxin or 4,15-diacetoxyscirpenol, while *F. graminearum* produces B-type trichothecenes, such as deoxynivalenol (DON), that have a carbonyl at C-8. These differences in side groups are due, at least, to the non-functional *Tri7* and *Tri13* in B-type trichothecene producers. In the search for the remaining trichothecene genes, the use of an EST library from a toxin over-producing strain carrying an altered *Tri10* has identified *Tri16*, a gene believed to be involved with trichothecene biosynthesis. We isolated and cloned this gene from *F. sporotrichioides*, then formed disruption vectors through insertional disruption and truncated disruption. Insertional disruption vectors produced only single cross-over events when the vector was transformed into the host protoplasts thus producing a transformant with both a disrupted as well as an intact copy of the gene. Transformants carrying the truncated disruption vector were also tested by PCR and Southern hybridization for disruption events and analyzed for toxin production. Disruption of *Tri16* does not affect toxin production. Northern analyses suggest that *Tri16* is regulated like a secondary metabolite as it is turned on in later cultures like several of the other toxin biosynthetic genes. *Tri16* is physically located on linkage group 2 whereas the main trichothecene cluster is on linkage group 1. Even though *Tri16* is found in the EST library, these studies show that *Tri16* is not necessary for toxin production.

A SYSTEMATIC APPROACH FOR IDENTIFYING ANTIFUNGAL PROTEINS WITH ENHANCED RESISTANCE TO SCAB

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OBJECTIVES

1) Identification and purification of antifungal proteins from apoplastic fluids of wheat plants. 2) Expression of recombinant antifungal proteins and test the effectiveness of the purified proteins singly or in different combinations against scab using *in vitro* assays. 3) Incorporate the desired antifungal genes into elite germplasm of wheat using the transgenic approach.

INTRODUCTION

One of the strategies to enhance disease resistance in plants is to make effective use of their natural defenses such as pathogenesis-related (PR-) proteins. Many genes for PR-proteins were shown to be induced upon scab infection of wheat indicating their importance in plant defense (Li *et al*., 2001; Pritsch *et al*., 2001). In the past, we have utilized antifungal genes with different cellular targets in wheat transformation studies without experimental evidence that these proteins are actually effective against *Fusarium graminearum* (Chen *et al*., 1999; Anand *et al*., 2001). Even though some of these transgenic plants were moderately resistant under greenhouse trials, field evaluation did not show any significantly improved resistance to scab suggesting that these lines could not withstand continuous pathogen pressure encountered in the field where both the type I and type II resistance is required for survival (Anand *et al*., 2002 manuscript communicated). In order to speed up the effort to obtain transgenic plants with enhanced scab resistance and to improve the chance of success within a limited period of time, it will be useful to identify the genes encoding proteins that are effective against scab in preliminary *in vitro* assays. Thus a multi-pronged approach relying on identification of genes that are likely to have antifungal activity, isolating these genes from appropriate sources, and introducing them into wheat or barley plants has been developed.

MATERIALS AND METHODS

Field testing of the transgenic wheat plants- Field testing of transgenic and control lines were carried out in spring 2002 at the Plant Pathology Experimental Farm located near Manhattan, KS, USA. A randomized complete block design was used with 20 replicates for each treatment. Corn kernels (93 gm^{-2}) colonized by *F. graminearum* were applied to the soil.

Extraction of the Apoplastic fluid from wheat leaves- Ten grams of fresh leaves from mature plants were vacuum infiltrated with 100 mM sodium phosphate buffer (pH 6.8). The

infiltrated leaves were dried on filter paper sheets and centrifuged at 5000 rpm for 30*'*. The supernatant representing the apopalstic fraction was collected and stored at -70°C.

Recombinant expression of antifungal genes- The wheat cDNA clones isolated from the fungus-infected plants of wheat (Li *et al*., 2001) or rice were used for expression. The coding region fragments (minus signal peptide) were moved into the *E. coli* expression vectors, pQE60 or pTOPO under the control of the *lac* promoter and induced with isopropyl thiogalactoside (IPTG) for different time intervals to optimize maximum expression.

RESULTS AND DISCUSSION

Greenhouse and field testing of transgenic plants- Greenhouse testing was carried out in spring 2001 and fall 2002 with 4 independent homozygous transgenic (see Table 1). The line over-expressing the 383 chitinase/ 638 glucanase transgenes (#32A) showed a delay in the development of disease symptoms, and was scored as moderately resistant, while three other transgenic lines did not show any elevated levels of resistance reaction to scab (Table 1). We suspect that there may be a requirement for a threshold level of PR-proteins in order to be effective against scab. The results of the field evaluation are presented in Table 2. The transgenic lines did not have any enhanced resistance against scab, suggesting that these lines could not withstand continuous pathogen pressure encountered in the field where both the type I and type II resistance is required for survival.

Table 1. Greenhouse trials with the homozygous progenies of different transgenic wheat lines.

Entry	Total no. plants inoculated	Days after inoculation	Mean infected spikelets / head
#32A	25	10	3.7[b]
#32C	31	10	6.6[a]
MN99112	24	10	2.0[c]
Bobwhite	42	10	6.8[a]
#32A	25	14	7.4[b]
#32C	31	14	12.5[a]
MN99112	24	14	4.2[c]
Bobwhite	42	14	13.8[a]
#76	44	10	6.5[bc]
#78	51	10	6.1[c]
#82	70	10	7.1[a]
MN99112	42	10	2.05[d]
Bobwhite	64	10	6.8[ba]
#76	44	14	11.8[d]
#78	51	14	14.0[ba]
#82	69	14	14.6[a]
MN 99112	42	14	3.1[e]
Bobwhite	62	14	13.0[c]

Homozygous plants of #32A (383 chitinase and 638 glucanase), #32C (silenced line) used as an epigenetic control, Line # 76 and # 78 (289 glucanase and 383 chitinase) and line #82 (638 glucanase) were tested along with MN99112 (resistant check) and non-transformed 'Bobwhite' plants (susceptible check) respectively.

Table 2. Field evaluation of transgenic plants and control plants in spring 2002

Entry	Symptom rating - 3rd day	Symptom rating - 6th day	Symptom rating - 10th day	Symptom rating - 14th day
BT-14-18	17.5[a]	25.6[a]	59.7[a]	65.0[ba]
32A	9.4[c]	23.0b[a]	51.0[b]	67.8[a]
32C	13.2[b]	16.5[c]	54.7[ba]	61.2[ba]
Bobwhite	12.6[b]	17.9[bc]	53[b]	60.8[b]
Wheaton	6.7[c]	12.2[c]	49[b]	52.7[c]
MN99112	0.5[d]	1.4[d]	9.0[d]	19.3[d]
Scab-7	0.85[d]	3.5[d]	33.5[c]	52.2[c]

BT-14-18, *TLP* transgenic line; 32A, transgenic line co-expressing 383 chitinase/638 glucanase; 32C, transgene-silenced line co-transformed with 383 chitinase/638 glucanase; 'Bobwhite', untransformed control; Wheaton, a susceptible check; MN99112, a resistant check; Scab-7, a resistant check.

Characterization of the apoplastic fluid and in vitro antifungal assays- Western blot analysis of apoplastic fluid from leaves of the line #32A (see Table 1, with lesion phenotype) indicated that in addition to the expected transgene-encoded chitinase and β-1,3-glucanase bands, these extracts contained several other PR-proteins, including TLP's. In the apoplastic (extracellular) fluid prepared from these leaves about 6-10 major bands could be detected (Fig. 1). Further analyses indicated that the majority of the PR-proteins (>85%) are secreted and are localized extracellularly. The apoplastic fluid from the *chi/glu* transgenic line, 32A, with about 100 μg of total protein showed a distinct inhibitory effect against *F. graminearum in ivitro* antifungal assays (data not shown). Less effective mycelial growth inhibition was detected with equivalent amounts of the apoplastic fluid of the *TLP* transgenic line, D34.

A spore germination inhibition assay using the conidial suspension of *F. graminearum* was utilized to confirm the results of the mycelial growth inhibition assay. No germination of the conidia could be detected in the presence of barley chitinase (4 μg) and apoplastic protein preparation (25 μg protein) after 4 h of incubation, while 75%-100% germination was detected in the presence of non-transgenic apoplast extracts and 4 μg of *M. sexta* chitinase (Fig. 2). It is likely that the inhibition of spore germination by the apoplastic fluid might be due an additive or synergistic effects between chitinase and other PR-proteins including β-1,3-glucanase and TLP, or other proteins present in the apoplastic fluid.

Recombinant expression of other PR-protein genes- The successful inhibition of *Fusarium graminearum* in the two *in vitro* assays has prompted us to use an alternative approach which involves identification of specific combinations of antifungal proteins effec-

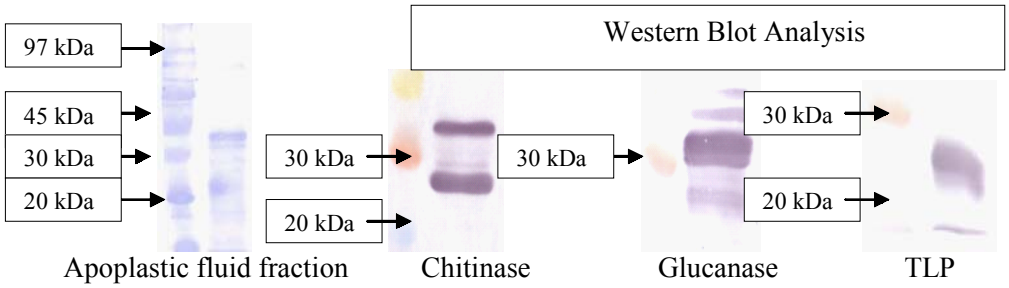

Figure 1. Detection of PR-proteins in apoplastic fluid prepared from the lesion-mimic transgenic wheat plant #32A. M = marker.

tive against scab using *in vitro* assays prior to the utilization of their genes in transgenic studies. The coding region fragments (minus signal peptide) were insertd into the *E. coli* expression vectors, pQE60 or pTOPO under the control of the *lac* promoter and colonies expressing TLP, LTP and 194 chitinase upon induction with isopropyl thiogalactoside (IPTG) were identified. The recombinant LTP protein appears to be soluble and could be detected in the supernatant after sonication of the cultures while the rice D34 TLP and 194 chitinase proteins were in the pellet fraction.

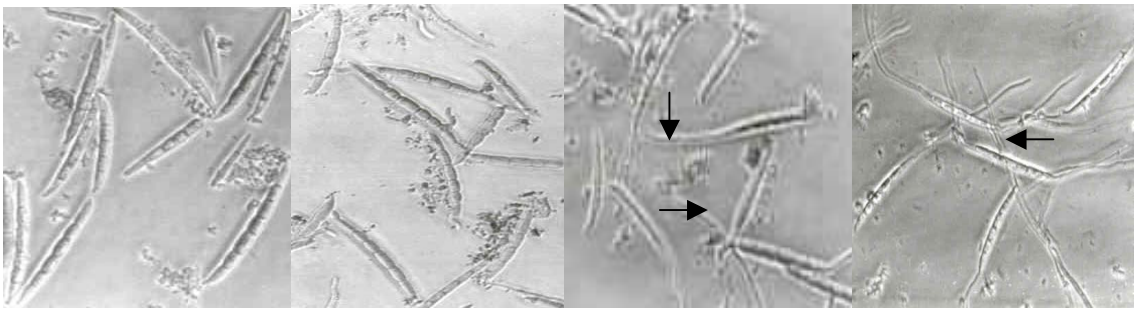

Figure 2. Spore germination inhibition assay with different protein preparations. Arrows indicate the germinating conidiophores.

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VERIFICATION OF MOLECULAR MARKERS LINKED TO FUSARIUM HEAD BLIGHT RESISTANCE QTLS IN WHEAT

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ABSTRACT

Molecular mapping led to the identification of QTL on chromosomes 3B and 5A of wheat (Anderson *et al*. 2001, Buerstmayr *et al*. 2002). The aim of this work was to verify molecular markers linked to these QTL in several spring by winter wheat crosses.

Crosses were initiated between FHB resistant spring wheat CM-82036 and several adapted European winter wheat genotypes. Populations of recombinant inbred lines in F4 to F8 generation were evaluated for resistance to FHB in replicated artificially inoculated field experiments.

The lines were genotyped with SSR markers mapping to one of the two putative QTL regions, 3B: GWM398, GWM533, GWM493, and 5A: GWM293, GWM304, GWM156.

Depending on testing year and population, markers on 3BS were more frequently associated with FHB resistance reaction than those on 5A, indicating that the QTL on 3BS has a larger and more consistent effect than the 5A QTL.

Not all 6 markers linked to a QTL in the model spring wheat population showed significant association in the verification populations. Further analysis should reveal the reasons for that.

Despite that, marker assisted selection for FHB resistance appears efficient in material segregating for resistance originating from CM-82036.

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WHEAT TRANSFORMATION FOR ENHANCED FUSARIUM HEAD BLIGHT TOLERANCE

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OBJECTIVES

To transform wheat lines with genes that may lead to improved tolerance of Fusarium head blight.

SUMMARY OF WORK

Fusarium graminearum is an important pathogen of wheat. Infection can result in significant yield losses and greatly reduce grain end-use quality due to detectable levels of the mycotoxin, Deoxynivalenol. To date, insufficient genetic resistance towards this pathogen has been identified within wheat germplasm. Biotechnology provides an avenue to introduce novel genes into elite wheat germplasm to complement an integrated program to manage *F. graminearum* pathogenesis. We have developed transgenic wheat with antifungal and anti-apoptotic genes using the *Agrobacterium*-transformation methods. Our preliminary data indicate that this approach will be effective in controlling FHB in wheat.

Antifungal genes: We have tested an animal lactoferrin gene for potential antifungal activity to *F. graminearum*. Lactoferrin (LF), an iron binding glycoprotein has long been reported to be active against a wide range of microorganisms including fungi (Zhang et. al. 1998). Lethal concentration of bovine LF, for instance, ranged from 18 to 100 μg/ml against yeast and 2 to 20 μg/ml against filamentous fungi (Bellamy et. al.; 1994). The lethal action of LF is believed to be due to the binding of the protein to the membrane and subsequent disruption of proton-gradient across the membrane. This results in membrane leakage and ultimately cell death. Lactoferrin contains an active antimicrobial domain lactoferricin (LFcin). Chong and Langridge (2000) recently demonstrated that LF protein expressed in potato tuber tissues has strong antimicrobial activities. The expression of non-plant antimicrobial genes such as LF in a transgenic plant has potential for broad-spectrum disease resistance.

In our previous work, the production of LF in tobacco cells indicated a potential for the development of disease resistant transgenic plants (Mitra and Zhang, 1994, Zhang et. al. 1998). In vitro analyses of total protein extracts from transgenic tobacco demonstrated strong anti *Fusarium graminearum* activity. Accordingly, transgenic wheat plants expressing the LF gene, were generated using both gene-gun and *Agrobacterium* mediated transformation methods. Among transgenic lines tested, five gene-gun generated lines and eight *Agrobacterium* generated lines consistently showed high levels of type II resistance (less than 10% infection) against scab in greenhouse experiments. Additional transgenic wheat

plants were generated expressing a short, synthetic LFcin gene (the 41 amino-acid long peptide from the amino-terminal end of LF) had a much stronger antimicrobial activity in tobacco than LF. Both LF and LFcin serve as membrane-disruptive agents when interacting with fungal membranes. Lactoferricin is substantially smaller and more cationic than LF; features that might help LFcin penetrate the fungal membrane more efficiently and protect it from further degradation by plant proteases. Nine western positive plants were obtained and 8-15 progeny of 3 transgenic lines were tested for type-II resistance in greenhouse. The plants were either 100% susceptible or had a high level of resistance (disease rating 10). The resistance correlated with the expression of the LFcin protein. In line 1, out of 15 plants, 6 were resistant (disease rating 10%) and 9 were susceptible (disease rating 100%); in line 2, out of 14 plants, 9 were resistant (disease rating 10%) and 5 were susceptible (disease rating 100%); and in line 3, out of 8 plants, 5 were resistant (disease rating 10%) and 3 were susceptible (disease rating 100%). Some PCR positive plants were susceptible, however, no lactoferrin protein could be detected in these plants. We have identified 9 LF and 6 Lfcin homozygous lines that showed consistent high level scab resistance in green house conditions.

The lactoferrin lines contained a human lactoferrin gene and the lactoferricin lines contained a synthetic human gene sequence. As a result, we decided to delay field trials of these lines and develop a parallel system using bovine lactoferrin gene. Significant amount of lactoferrin is present in milk and traces are also found in beef. Bovine lactoferrin will be safer and more acceptable to consumers and farmers. Accordingly, we have generated 18 primary wheat transformants containing the bovine lactoferrin (blf) gene and 7 primary wheat transformants with bovine lactoferricin (blfcin) gene. A preliminary assay showed anti-*Fusarium* activity of total protein extract from transgenic wheat with bovine constructs. Homozygous plants of these lines are being created for testing in green house and in field trials (pending authorization). As for intellectual property rights, we are authorized to use the full-length LF and the synthetic LFcin, and A-16 promoter belongs to us.

Antiapoptic genes: Our second major set of genes relates to apoptosis, or programmed cell death (PCD). Apoptosis is a highly regulated process whereby individual cells of multicellular organisms undergo systematic self-destruction in response to a wide variety of stimuli. Programmed cell death regulates normal cellular turnover, the immune system, embryonic development, metamorphosis, hormone dependent atrophy, and chemical-induced cell death (Pennell and Lamb, 1997; Ryerson and Heath, 1996; Jones and Dangl, 1996). It is believed that cell suicide responses evolved in response to viruses, providing a mechanism for limiting viral replication and spread. Most viruses have evolved genes encoding proteins that effectively suppress or delay PCD long enough for production of sufficient progeny. In addition, a growing number of viruses can induce PCD late in the infection process, which may promote spread of progeny to surrounding cells, while evading host immune inflammatory responses and protecting progeny virus from host enzymes and antibodies.

We have evidence that members of an animal anti-apoptotic gene family (Bcl-2) function in plants (Dickman et. al., 2001). Transgenic tobacco lines were generated harboring various anti-apoptotic proteins (human Bcl-2 and Bcl-X_L, nematode CED-9, and baculovirus Op-IAP). When several economically important fungal and viral pathogens were inoculated

onto tobacco harboring these transgenes, the plants were highly tolerant and in most cases, completely resistant. We have now extended these findings to wheat, including having a number of elite lines expressing resistance to scab. We believe the transgenes are functioning as expected and as they do in other plants for the following reasons. The observed resistance to the necrotrophic fungal (scab) pathogen is consistent with our previous observations in tobacco (Dickman *et al*, 2001). In addition, we have resistance in wheat to heat and salt stress which is also in accordance with results in tobacco. Thus, we are eager to evaluate these lines under field conditions. We now have homozygous (T5) lines of wheat carrying Op-IAP. We are creating homozygous lines for Bcl-xl and a mutant transgene Bcl-xl (G138A). The advantages of Bcl-xl, are: (i) the structure and mode of action is different then Op-IAP, although both genes are cytoprotective (anti-apoptotic); (ii) antibodies are commercially available for Bcl-xl, and (ii) we have a null mutant construct for comparative purposes. In addition, we are generating new wheat lines harboring Sf-IAP (Huang *et al*, 2000). Sf-IAP from the insect *Spodoptera frugiperda* is in the same class of proteins as OP-IAP. However, other transgenic plants (tomato and *Arabidopsis*) harboring this gene exhibit a number of interesting phenotypes (eg. delayed fruit ripening, delayed senescence) as well as fungal disease resistance.

Our Transformation Protocol: Introduction of a maize RIP into wheat as an example: A maize ribosomal inactivating protein (Genbank accession No. AF233881) was isolated from maize leaf (cv A188) via PCR. The PCR product was sequenced for authenticity and subsequently subcloned down stream of the maize ubiquitin promoter element coupled with its 5' intron. The resultant cassette was dropped into the binary plasmid pPZP212 (Hajdukiewicz *et al*., 1994). The final binary vector is referred to as pPTN285. The binary plasmid was mobilized into *A. tumefaciens* strain C58C1 carrying the Ti plasmid pMP90 (Koncz and Schell, 1986) via tri-parental mating (Ditta *et al*., 1980). Wheat transformations (cv Bobwhite) were set-up following a modification of the protocol described by Cheng *et al*. (1997). A total of ten lines representing eight independent transformants were recovered. Ten T1 seed per line were sown in the greenhouse. An npt II ELISA was conducted on the individuals approximately 20 day after planting using a commercial kit (Agdia Cat# 73000/0480) following the manufacturer's instructions. Northern blot analysis was conducted on a

Table 1. Segregation & Northern blot (N. blot) data in T_1 generation of wheat lines transformed with pPTN285

Line	Npt II Pos.	Npt II Neg.	N. blot
130-02-02-01	10	0	Pos
130-02-05-01	7	3	Pos
130-02-06-01	5	5	Pos
130-03-01-01	4	6	Pos
130-03-02-01	8	2	Pos
ye03-04-02-01	6	4	Neg
ye10-06-01-01	10	0	Pos
ye10-06-01-02	5	5	Pos
ye10-06-01-03	6	4	Pos
ye10-03-01-01	9	1	Pos

Npt II Pos and & Neg. columns refer to the total number of T_1 individuals positive for npt II expression as determined by ELISA. N. blot column indicates whether the subset of npt II positive T_1 individuals derived from the respective line were expressing the RIP transcript.

subset of the npt II positive individuals per line. A summary of the data is given in Table 1 below. T2 seed is being increased to identify homozygous lines for future screening.

Field Testing: In 2002, we undertook our first field trials of transgenic wheat. The trials were done at Mead, NE with APHIS approval. While the lines were misted to induce FHB infection, the climate was extremely hostile with severe heat after planting which "pushed" the plants to mature early and produce poor quality seed. We are currently evaluating the data, but it is expected that the main outcomes of this year's experiments were increase our seed and to learn how to meet the necessary regulations to take transgenic lines to the field.

Transfer of transgenes to elite wheat varieties: We are crossing the most FHB tolerant transgenic lines to Alsen (elite FHB tolerant line), to Wheaton (elite FHB susceptible line), and to Wahoo or Millennium, and Wesley, popular recent releases. Provided these efforts show promise, we will expand our crosses to additional lines and market classes. We are also making crosses among and between our elite antifungal genes and our elite antiapoptotic transgenic lines. We are interested in determining if pyramiding genes having similar mechanisms, and transgenes affecting the two mechanisms of antifungal activity that we are studying in combination may provide added protection against FHB.

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MOLECULAR CHARACTERIZATION OF SCAB RESISTANCE QTL IN WHEAT

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Profiling of scab resistance gene expression in wheat

The goals of this project are to discover new genes for scab resistance, identify the pathway involved in wheat resistance to scab infection and understand the genetic mechanism of Type II resistance in wheat. Three pairs (forward and reverse) of suppression subtractive hybridization libraries have been developed from infected spikes harvested 6, 36 and 72 hours after inoculation (HAI) by subtracting cDNA of bulked infected susceptible recombinant inbred lines (RIL) from bulked infected resistant RILs. The RILs generated from the cross between the resistant cultivar Ning 7840 and the susceptible cultivar Clark. So far, 2,306 differentially expressed sequence tags (ESTs) have been cloned and printed in glass slides for microarray analysis. About 80 clones were sequenced. For microarray analysis, several labeling and hybridization kits or protocols were experimented and only the kit from Genisphere produced reliable result with low background. Infected wheat spikes collected from both bulks at various time courses after inoculation will be used as probes for array hybridization to profile the scab resistance gene expression.

Mapping QTL from Wangshuibai

The objectives of this project are to: (1) discover new QTL for scab resistance from the Chinese landrace Wangshuibai which does not relate to Sumai 3, (2) investigate QTL effects on type II resistance in Wangshuibai, and (3) develop selectable markers for marker-assisted selection. F_8 recombinant inbred lines derived from the cross between Wangshuibai and the susceptible cultivar Alondra's were evaluated for Type II resistance in the greenhouse experiment. Total 15 plants were evaluated for each line. The frequency distribution of percentage of scabbed spikelets among 104 F8 RILs showed continuous distribution with one peak skewed toward resistant parent (Wangshuibai). The same distribution was observed for DON yield which was evaluated by Dr. Hart from Michigan State University. About 200 pairs of SSR primers were screened for the parents and about 30% showed polymophism. Based on the greenhouse scab evaluation, bulked resistant and susceptible lines were selected and are being used for SSR primers screening. The RILs will be further evaluated for scab resistance and DON yield next year and more SSR markers will be screened to identify closely linked markers to the QTL for scab resistance in cultivar Wangshuibai.

Marker-assisted selection

The goals of this project are to increase throughput of marker screening, reduce marker analysis cost, and develop breeder-friendly STS markers to facilitate marker-assisted selection (MAS). To improve efficiency of MAS, we optimized a fast DNA isolation protocol. In this

protocol, NaOH is the DNA extraction buffer and Tris is used as DNA storage buffer. The FastPrep system from Q.Biogene and a mini centrifuge are used for tissue preparation. DNA can be isolated with this method in any location where electricity is available. This method is suitable for MAS in conventional breeding programs since costs of equipment and reagents are low. With this method, about 200 DNA samples can be isolated daily by one person. Isolated DNA is good for STS and SSR even after 1 year of storage at –20°C. A Li-Cor Sequencer is used for SSR analysis. To increase throughput and reduce cost, primers of two flanking markers are labeled with two different fluorescence dyes (IR800 and IR700) and combined in one PCR reaction. The PCR is analyzed in one gel and produces images of both flanking markers. Each gel can be reused for 3-4 times. This method significantly reduces cost of MAS and has been successfully used for marker-assisted backcross to transfer 3BS QTL to Clark background.

To develop breeder friendly marker, one STS marker tightly linked to 3BS scab resistance QTL was converted from an AFLP marker and has been released to several breeding programs. This STS explained about 50% of phenotypic variation for Type II resistance in the population from the cross of Ning7840/Clark. It can be amplified with crude DNA isolated with the simplified method. An additional STS was developed from another AFLP marker, but the PCR condition still needs to be optimized before it can be used for MAS.

GENETIC DIVERSITY OF NEW FUSARIUM HEAD BLIGHT RESISTANT BARLEY SOURCES

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OBJECTIVE

To determine the genetic relatedness of new sources of Fusarium head blight resistance in barley to those already in use by the Minnesota barley breeding program.

INTRODUCTION

Since the outbreak of FHB in the Upper Midwest, there has been a major effort to identify FHB resistant sources and breed for FHB resistant malting barley. A number of barley varieties from around the world have been identified as potential sources of FHB resistance. All of these varieties exhibit partial resistance to FHB and, based on several mapping studies, it is known that resistance to FHB in barley is a quantitative trait controlled by a number of genes (Kolb *et al*, 2001). Thus, to obtain durable disease resistance, the best breeding strategy is to pyramid genes for FHB resistance from multiple resistant sources into one or more varieties. Many of the known FHB resistant barley sources are currently in use in variety development and/or genetic mapping studies. To date, no single source appears to provide sufficient resistance and additional new sources of resistance with novel FHB resistance genes are needed.

A major effort to identify new sources was undertaken by researchers at North Dakota State University (NDSU) who screened of over 8,200 accessions of six-rowed spring barley from the USDA Small Grains Germplasm Collection (Steffenson, 2002). These researchers identified some accessions with FHB resistance equal to or greater than that of Chevron; currently the standard six-rowed resistant check in most barley FHB research (Table 1). To more effectively utilize these new sources we wanted to determine how related these new sources are to those already in use.

MATERIALS AND METHODS

The objective of this research was to use SSR markers to determine the genetic relatedness of new sources of FHB resistance in barley (Table 1) to sources already in use by the Univ. of MN barley breeding program (Table 2). We obtained seeds of new accessions from Dr. Brian Steffenson at the Univ. of MN. Dr. Steffenson originally obtained seed from the USDA's Germplasm Resources Information Network (GRIN) and performed single head selection on each line. Plants were grown in the greenhouse and DNA extracted from a single plant. A genetic diversity study, previously conducted in our lab, included many of the parents our barley program has used in breeding for FHB resistance (Wingbermuehle, 2002). To combine the information from the Wingbermuehle study with the accessions screened here for the first time, we selected, for each SSR primer, a representative geno-

type for each allele that had been previously identified. Then, for each primer, we re-ran these representatives along with the new genotypes. In this way, we could unambiguously assign SSR allele genotypes to each new accession. We used sixty-nine SSR markers in this study. For two of these markers (HvLTPPB and Ebmatc0028), two loci were used in analysis for a total of 71 polymorphic loci analyzed. Primers were chosen that both provided coverage spanning the barley genome and included eight markers that have been previously linked to FHB resistant quantitative trait loci (QTL) (de la Pena *et al*., 1999; Mesfin *et al*., 2003).

Table 1. Recently identified FHB resistant accessions from the spring six-rowed barley world collection.

Accession	Origin	Accession	Origin
CIho 2492	Sweden	PI 328642	Romania
PI 467654	Finland	CIho 9625	Ethiopia
CIho 6613	United States	PI 383090	Ethiopia
PI 371317	Switzerland	CIho 4530	China
PI 371308	Switzerland	PI 565567	China
PI 370919	Switzerland	PI 565583	China
PI 370984	Switzerland	PI 565854	China

Table 2. Midwest varieties, FHB resistant breeding lines, and FHB resistant varities.

Line	Description		Line	Description
AC Oxbow	FHB resistant variety, 2-row, covered hull, origin - Canada		FEG4-98	U of MN breeding line, 6-row; FHB resistance derived from Atahualpa
Atahualpa	FHB resistant variety, 2-row, hulless, origin - Ecuador		FEG6-28	U of MN breeding line, 6-row; FHB resistance derived from Kitchin
Chevron	FHB resistant variety, 6-row, covered hull, origin - Switzerland		Foster	6-row malting variety developed at NDSU
Frederickson	FHB resistant variety, 2-row, covered hull, origin - Japan		Lacey	6-row malting variety developed at the U of MN
Hor211	FHB resistant variety, 6-row, hulless, origin - Ukraine		Legacy	6-row malting variety developed by Busch Agricultural Resources, Inc.
Kitchin	FHB resistant variety, 2-row, covered hull, origin - USA		M100	U of MN advanced breeding line, 6-row
Zhedar1	FHB resistant variety, 2-row, covered hull, origin - China		M104	U of MN advanced breeding line, 6-row
CIho 9831	2-row, covered hull, origin – Japan		M105	U of MN advanced breeding line, 6-row
Conlon	2-row variety developed by North Dakota State University (NDSU)		M81	U of MN advanced breeding line, 6-row
Drummond	6-row malting variety developed at NDSU		M84	U of MN advanced breeding line, 6-row
Excel	6-row malting variety developed at the University of Minnesota (U of MN)		MAS2-002	U of MN breeding line, 6-row; FHB resistance derived from Kitchin
FEG14-119	U of MN breeding line, 6-row; FHB resistance derived from AC Oxbow		MAS2-054	U of MN breeding line, 6-row; FHB resistance derived from Kitchin
FEG2-26	U of MN breeding line, 6-row; FHB resistance derived from Zheddar #1		MNBrite	6-row variety developed by the U of MN, moderately resistant to FHB
FEG4-66	U of MN breeding line, 6-row; FHB resistance derived from Atahaulpa		Robust	6-row malting variety developed at the U of MN

All gels were run using the Global IR2 collection system with gel images recorded by E-seq software (LI-COR, Inc). GeneImagIR software (LI-COR, Inc) was used to size bands on gel images. Final band scores were determined visually with the aid of GeneImagIR. Data from the two studies were combined and organized using Microsoft Excel. Cluster and principal coordinate (PCO) analyses were conducted using NTSYSpc software (Exeter Software). Cluster analyses were calculated using the unweighted pair-group method, arithmetic average (UPGMA) algorithm.

RESULTS AND DISCUSSION

Using the full set of 71 SSR loci spanning the barley genome, we determined the genetic relationships of new FHB resistant barley accessions to resistant parent sources already in use and to existing varieties and breeding lines. From this analysis, larger clustering of groups is most evident with the PCO (Figure 1), while relationships between subsets of samples are easier seen with the cluster diagram (Figure 2). Both PCO and cluster analysis show genotypes falling into two major groups. The first group contains all of the six-rowed varieties and breeding lines. The second group contains the new resistant sources, the resistant sources already in use, and the two two-rowed varieties CIho9831 and Conlon.

Particularly interesting with respect to FHB breeding efforts are the relationships in the second group – those within and between the new resistance sources and those already being used. Within this cluster there is clearly more variation than within the variety and breeding line group (Figure 2). In addition, overall clustering does not appear to group genotypes by origin of accession. Toward the goal of combining different sources of resistance into a common variety, choosing new parent sources from those most different to any already being used is likely the best option.

Of the FHB resistance sources already in use as parents, Frederickson and Zhedar 1 (both two-rowed), are highly similar at 97%. All other parent sources appear to be relatively different. Based on a similarity index using the 71 SSR marker loci set, all other parent sources are less than 40% similar to one another (data not shown). Overall, the new FHB resistant accessions are also not highly similar to one another. This is with the exception of PI371308 and PI383090, which are 94% similar. Between the sources already in use and the new sources, two relationships stand out. First, Hor211 clusters closely with PI370919 and PI467654. Second, Chevron is most like accession CIho6613 at 74% similarity. While genetic dissimilarity does not insure that accessions will contain different genes for FHB resistance, maximizing the dissimilarity between new parents and those already in use, should increase the chance of identifying and combining different FHB resistance.

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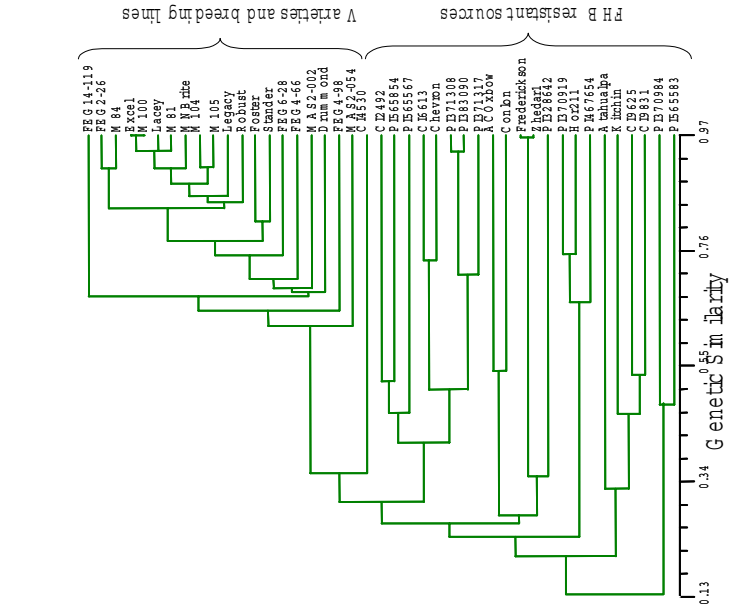

Figure 2. UPGMA cluster analysis of barley lines and Fusarium head blight resistant sources, conducted using 71 SSR loci. Cophenetic correlation r = 0.98

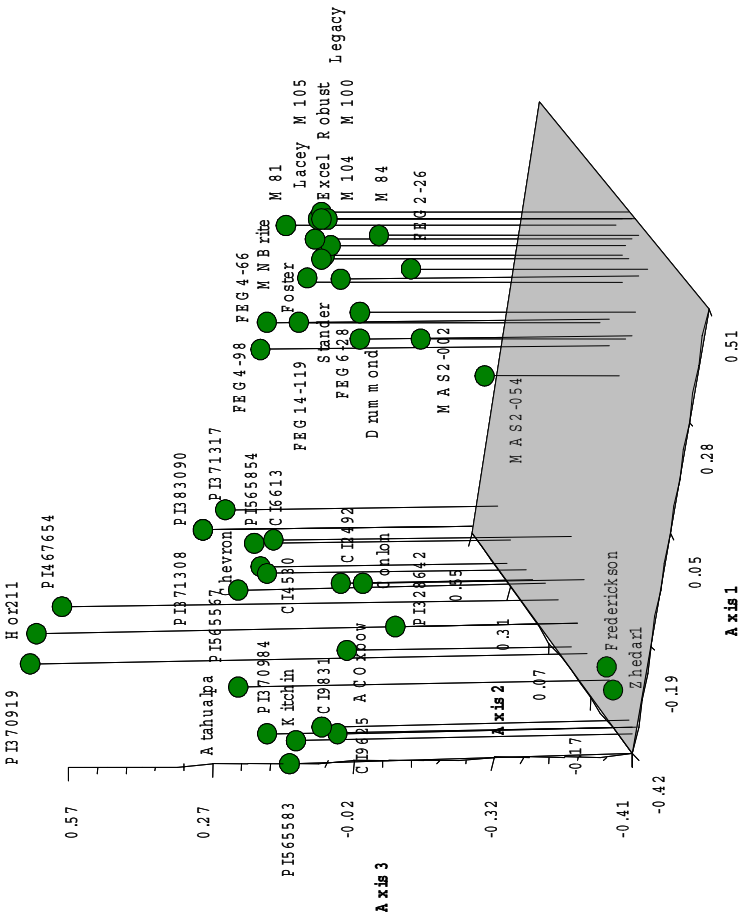

Figure 1. Principal coordinate analysis of barley lines and Fusarium head blight resistant sources, conducted using 71 SSR loci. First three axes account for 38.16% of total variation.

MAPPING FUSARIUM HEAD BLIGHT RESISTANCE QTL IN THE CHINESE WHEAT LINE FUJIAN 5114

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ABSTACT

Breeding for resistance to Fusarium head blight (FHB) is facilitated by the identification of different resistance lines and resistance QTL. A population of 78 recombinant inbred lines (RIL) was developed from the cross Fujian 5114/Norm, and was screened for FHB resistance in three field and two greenhouse experiments. Fujian 5114 is a spring wheat cultivar from the Fujian Province of China. Fujian 5114 has levels of FHB resistance similar to 'Sumai 3', but putatively differs from Sumai 3 in some resistance loci. The RIL population was evaluated for FHB severity and visually scabby kernels (VSK) in mist-irrigated, inoculated field trials in the summers of 2000 and 2001. The population was also evaluated for spread within the spikelet from point inoculations in two greenhouse trials in 2001. The results generally correlate well (r = 0.29-0.82 for correlations with p<0.05), with the best correlations resulting from the greenhouse experiments. In the field study, the proportion of variance due to RIL was 29% and 30% for field severity and VSK, respectively, and variance due to RIL X Environment was 34% and 12%. Heritability on an entry mean basis ranged from 0.90 in the greenhouse to 0.66 in the field FHB severity evaluations. Sixty microsatellite markers were mapped on the entire population and this information was combined with phenotypic data for QTL analysis. Interval analysis confirmed the presence of the 3BS resistance QTL in Fujian 5114. This QTL explained up to 28% of the phenotypic variation in FHB. An additional QTL was identified on chromosome 5BL, explaining up to 25% of the variation in FHB severity. The R^2 values of the two QTLs are higher for the two greenhouse experiments than those of the field experiments. The QTL on 5BL appears to be associated with delayed spread of the disease, as the corresponding R^2 values were reduced from the 15 to the 21 day greenhouse evaluations. These results indicate that Fujian 5114 contains some FHB resistance loci that differ from Sumai 3. Additional investigation of the 5BL QTL for breeding of increased resistance to FHB is warranted.

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

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INTRODUCTON

Aim of this work was to detect QTL for combined type I and type II resistance against FHB and estimate their effects in comparison to the QTL identified previously for type II resistance (Bai *et al*. 1999, Waldron *et al*. 1999, Anderson *et al*. 2001, Buerstmayr *et al*. 2002).

MATERIALS AND METHODS

Plant materials

The population of F_1 derived doubled haploid (DH) lines which was described in Buerstmayr *et al*. (2002) was used for this research. The resistant parent was 'CM-82036' (originating from Sumai3 x Thornbird) and the susceptible parent was 'Remus'. In total 364 DH lines were available.

Field experiments for evaluation of Fusarium head blight resistance

The lines were evaluated during two seasons (1999 and 2001) at the experimental field of IFA-Tulln. Trial location, seed treatment, plot size, sowing density and crop management were the same as described in Buerstmayr *et al*. (2002). Inoculation was done in separate experiments by spraying heads at anthesis with one *F. culmorum* or one *F. graminearum* isolate as described in Buerstmayr *et al*. (2000). Resistance reaction was assessed out in percent diseased spikelets per inoculated plot on days 10, 14, 18, 22 and 26 after inoculation.

Genotyping of the DH population with molecular markers

Genotyping of 239 DH lines was performed using 28 RFLP, 267 AFLP, 112 SSR, 3 storage proteins and one morphological marker. With the markers Barc75, Gwm389, Gwm1034, Gwm533, Barc133, Gwm493, Barc141, Barc40, Gwm304, Gwm293, Barc117, Barc186, and Barc1, which appeared to be close to one of the putative QTL regions, additional 122 DH lines were genotyped and included in the QTL mapping.

Statistical analysis

The FHB severity data were analyzed by ANOVA. Linkage maps were constructed using MAPMAKER 3.0b for MS-DOS (Lander *et al*. 1987). QTL analysis was done by simple interval mapping and composite interval mapping using PLABQTL (Utz and Melchinger 1996).

RESULTS AND DISCUSSION

The population showed significant quantitative variation for FHB severity readings (Figure 1). The genotype by isolate interaction was non-significant underlining the horizontal nature of FHB resistance in wheat.

QTL analysis revealed that two genomic regions were significantly associated with FHB resistance in that population, mapping to chromosomes 3B (*Qfhs.ndsu-3BS*) and 5A (*Qfhs.ifa-5A*) respectively (Table 1). The two QTL explained together 47 % of the phenotypic variance for visual disease severity. The peaks of the LOD profiles obtained by simple and by composite interval mapping were in the same regions (Figure 2). The two QTL on 3B and 5A mapped to the same genomic regions as in our previous study for type II FHB resistance (Buerstmayr *et al*. 2002), with the exception that we did not find a QTL after spray inoculation on chromosome 1B. Our results concerning the *Qfhs.ndsu-3BS* locus are in full agreement with Waldron *et al*. (1999), Anderson *et al*. (2001) and Zhou *et al*. (2000). A significant QTL in the *Qfhs.ifa-5A* region was also detected by D. Somers (AG Canada, Winnipeg, pers. comm.). In the present study using spray inoculation, the effects of the two QTL were in a comparable range. On the contrary, after single floret inoculation, the 3B QTL had a much larger effect than the 5A QTL (Buerstmayr *et al*. 2002). This is an indication that *Qfhs.ifa-5A* may contribute more towards type I resistance and to a lesser extent to type II resistance whereas *Qfhs.ndsu-3BS* appears to play a role primarily in type II resistance.

For both QTL the allele conferring resistance originated from the resistant parent 'CM-82036'. The association of the two QTL on 3B and 5A with the phenotype is shown in Table 4. Lines with the 'resistant' allele (originating from 'CM-82036') at both QTL regions had a mean FHB severity of only 20 % compared to lines with the alleles from susceptible 'Remus' which reached on average of 58 % bleached spikelets after 26 days (Table 2).

Both QTL regions are already well covered by SSR markers. Marker assisted selection for the two major QTL appears therefore feasible and should help breeders to select for improved lines with combined type I and type II resistance.

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Table 1. QTL estimate for mean values of percentage of infected spikelets on day 26 after inoculation (FHB-26) over two years of the experiments in Tulln. QTL are described by chromosome location, logarithm of odds (LOD) and percentage of explained phenotypic variance (R^2). QTL analysis was carried out by composite interval mapping.

		FHB-26	
Map interval	QTL	LOD	R^2
Xgwm533 - Xgwm493	*Qfhs.ndsu-3BS*	29.1	31.6
Xgwm293 – Xgwm156	*Qfhs.ifa-5A*	20.5	23.2
Simultaneous fit			46.9

Table 2. Effect of alternative alleles at two QTL regions for mean percentage of infected spikelets 26 days after inoculation (FHB-26) for line means obtained in Tulln over two years.

QTL*)			FHB-26		
Qfhs.ndsu-3BS	*Qfhs.ifa-5A*	Number of lines	Median	Mean	Stderr.
CM-82036	CM-82036	87	19.8	21.9	8.5
CM-82036	Remus	74	34.1	34.8	13.6
Remus	CM-82036	73	37.5	39.6	13.1
Remus	Remus	110	58.3	57.7	16.1

*) Only lines with non-recombined *Xgwm533 – Xgwm493* (*Qfhs.ndsu-3BS*) and *Xgwm293 – Xgwm156* (*Qfhs.ifa-5A*) intervals were included in these calculations.

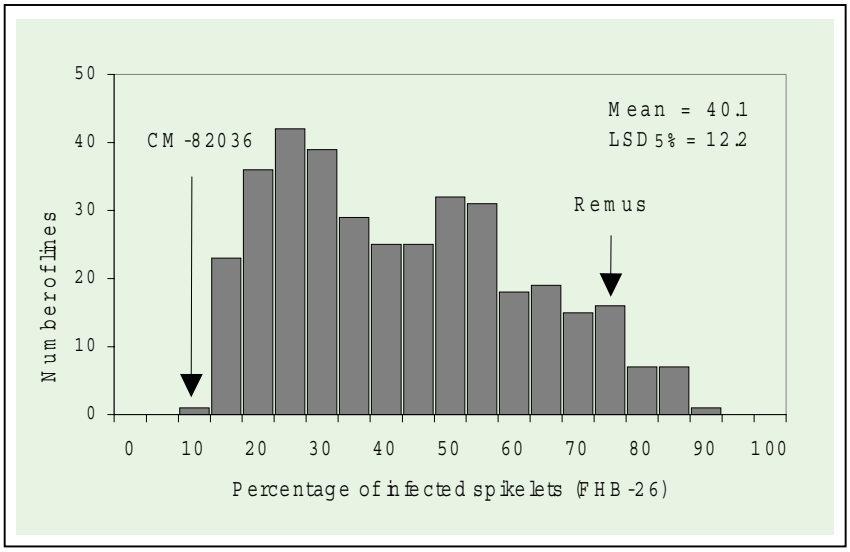

Figure 1. Frequency distribution of 364 DH-lines for mean values of FHB severity on day 26 after inoculation (FHB-26). Arrows indicate values of the parental lines. The overall population mean and the least significant difference for comparison of line means ($\alpha = 0.05$) using the genotype by year interaction mean square as an error term are given also.

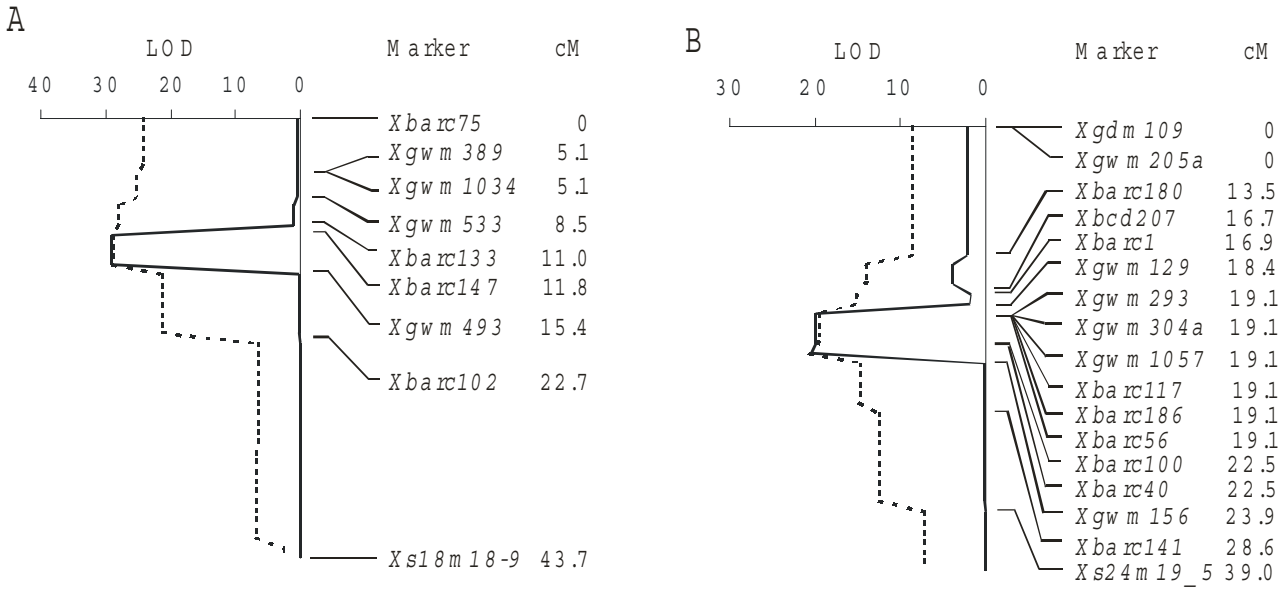

Figure 2. Interval analysis of QTL for percentage of infected spikelets on day 26 after inoculation (FHB-26) on linkage groups corresponding to chromosomes 3B (**A**) and 5A (**B**). LOD values were calculated by composite interval mapping (solid line) and simple interval mapping (dotted line).

QTL MAPPING AND SSR GENOTYPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN VIRGINIA TECH WHEAT BREEDING PROGRAM

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ABSTRACT

Mapping of quantitative trait loci (QTLs) associated with Fusarium head blight (FHB) resistance and application of marker assisted selection (MAS) can be used to accelerate development of FHB resistant wheat varieties and to provide for a better understanding of the mechanisms governing resistance. Two F_2 and two corresponding F_1-derived doubled haploid populations of Pion2684 x W14 and Madison x W14 were used in discerning the inheritance and identity of QTLs associated with FHB resistance in wheat line W14. In the F_2 populations, two complementary genes with major effects were postulated to govern FHB resistance. This was confirmed upon subsequent evaluation of doubled haploid populations in two independent experiments in 2001 and 2002. Microsatellite markers (SSRs) were used to identify QTLs associated with FHB resistance. Seventy six percent (152 out of 200) of SSRs detected polymorphism between parents. Among 36 pairs of primers used to date, a total of 45 loci on three chromosome regions (2BS, 3BS, and 5AL) have been comparatively mapped in one F_2 of W14 x Pion2684 and two doubled haploid populations of W14 x Pion2684 and W14 x Madison. Fifteen markers were significantly ($p < 0.05$) associated with FHB resistance, and explained 21%, 36% and 31% of the total variation of disease severity in F_2, $F_{2:3}$, and DH populations of W14 x Pion2684, respectively. These markers also explained 43% of total variation of disease severity in DH population of W14 x Madison. Nine of the 15 SSRs were used to genotype in 27 FHB resistant soft red winter wheat lines to determine the putative contribution of QTLs on these chromosomes to FHB resistance and the potential for using these SSRs in MAS. Among the nine SSRs loci genotyped, Xgwm 493 in the 3BS QTL region and Xgwm156 in the 5AL QTL region were the most commonly detected loci having the same fragment size as the FHB resistant wheat lines Sumai 3 and W14. In contrast, Xgwm 533 was detected in only five of 27 lines, while the other 22 lines have alleles at 2BS and/or 5AL QTL loci. The contribution of individual QTL towards FHB resistance will be evaluated further in doubled haploid and near-isogenic line populations.

INSIGHT IN THE DIFFERENTIALLY EXPRESSED GENES IN RESPONSE TO *FUSARIUM* MYCOTOXINS IN FHB RESISTANCE WHEAT NOBEOKABOUZU-KOMUG

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ABSTRACT

Fusarium head blight (FHB, scab) is a fungal disease of wheat and other small cereals that is found in both temperate and semi-tropical regions. FHB causes severe yield and quality losses, but the most-serious concern is the possible mycotoxin contamination of cereal food and feed. Breeding for FHB resistance by conventional selection is feasible, but tedious and laborious. This study was conducted to identify the response genes to *Fusarium* mycotoxins believed to be held by the Japanese variety Nobeokabouzu-komugi, which is highly resistant to FHB, and to construct a stressed ESTs library from this bread wheat variety. For this purpose, suppression subtractive hybridization (SSH) technique was used as it combines normalization (*suppression of abundant transcripts and enrichment of rare transcripts*) and subtraction (*isolation of differentially expressed transcripts*) and as it is a powerful approach to identify and isolate genes which are transcribed under a certain stress. It may as well help in understanding the complex regulating mechanism of resistance to FHB. Nobeokabouzu-komugi seeds were germinated in water; and then transplanted in a phosphorous solid solution with and without metabolite of *Fusarium graminearum* including 10 ppm DON. The root meristems were collected and used for total RNA extraction. cDNA was synthesized using Clontech kit and the SSH method was applied to generate differentially regulated cDNA probes. These cDNA probes were cloned by using TA cloning method. Approximately one thousand clones were isolated from the subtracted stressed plants. Out of these clones, 80 random ones were sequenced. Only one duplication was found, meaning that more that 98% are singletons. Further, sequence homology search using BLAST program from NCBI showed that 19 clones present high homology with some ESTs from Fusarium infected spike of another FHB resistance variety, Sumai 3; whereas others show homology to ESTs induced by different stresses specially (vernalization, ABA, dehydration, salt, etiolating, cold, and drought). These clones will be used for the differential display analysis by using micro array.

CONTROL OF SCAB WITH PUROINDOLINE-CONTAINING TRANSGENIC WHEAT

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ABSTRACT

Wheat and barley head blight or scab is a fungal disease caused by two species of *Fusarium* (*F. graminearum* and *F. culmorum*) causing premature ripening and white heads. The wheat puroindoline proteins (PINA and PINB), which are endosperm-specific and contribute to grain softness, also have *in vitro* and *in vivo* anti-fungal properties. These studies have been extended to include wheat *Fusarium* scab. The growth of both *Fusarium* species was negatively affected 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. culmorum* in both field (summer 2001) and green-house (2001-2002) studies. The plants were analyzed for scab by visual inspection of the heads. The majority of Hi-Line control plants had between 40-70% infected spikelets/head. *PinB*-transgenic lines showed a large increase in plants with only 0-20% infected spikelets/head, a decrease in both the moderately and severely infected heads, and a decrease of the percentage of tombstones, when compared to the control. Experiments are in progress using *F. graminearum* as the fungal pathogen causing the scab on Hi-Line controls and on the *pinB*-containing transgenic wheat. These data suggest that PIN proteins may provide protection to wheat and barley against *Fusarium* scab.

GENETIC ANALYSIS OF TYPE II FUSARIUM HEAD BLIGHT (FHB) RESISTANCE IN THE HEXAPLOID WHEAT CULTIVAR 'WANGSHUBAI'

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ABSTRACT

The dramatic impact of FHB on the wheat production throughout the US has driven breeding and germplasm enhancement projects to search for new potential resistance sources. Chinese introductions show the most potential for resistance to the spread of the infection through the head (Type II). 'Sumai 3' is the most widely used of these introductions, being utilized for genetic studies and breeding purposes. In the Wheat Germplasm Enhancement project at NDSU we are interested in additional resistance sources that can be used in place of or in conjunction with Sumai 3. One of these new sources is the hexaploid wheat cultivar 'Wangshubai'. The level of resistance shown by Wangshubai in our greenhouse evaluations is 7-11%, compared to 15% for Sumai 3; this is consistent with reported results of others. Previous genetic diversity studies had detected no genetic relationship between Sumai 3 and Wangshubai, suggesting different loci or alleles for resistance. In order to dissect the genetic components of Wangshubai resistance to FHB spread, we have developed a population of 388 F6-derived recombinant inbred lines developed from a cross between Wangshubai and ND671 (a susceptible elite line from the HRSW breeding program at NDSU). We have phenotypic data from 4 greenhouse and 2 field evaluations. Infection in the greenhouse experiments was achieved through single floret inoculations at flowering, while the field experiments relied on natural infection. We are using a subset of 88 lines for preliminary QTL analysis. The molecular markers used for this purpose are SSRs previously mapped to specific wheat chromosomes. For confirmation purposes, the chromosomes will be anchored using RFLP markers. The remaining lines will be used for validation of QTL. Preliminary QTL analysis results using 75 SSR markers covering 13 wheat chromosomes shows the presence of major QTL in chromosome 3BS located close to the SSR locus *Xgwm533*. This QTL explained about 25% of the phenotypic variation, and its location is similar to that found in Sumai 3. The amount of phenotypic variation explained is comparable to that explained by the major QTL in Sumai3. However, the level of resistance in Wangshubai (7 to 11% of spread) is better than in Sumai 3 (15% of spread). This fact could be because: 1) both sources have different alleles of the gene/s for the QTL found in chromosome 3BS, or 2) Wangshubai has additional genes contributing to a higher level of resistance. Our plans include completing the genetic map for the population in this study to search for additional QTLs explaining more of the phenotypic variation and to study the possible relationship of resistance to FHB with other traits.

IDENTIFICATION OF SCAB RESISTANCE GENE EXPRESSION IN WHEAT FOLLOWING INOCULATION WITH *FUSARIUM*

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ABSTRACT

Fusarium head blight (scab), caused by fungus *Fusarium* species, is a worldwide disease of wheat (*Triticum aestivum* L.). Chinese cultivar, Ning 7480, is one of few wheat cultivars with resistance to scab. To identify the differentially expressed genes corresponding to scab resistance of Ning 7840, the pooled cDNA libraries at different time-points, 2hr., 4hr., 6hr., 12hr., 24hr., 36hr., 72hr. and 96hr., after inoculation with *Fusarium* were constructed using glume mRNAs from Ning 7480. We performed a PCR-selected cDNA subtraction using the pooled glume mRNAs in the tester (Ning 7480 inoculated with *Fusarium*) and the driver (Ning 7480 inoculated with water). The cDNA libraries were differentially screened by the forward subtracted cDNAs (the tester subtracted against the driver) and the reverse subtracted cDNAs (the driver subtracted against the tester) as probes. 24 cDNA clones were isolated based on their specific hybridization only with the forward subtracted cDNAs, and not with the reverse subtracted cDNAs. Real-time quantitative PCR showed that the known defense response protein, chitinase, was induced at 24 hours and reached maximal induction at 72 hours after inoculation with *Fusarium*. Also, the hypothetical defense response protein, XP_104345, was induced at 12 hours and showed high levels of induction at 72 hours. Two putative defense response genes, Sigma-E factor and a retroelement, were down-regulated early from 2 hours after inoculation in the treated tissue with maximal induction occurring around 72 and 96 hours. The slot-blots containing the above putative defense response genes were probed respectively with the cDNA pools from the tester and driver. The slot-blot analysis confirmed the presence of the cDNA induced with *Fusarium* in all of the four putative defense response genes. The location for these putative genes is proceeding based on nulli-tetrasomics analysis in our lab.

MAPPING GENES CONFERRING FUSARIUM HEAD BLIGHT RESISTANCE IN C93-3230-24

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ABSTRACT

The six-rowed line C93-3230-24, from the cross B2912/Hietpas 5, was identified by researches at Busch Agricultural Resources, Inc. (BARI) to have (FHB) resistance similar to Chevron, and better FHB resistance than either of its parent in a greenhouse test. The genetic background of C93-3230-24 appears to be completely different than that of any of the FBH resistant accessions identified. Thus, this line may have alleles for FHB resistance and DON accumulation not currently identified. The objectives of this study are: 1) to construct skeletal maps that includes RFLP and SSR markers for an F_1-derived DH mapping population developed from the cross Foster/C93-3230-24 and 2) determine the position of QTL controlling FHB resistance, DON accumulation, days to heading and maturity, plant height, and spike nodding angle. Field experiments were conducted in mist-irrigated FHB nurseries in 2001 and 2002 in North Dakota and Zhejiang Province China using 118 DH lines and parents. Single locus analysis using available marker data identified six regions in five chromosomes associated with FHB resistance. The regions are located in chromosomes 2H, 4H, 5H, 6H, and 7H. The region with the largest effect on FHB resistance appears to be in chromosome 2H. Associations between the markers and maturity and/or plant height were found in the same regions as FHB resistance. Results in this study are similar to those obtained in studies using the resistant six-rowed cultivar 'Chevron' and the ICARDA/CIMMYT cultivar 'Gobernadora'. Thus, preliminary results suggest that C93-3230, Chevron, and Gobernadora may have similar alleles for FHB resistance.

TARGETED SATURATION MAPPING OF *QFHS.NDSU-3BS* USING WHEAT ESTS AND SYNTENY WITH THE RICE GENOME

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ABSTRACT

A major QTL, *Qfhs.ndsu-3BS*, for resistance to Fusarium head blight (FHB) has been identified and verified by several research groups. However, the DNA marker density near this major QTL is less than required for map-based cloning. The objective of this project was to develop STS (sequence tagged site) markers from wheat ESTs to increase the marker density near this major QTL. On the basis of synteny between wheat chromosome 3BS and rice chromosome 1S, we initiated a strategy to identify wheat ESTs likely near this QTL. The sequences of BAC/PAC clones located on the distal portion of rice chromosome 1S were compared with wheat ESTs in GenBank using BLASTN search. Primers of STS markers were designed for non-redundant wheat ESTs with E values equal or less than e-15 and the length of identity greater than 100bp. Using wheat deletion lines for chromosome 3BS, 25 out of 79 STS markers were located to the chromosome bin 3BS 0.78-0.87, where this QTL is most likely located. Nine STS markers were mapped in a previously reported Sumai 3/ Stoa mapping population. The STS marker *XSTS3B-138* explains 55% of the FHB variation of this mapping population. Therefore, this research strategy is useful for developing a high resolution map of this major QTL region, and may have broad applications for targeted mapping of other traits in cereal crops.

IDENTIFICATION OF QTL ASSOCIATED WITH SCAB RESISTANCE IN ERNIE

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ABSTRACT

Fusarium head blight (scab) in wheat is a major problem worldwide. No source of complete resistance is known. Sumai 3, a cultivar from China, is the major resistant resource across different breeding programs in US. A major QTL conditioning scab resistance in Sumai 3 has been identified on 3BS. Identification of different sources of resistance is critical to breeding scab resistant wheat to reduce the potential for genetic vulnerability. Ernie, a scab resistant cultivar, released from the University of Missouri, appears to have a different set of resistance genes. Using AFLP and SSR markers we have mapped the scab resistant QTL by 300 F_8 recombinant inbred lines (RILs) developed from a cross between Ernie and MO 94-317, a highly susceptible Missouri variety. The scab index (the ratio of infected spikelets to total spikelets of the inoculated head) in these lines ranged from 15.7 to 75.7%. Eight *Eco*RI and 8 *Mse*I primers forming 64 primer pairs were used to screen the parents. Over 80% of these pairs had polymorphic bands. The average number of polymorphic bands was 7 with a range of 2 to 21. Two hundred AFLP and SSR loci were used to construct a linkage map. MapMaker version 3.0 for Unix was used to construct the linkage map. QTL analysis was performed on the scab data using QTL Cartographer version 1.16. Two SSR markers per chromosome were used to anchor the AFLP markers to chromosomes. The QTL information will be useful in developing resistant materials by gene pyramiding.

OVER-EXPRESSION OF ANTI-FUNGAL PROTEIN GENES INCREASES RESISTANCE OF TRANSGENIC WHEAT TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Anti-fungal proteins (AFPs) such as ß-1,3-glucanases, chitinases, thaumatin-like proteins (tlps), thionins and ribosome-inactivating proteins (RIPs) are known to inhibit fungal growth via different mechanisms. Glucanases and chitinases degrade fungal cell walls, tlps and thionins degrade fungal cell membranes and RIPs inhibit fungal protein synthesis. Transgenic wheat (cv. Bobwhite), over-expressing these AFPs, has been generated using micro-projectile bombardment. We have developed 25, 25, 31, 24 and 15 transgenic wheat lines carrying a wheat -puro-thionin, a barley tlp-1, a barley ß-1,3-glucanase, a barley RIP and a barley chitinase, respectively. In addition, we have developed 10, 11 and 11 transgenic wheat 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 resistance to Fusarium head blight. Four independent glasshouse disease screens have been conducted on the tlp-1 lines and two of those lines consistently demonstrated an increase in resistance when compared to non-transgenic controls. Similarly, three disease screens have been conducted with our glucanase lines and four of these lines have performed well. In four disease screens, one α–puro-thionin line performed well in three of the screens, and two more lines performed well in two of the screens, and therefore, have been evaluated further. Molecular characterization of our lines shows that they are genetically independent and that they accumulate the appropriate AFP. In addition, preliminary disease screen data on the remainder of our AFP transgenic lines will be presented.

EFFECT OF CHEVRON ALLELES AT TWO FUSARIUM HEAD BLIGHT RESISTANCE QTL DETERMINED USING NEAR-ISOGENIC LINES

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OBJECTIVES

To evaluate near-isogenic lines, developed for chromosome 2H and chromosome 6H target QTL-regions, for FHB resistance and other confounding traits (heading date and plant height) associated with the disease severity.

INTRODUCTION

Fusarium head blight (FHB), caused primarily by *Fusarium graminearum*, has caused significant yield and grain quality losses in barley (*Hordeum vulgare* L.) since 1993. Phenotypic selection for FHB resistance has been only modestly effective largely because FHB resistance is highly influenced by the environment and screening methods are laborious and expensive. Marker-assisted-selection (MAS) is a promising tool to augment current methods to breed for FHB resistance.

To utilize MAS for FHB resistance, the barley-breeding and genetics program at the University of Minnesota is engaged in mapping and validating QTL for FHB. We have previously identified FHB resistance QTL distributed across the genome using the source of resistance Chevron (de la Pena *et al*., 1999; Canci *et al*., 2000). We have also used Chevron-derived populations to validate 3 of the QTL; 2 on chromosome 2H and 1 on chromosome 6H (Canci, 2001; Gustus *et al*., 2001). These QTL regions are now candidate targets for MAS, but a significant problem is that they are coincident with QTL late heading date (HD) and tall plant heights (HT).

To further understand the association between FHB resistance and these two other traits (HD and HT) and also elucidate the genetic basis of FHB resistance, it is important to fine map the associated regions. For this purpose, we developed near-isogenic lines (NILs) for chromosome 2H and chromosome 6H target QTL-regions using both molecular marker-assisted backcrossing and heterogeneous inbred families (HIF) procedures. These lines will be useful for studying disease resistance. We have also used the BC-derived NILs as parents to create fine mapping populations.

MATERIALS AND METHODS

Development of the BC_3 NILs: We initiated the development of NILs using donor parents selected from the 101 $F_{4:7}$ progenies previously used for linkage mapping (de la Pena *et al*., 1999). The recurrent parent was the elite line M69. A marker-assisted backcrossing scheme was used to advance selected lines to the BC_3F_2 generation. Six BC_3F_2 lines

carrying the FHB-resistance Chevron alleles at each target QTL region were selected and advanced by selfing to the BC_3S_4 generation.

Development of HIFs: Among the 101 $F_{4:7}$ families de la Pena *et al*. used for mapping FHB-resistance QTL in 1999, we selected 12 families segregating at marker loci associated with each target QTL region as HIFs. Ten segregating progeny lines grown from each selected HIF were genotyped with all available SSR markers in the target QTL region. Based on the marker data, two NILs contrasting at a specific marker locus were identified from each HIF and selected as pairs for field evaluation.

Field Evaluations of NILs: The 30 NILs comprising six chromosome 2H BC_3-derived NILs, six chromosome 6H BC_3-derived NILs, and 18 HIF-derived NILs together with parental lines, Chevron and M69, were evaluated at St. Paul and Crookston, Minnesota in the summer of 2002. At each location, entries were planted in 2.4 m long single-row plots spaced at 30 cm apart. The experimental design was a randomized complete block design with three replications. Nurseries at St. Paul were inoculated using the macroconidia inoculation technique (Dill-Macky, 2002). The initial inoculation was performed at heading so as to avoid confounding effect of differences in heading date and potential escape of the pathogen. A second inoculation was repeated three days after initial spraying. At Crookston, a grain spawn inoculation technique was used (Dill-Macky, 2002). Nurseries were mist-irrigated daily after inoculation until soft dough stage. We measured FHB severity, plant height and heading date. To measure FHB severity, 10 random spikes from each plot were examined and the number of infected spikelets from each spike counted and expressed as a percent of the total spikelets present. Heading date was determined as the number of days after planting to 50% emergency from the boot.

Statistical Analysis: Since all NIL pairs have different alleles only at the target QTL region, differences in phenotype can be attributed to the genes in those segments. To determine the effect of each NIL pair, the data were subjected to ANOVA using Proc GLM procedure (SAS Institute, 2000). Means were separated using LSD. A combined analysis across locations was conducted to determine genotype x environment interaction. The combined analysis showed that all measured traits had significant G x E effect. Therefore, results were presented for individual locations. The magnitude of the effect of alleles segregating at the target QTL regions were determined by comparing the means of NILs carrying different alleles at each locus.

RESULTS AND DISCUSION

Results of mean separations for the BC_3-derived NILs showed that NILs carrying the Chevron allele at the chromosome 2H QTL region reduced FHB by 44% for St. Paul and 41% for Crookston (Table 1). This same QTL region increased HD by six days confirming previous studies indicating that the chromosome 2H QTL region is associated with *Eam6*; a maturity gene that affects HD. On the contrary, there was no significant effect of the chromosome 6H region on either FHB or HD. The results for chromosome 2H QTL region are in agreement with Gustus *et al*. (2001), however Gustus found a small but significant reduction in FHB severity with the Chevron allele at the chromosome 6H QTL.

Based on the HIF-derived NILs, the Chevron allele at the marker *Bmag0140* on chromosome 2H had a similar effect as the BC-NIL reducing FHB by ~40% (Table 2). However, these NILs for this marker did not differ for heading date across the 2 locations (data not shown). Marker *Bmag0807* on chromosome 6H was associated with a 31-35% reduction in FHB. There was no association between HD and any of the analyzed markers on chromosome 6H. However, marker *Bmag0807* was associated with plant height in the two locations (data not shown).

The difference in results between the BC_3 and HIF NILs for chromosome 6H suggest that *Bmag0807* is closer to the FHB QTL than *Bmac0218*, which was used to develop the BC NILs. The general conclusion from this study is that the BC NILs for chromosome 2H should be useful for fine mapping FHB and HD. Since the BC NILs developed for chromosome 6H using *Bmac0218* did not appear to carry FHB resistance, we are looking back at BC2 lines generated in this project to see if they carry the Chevron allele at marker locus *Bmag0807*.

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Table 1. Means for Fusarium head blight (FHB), heading date (HD) and plant height (HT) for backcross-derived NILs and parents.

		FHB Severity (%)		Heading Date		Plant Height (cm)	
Genotype	No. of Lines	St. Paul	Crookston	St. Paul	Crookston	St. Paul	Crookston
Chevron	1	4.4c	1.7c	56.7a	54.7a	85.3a	105.5a
M 69	1	55.6a	23.2a	48.0c	48.0b	75.3b	82.5c
BC_3Chr.2	6	30.9b	11.2b	54.6b	54.2a	76.9b	89.0b
BC_3Chr.6	6	65.7a	22.9a	49.6c	47.7b	75.6b	88.9b

Means within the same column followed by the same letter are not significantly different (P • 0.05).

Table 2. Mean FHB severity of NILs derived from HIFs and contrasting at 5 marker loci

				FHB Severity (%)	
Chromosome	No. of NILs	Marker	Genotype	St. Paul	Crookston
2H	6	Bmag0125	A	31.5b	6.9b
			B	41.8a	10.8b
2H	6	Bmag0140	A	34.4b	8.3b
			B	61.5a	14.1a
2H	6	Bmac0093	A	36.1a	8.8a
			B	37.6a	8.5a
6H	6	Bmag0807	A	28.1b	7.0b
			B	43.5a	10.2a
6H	6	Bmag0870	A	26.2b	7.8a
			B	40.0a	9.1a

A=Chevron; B=M69.
Means within the same column followed by the same letter are not significantly different (P□0.05).

SATURATION GENETIC AND PHYSICAL MAPPING OF CHROMOSOME 3 FUSARIUM HEAD BLIGHT QTL REGION

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ABSTRACT

Complete resistance to Fusarium head blight (FHB), caused by *Fusarium graminearum* in the USA, has been a difficult goal to attain. To date, no single gene-for-gene resistance mechanisms have been discovered. Quantitative trait loci (QTL) for resistance against FHB have been mapped in several segregating populations. Eighteen of the 21 chromosomes in wheat and all 7 chromosomes in barley have been reported to be associated with resistance. In three or more mapping studies conducted using Chinese wheat cv. Sumai 3 as the resistant parent, QTL on chromosomes 3BS and 6BL were discovered. QTL for FHB resistance have also been mapped to chromosomes 2H, 3H, and 1H in several different barley populations. Because of the high level of synteny between grass species, it was determined that the QTL on 3BS and 3H reside in a syntenous position, between restriction fragment length polymorphism (RFLP) markers BCD907-ABG471. Using the resources provided by the Rice Genome Project sequencing effort, we have targeted phage artificial chromosomes (PACs) from rice chromosome 1 that are located in a syntenous position to 3HS in barley. By using the blastn function on the NCBI web site and limiting the search to genus *Hordeum*, barley expressed sequence tags (ESTs) can be identified with homology to the individual PACs. Eighty-seven unique barley ESTs were identified that covered 18 PACs. To date, 24 ESTs have been screened against the cv. Morex bacterial artificial chromosome (BAC) library. These 24 ESTs identified 193 BAC clones. We have genetically mapped 12 ESTs to date and 7 mapped in the target region on 3HS in the Steptoe/Morex DHL population and the Foster/CI4196 RIL population. The ratio of 7 out of 12 mapping to the target region is sufficient to expect to saturate the region since additional PAC clones are available from the target region. Another source of ESTs that we are experimenting with are the wheat ESTs mapped to group 3 using the wheat deletion lines (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi).

MICROSATELLITE GENETIC MAP IN WHEAT

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ABSTRACT

Genetic maps saturated with informative markers are of great importance for localizing and manipulating important genes or 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 2000; Salina *et al* 2000). To date, we have developed more than 400 new SSR primer pairs, 209 of which generate PCR products which map to 225 loci[(a)] on the ITMI population. PCR products from an additional 137 primer pairs enable physical mapping of 142 loci. The poster associated with this abstract displays the latest version of a genetic/physical map containing over 1400 total loci including 367 Xbarc loci. Detailed information about primer pairs and the loci they amplify will be posted at: http://wheat.pw.usda.gov/ggpages/genomics.shtml

[(a)]Designated with the prefix “Xbarc”, where “barc” in the acronym for “Beltsville Agricultural Research Center”.

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STRATEGIES FOR COMBATING FUSARIUM IN BARLEY THROUGH GENE EXPRESSION TARGETING, METABOLIC PROFILING AND SIGNALING ANALYSIS

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ABSTRACT

Several basic studies must be undertaken in order to understand the interactions between *Fusarium graminearum* and its barley and wheat hosts: 1) Gene promoters are needed to target the expression of antifungal protein genes to organs that are initially colonized by *F.g.*, 2) Metabolic profiling must be developed to determine which metabolites are extracted from host tissues, and 3) It is important to understand the signaling pathway involved in host perception of *F.g.* invasion and attempts to mount an effective response. We have previously produced a gene promoter (*Lem1*) that is specific for the young lemma/palea. More recently, Tilahun Abebe has used the suppressive subtractive hybridization method to identify genes expressed in lemmas/paleas but not in flag leaves. This led to the development of the *Lem2*, which is specific to the lemma/palea of developing seeds during the period from endosperm elongation through the dough stage. Maria Laura Federico has developed a promoter (*EpiLTP*) that has preferential activity in the pericarp epithelium. A vector (*Ala/gfp*) was developed by Jianming Fu to test coding sequences of genes in a transient system, prior to their use in stable transformation. This has been applied to the expression of the anti-Fusarium gene *Hth1* of barley. Portions of the *Hth1* coding sequence were linked to a polyalanine bridge, followed by *gfp*. This showed that the failure of this endosperm protein to be produced in lemmas resides with sequences encoding the mature peptide. GC-MS by Cynthia Henson showed that early infection of the lemma and pericarp involves accumulation of metabolites that could be essential to fungal metabolism. In particular, metabolites known to be involved in appressorium turgor pressure (trehalose, mannitol and glycerol) were found. Our studies have shown that no alpha-amylase accompanies infection, even when infections are very heavy. We are examining whether the most obvious substrate (starch) is ever mobilized during infection, and we are attempting to develop a metabolic profile for infected tissue. Finally, it is not clear how barley reacts to the F.g. in the early stages of infection. Initial studies have shown that H2O2 is produced at the site of F.g. inoculation on the pericarp. Thus, barley may have the beginnings of a productive response that could be strengthened through breeding/molecular approaches.

TRANSGENE EXPRESSION IN SPRING WHEAT (*TRITICUM AESTIVUM* L.) TRANSFORMED WITH CANDIDATE ANTI-*FUSARIUM* GENES

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OBJECTIVE

To create transgenic wheat lines carrying novel co-dominant loci with the potential for conferring effective and durable resistance to Fusarium head blight (FHB).

INTRODUCTION

Host plant resistance is the most efficient and cost-effective way to protect the wheat crop from FHB. Our aim is to create new germplasm sources of *Fusarium* resistance by using genetic engineering to introduce novel anti-*Fusarium* (AF) genes into wheat. We have designed and constructed a set of transformation vectors that fuse the maize *Ubi*1 promoter to AF genes that target either the *Fusarium* cell walls or membranes or that mitigate the cellular toxicity of the mycotoxins synthesized by the fungus during infection (Table 1). To give our candidate AF genes the best chance of protecting the plant, they must be expressed at high levels in tissues encountered by fungus as it invades and spreads, i.e., young florets. Here we report semi-quantitative and tissue-specific data for expression of these transgenes in wheat. We also use a new technique to visualize transgene expression in wheat tissues and organs.

MATERIALS AND METHODS

Vector and plasmid constructs: The monocot expression vector pUBK (Okubara *et al*., 2002) consists of the *bar* gene conferring resistance to the herbicide bialaphos regulated by the promoter, first exon, and first intron of the maize *Ubi*1 gene (UBI) (Christensen and Quail, 1996). In the AF constructs, the *bar* gene was replaced by candidate AF sequences (Table 1).

Generation, selection and progeny analyses of transformants: Transformants made by particle bombardment of immature embryos of cv. Bobwhite were identified as described (Okubara *et al*., 2002). Transgene inheritance was followed and homozygotes were identified using PCR amplification of genomic DNA with a forward primer from the UBI region and reverse primers specific for each AF coding region.

Transgene expression analyses*:* Semi-quantitative RT-PCR was carried out with 5-600 ng of total RNA from endosperm (data in Table 1) or other organs (Fig. 1) as described (Okubara *et al*., 2002). Transcript-derived cDNA was amplified using a primer specific for the first exon of the *Ubi*1 gene and a reverse primer specific for each AF sequence. Actin amplification from 5 to 40 ng of total RNA served as the internal standard for RNA integrity

(Okubara *et al.*, 2002). For visual localization of AF and actin transcripts, we modified a method for *in situ* RT-PCR (Kolti and Bird, 2000), adapting it for whole mounts of cereal organs and tissues (Somleva, unpublished).

RESULTS AND DISCUSSION

We used semi-quantitative RT-PCR to measure transgene expression in endosperm of hemizygotes and homozygotes from early generations of our initial set of transgenic lines (Table 1). Levels of transgene steady state mRNA varied among independent transformation events. In all, 16 hexaploid wheat lines have shown detectable levels of expression of the AF genes. Lines AB5-126 and C3-9 and C3-10, C1-3 and C9-25, C17-20, and AB8-7 and AB8-50 express the highest amounts of transgene constructs for *tlp-1*, FvExo, FvGlu and FvEndo, respectively. The highest *TRI101* transcript accumulation was observed in lines 156 and 176 (Okubara *et al.*, 2002). Transgenes with coding regions of fungal origin were expressed at least 10-fold lower, on average, than the wheat *tlp1* transgenes from the same promoter. FvGlu exhibited about 100-fold lower expression than wheat *tlp* transgenes or endogenous actin.

Because of the possibility of gene silencing, there is no guarantee that primary transformants showing strong expression will produce progeny with the same characteristics. Therefore, we used semi-quantitative RT-PCR to measure mRNA levels in T_4-T_7 endosperm from homozygous progeny of some of our lines (Table 1). Nearly all of those tested had expression levels as high or higher than in earlier generations. Only one line, C17-21, had lost expression. Evidently transgene silencing did not occur in the majority of our lines, either in later generations or in the transition from the hemizygous to the homozygous state. Even transgenics containing the wheat *tlp* gene, which is completely homologous to wheat endogenous genes, maintained their expression levels. The increases in transgene expression in lines AB8-7 and C1-3 are higher than can be accounted for by the two-fold increase in gene copy number in homozygotes compared to early generation hemizygotes. A similar additive effect of transgene expression has been observed in homozygous rice plants (James *et al.*, 2002).

To compare transgene expression levels among different parts of the plant, semi-quantitative RT-PCR was performed on mRNA from leaves, endosperm, anthers and ovaries of two FvExo lines (Fig. 1). Transcript levels were highest in endosperm and lowest in ovaries. This result shows that measurements of endosperm mRNA levels are not necessarily predictive of transgene expression in other organs. We plan to measure transcript levels in the outer tissues of the floret, since that is the first part of the plants encountered by the fungus. In addition, we are exploring the potential utility of other promoters to support stronger AF gene expression in floral tissues.

To more precisely localize expression from the UBI promoter, we have adapted a method of *in situ* RT-PCR (Koltai and Bird, 2000) for whole mounts of wheat tissues and organs. UBI-driven expression of various AF genes can be detected in lemma, pollen and stigma, but not in anthers (Fig. 2). These results agree with experiments using GUS fusions to report UBI activity in transgenic wheat (Stoger *et al.*, 1999). However, the *in situ* method has the potential for more precise and construct-specific localization.

Table 1. AF gene expression in transgenic wheat.

Anti-*Fusarium* coding regions fused to UBI	Line name	Expression results[6]	
		RNA analysis in T_1-T_2 generations	RNA analysis in T_4-T_7 generations[7]
wheat *tlp1*[1]	AB5-126	High[8]	n. a.
	C3-9	Medium[9]	High (T_5)
	C3-10	High	High (T_5)
Fs *TRI101*[2]	AB6-74	Low[10], some unspliced	n. a.
	AB6-176	Low-Medium, some unspliced	n. a.
	AB6-156	Low-Medium, some unspliced	n. a.
	B65-49	Low, some unspliced	n. a.
FvEndo[3]	AB8-7	Low	High (T_7)
	AB8-15	Low	n. a.
	AB8-50	Medium	High (T_5)
	AB8-108	Medium	n. a.
FvExo[4]	C1-3	Medium	High (T_5)
	C9-25	Medium	Medium-High (T_5)
FvGlu[5]	AB9-59	Low	n. a.
	C17-20	Low	Low (T_4)
	C17-21	Low	n. d.

[1]*T. aestivum* leaf cDNA encoding a thaumatin-like protein (Rebmann et al., 1991); [2]*Fusarium sporotrichioides* gene encoding DON acetyltransferase (McCormick et al., 1999); [3, 4, 5]*F. venenatum* cDNAs encoding an endochitinase, exochitinase, and glucanase (Berka, unpublished); [6]Relative levels based on RT-PCR of endosperm mRNA. [7]Homozygous plants. [8]RT-PCR amplification products of similar intensity to those from actin in 5-40 ng total RNA. [9]Detectable expression in 50-200 ng total RNA. [10]Expression was only detected in 600 ng RNA. n. a. = not analyzed; n. d. = not detected.

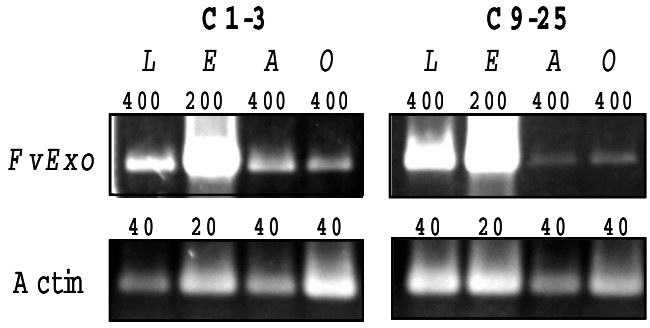

Figure 1. RT-PCR analyses of *FvExo* expression in four wheat organs. (*L* leaves, *E* endosperm, *A* anthers, *O* ovaries). Actin amplification was used as a standard for RNA integrity. Total RNA amount [ng] used in each assay is indicated above the bands.

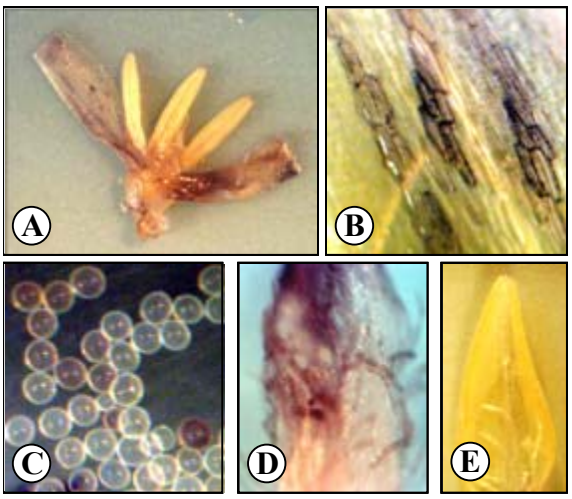

Figure 2. Localization of transgene mRNA by whole-mount *in situ* RT-PCR. Gene expression is visible as a *purple* (dark) precipitate. (**A**) Positive control - actin expression in a floret from a non-transformed plant (7x); (**B**) Wheat *tlp-1* expressing cells in the lemma (50x); (**C**) *TRI101* mRNA in pollen grains (90x); (**D**) *TRI101* transcripts in the stigma (90x); (**E**) A negative control – no expression of *FvEndo* was detected in palea after *in situ* RT-PCR without reverse transcription (7x).

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MOLECULAR MAPPING OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN THE SPRING WHEAT CULTIVAR FRONTANA

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ABSTRACT

Fusarium head blight (FHB, scab) may cause severe yield losses, but the most serious concern is the mycotoxin contamination of cereal food and feed. Breeding for FHB resistance by conventional selection is feasible but tedious and expensive. Despite that resistance originating from Sumai 3 is already well characterized (Anderson *et al*. 2001, Buerstmayr *et al*. 2002) only limited molecular genetic information is available on other sources of resistance.

A population of 210 doubled haploid (DH) lines originating from the F1 of the cross Frontana (moderately resistant) by Remus (susceptible) were evaluated for the expression of Fusarium head blight resistance traits in field trials in the seasons 1999 and 2001. Inoculation and evaluation methods used were similar to Buerstmayr *et al*. (2000).

The population was genotyped with more than 560 markers (SSR, AFLP, RFLP). QTL analysis revealed significant association of several genomic regions with FHB severity. The most prominent and consistent QTL effect was detected on chromosome 3A (LOD=5.3 R-square=13.3), associated with the SSR markers GWM1110 and GWM1121, and tentatively named *Qfhs.ifa-3A*.

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EXAMINATION OF MOLECULAR VARIABILITY OF *FUSARIUM CULMORUM* ISOLATES

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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 and molecular variability of *Fusarium culmorum* isolates using different techniques. Altogether 11 Hungarian and 28 other *F. culmorum* isolates were involved in this study, together with *F. graminearum*, *F. crookwellense* and *F. pseudograminearum* strains. Mycotoxin producing abilities of the isolates were tested by thin layer chromatography. The mycotoxins tested involved deoxynivalenol (DON) and its acetylated derivatives, nivalenol (NIV), zearalenone and fusarenone X. Most of the isolates produced zearalenone. 28 isolates were found to belong chemotype I (producing DON and 3-acetyl-DON), while 8 represented chemotype II (producing NIV and/or fusarenone X) according to Miller *et al*. (1991). Among the Hungarian isolates, one produced NIV, while all other isolates belonged to chemotype I. Pathogenicity tests were carried out as described previously (Mesterházy, 1985). Isolates belonging to chemotype I were in general found to be more pathogenic in *in vitro* tests than those belonging to chemotype II. Phylogenetic analysis of random amplified polymorphic DNA (RAPD) profiles of the isolates obtained by using 40 different random decamers let us cluster the isolates into different groups, although the variability observed was relatively low. Most Hungarian isolates formed a well-defined cluster on the dendrogram. Sequence analysis of a putative reductase gene fragment of the isolates was also carried out. Strong correlation was observed between the geographic origin of the isolates, and their position on the cladogram produced based on sequence data. These observations are in agreement with the previous finding, that a similar correlation between geographic origin and sequence data exists in the case of *F. graminearum* isolates (O'Donnell *et al*., 2000). Correlation was not observed between sequence relationships and mycotoxin producing abilities or pathogenicity of the strains. Double-stranded RNA elements indicative of mycovirus infection were detected for the first time in 5 *F. culmorum* isolates. The sizes of the dsRNA elements varied between 0.6-3.9 kbp. Correlation was not observed between the presence of mycoviruses and geographic origin, mycotoxin production or pathogenicity of the isolates. Further work is in progress in our laboratory to reveal the structure of Hungarian *F. culmorum* populations, and to further characterize their mycoviruses.

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A NON-CODING WHEAT RNA MAY PLAY AN IMPORTANT ROLE IN WHEAT RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

To understand the molecular events underlying Fusarium head blight (FHB) resistance in wheat (*Triticum aestivum* L.), gene expression profiles (GEP) were compared between the *Fusarium graminearum*-inoculated and the water-inoculated (mocking inoculation) spring wheat cultivar Sumai 3 (FHB—resistant). One GEP, designated as G12 which is specifically expressed in the pathogen-inoculated Sumai 3, was identified, cloned and sequenced. Southern blot verified that G12 represented a wheat gene. The corresponding full-length cDNA, designated as G12-S, was cloned with 5'RACE technology and sequenced. Bioinformatic analyses indicated that the sequence of G12-S is similar to the minus strand of the wheat chloroplast gene encoding ATP synthase CF-O subunit I. No significant open reading frame is found in G12-S sequence, indicating that it may function at RNA level by directly targeting the complementary transcripts of the chloroplast ATP synthase CF-O subunit I gene and/or its likes to neutralize their ability to translation.

A PUTATIVE ACYL-COA-BINDING-PROTEIN OF *FUSARIUM GRAMINEARUM* MAY PLAY AN IMPORTANT ROLE IN THE FHB PATHOGENESIS IN WHEAT

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ABSTRACT

To identify the genes important to the pathogenesis of Fusarium head blight (FHB) in wheat, we compared the gene expression profiles (GEP) between *Fusarium graminearum* (isolate Fg4) infected and healthy spikelets, as well as between FHB-resistant cultivar Sumai 3 and FHB susceptible cultivar Wheaton. Several GEPs specific to *Fusarium*-infected Sumai 3 were identified, cloned and sequenced. Southern analysis indicated that GEP 4CL represents a *F. graminearum* gene. With 5'RACE technology, corresponding full-length cDNAs were cloned from the FHB-infected spikelets of Sumai 3 and Wheaton and from *F. graminearum* culture, respectively. Sequence polymorphisms were observed in the 5' untranslated region among the three full-length cDNA clones. Bioinformatic analyses indicated that the cognate gene may encode an acyl-CoA-binding-protein (ACBP) protein. The possible role of the putative ACBP protein in *F. graminearum*'s pathogenicity and the importance of the differential mRNA editing to FHB pathogenesis in wheat were discussed.

IDENTIFICATION OF CHROMOSOME REGIONS ASSOCIATED WITH FUSARIUM HEAD BLIGHT RESISTANCE IN BREAD WHEAT CULTIVAR SUMAI 3 WITH ITS SUSCEPTIBLE NILS BY USING DNA MARKERS

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ABSTRACT

Fusarium head blight (FHB) is one of the most destructive diseases of wheat by reducing the grain yield and quality. Several types of the host resistance to FHB have been described: resistance to initial infection (Type I), resistance to spreading of infection (Type II), and degradation the mycotoxin (Type III). Sumai 3, a Chinese wheat cultivar, is one of the most widely used resistant resources for FHB resistance in wheat breeding around the world. On the basis of molecular mapping, chromosomes 5A, 3BS, and 6BS seem to be likely locations for FHB resistant gene from Sumai 3. In this study, we reported the identification of chromosome regions associated with FHB resistance in Sumai 3 by using its susceptible near-isogenic lines (NILs).The plant materials used in this study were Sumai 3 and its four NILs (NILs-1, NILs-2, NILs-3, and NILs-4). The NILs were derived from a cross between Sumai 3 and Chuan980, a susceptible cultivar, followed by seven-times backcross with Sumai 3 and screened FHB susceptible lines in each generation by artificial inoculation. SSR and AFLP analyses were applied to screen the polymorphism between Sumai 3 and its four NILs. The detected polymorphic markers were mapped using a mapping population of double haploid lines (DHLs) derived from a cross between Sumai 3 and Gamenya. We examined 84 SSRs and 107 AFLP primer combinations that produce approximately 900 AFLP markers. Of these markers, two SSR (*Xgwm533-a* and *Xgwm389*) and five AFLP (ACT/CGAC118, AGA/CGAC136, AAC/CGAC285, AGT/CTGA225, and AGA/CTAT304) markers showed polymorphism between Sumai 3 and its four NILs. The NILs-1, NILs-2, and NILs-3 have different band pattern from Sumai 3 at all of the seven polymorphic markers while NILs-4 is different from Sumai 3 only at the AGA/CTAT304 marker, indicating that the genotypes of the different Sumai 3 NILs with susceptible to FHB is different. Six of the seven-polymorphism markers were mapped on chromosome 3BS where the resistance QTLs has been consistently detected in the populations including Sumai 3 or their derivatives. The AGA/CTAT304, which differ the NILs-4 from Sumai 3, was located on chromosome 2AL. The present study revealed that one FBH resistance gene locates on chromosome 3BS in Sumai 3 and Sumai 3 may have other genes that affect the FHB resistance.

TRANSPOSON-MEDIATED GENERATION OF MARKER-FREE BARLEY PLANTS EXPRESSING PUTATIVE ANTIFUNGAL PROTEINS

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ABSTRACT

The use of transposon-mediated repositioning of transgenes has been proposed as an attractive strategy to generate transgenic plants free of selectable marker genes (Yoder and Goldsbrough, 1994). In barley, previous research has demonstrated high transposition frequencies of a *Ds* element resulting from crosses of two transgenic plants, one containing *Ds-bar* and the other expressing *Ac* transposase. Expression of the relocated transposon-borne transgene is less prone to gene silencing than that of the transgene integrated at the original site as a result of bombardment (Koprek *et al*., 2001). Characterization of *Ds*-delivered transgenes in rice confirmed the stability of insertion site and the expression of the Cry 1B protein during generation advance (Cotsaftis *et al*., 2002). To date transposon-mediated repositioning of a value-added transgene has not been demonstrated in barley, although functionality of the maize As/*Ds* system as a gene-tagging tool has been described (Koprek *et al*., 2001).

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe (*teleomorph Gibberella zeae*) is a major disease for barley and wheat throughout the world (Parry *et al*., 1995). Introduction of putative, recombinant antifungal proteins into barley offers the potential to limit pathogen infection and growth. Transformation technologies for barley have been developed and subsequently improved (Wan and Lemaux, 1994; Cho *et al*., 1998; Bregitzer *et al*., 2000), setting the stage for the introduction of putative antifungal genes. To exploit potentially useful aspects of the maize *Ac/Ds* system, we are using this system to produce transgenic barley plants containing independent insertions of genes encoding putative antifungal proteins. Crossing of these plants to plants expressing *Ac* transposase will result in the excision and reintegration of *Ds*-bordered transgenes into new locations. This movement will result in the stabilization of transgene expression and the unlinking of the plasmid and selectable marker sequences needed to identify transgene-containing tissue from the transgene itself.

The putative antifungal genes chosen, *tlp1* (thaumatin-like protein) and *tlp4* from oat and two of the trichothecene pathway genes, *Tri101* and *Tri12*, isolated from *Fusarium sporotrichioides*, were placed in a *Ds*-bordered, maize *ubiquitin*- or rice *actin* promoter-driven expression cassette. The resultant *tlp* constructs, together with pAHC20 (*ubiquitin* promoter-*bar-nos*) or pActHpt4 (*actin* promoter-*hpt-nos*), were introduced via bombardment into scutellar cells of immature embryos or green, regenerative tissues of two spring cultivars of barley, Golden Promise, a 2-rowed variety, and Drummond, an elite 6-rowed variety. Plants derived from 3 putative *DsUbiTlp*1 lines and 3 *DsUbiTlp*4 transgenic GP lines were

positive for *bar* and further analyses for the presence of *tlp* and its expression is ongoing. Three, one, and five hygromycin-resistant lines were obtained from *DsActTlp*1-, *DsActTlp*4- and *DsActTri101*- transformed Drummond green tissue, respectively; these lines have been transferred to regeneration medium. During the transformation process, we found that bialaphos, used to identify *bar*- expressing tissue, is not suitable for selection of immature embryo and green tissue transformants in Drummond. Subsequently, hygromycin was used for selection. In addition, the *Ac*-transposase gene driven by its own promoter was transformed into Drummond green tissue; five hygromycin-resistant *AcTPase* lines were obtained and are under regeneration. *Ac*TPase was also introduced into Drummond by backcrossing *ubiquitin*- and *Ac* promoter-driven *Ac*TPase-containing Golden Promise lines that were previously isolated (Koprek *et al.*, 2001).

To assist in characterization of the level of transgene expression, antibodies to TLP1, TLP4, and the Tri101proteins are being developed. Genes for *tlp1, tlp4, tri101* and *tri12* were inserted in vector pGEX-4T3 and TLP1, TLP4 and Tri101 proteins were purified for antibody preparation. These three antibodies were tested for experimental efficiency. Antibody to Tri101 was efficient in detecting the Tri101 protein in western blots, while antibodies to TLP1 and TLP4 showed nonspecific binding to barley proteins. Nonspecific antibodies will be removed by passing serum through an affinity column bound with purified TLP1 and TLP4 proteins produced in *E. coli*. The *Tri12* gene was also cloned into pGEX-4T3, but protein expression was low. Subsequently the gene was inserted into other vectors pMAL-c2X and pMAL-p2X; however, the resultant MAL-Tri12 fusion protein expression was still weak. Low expression of the *Tri12* gene product may be due to its 14 transmembrane domains.

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EFFECT OF BACTERIAL GROWTH MEDIUM COMPOSITION ON ANTIFUNGAL ACTIVITY OF *BACILLUS SP.* STRAINS USED IN BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT

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ABSTRACT

Several microbial strains belonging to different taxa, isolated from various parts of the world, have been shown to have the ability to antagonize *Fusarium graminearum* to different extents under various conditions. Some of these microbial strains are being developed as biological control agents (BCAs) for control of FHB. Different BCAs have different mechanisms of antagonizing FHB, such an enzymes, antibiotics, parasitism, and/or competition for nutrients. We have studied four different *Bacillus sp.* strains that show promise for use as BCAs to control FHB. All these strains seem to belong to a phylogenetic group designated as the *Bacillus subtilis* group (group II). Among the many antibiotics that *B. subtilis* and its relatives are known to make are cyclic lipopeptides such as iturin. If one or more iturin-like antibiotics are needed for these bacterial strains to control FHB, it is important that a growth medium be used for culturing the BCAs that encourages production of such antibiotics. In previous studies, we have usually grown the four BCAs in potato-dextrose broth (PDB), which may not have been an optimal growth medium for production of iturin-like antibiotics. Other researchers working with *B. subtilis* have found that dextrose (glucose) is not an optimal carbon source for iturin production, and that the nitrogen source in the growth medium also has a large influence on the amount of iturin produced. All four of our BCAs grew well in a defined growth medium previously described in the literature that may stimulate antibiotic production of our BCAs more than does PDB. The defined medium contains mannitol as a carbon source, and glutamic acid as a nitrogen source, along with inorganic salts. We have conducted studies with both the broth and agar-solidified form of this medium, finding that the bacteria grow well in both. Plate assays were conducted to test the ability of the BCAs to antagonize *F. graminearum* on the agar-solidified form of this growth medium. Antagonism against the fungus was apparent, suggesting that antibiotic was being produced in the medium. Presence of iturin in the growth medium will be tested for chromatographically, and compared to amounts produced in PDB. In addition, greenhouse groundbed trials will compare the effect that BCA cells grown in the defined broth medium have upon wheat challenged with FHB, to the effect that BCA cells grown in PDB have upon wheat challenged with FHB. In uniform field trials to compare the ability of different microbial BCAs to control FHB, it should be recognized that different microbial BCAs can have different mechanisms of antagonism, and that different growth media may promote these mechanisms to varying degrees. Formulation and optimization of growth media for commercial production and application of BCAs to control FHB should also bear this in mind.

TAXONOMIC AFFILIATION OF BACTERIAL STRAINS USED IN THE BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT SUGGESTS POSSIBLE ROLE OF LIPOPEPTIDE ANTIBIOTIC IN FUNGAL ANTAGONISM

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ABSTRACT

For the last several years, our laboratory has been working with four endospore-forming bacterial strains (designated as 1B-A, 1B-C, 1B-E, and 1D-3) isolated from South Dakota wheat foliage and residue that can antagonize *Fusarium graminearum* in laboratory plate assays and in greenhouse and field plot trials. We have attempted to identify these bacterial strains by different techniques, with different identification methods resulting in different genus affiliations for the strains. In previous work, analysis of membrane fatty acid methyl esters (FAME analysis) indicated that strains 1B-A and 1D-3 were *Bacillus lentimorbus*, and that strains 1B-E and 1B-C were *Bacillus subtilis*. Sequence analysis showed that all four strains had identical sequences in the first 500 base pairs of their 16S rDNA genes, and all were most closely related to *Bacillus amyloliquefaciens* with less but significant relatedness to *Bacillus atrophaeus*. The strains differed in the total number and amount of antibiotic compounds produced, and their growth curves in potato dextrose broth also differed. In the work presented here, colonial morphology, microscopic appearance, and 20 different phenotypic traits were evaluated and used to arrive at suggested identities for the strains. Strains 1B-A and 1B-C had similar colonial morphology, with a shiny and wrinkled appearance, whereas colonies of strain 1B-E were shiny but not wrinkled, and colonies of strain 1D-3 were a dull color with bumps instead of wrinkles. All strains had oval endospores which did not cause swelling of the sporangium. Results of 20 different phenotypic tests suggested that all four strains were most closely related to *Bacillus firmus*. These attempts to identify the four strains strongly suggest that they are tied to a phylogenetically and phenetically coherent *B. subtilis* group (group II). However, the four strains may all belong to a previously uncharacterized taxon with relatedness to *B. amyloliquefaciens* and *B. atrophaeus*, taxa which were split out of the old *Bacillus subtilis* taxon. There is a good amount known about the antibiotics produced by members of the *B. subtilis* group (group II). Among the many antibiotics that are known to be produced by *B. subtilis* and its relatives are cyclic lipopeptides such as iturin. We are hypothesizing that one or more cyclic lipopeptides such as iturin are responsible for a significant amount of the biological control these bacterial strains exert against *F. graminearum*, and we are presently engaged in experiments to test this hypothesis.

JAU 6476 FOR THE CONTROL OF *FUSARIUM GRAMINEARUM* AND OTHER DISEASES IN CEREALS

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ABSTRACT

JAU 6476 (tested under the code AMS 21619) is a novel broad-spectrum fungicide belonging to the new chemical class of triazolinthione discovered and developed worldwide by Bayer CropScience. The common name for this molecule is prothioconazole. JAU 6476 is a systemic fungicide showing excellent efficacy against a broad range of diseases in different crops, including wheat, barley, peanuts, canola, etc. In cereal crops, JAU 6476 provides excellent activity against most major diseases, including Fusarium head blight (*Fusarium spp*.), leaf blotch diseases (*Septoria tritici, Leptosphaeria nodorum, Pyrenophora spp., Rhychosporium secalis*), rust (*Puccinia spp*.), powdery mildew (*Erysiphe graminis*) and eyespot (*Pseudocercosporella herptrichoides*). Trial results indicate that JAU 6476 is more effective than currently tested products for the reduction of deoxynivalenol (DON), a mycotoxin caused by *Fusarium graminearum.* JAU 6476 applications provide outstanding cereal disease control along with excellent crop safety to ensure high quality yields.

EFFECT OF FUNGICIDE TREATMENTS ON FUSARIUM HEAD BLIGHT AND LEAF DISEASE INCIDENCE IN WINTER WHEAT

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OBJECTIVES

The objectives of this study were to compare control of Fusarium head blight and leaf diseases of winter wheat with the application of different fungicides and combinations of fungicides.

INTRODUCTION

Winter wheat cultivars grown in western Canada are susceptible to most leaf diseases and Fusarium head blight (FHB). These diseases can cause losses in yield and quality, which affects producers and end-users of the grain. Winter wheat producers routinely apply a fungicide treatment for control of leaf diseases, but not for FHB control. Few fungicides are registered for FHB control. Those that are registered require different application times for control of FHB compared to leaf diseases. Timing of fungicide application for FHB control is critical due to the specific period of host susceptibility. Producers are interested in the efficacy of fungicides for FHB control, and have questioned whether delaying fungicide applications to control FHB would compromise their ability to control leaf diseases.

MATERIALS AND METHODS

Trials were conducted at one location in 1999 (Carman, Manitoba), and two locations (Carman and Winnipeg, Manitoba) in 2000, 2001 and 2002. In 1999 ten cultivars (only cultivar means will be reported) and eight treatments (Table 1) were evaluated in a split-plot, four replicate trial. Treatment was the main plot effect and cultivar was the sub plot effect. In 2000, 2001 and 2002 trials with the same treatments were conducted on a single winter wheat cultivar, “CDC Falcon”, at Carman and Winnipeg, MB.

Where appropriate fungicide treatments were based on label recommendations for FHB control. Tilt was applied at the boot stage for control of leaf diseases since there is no label recommendation for FHB control. Bravo and Folicur were applied according label instructions for FHB control. In the Bravo x 2 treatment the first application was made at the recommended time for FHB control and the second application was made two weeks later. All fungicide treatments preceded FHB inoculation by at least three days.

All plots except for the un-inoculated control were inoculated with a macroconidial suspension of *Fusarium graminearum* at anthesis and four days after the first inoculation. Mist irrigation was applied for 5 min./h for 12 h after each inoculation. Eighteen to twenty-one days after inoculation, 50 spikes/plot were collected from all plots for evaluation of FHB reaction. The number of infected spikes was determined. Of the infected spikes, the per-

centage of infected spikelets was determined. From this the FHB index was calculated as (% infected spikes x % infected spikelets)/100.

Percent leaf area affected by leaf spot diseases and leaf rust was evaluated visually on the flag leaf on a per plot basis in 1999. In 2000-2002, leaf area affected by disease was determined by collecting 20 flag leaves and 20 penultimate leaves per plot and evaluating percent disease through digital imaging technology.

Plot yield was measured at maturity.

Table 1: Treatments and timing of treatment application to field trials conducted in 1999, 2000, 2001 and 2002. FHB inoculum was applied to all plots treated with fungicides.

Treatments and Time of Application
Un-inoculated control
FHB inoculated control - Anthesis + 4 days later
Tilt - Boot stage
Bravo - Heading
Folicur - Heading
Bravo x 2 - Heading + 2 weeks later
Tilt (Boot Stage) + Bravo - Heading
Tilt (Boot Stage) + Folicur - Heading

RESULTS AND DISCUSSION

1999

Disease levels were high in 1999. Cultivars differed in susceptibility to FHB, leaf rust and leaf spot (data not reported). The FHB index was higher than the un-inoculated check in all fungicide treated plots (Figure 1a). Treatment with either Bravo or Folicur reduced the FHB Index relative to the inoculated check. Plots treated only with Tilt did not differ in FHB Index compared to the inoculated check. Treatments which included Tilt provided the best control of leaf spot diseases (Figure 1b) and leaf rust (Figure 1c). All fungicides increased yield relative to the untreated checks (Figure 1d). The highest yields were obtained with plots treated with Tilt or combinations of Tilt+Bravo and Tilt+Folicur. Yield was highly negatively correlated with %leaf spot (-0.98) and %leaf rust (-0.90), but was not significantly correlated with FHB Index (0.20).

2000-01

Disease levels in trials conducted in 2000 and 2001 were low at both locations. Significant differences in yield and FHB Index were observed at Winnipeg in 2001, while significant differences in leaf spot were observed at both locations in 2000. Folicur and Bravo provided similar levels of FHB control. All fungicide treatments reduced leaf spot relative to the untreated control. Fungicide treatments did not provide a significant yield advantage compared to the untreated checks.

2002

FHB levels were high at both locations in 2002. Leaf disease data has yet to be analysed. At Winnipeg all treatments had higher levels of FHB than the un-inoculated control (FHB Index =7). The FHB Index of the inoculated control was 41. Folicur (FHB Index = 29) and Bravo (FHB Index = 26.5) significantly reduced the FHB Index relative to the inoculated control and were not statistically different from each other. Other treatments were not significantly different from the inoculated control. In Carman, the FHB Index of the fungicide treatments did not differ significantly from the inoculated check (FHB Index = 26). There were no significant differences for yield.

The results from these trials show that even when plots are inoculated and mist irrigation is applied to increase humidity it is difficult to get consistently high levels of FHB on winter wheat in Manitoba. Lower June temperatures (data not shown) in 2000 and 2001 relative to 1999 appeared to be the main reason for lower disease incidence. Disease forecasts would be beneficial in this situation.

Under high disease pressure fungicide treatments with either Bravo or Folicur reduced FHB index. However, the FHB Index of these treatments was still high relative to the un-inoculated control. When either leaf rust or leaf spotting diseases were present, treatments with Tilt and to a lesser extent, Folicur reduced these diseases. Under low disease pressure, fungicide treatments provided little advantage. Overall, there was no association between yield and FHB Index. Leaf diseases appeared to be the main cause of yield differences observed.

CONCLUSIONS

Under high disease pressure fungicide treatments reduced both FHB and leaf diseases. Yield differences were primarily associated with differences in leaf disease control. Tilt provided the best control of leaf diseases. Folicur applied at heading provided some level of leaf disease control. Folicur and Bravo appear to provide similar levels of FHB control. Weather conditions during flowering of winter wheat are often not conducive the FHB development. Disease forecasts would be useful to determine whether fungicide application is necessary in winter wheat.

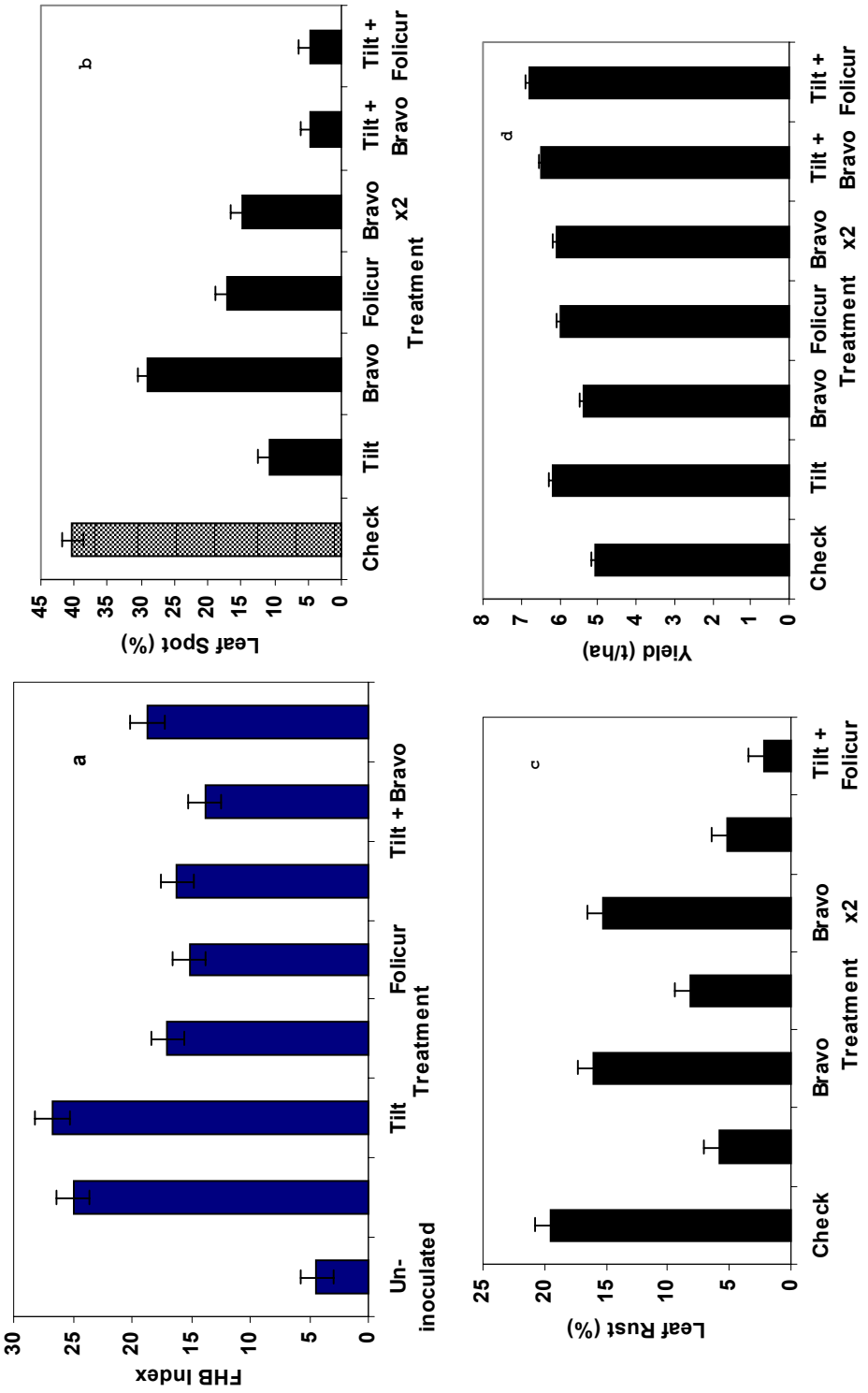

Figure 1. Effect of fungicide treatment on a) FHB Index, b) % leaf spot, c) % leaf rust and d) yield of winter wheat grown in trials conducted at Carman, MB in 1999.

POPULATION DYNAMICS IN THE FIELD OF A BIOCONTROL AGENT FOR FUSARIUM HEAD BLIGHT OF WHEAT

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ABSTRACT

Gibberella zeae (anamorph *Fusarium graminearum*) is the major causal organism of Fusarium head blight (FHB) on wheat and barley. Wheat is generally most susceptible to infection at anthesis due to exposed anthers being an important site of infection. Application of a *Crytococcus* strain, OH 182.9, originally isolated from wheat anthers in Wooster, Ohio has reduced disease severity by 56% and increased the 100-kernel weight by as much as 100% in field trials. The goal of this research was to determine the ability of OH 182.9 to survive and possibly reproduce on the anthers in the field. Heads of the soft red winter wheat cultivar Freedom were marked to distinguish those that had extruded anthers and those that had no visible anthers. Cells of the yeast antagonist were produced and harvested after a 48-hour growth in a semi-defined liquid medium at 25ºC in 250 rpm and applied ($1x10^7$colony forming units (CFU)/ml) to thoroughly wet the wheat heads. Non-antagonist/buffer treated plants served as controls. Pathogen inoculum consisted of *F. graminearum* colonized corn kernels scattered throughout the plots 3 weeks prior to flowering. Plots were under mist irrigation twice daily throughout anthesis and early grain development growth stages. Anthers were collected for up to 10 days after applying yeast antagonists and CFU per 100 anthers in 0.5 ml buffer were determined. Initial OH 182.9 populations on anthers, at day 0, were $2.6x10^4$ CFU/ml. OH 182.9 population increased to $2.1x10^6$ CFU/ml (80 times) by 6 days after applying the cell suspension. The yeast population was $2.2 x10^6$ by 10 days after application. The population levels were significantly ($P \leq 0.05$) greater than those on the control plants on the heads with exposed anthers and heads with no visible anthers at 6,8,10 days and 8 days, respectively after inoculation. There was no significant difference in disease severity between OH182.9 treated and untreated plants. This one season test will be repeated in 2003 to further determine the population dynamics of OH182.9 on wheat floral structures.

VARIATIONS IN FUNGICIDE APPLICATION TECHNIQUES TO CONTROL FUSARIUM HEAD BLIGHT

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ABSTRACT

The frequency and severity of Fusarium head blight (FHB) on wheat have been on increase during the past decade in Uruguay. Given low level of resistance in the commercial cultivars, chemical control of the disease is widely adopted. The application of Folicur 430 SC (tebuconazol, 432 g/l) or Caramba (metconazol, 90 g/l) at the rate of 450 cc/ha and 1000 cc/ha, respectively, is recommended at the beginning of flowering. In order to increase the efficiency of fungicide application, variations in the spray nozzles and angles were tried.

The treatments, two fungicides (Folicur and Caramba), two types of spray nozzles (hollow cone spray tips, *ConeJet*, and twin even flat spray tips, *TwinJet*, mounted at 0° and 30° angle on the bar), and two application times (beginning of flowering, Zadoks 61 and mid flowering, Zadoks 65), were combined in a factorial design with complete blocks replicated four times. All treatments were applied with a CO_2 backpack type sprayer. Grain yield, test weight, thousand kernel weight, visual disease note on a 1-5/1-5 scale, incidence (percentage of diseased spikes) and percentage of scabby grains were evaluated (Table 1).

The results show that overall Caramba gave better control of the FHB than Folicur. In general, the early control of the disease at Z61 was superior to FHB control in Z65. The utilization of *TwinJet* improved the spike coverage significantly thereby, resulting in better visual score of infection. However, spike infection in the field and grain infection evaluated after harvest demonstrated these differences more clearly in the case of Folicur than Caramba. Although some advantage in using the *TwinJet* at an angle of 30° on the bar was observed, these results need further testing and confirmation. In spite of the fact that grain yield, test weight and thousand kernel weights were affected by moderate infection of foliar diseases, the utilization of Caramba early on and especially using *TwinJet* spray nozzles gave significantly higher grain yield compared to other treatments.

Table 1. Effect of chemical control treatments on FHB infection and grain yield.

Treatments				Fusarium infection (%)			Grain yield	TKW	Test weight
Application Time	Fungicide	Spray nozzle	Degree/ vertical	Visual score	spike	grain	kg/ha	g	kg/hl
Z-61	Caramba	Twin	0º	**22 e**	**37e**	**7**	**2415a**	**27.1ab**	**80.2a**
Z-61	Caramba	Cone	0º	25 de	39cde	7.8	**2474a**	**28.1a**	**79.6ab**
Z-61	Caramba	Twin	30º	**15 f**	38de	7.3	**2083ab**	26.8ab	**79.6ab**
Z-65	Caramba	Twin	0º	28 cd	**37e**	**6**	1599bc	24.9b	79.5ab
Z-65	Caramba	Cone	0º	28 cd	41cde	6.9	1863bc	25.7ab	78.5bc
Z-65	Caramba	Twin	30º	**15 f**	**36e**	**5.6**	2012ab	**27.1ab**	79.5ab
Z-61	Folicur	Twin	0º	25 de	55bc	8.8	1820bc	26.2ab	78.2bc
Z-61	Folicur	Cone	0º	35 b	53bcd	9.6	1759bc	24.4bc	77.6cd
Z-61	Folicur	Twin	30º	35 b	55bc	10.4	1767bc	24.8b	78.6abc
Z-65	Folicur	Twin	0º	35 b	49bcde	9.4	1644bc	24.3bc	77.0cd
Z-65	Folicur	Cone	0º	32 bc	64ab	6.8	1691bc	24.4bc	77.3cd
Z-65	Folicur	Twin	30º	35 b	52bcd	7.2	2045ab	26.4ab	78.6abc
Check without fungicide				52 a	78a	10	1415c	21.5c	76.4d

AERIAL SPRAY COVERAGE TRIALS IN SOUTH DAKOTA – 2002

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INTRODUCTION AND OBJECTIVES

In a state such as South Dakota, wheat fields are typically very large and with 2.5 M acres of wheat and another 400,000 acres of barley, the only practical means of applying fungicide is from the air. As such it is critical to identify methods whereby applicators and producers can optimize the application for efficacy and cost effectiveness. These trials were intended to take initial steps in accomplishing those goals.

MATERIALS AND METHODS

The trial was conducted on August 28, 2002 in collaboration with MJ Aviation, Inc. at Letcher, SD. Treatments, listed in Table 1 were a comparison of three different brands of nozzles with varying combinations of modifications. Each treatment was repeated three times. Airplanes were loaded with water and fluorescent rhodamine dye blended with a pink foam marker dye.

Table 1: Treatment list of nozzle and spray configurations for aerial coverage trial.

Trt #	GPA	Nozzle	Nozzle Spacing	Nozzle Modifications	Boom Ht.	Drop Used	Check Valve	Expected Swath	Spray pressure (PSI)	Airplane Speed
1	5	Lund	14”	None	16’, 19’, 22’	No	Brass TeeJet	60’	39#	130, 130, 130
2	5	CP	7”	Straight Stream	18’, 15’, 14’	No	TeeJet	60’	22#	128, 128, 124
3	5	CP	7”	15º Deflection	16’, 18’, 19’	No	TeeJet	60’	22#	129, 129, 129
4	5	CP	7”	30º Deflection	18’, 18’, 19’	No	TeeJet	60’	22#	125, 128, 128
5	5	Accu-Flow 0.028	7”	3/32 Restrictor	16’, 13’, 18’	No	TeeJet	35’	40#	127, 127, 122
6	5	Accu-Flow 0.028	14”	3/32 Restrictor	16’, 15’, 18’	No	TeeJet	35’	30#	128, 123, 126
7	5	Accu-Flow 0.028	7”	1/8 Black Restrictor	18’, 19’, 16’	No	Internal	35’	30#	128, 131, 131
8	5	Accu-Flow 0.028	7”	3/32 Restrictor	12’, 14’, 14’	6”	TeeJet	35’	30#	126, 125, 125
9	5	Accu-Flow 0.028	7”	3/32 Restrictor	22’, 24’, 23’	6”	TeeJet	35’	30#	125, 125, 125

Measurements were taken of spray pattern deposition on a string line and measurement of drift on a string line suspended from an 18 m high drift tower positioned at 46 m from the center of the spray swath, perpendicular to the prevailing wind. Measurements were also taken for droplet patterns on water sensitive and chrome coat papers. The rhodamine dye was used for measurements on the string line tests and the pink foam marker dye was used for droplet deposition on the chrome coat paper.

Treatment one was applied with an Air Tractor AT-402B Turbo with nozzles spaces every 14 in. and treatments two through nine were applied with an Air Tractor AT-401B radial engine with nozzles spaces every 7 or 14 in. across the boom.

String line patter and drift tower data were read and analyzed by String Analysis/Graphics (WRK) and water sensitive and chromecoat paper data was analyzed by Dropletscan (WRK and DSI). Additional analysis was complied in Excel (Microsoft).

RESULTS AND DISCUSSION

This trial was initially planned for May, but excessive winds through the month prevented completion of the trial at that time. During the period of the August trial, the wind speed ranged from two to nine mph and no deposition was measured on the drift tower string line.

One of the most serious problems encountered with aerial application has been incomplete coverage of the head. One side of the head may receive reasonable coverage while the opposite side may receive no product. In an earlier trial (Draper, unpublished) CP nozzles were compared with hollow cones at five or ten gallons of water delivered. In that trial, CP nozzles gave poor performance for droplet uniformity and head coverage, but increasing the gallons delivered helped offset the coverage deficiency. Nonetheless, CP nozzles are preferred by aerial applicators in South Dakota because they work well for herbicide applications. If we are to improve fungicide application by air, we must identify a preferred configuration for optimized coverage. CP nozzles were retained in this study because of their common usage, Lund nozzles were in place on one of the cooperators airplanes, and the Accu-Flow nozzles (Bishop Equipment, Inc.) were tested because of their use in orchards and that they are noted for good patterns with little drift.

No nozzles tested in this trial overcame the problem of poor deposition on the back of the head.

All Accu-Flow nozzle configurations deposited a slightly narrower swath with less off target movement than the CP or Lund nozzles.

All Accu-Flow nozzle configurations deposited a more uniform droplet pattern than the Lund or CP nozzle configurations.

Additional treatments will be competed in the coming year, looking at different orifice size and other nozzles designed to produce small droplet size with minimal drift.

UNIFORM TRIALS FOR BIOLOGICAL CONTROL AGENT PERFORMANCE IN THE SUPPRESSION OF FUSARIUM HEAD BLIGHT IN SOUTH DAKOTA – 2002

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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' and 'Ingot' hard red spring wheat were planted in a factorial randomized complete block design with six replications, both at Brookings, SD. Barley was protected with isolates of *Bacillus subtilus*-type isolates SDSU-1BA and SDSU-1BC, *Cryptococcus nodaensis* OH 182.9, *Bacillus*-type isolate TrigoCor 1448, *Lysobacter* sp. strain 'C3', *Bacillus*-type isolate TrigoCor 2, and *Bacillus*-type isolates BHWJ 4-1 and BHWJ 4-2B. The BCAs were compared to a standard chemical treatment of Folicur (4 fl/oz/A) with Induce non-ionic surfactant (0.125%). Spring wheat was protected with Folicur + NIS, *Cryptococcus nodaensis* OH 182.9, *Bacillus*-type isolate TrigoCor 1448, isolates of *Bacillus subtilus*-type isolates SDSU-1BA and SDSU-1BC, *Lysobacter* sp. strain 'C3', *Bacillus*-type isolate TrigoCor 2, and *Bacillus*-type isolates BHWJ 4-1 and BHWJ 4-2B

At the time that the heads were completely emerged from the boot, a misting cycle was started for 5 minutes out of every 20minutes, 24 hours a day. The mist system was turned off and the BCAs were applied to the heads and allowed to dry before the misting was turned on again. Two days following inoculation with the BCAs, the crop was challenge inoculated with 10^4 macroconidia/ml of *Fusarium graminearum* 'Fg4'. The barley plots were misted for seven days total and the spring wheat plots were misted for three days following anthesis.

RESULTS AND DISCUSSION

During the inoculation period, the environment was very hot and dry. And it was difficult to retain free moisture between misting periods. Very little FHB developed in the either the wheat or barley plots and no significant differences were detected among the barley treatments for FHB incidence, FHB severity, FHB index (incidence x severity), yield, test weight, protein, or deoxynivalenol (DON) levels in the harvested grain, even among the challenge inoculated plots. Only Folicur + NIS resulted in a reduction of any disease component on spring wheat, although there was no significant FHB that developed on the spring wheat either. While Folicur reduced overall leaf disease and leaf rust significantly, no biological controls had a significantly measurable effect on any leaf disease.

UNIFORM FUNGICIDE PERFORMANCE TRIALS IN SOUTH DAKOTA – 2002

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INTRODUCTION AND OBJECTIVES

Fusarium head blight (FHB – scab) has been a serious concern for wheat producers in South Dakota for the past several years. FHB and low market prices are the two reasons most often cited by producers as they decrease the number of acres they plan to plant to wheat. Fungicide alternatives for disease control are available to local producers on special year-to-year labels.

The objectives to this study were to evaluate the efficacy of various fungicides, fungicide combinations, or biological controls for the suppression of Fusarium head blight and other wheat diseases.

MATERIALS AND METHODS

Three South Dakota locations were planted to hard red spring wheat and two locations were planted to hard red winter wheat for inclusion in the Uniform Fungicide Trial for the suppression of FHB.

Two hard red spring wheat cultivars, Oxen and Ingot, were planted at three South Dakota locations (Brookings, Groton, and South Shore/Watertown). Two hard red winter wheat cultivars, Wesley and Arapahoe, were planted at Selby and South Shore/Watertown. Trials were planted in a factorial randomized complete block design. There were six replications of spring wheat and four replications of winter wheat. At anthesis, the trial treatments were applied. The following day, the crop was challenge inoculated with 10^4 macroconidia/ml of *Fusarium graminearum* 'Fg4'. The plots were misted for three days total.

Sixteen days following treatment, plots were evaluated for leaf diseases, FHB incidence, FHB head severity, and FHB field severity, *Fusarium* damaged kernels (FDK), deoxynivelanol (DON), grain yield, test weight, and protein.

RESULTS AND DISCUSSION

The weather in 2002 was very hot and dry in South Dakota. Grain yields were about half of normal in much of the state and yields were progressively lower the farther west the fields were located. In the spring wheat trials at Groton and South Shore/Watertown, very little disease developed and there were no significant differences among treatments. Similar

results occurred in the winter wheat trial locations. With the supplemental mist irrigation, while no significant FHB developed, leaf diseases were enhanced and some treatments did significantly improve results over the untreated control.

No significant differences were detected for FHB incidence, FHB head severity, FHB field severity, *Fusarium* damaged kernels (FDK), deoxynivelanol (DON), grain yield, test weigh, and protein. No significant disease response resulted from challenge inoculation with *Fusarium* conidia. However, in greenhouse trials the strain used has been shown to be highly virulent. Presumably, extremely high temperatures and dry conditions minimized the conditions for infection. FHB rating was done at five days earlier than normal due to the dry conditions leading to a rapidly maturing crop.

The presence of a fungicide in the treatment generally resulted in a significant reduction in leaf disease from the untreated (Table 1). Folicur, BAS 505, and AMS 21619 all resulted in a reduction in leaf disease. However, BAS 505 and AMS 21619 did not reduce leaf rust in the trial. The biological control treatments in the trial did not result in reduced disease unless they were co-applied with Folicur or AMS 21619.

Table 1. Disease categories with a significant response to treatments at Brookings[1].

Treatment	Whole Plot Leaf Disease Rating[2]	Leaf Disease (% leaf area)	Leaf Rust (% leaf area)
Untreated	6.25	65.33	9.18
Folicur + NIS	5.08	36.30	1.12
AMS 21619 + NIS	5.25	34.08	6.63
BAS 505 + NIS	5.50	38.92	9.37
OH 182.9	6.33	62.00	11.77
TrigoCor 1448	6.42	55.08	7.97
TrigoCor + Folicur + NIS	5.08	27.03	0.87
AMS 21619 + Folicur + NIS	5.08	28.02	1.25
LSD (P=0.05)	0.57	14.01	3.86

[1]Other measurements of disease and yield were not significant.
[2]Green leaf evaluation based on a scale of 0-9 where 0 is disease free and 9 is completely necrotic.

FUSARIUM HEAD BLIGHT: EPIDEMICS AND CONTROL

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OBJECTIVES

i) To document the effect of two biocontrol agents (TrigoCor 1448, and OH182.9) and three fungicides (Folicur, AMS21619, and BAS 505) on disease development, ii) To evaluate the effect of these materials for managing Fusarium head blight, and iii) To determine the relationships between the disease, DON and yield.

INTRODUCTION

Fusarium head blight (FHB) or scab, caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*) is a major disease in many wheat and barley production regions of North America, including Ohio, and throughout the world (Bai and Shaner 1994; Parry *et al*.1995; McMullen *et al*. 1997). This disease has been difficult to control. Although recent advances in host resistance are beginning to improve disease management in some wheat production regions, many wheat and barley producers have few management options. Commonly used methods of disease management including tillage and crop rotations, have not been effective in eliminating wide spread disease epidemics (McMullen *et al*. 1997). Controlling Fusarium head blight will require multiple disease management strategies, coupled with greater understanding of the epidemiology of the disease (Bai and Shaner, 1994; Parry, *et al*., 1995; Shaner and Buechley, 2000).

Effective fungicides could provide growers with management options when susceptible cultivars are grown, and may help protect yield and grain quality of cultivars with partial resistance under conditions favorable for disease. Although a few fungicides have shown some efficacy against scab, their results have been inconsistent over locations and years (Parry, *et al*., 1995; McMullen *et al*. 1997; Shaner and Buechley, 1999; Gilbert and Tekauz, 2000). Treatment with some fungicides reduced DON contamination of grain, but others caused an increase in DON levels (Shaner and Buechley, 1997, 1999 and 2000; Gilbert and Tekauz, 2000).

MATERIALS AND METHODS

Seeds of wheat cultivar Elkhart treated with Raxi-Thiram, were planted using 24 seeds/ft of row on 11 Oct., and 27 Sep., 2000 and 2001, respectively, in Ravenna silt loam soil at the Ohio Agricultural Research and Development Center, Wooster. For each treatment, there were three replicate plots. Each plot was 15-ft long, and consisted of 7-rows with 7 in. between rows. Plots were inoculated by broadcasting colonized corn kernels (0.12 oz/sq ft) over the plot surface on 14 May in 2001, and 30 Apr. in 2002. Plots were misted each day from one week prior to flowering to two week after flowering. Biological agents and fungicides were applied as sprays in 26.2 gal. water/A with a CO- pressurized back pack sprayer at flowering growth stage (GS) 10.5.1. Disease assessments were made twice a week (June 11 - June 26) in 2001 and three times a week (June 07 - June 21) in 2002 for both incidence and severity in one ft. of row at 15 locations in each plot. Plots were harvested on 17 of July in 2001 and on 11 July in 2002. Yield (bu/A) was determined from harvested grain adjusted to 13.5% moisture, and grain was analyzed for DON content.

RESULTS AND CONCLUSIONS

Disease development varied greatly among the different fungicides and biological control treatments tested in the two years. Based on the coefficient of determination (R^2), evaluation of the residual plots, standard error of estimates (SE) and mean square errors (MSE), the Gompertz model was appropriate for describing the disease incidence and severity data sets (R^2 ranged from 82 to 96%). The various treatments had a significant effect on disease development. Rates of disease increase for the various treatments and the control ranged from 0.138 to 0.229 and from 0.054 to 0.129 per day based on disease incidence, and from 0.093 to 0.172 and from 0.066 to 0.125 per day for disease severity in 2001 and 2002, respectively (Table 1). Area under the disease progress curve based on disease incidence (AUDPCI) ranged from 418.0 to 804.2 in 2001, and from 605.2 to 911.8 in 2002; when based on disease severity (AUDPCS) ranged from 125.1 to 315.7 in 2001, and from 176.6 to 383.3 in 2002 (Table 1). Maximum disease incidence (*Ymax*) for the various treatments ranged from 55.0 to 89.6%; from 55.1 to 82.5% and Maximum disease severity ranged from 23.9 to 57.9%; from 27.9 to 54.0% in 2001 and 2002, respectively (Table 2).

Plots treated with AMS21619 or BAS 505 had significantly lower rates of disease increase, low maximum disease, AUDPCI , and AUDPCS values than the untreated control in both 2001 and 2002 (Tables 1and 2). Additionally, plots treated with Folicur had significantly lower rates of disease progress, low maximum disease, AUDPCI, and AUDPCS values than the untreated control plots in 2002.

Plots treated with AMS21619, and BAS 505 had significantly higher yield in both years, higher test weight, and lower DON levels than grain from the untreated control plots in 2001 only. However, plots treated with Folicur had significantly higher yield in 2002. Although the biocontrol agent OH182.9 did not have a significant effect on reducing disease development, grain harvested from plots treated with this biocontrol agent had significantly lower DON than grain from the untreated control plots in 2001. No differences were found among treatments in DON levels, damage kernels, or test weight in 2002.

There were positive correlations between DON and final disease severity, AUDPCI, AUDPCS. On the other hand, there were negative correlations between yield and maximum disease severity, AUDPCI, and AUDPCS.

In conclusion, the treatments exhibited different effects on Fusarium head blight development and control. Treatments AMS21619 and BAS 505 had low maximum disease, low epidemic rates, and small AUDPCI and AUDPCS values that were significantly different from the control. On the other hand, treatments TrigoCor 1448 and OH182.9 had high maximum disease, fast epidemic rates, and large AUDPCI and AUDPCS values that were not significantly different from untreated control. These results indicate the AMS21619 and BAS 505 fungicides have greater potential for management of Fusarium head blight than the other treatments tested.

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Table 1. Fit of models, epidemic rates, and area under disease progress curve of Fusarium head blight incidence (AUDPCI) and severity (AUDPCS) for fungicides and biocontrol agents tested in Ohio, in 2001 and 2002.

Year	Treatment & rate/A	Incidence			Severity		
		Model Fits	Rate	AUDPCI	Model Fits	Rate	AUDPCS
2001	Control	Gompertz	0.212	759.3	Gompertz	0.159	291.9
	Folicur 3.6 EC 4.0 fl oz Induce (0.125%, v/v)	Gompertz	0.194	634.1	Gompertz	0.141	235.9
	AMS21619 480SC 5.7 fl oz Induce (0.125%,v/v)	Gompertz	0.138*	418.0*	Gompertz	0.093*	125.1*
	BAS 505 50G 6.2 oz	Gompertz	0.143*	469.2*	Gompertz	0.117*	159.4*
	TrigoCor 1448	Gompertz	0.231	798.4	Gompertz	0.169	315.7
	OH182.9	Gompertz	0.229	804.2	Gompertz	0.172	307.5
2002	Control	Gompertz	0.114	911.8	Gompertz	0.125	383.3
	Folicur 3.6 EC 4.0 fl oz Induce (0.125%, v/v)	Gompertz	0.068*	655.4*	Gompertz	0.092*	214.7*
	AMS21619 480SC 5.7 fl oz Induce (0.125%,v/v)	Gompertz	0.054*	605.2*	Gompertz	0.066*	176.6*
	BAS 505 50G 6.2 oz	Gompertz	0.068*	668.6*	Gompertz	0.087*	235.0*
	TrigoCor 1448	Gompertz	0.129	819.2	Gompertz	0.124	330.0
	OH182.9	Gompertz	0.102	912.7	Gompertz	0.119	374.2

* Indicates means significantly different ($P \# 0.05$) from untreated control based on Fisher's LSD.

Table 2. Maximum disease (*Ymax*) of Fusarium head blight, yield, and DON content of grain for fungicides and biocontrol agents tested in Ohio in 2001and 2002.

Year	Treatment & Rate/A	*Y max*		Damage Kernels	Yield (bu/A)	DON (ppm)	Test Weight
		Incidence (%)	Severity (%)	(%)			
2001	Control	82.5	50.9	61.7	62.3	16.6	56.1
	Folicur 3.6 EC 4.0 fl oz Induce (0.125%, v/v)	75.8	41.5	33.3	66.6	12.0	57.7
	AMS21619 480SC 5.7 fl oz Induce (0.125%,v/v)	55.0*	23.9*	4.3	74.0*	7.2*	59.5
	BAS 505 50G 6.2 oz	60.1*	28.6*	6.7	77.1*	8.4*	60.0
	TrigoCor 1448	89.6	57.9	51.7	56.0	24.0*	54.7
	OH182.9	87.5	51.8	56.7	62.0	13.4	56.7
2002	Control	81.9	54.0	28.8	43.7	23.0	53.2
	Folicur 3.6 EC 4.0 fl oz Induce (0.125%, v/v)	60.7*	33.5*	23.3	50.3*	13.5	56.0
	AMS21619 480SC 5.7 fl oz Induce (0.125%,v/v)	55.1*	27.9*	16.8	52.9*	13.5	55.8
	BAS 505 50G 3.1 oz	62.0*	30.6*	28.3	51.2*	15.5	53.1
	TrigoCor 1448	82.5	49.7	51.3	48.9	26.0	49.5
	OH182.9	81.3	52.3	33.8	43.7	24.0	53.0

* Indicates means significantly different ($P \# 0.05$) from untreated control based on Fisher's LSD.

EFFECT OF THREE *BACILLUS SP.* FROM WHEAT ON FHB REDUCTION

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INTRODUCTION

Fusarium head blight (FHB) of wheat caused by *Fusarium graminearum* Schwabe [Teleomorph = *Gibberella zeae* (Schwein.) Petch] is becoming one of the most devastating crop diseases in Canada (Gilbert and Tekauz, 2000). Many reasons contribute to this and the most important one is the rotations (Dill-Macky and Jones, 2000). In addition, no-till and minimum till practices also contribute to persistence of the pathogen, and disease spread (Fernando, 1999). In 2000, FHB damaged 8.5 percent of the total wheat crop in Manitoba and caused yield losses of about $40 million in total (Tekauz, 2001). At present, available and affordable traditional disease control options, such as resistant varieties, cultural practices (crop rotations, tillage to destroy residues) and foliar fungicides, are only partially effective (McMullen *et al*., 1997). Biological control is an environment-friendly alternative strategy in FHB management and shows considerable promise for reducing FHB (Khan *et al*. 2001). In a bio-ecological view, the understanding of the interactions between FHB and wheat phyllosphere microbes can be a requisite to finding effective antagonist(s) to the pathogen.

The objectives of this study are (a) to screen microbes from various plant parts of wheat and test their ability to inhibit the growth of the pathogen *in vitro*; (b) to investigate the interaction between bacterial isolates and the FHB pathogen in plant assays in the greenhouse.

MATERIALS AND METHODS

Microbes originated from the rhizosphere, leaves, leaf sheaths and heads of field wheat. The bacteria were isolated by serial dilution and single colonies were purified. Bacteria were identified using the MicroLog system (Biolog™ Inc., Hayward CA94545, USA). The ability of isolates to inhibit radial mycelial growth of *Fusarium graminearum* was assayed on PDA and NA plates and percent mycelial inhibition was calculated. Based on *in vitro* test results, three *Bacillus* strains were selected for greenhouse work. In greenhouse (25°C, 14 hrs photoperiod/day), potential FHB antagonistic bacterial strains were individually applied onto the seeds and heads of highly susceptible cultivar AC-Teal in order to investigate the microbial interaction between antagonists and the pathogen *in vivo*. For seed-coating treatment, germinated seeds were immersed into bacterial suspension (4.5×10^8 cfu/ml) for 30 minutes before seeding. When wheat was at 50% flowering, 5 µl of each bacterial suspension was applied onto heads by injecting directly onto the floret. The pathogen macroconidia (5×10^5/ml) was inoculated into the same spot either before or after bacterial inoculation. Head inoculation was undertaken as follows: one floret in the middle spike of head was injected with 2 µl of *Fusarium* macroconidia suspension (5×10^5 macroconidia/ml and 0.04% Tween 80). After inoculation, wheat plants were incubated in a mist chamber for 72

hours at 22°C and transferred to a greenhouse bench. There were six treatments (10 pots/replicate and 5 plants in each pot): (1) seed coating with bacteria and bacterial application on head 4 hrs prior to *Fusarium* inoculation (BST-BBI); (2) seed coating with bacteria and bacterial application on head 4 hrs post *Fusarium* inoculation (BST-BAI); (3) seed coating with bacteria and no bacterial application on head (BST) prior to *Fusarium* application; (4) bacterial application on head 4 hrs prior to fusarium inoculation on head and no seed coating of bacteria (BBI); (5) bacterial application on head 4 hrs post *Fusarium* inoculation and no seed coating of bacteria (BAI) and (6) no seed coating of bacteria and no bacterial application on head prior to *Fusarium* application (CK). The FHB incidence (the number of heads infected) and severity (the number of diseased spikes on each head) were estimated at 16 days after inoculation.

RESULTS AND DISCUSSION

Sixty-one bacterial and five fungal strains were isolated from various parts of the wheat plant. Forty-nine percent were from rhizosphere, thirty-seven percent from leaves, nine percent from leaf sheaths and five percent from heads. Only 7% of bacterial isolates inhibited the growth of *F. graminearum*. Only one phyllosphere fungus, strain L-07-12, inhibited the growth of the pathogen up to 74%. The inhibitory ability (*in vitro*) of three bacterial isolates, *Bacillus subtilis* strain H-08-02 from the head, *B. cereus* strain L-07-01 from the leaf and *B. mycoides* strain S-07-01 from the rhizosphere was 60%, 52% and 55%, respectively.

Microbial interactions *in vivo* (Table 1) showed that seed coating plus application of bacteria on head prior to fusarium inoculation (treatment #1) gave the best disease reduction results for all three bacteria, of which strain H-08-02 performed the best (49.1%). The treatments with *B. subtilis* strain H-08-02 significantly reduced disease severity (treatments 1-5). This means that it will be beneficial if we select the antagonists from wheat heads because the pathogen and beneficial microorganisms may have co-evolved on heads or the bacterium is capable of using the head as a niche. This is consistent with other studies on bacterial population dynamics. In addition; data suggests bacterial application should be done prior to fungal spore landing and subsequent infection for effective control of the FHB fungus on heads.

Why do we think biocontrol will work? The wheat plant is most susceptible at anthesis. As the window of infection that will lead to economic loss is quite narrow, an application of a biocontrol agent onto heads at or just prior to anthesis should work well. Our results suggests, the antagonist should be applied on heads (infection court) to abort, curtail or delay germination of spores (mainly ascospores), to achieve control. Though the window of infection in the barley plant is supposedly a little longer, if optimum conditions and timing of application are perfected, biocontrol should work. Therefore, our target is to develop a foliar bio-fungicide that will be effective as a chemical fungicide application in reducing the FHB incidence and severity on heads, and in turn reduce DON levels. A biological pesticide capable of reducing initial infection and disease progress should reduce the present economic impact caused by FHB.

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Table 1. Effect of three Bacillus strains on FHB infection in vivo

	B. mycoides **(S-07-01)**		***B. cereus*** **(L-07-01)**		***B. subtilis*** **(H-08-02)**	
	severity(%)	RB (%)*	severity(%)	RB (%)*	severity(%)	**RB (%)***
1	49.3 ^{c}	48.1 ^{a}	33.3 ^{b}	48.1 ^{a}	45.4 ^{c}	**49.1** ^{a}
2	90.1 ^{ab}	5.9 ^{bc}	40.6 ^{ab}	36.7 ^{ab}	58.9 ^{bc}	**34.0** ^{abc}
3	92.8 ^{a}	3.0 ^{c}	50.2 ^{ab}	21.8 ^{ab}	72.0 ^{b}	**19.3** ^{c}
4	63.0 ^{bc}	34.2 ^{ab}	35.5 ^{ab}	44.7 ^{ab}	55.6 ^{c}	**37.7** ^{ab}
5	83.6 ^{ab}	12.6 ^{bc}	59.9 ^{ab}	6.7 ^{ab}	64.3 ^{bc}	**27.9** ^{bc}
6	**95.7** ^{a}	**0.0** ^{c}	**64.2** ^{a}	**0.0** ^{b}	**89.2** ^{a}	**0.0** ^{d}

Note: * RB = relative control
1 — BST-BBI; 2 — BST-BAI; 3 — BST; 4 — BBI; 5 — BAI; 6 — CK.
The data with the same letter within a column are not significantly different based on Fisher's LSD test.

AN EXTENSION AGRONOMIST'S EXPERIENCES WITH FUNGICIDE APPLICATION TECHNIQUES TO IMPROVE CONTROL OF FHB

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ABSTRACT

Fusarium head blight has over the years caused billions of dollars in damage to small grain crops in the U.S. and Canada. Small grain acreage in Northeast North Dakota has declined 38% since 1992 including a 70% decline in durum and barley acres. Genetic resistance is a few years away and cultural control methods of rotation, residue management and choosing tolerant varieties have not prevented disease occurrence. Fungicide use has been shown to significantly reduce *Fusarium* infection when weather conditions are favorable to disease development. Fungicides have shown effectiveness in lab and greenhouse and field situations but *Fusarium* control is often inconsistent and disappointing for growers. Much research has been done since 1993 to improve the effectiveness of fungicides. New fungicides have been labeled for heading application and application techniques have been examined in detail. Application parameters studied have included the following variables for ground application of fungicides.

A. Application timing including split application
B. Spray application angle
C. Spray pressure: 30 psi to 90 psi in 10 psi increments.
D. Spray nozzles: Various nozzles studied. Generally smaller orifice nozzles have better coverage than nozzles providing coarse sprays.
E. Gallons of water per acre (gpa); 9 to 54 gpa.
F. Effects of dew
H. Adjuvants

Techniques learned from these studies and the labeling of more effective fungicides has led to recommendations that have improved fungicide effectiveness for growers. Fungicide use has increased as growers have experienced profitable results from fungicide application in hard red spring wheat. Fungicide effectiveness has been marginal in durum and barley. Achieving an economic reduction in Deoxynivalenol (DON) content with fungicides is a continuing problem as reductions in DON are generally small.

BARLEY CULTIVAR RESPONSE TO FUNGICIDE APPLICATION FOR THE CONTROL OF FUSARIUM HEAD BLIGHT AND LEAF DISEASE

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OBJECTIVES

To determine if efficacy of fungicides Headline and AMS21619 for the control of Fusarium head blight (FHB) and leaf disease is different among barley cultivars.

INTRODUCTION

Barley producers in traditional barley producing areas have been frustrated by the inconsistent performance of fungicides applied to barley. As a result barley acreage has shifted to different regions of the state where diseases have not been as prevalent. However, diseases are developing in these regions and disease levels increasing when environmental conditions are appropriate.

Barley is particularly difficult to research because obtaining adequate disease levels for effective fungicide evaluation has been inconsistent. The extended period of heading among barley main stem and tillers, lack of yield loss due to FHB, and an inability to distinguish losses between leaf diseases and FHB, and near zero tolerance for the presence of the toxin deoxynivalenol (DON) by the malting industry complicate prioritization of research goals.

Fungicides are often evaluated on a specifically selected cultivar. Often the first priority of the cultivar selection is susceptibility to diseases. Little data is available to show that fungicide performance on a particular cultivar will be similar on all cultivars.

MATERIALS AND METHODS

Five cultivars, Conlon, Drummond, Lacey, Legacy, and Robust were selected for evaluation in a field at the Langdon Research Extension Center in spring 2002. Seven rows spaced 6-inches apart were planted with a double-disk Hege drill in plots 16 ft. long in a RCB design arranged as a factorial with four replicates. Border plots of Robust barley were planted between treatment plots to minimize drift potential to adjacent plots. Nutrients were added to attain a yield goal of 120 bu./acre and recommended production practices were followed. Three weeks prior to heading a *Fusarium* spawn grown on spring wheat was hand broadcast at a rate of approximately 200 grams/plot.

Fungicides and fungicide combination treatments included:

1. AMS 21619 5.7 oz/acre (triazole) and Induce 0.125 % v/v (adjuvant).

2. Quadris (azoxystrobin) 12.3 oz/acre + AMS21619 5.7 oz/acre and Induce 0.125 % v/v.

3. AMS 21619 5.7 oz/acre and Induce 0.125 % v/v + AMS 21619 5.7 oz/acre and Induce 0.125 % v/v.

4. Untreated check.

5. Caramba (metconazole) 13.5 oz./acre and Induce 0.125% v/v.

6. Caramba 13.5 oz./acre and Induce 0.125% v/v + AMS 21619 5.7 oz/acre and Induce 0.125 % v/v.

7. Quadris 12.3 oz/acre.

Treatments were applied by CO_2 backpack sprayer at 18 gpa with hydraulic nozzles XR8002 oriented downward from horizontal at Zadoks growth stage 40 and XR8001 nozzles mounted on a double swivel angled 30 degrees downward and oriented forward and backward to improve coverage of the target at Zadoks 59. Visual estimation of flag leaf necrosis, three samples per plot, and FHB incidence and field severity, 20 samples per plot, (spikelet count per individual head multiplied times FHB infected spikes per head) were determined. Each plot was harvested with a Hege plot combine and the grain sample cleaned and processed for yield, plump, and test weight measurement. A sample was ground for DON analysis at NDSU. Data was analyzed with SAS GLM.

RESULTS AND DISCUSSION

Most of the disease present was Septoria speckled leaf blotch, *Septoria passerinni* Sacc. and *Stagonospora avenae* F. sp. tritica T. Johnson, on the six-row cultivars, Drummond, Lacey, Legacy, and Robust. Spot blotch, *Cochliobolus sativus* (Ito & Kirivayashi), was the most common disease on two-row Conlon. Leaf disease levels on all cultivars were small and probably did not contribute significantly to yield differences. Lacey had greater levels of leaf disease than Conlon, Drummond, and Robust (Table 2). Caramba applied alone and two applications of AMS21619 reduced flag leaf necrosis to levels smaller than the check. Although there were differences among cultivars and fungicides in % plump, all levels were excellent.

When yield was compared cultivars responded very differently to fungicide combinations (Table 1 and Figure 1). Drummond had no significant differences among treatments. However, Quadris was the only treatment significantly different than the untreated. Cultivars Lacey and Legacy had a variable response to fungicide. The AMS21619 combination significantly improved yield over the untreated while other fungicide treatments did not. Yield of Robust was improved above the untreated by Quadris and the Quadris-AMS21619

combination treatments. Conlon had the greatest yields and responded well to fungicide treatments. Caramba alone and all other fungicide combinations increased yield over the untreated in Conlon.

Cultivars had significantly different levels of FHB incidence and severity in untreated plots but there were no differences among fungicide treatments (Table 2). Lacey had both the greatest levels of FHB incidence and severity among cultivars. Drummond had the smallest FHB field severity levels.

Conlon had significantly smaller DON levels than other cultivars (Table 2). Legacy had smaller DON levels than Drummond, Lacey, and Robust. Drummond had the greatest DON levels at 27.1 ppm. Caramba applied at Zadoks 59 had DON level of 23.8 ppm, significantly higher than fungicide combinations that included the AMS21619 fungicide applied at Zadoks 59. Fungicides applied at Zadoks 40 with AMS21619 at Zadoks 59 tended to reduce DON levels compared to similar fungicides applied alone. Reduction in DON due to fungicide application was small (less than 20%) and the reductions would not produce acceptable malting quality.

SUMMARY

In this trial, cultivars without fungicide treatment had significant differences in leaf disease and FHB resulting in differing yields, test weights, and % plump. DON levels were different among cultivars without treatment. Fungicide treatments performed similarly among varieties for all measured factors except yield. More years of research will be needed to confirm yield and fungicide response trends among varieties.

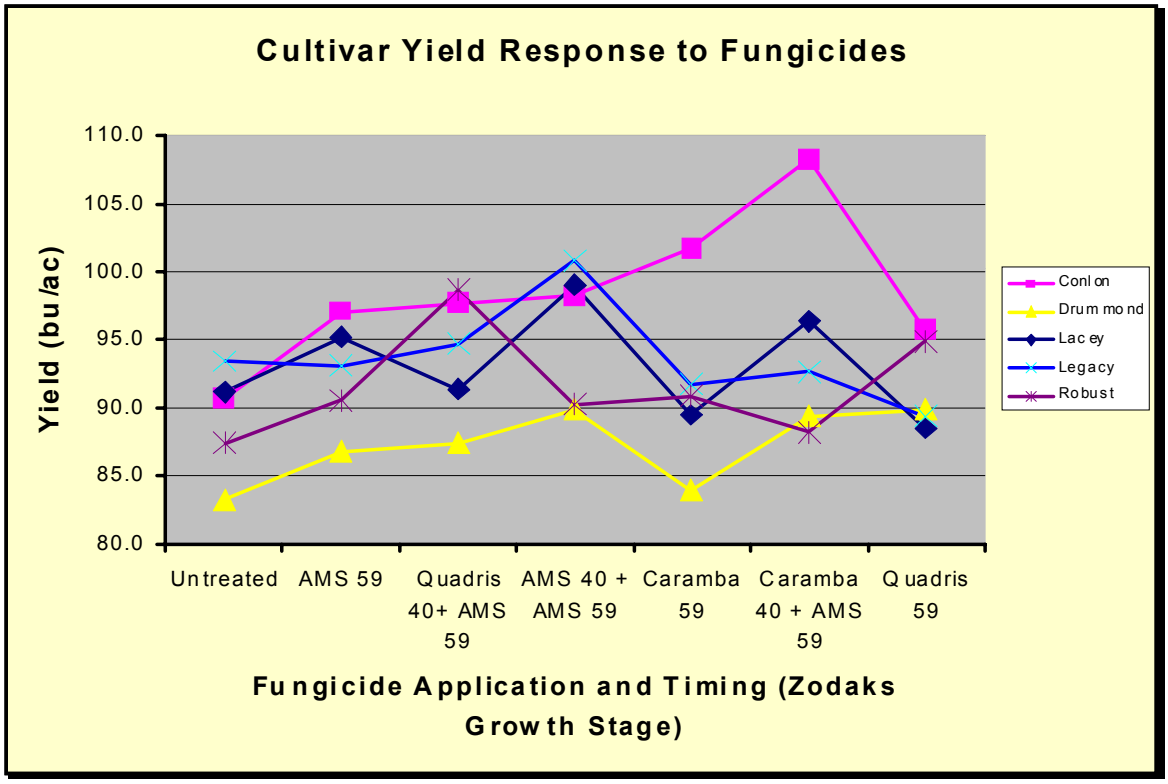

Figure 1. Cultivar yield by fungicide treatment (2002).

Table 1. Disease and quality parameter responses to fungicide treatments by cultivar (2002).

Cultivar	Fungicide	Flag Leaf	FHB		Yield	Test Wt.	Plump	DON*
		Necrosis	Incidence	Field Severity				
		%	%	%	bu/acre	lb/bu	%	Ppm
Conlon	1	1.8	51	7.5	97.1	50.5	96.2	8.7
	2	1.5	55	6.4	97.7	50.4	95.0	8.0
	3	2.3	50	7.5	98.3	50.4	95.2	9.2
	4	10.0	58	8.2	90.7	50.0	94.4	10.2
	5	4.3	48	6.6	101.7	49.5	95.5	9.6
	6	1.3	56	5.9	108.2	50.1	95.2	9.8
	7	4.3	51	8.1	95.7	49.9	94.4	8.6
Drummond	1	1.5	54	4.5	86.8	44.0	82.7	27.3
	2	9.0	55	4.8	87.4	45.0	83.0	29.3
	3	1.3	61	5.6	89.9	45.0	85.7	22.8
	4	2.3	63	7.6	83.2	44.2	76.9	30.8
	5	1.8	54	4.3	83.9	44.3	83.6	32.1
	6	1.8	50	3.2	89.4	44.4	80.2	23.8
	7	1.5	41	3.1	89.9	45.0	81.1	23.7
Lacey	1	3.0	91	18.0	95.2	46.4	88.6	23.5
	2	14.3	90	21.2	91.4	46.2	88.0	20.3
	3	3.0	75	13.8	99.0	47.0	91.2	16.9
	4	11.3	74	20.8	91.1	46.6	83.4	25.2
	5	5.8	93	22.8	89.5	45.8	86.2	35.1
	6	7.5	83	14.8	96.4	46.7	85.4	19.0
	7	7.0	89	21.5	88.5	46.9	87.5	22.2
Legacy	1	5.0	48	4.7	93.1	44.7	87.8	17.4
	2	3.8	71	9.1	94.7	44.7	89.6	12.7
	3	2.5	53	5.2	100.7	44.3	90.5	13.5
	4	9.1	71	14.5	94.9	44.6	89.6	19.6
	5	1.3	69	8.6	91.7	44.4	86.9	21.8
	6	3.0	66	9.8	92.7	44.8	86.2	11.9
	7	12.3	81	16.6	89.4	44.5	84.1	24.1
Robust	1	10.5	59	6.3	90.5	45.9	88.5	20.6
	2	5.5	65	6.9	98.6	47.0	86.4	15.4
	3	2.0	70	9.2	90.2	47.0	89.0	25.2
	4	3.0	60	6.1	87.4	46.5	85.8	18.1
	5	1.5	56	11.0	90.8	46.8	85.4	20.5
	6	2.8	65	10.8	88.2	46.7	87.7	21.8
	7	2.8	70	10.0	94.9	46.0	86.3	25.3
Cult*Trt		NS	NS	NS	6.6**	NS	NS	NS
CV %		120	21	47	6	2	4	32

Treatment 1 AMS 21619 applied at Zadoks 59 growth stage
Treatment 2 Quadris applied at Zadoks 40 + AMS 21619 at Zadoks 59 growth stage
Treatment 3 AMS 21619 applied at Zadoks 40 + Zadoks 59 growth stage
Treatment 4 Untreated
Treatment 5 Caramba applied at Zadoks 59 growth stage
Treatment 6 Caramba applied at Zadoks 40 + AMS 21619 at Zadoks 59 growth stage
Treatment 7 Quadris applied at Zadoks 59 growth stage
* Tacke, B.K. and Casper, H.H. Determination of Deoxynivalenol in Wheat, Barley, and Malt by Column Cleanup and Gas Chromatography with Electron Capture Detection: Journal of AOAC International Vol. 79, No. 2. 1996 (p.472-8309)
** Significant at 0.05 probability level for mean comparisons.

Table 2. Disease and quality parameter responses by cultivar and fungicide treatment across cultivars (2002).

Cultivar	Fungicide	Flag Leaf Necrosis	FHB		Yield	Test Wt.	Plump	DON*
			Incidence	Field Severity				
		%	%	%	bu./acre	lb./bu.	%	Ppm
Conlon		3.6	52.7	7.2	98.5	50.1	81.9	9.2
Drummond		2.7	53.9	4.7	87.2	44.5	95.1	27.1
Lacey		7.4	84.8	19.0	93.0	46.5	87.2	23.2
Legacy		5.3	65.6	9.8	93.9	44.6	87.8	17.3
Robust		4.0	63.6	8.6	91.5	46.6	87.0	21.0
	1	4.4	60.5	8.2	92.5	46.3	88.8	19.5
	2	6.8	67.3	9.7	94.0	46.7	88.4	17.1
	3	2.2	61.8	8.3	95.6	46.8	90.3	17.5
	4	7.1	65.0	11.5	89.5	46.4	86.0	20.8
	5	2.9	63.8	10.7	91.5	46.2	87.5	23.8
	6	3.3	64.0	8.9	95.0	46.5	86.9	17.3
	7	5.6	66.5	11.8	91.7	46.4	86.6	20.8
CultLSD **		3.2	6.6	2.2	2.8	0.4	1.6	3.8
TrtLSD **		4.0	NS	NS	4.0	NS	2.2	4.5 ***
CV %		120	21	47	6	2	4	32

Treatment 1 AM S21619 applied at Zadoks 59 growth stage
Treatment 2 Quadris applied at Zadoks 40 + AM S21619 at Zadoks 59 growth stage
Treatment 3 AM S21619 applied at Zadoks 40 + Zadoks 59 growth stage
Treatment 4 Untreated
Treatment 5 Caramba applied at Zadoks 59 growth stage
Treatment 6 Caramba applied at Zadoks 40 + AM S21619 at Zadoks 59 growth stage
Treatment 7 Quadris applied at Zadoks 59 growth stage
* Tacke, B.K. and Casper, H.H. Determination of Deoxynivalenol in Wheat, Barley, and Malt by Column Cleanup and Gas Chromatography with Electron Capture Detection: Journal of AOAC International Vol. 79, No.2. 1996 (p.472-8309)
** Significant at 0.01 probability level for mean comparisons.
*** Significant at 0.05 probability level for mean comparisons.

ANALYSIS OF THE 2002 UNIFORM WHEAT FUNGICIDE AND BIOCONTROL TRIALS ACROSS LOCATIONS

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OBJECTIVES

Evaluate a common set of foliar fungicide and biological control agent (BCA) treatments, across a wide range of environments, for effectiveness in managing Fusarium head blight (FHB) and associated yield and seed quality parameters.

INTRODUCTION

Identifying fungicides and BCA's that significantly reduce the incidence and severity of FHB in the field, and mycotoxins in the grain, would have widespread benefits to growers and end-users of all market classes of wheat. The Uniform FHB Fungicide and BCA Test was established as a means of rapidly identifying fungicide and/or BCA treatments that are effective, economical and environmentally safe to use in FHB management programs across the United States.

MATERIALS AND METHODS

Plant pathologists from 12 states (Table 1) conducted 22 trials across a range of wheat classes, including durum, hard red spring, soft red winter, and soft white winter wheat. Each trial evaluated eight uniform treatments (Table 2), including two advanced BCA's, OH 182.9 Yeast [USDA/ARS] and TrigoCor 1448 bacterium [Cornell University]; three foliar fungicides (AMS 21619A [Bayer], BAS 505H [BASF], and Folicur [Bayer]); and a non-treated control.

All treatments were applied at early flowering stage using a CO_2 pressurized sprayer equipped with twinjet XR8001 nozzles, mounted at a 60-degree angle forward and backward. Details such as plot size, crop husbandry, spray volume and pressure, sprayer type, and number of treatment replications varied from location to location. Consult individual state trial reports for specific details.

Data from individual trials were grouped and statistically analyzed with other winter wheat or spring wheat trials, respectively. This was done in order to detect any treatment differences that may be linked to production of winter vs. spring wheat, respectively. Treatment means from each location served as treatment replications. Data summary tables include treatment means, in actual units measured, as well as means of treatment rankings from within individual tests. Treatment rankings are provided as an alternative approach to treatment comparison.

RESULTS AND DISCUSSION

Winter wheat trials– Of 13 winter wheat trials conducted, data are presented for 12 trials (Tables 3-5); circumstances precluded the collection of disease data at one location in VA. Table 3 summarizes all FHB data, including a ranked treatment means. Table 4 presents all yield and seed quality parameters. Table 5 summarizes analyzed disease, yield and seed quality means from trials that had moderate to severe FHB. All three tables indicate that treatments involving AMS 21619A were generally superior to other treatments when compared with the check. Most fungicide treatments reduced disease levels compared to the check plots, but only the treatments involving AMS 21619A translated into statistically significant yield results in the absence of foliar disease (Tables 4 and 5). Treatments involving Folicur or BAS 505H, although not always as effective as treatments involving AMS 21619A, were often superior to the BCA treatments. Neither of the BCA's tested, when applied alone, were statistically different than the check for any parameter except for a more favorable plot severity ranking (Table 3). Test weights were statistically similar among all treatments (Table 4). Percent VSK was significantly lower than the check only when AMS 21619A was applied (Table 4, 5). Similarly, DON levels tended to be lowest in treatments involving AMS 21619A, but differences among treatments were not always significantly (Tables 4, 5).

Spring wheat trials – Of nine spring wheat trials conducted, four had extremely low levels of FHB and/or no FHB ratings were collected. These four tests were excluded from this summary. The results of the remaining five trials (all from North Dakota, and with moderate to severe FHB levels) are summarized in Tables 6 and 7. When actual data are considered (Table 6), all solo fungicide treatments provided similar levels of FHB control. In contrast, no treatment resulted in significantly higher yields compared to the check. Similar results were seen with treatment mean rankings (Table 7) except that crop yields associated with the above fungicide treatments ranked significantly higher than the check plots. This may be an artifact of foliar disease management in those tests, rather than any specific activity against FHB. Test weights tended to be significantly improved when fungicides were applied. There was insufficient VSK or DON data collected to make any general comments in regard to treatment effectiveness. Consult individual state trial reports for further details on VSK and DON data that was collected.

Summary - In winter wheat trials, treatments involving AMS 21619A were generally superior to the other treatments tested. Neither BCA tested provided control of FHB when compared with the check. Treatments involving Folicur or BAS 505H tended to provide an intermediate level of FHB control. In spring wheat trials, all fungicide treatments performed more or less similarly. Better results in spring wheat trials for Folicur and BAS 505H may be related to differences in demands placed on treatments between winter and spring wheat. BCA's tested in spring wheat trials were ineffective in managing FHB. Overall, seed quality parameters associated with FHB from both winter and spring trials were less impacted by foliar fungicides than were FHB symptoms expression. DON levels were reduced by fungicide treatments in winter wheat trials, but levels were often unacceptably high where moderate to severe FHB existed.

Table 1. States, principal investigator (PI), institution, number of uniform trials conducted, and wheat class evaluated.

State	PI	Institution	No. trials	Wheat Class
AR	Gene Milus	University of Arkansas	1	SRWW *
IL	Wayne Pederson	University of Illinois	1	SRWW
IN	Greg Shaner	Purdue University	2	SRWW
KY	Don Hershman	University of Kentucky	1	SRWW
MD	Arv Grybauskas	University of Maryland	1	SRWW
MI	Pat Hart	Michigan State University	1	SRWW
MO	Laura Sweets	University of Missouri	2	SRWW
ND	Marcia McMullen	North Dakota State University	6	HRSW
NY	Gary Bergstrom	Cornell University	1	SWWW
OH	Pat Lipps	Ohio State University	1	SRWW
SD	Marty Draper	South Dakota State University	3	Durum, HRSW
VA	Erik Stromberg	VPI and SU	2	SRRW

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

Table 2. Treatment, rate, and adjuvant used in the uniform trials in 2002.

#	Treatment	Rate of Product/A	Adjuvant
1	OH 182……	varied among locations	
2	Folicur 3.6F…….	4 fl oz	0.125% Induce
3	AMS 21619A 480SC……..	5.7 fl oz	0.125% Induce
4	AMS 21619A 480 SC …… + Folicur 3.6F	3.6 fl oz + 4 fl oz	0.125% Induce
5	BAS 505F 50WG ……	6.4 fl oz	0.125% Induce
6	TrigoCor 1448…….	varied among locations	
7	TrigoCor 1448……. + Folicur 3.6F	varied + 4 fl oz	0.125% Induce
8	Non-treated check		

Table 3. Treatment and rank means for FHB incidence, head severity, and plot severity from winter wheat trials[a].

Treatment	Incidence (%)	Incidence Rank	Head severity (%)	Head severity Rank	Plot severity (%)	Plot severity Rank
OH 182.9……..	26.4ab[b]	5.5ab	25.5ab	4.6ab	13.2ab	4.2b
Folicur……..	22.5ab	3.5cd	24.1ab	3.7bc	9.2bc	3.6b
AMS 21619A…….	18.4b	1.8e	19.8b	3.1bc	6.8c	1.9c
AMS 21619A + Folicur…	18.5b	2.5de	18.8b	2.1c	7.6c	2.1c
BAS 505……	21.5ab	2.6de	24.1ab	4.0bc	9.6bc	3.1bc
TrigoCor 1448……...	28.9ab	4.8a-c	25.4ab	4.6ab	13.9ab	4.4b
TrigoCor 1448 +Folicur…	25.5ab	4.2b-c	22.4ab	4.3a-c	10.2bc	4.1b
Non-treated……	30.4a	6.2a	29.7a	6.4a	16.4a	6.1a

[a]AR, IL, IN (2 trials), KY, MD, MI, MO (2 trials), NY, OH, VA.
[b]Means within a column followed by a common letter are not significantly different P=0.05, Student-Newman-Keuls; arcsine-transformed percentage data were used in statistical analyses.

Table 4. Treatment and rank means for yield, test weight, visually scabby kernels (VSK), and DON from winter wheat trials.

Treatment	Yield[a] (bu/A)	Yield Rank	Test Weight[b] (lbs/bu)	Test Weight Rank	VSK[c] (%)	VSK Rank	DON[d] (ppm)	DON Rank
OH 182.9…….	59.2ns[e]	5.9a	54.8ns	5.8ns	35.0a	5.2a	11.0ns	5.7a
Folicur……..	62.2	4.2ab	55.6	3.7	30.3ab	4.2ab	8.3	2.7cd
AMS 21619A……...	75.4	2.0c	56.2	2.4	24.0b	2.2c	4.5	1.3d
AMS 21619A + Folicur...	63.1	3.4bc	56.2	3.7	24.3b	2.8c	5.0	2.3d
BAS 505……...	60.9	4.9ab	55.6	3.8	28.6ab	3.6a-c	7.6	2.8cd
TrigoCor 1448……..	61.9	4.4ab	54.8	4.2	34.1a	4.6ab	11.3	6.0a
TrigoCor 1448 + Folicur..	62.5	3.8a-c	55.5	3.7	32.3a	5.4a	9.2	4.0bc
Non-treated……..	58.2	5.9a	54.8	4.9	32.8a	4.8ab	10.2	4.3b

[a]data from AR, IL, IN, KY, MD, MO(2 trials), NY, OH, VA.
[b]data from AR, IN (2 trials), KY, MD, MO (2 trials), NY, OH, VA.
[c]data from AR, KY, MO(2 trials), NY, OH, VA.
[d]data from IN (2 trials), KY, MD, MO (2 trials), NY, OH, VA.
[e]Means within a column followed by a common letter are not significantly different P=0.05, Student-Newman-Keuls; ns = not significant; VSK percentages were arcsine-transformed for statistical analysis.

Table 5. Treatment means for FHB incidence, head severity, plot severity, yield, visually scabby kernels (VSK), and DON from winter wheat trials in AR, IL, KY, MI, NY, and OH that had moderate to severe levels of FHB and little interference from other diseases.

Treatment	Incidence (%)	Head severity (%)	Plot severity (%)	Yield[a] (bu/A)	VSK (%)	DON[b] (ppm)
OH 182.7…….	50.4a[c]	43.0ab	28.3ab	54.3c	38.3a	19.8a
Folicur……	42.4ab	37.0b	27.5ab	61.0a-c	31.8ab	14.9ab
AMS 21619A…….….	33.6bc	32.7b	14.0c	66.0a	24.5b	7.8b
AMS 21619A + Folicur…	28.6c	31.8b	15.8bc	62.7ab	24.3b	8.5b
BAS 505……..….	40.0ab	39.0b	20.3a-c	60.0a-c	30.5ab	13.7ab
TrigoCor 1448……..	49.6a	44.2ab	29.3ab	56.0bc	37.8a	20.4a
TrigoCor 1448 + Folicur..	41.2ab	37.6b	21.0a-c	61.7a-c	34.0ab	16.7ab
Non-Treated……....	51.2a	50.8a	33.3a	54.3c	37.6a	19.7a

[a]No yield data for MI.
[b]No DON data from AR, IL, and MI.
[c]Means within a column followed by a common letter are not significantly different P=0.05, Student-Newman-Keuls; arcsine-transformed percentage data were used in statistical analyses.

Table 6. Treatment means for FHB incidence, head severity, plot severity, yield and test weight from five of nine spring wheat trials that had moderate to severe levels of FHB and foliar diseases.

Treatment	Incidence (%)	Head severity (%)	Plot severity (%)	Yield (bu/A)	Test weight (lbs/bu)
OH 182.7……..….	71.0ab[a]	34.5ns	26.5a	40.8ns	56.8bc
Folicur……..….	66.2a-c	22.8	16.8b	47.0	58.0ab
AMS 21619A……...….	57.2bc	22.8	16.3b	38.0	58.3ab
AMS 21619A + Folicur...	57.6bc	23.0	17.0b	50.3	58.3ab
BAS 505……..….	51.6c	22.5	15.0b	51.3	59.0a
TrigoCor 1448…….…….	76.8a	32.8	25.8a	41.5	57.0bc
TrigoCor 1448 + Folicur...	71.0ab	32.0	23.5ab	47.8	57.8a-c
Non-treated……....	80.8a	34.8	29.3a	39.8	56.3c

[a]Means within a column followed by a common letter are not significantly different P=0.05, Student-Newman-Keuls; arcsine-transformed percentage data were used in statistical analyses; arcsine-transformed percentage data were used in statistical analyses; ns = not significant.

Table 7. Average rankings for FHB incidence, head severity, plot severity, yield, and test weight from five of nine spring wheat trials that had moderate to severe levels of FHB and foliar diseases.

Treatment	Incidence	Head severity	Plot severity	Yield	Test weight
OH 182.7…….....…..	4.2ab[a]	4.7ns	4.6ab	4.5a	2.8ab
Folicur……...…...	2.8b	2.4	2.2c	3.5ab	2.0a-c
AMS 21619A……....	2.4b	3.3	2.8bc	1.5b	1.8bc
AMS 21619A + Folicur...	2.6b	2.3	3.4bc	2.0b	1.5bc
BAS 505…….……..	2.2b	3.4	2.2c	1.8b	1.3c
TrigoCor 1448……...…	4.8ab	3.9	4.4ab	5.0a	2.8ab
TrigoCor 1448 + Folicur..	3.8ab	3.4	3.6bc	2.5b	2.3a-c
Non-treated……...	5.8a	5.0	5.8a	5.0a	3.3a

[a]Means within a column followed by a common letter are not significantly different P=0.05, Student-Newman-Keuls; ns = not significant.

MANAGEMENT OF FUSARIUM HEAD BLIGHT IN WHEAT USING SELECTED BIOLOGICAL CONTROL AGENTS AND FOLIAR FUNGICIDES, 2002

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OBJECTIVES

Evaluate selected foliar fungicides and biological control agents (BCA) for potential use in soft red winter wheat Fusarium head blight (FHB) management programs in Kentucky. Also, to generate data as a cooperator in the 2002 National Fusarium Head Blight Uniform Fungicide and Biocontrol Trial.

INTRODUCTION

FHB is a significant disease concern in all wheat and barley producing regions of the United States. FHB epidemics are rare in Kentucky, but each year some fields are severely damaged by the disease. Currently, the only options available for the management of FHB are the use of cultural practices that encourage escape from disease. These include the use of multiple planting dates and varieties representing different flowering dates and periods. Moderate resistance is also available in several different wheat varieties, but severe FHB will occur under conditions that favor FHB. Preliminary studies conducted in various states indicate that foliar fungicides (Milus, Hershman, and McMullen, 2001) and BCA's may be capable of providing safe, effective and economical management of FHB. Nonetheless, specific and consistent data are lacking in regards to which products and rates are most suitable for use in FHB management programs. The National FHB Uniform Fungicide and Biocontrol Test was established as a means of addressing this deficiency in data. This test involves cooperators at various locations across the county, the use of a standard set of promising treatments, and a reasonably standardized testing protocol. Each state, including the one in Kentucky during 2002, also evaluates unique treatments of local interest.

MATERIALS AND METHODS

The test site was established at the University of Kentucky Research and Education Center in Princeton, KY. The core set of treatments evaluated was determined by collective agreement of the scientists involved in the National FHB Uniform Fungicide and Biocontrol Test. Treatments included a variety of foliar fungicides and two BCA's. An additional fungicide treatment of local interest was also included at the Kentucky trial location. The test site was planted in a conventionally-tilled seed bed on October 22, 2001. Plots were maintained according to standard crop husbandry practices for soft red winter wheat production in west Kentucky (Bitzer and Herbek, 1997). The wheat variety planted was 'Patton'. This variety expresses FHB "Type 2" resistance, which is resistance to spread of FHB within a spike. Maize was the previous crop grown in the test site.

Plots were inoculated on April 1, 2002 with sterilized, cracked corn infested with a mixture of several highly pathogenic isolates of *Fusarium graminearum*, the primary causal agent of FHB. Test plots were mist-irrigated according to a strict regime in order to encourage the causal fungus to produce infectious spores and infect the test plots. Between inoculation and the onset of flowering, plots were mist-irrigated for two hours daily, between 7 pm and 9 pm. Following the onset of flowering, plots were mist-irrigated eight times each day for 15 minutes each misting cycle. Fungicides were applied to plots on April 30, 2002 when the crop was in the early flowering. Treatments were applied using a CO^2-propelled hand-held sprayer delivering at 40 PSI in 18-20 GPA. The spray boom was equipped with twinjet XR8001 nozzles oriented at a 60-degree angle forward and backward. FHB incidence, severity, and field severity data were obtained by collecting, and visually rating, 100 heads from each test plot. Plots were harvested with a small plot combine and grain yield and test weight where calculated. Deoxynivalenol (DON) levels were determined for 50-gram grain subsamples collected from each test plot. DON analyses were conducted at the Michigan State University Don Testing Laboratory. Tests to ascertain percent seed infected by *Fusarium* spp., as determined by plating seed, were conducted at Dr. TeKrony's Seed Technology Laboratory in Lexington, KY. Percent visually scabby kernel (VSK) percentages were determined by segregating healthy from scabby kernels for two sets of 100-seed samples for each treatment replication.

RESULTS AND DISCUSSION

Test conditions were favorable for FHB. Plot yields and test weights were significantly reduced by excess soil moisture. The two treatments involving AMS 21619A and TrigoCor 1448 when applied alone, significantly reduced FHB incidence compared the check. The same treatments, plus TrigoCor 1448 + Folicur also significantly reduced FHB plot severity. No treatment significantly reduced FHB head severity compared with the check. Only TrigoCor 1448 applied alone, resulted in significantly higher yield compared with the check. No treatment significantly impacted crop test weight, % visually scabby kernels (VSK), *Fusarium* spp. colonization of grain or DON levels. There were no foliar diseases of consequence in this trial.

REFERENCES

Bitzer, M. and Herbek, J. 1997. A comprehensive guide to wheat management in Kentucky. University of Kentucky Extension Service Publication ID-125, University of Kentucky Press.

Milus, E., Hershman, D., and McMullen, M. 2001. Analysis of the 2001 uniform wheat fungicide trials across locations. Pages 75-79 in Proceedings of the 2001 Fusarium Head Blight Forum, Erlanger, KY, University Press, Michigan State University.

Table 1. Effect of foliar fungicides and biological control agents on FHB, yield, seed quality in Kentucky, 2002.

	FHB Ratings[@]							
Treatment and rate	Plot Inc (%)	Head Sev (%)	Plot Sev (%)	Yield (bu/A)	Test wt (lbs/bu)	VSK* (%)	FC** (%)	DON+ (ppm)
OH 182.7 variable……	29.8 ab[#]	10.3ns	3.0ab	41.5ab	50.6ns	28.6ns	66.0ns	1.8b
Folicur 4.0 fl oz Induce 0.125% v/v……	27.0ab	10.8	3.0ab	45.3ab	50.2	26.3	76.7	1.8b
AMS 21619A 5.7 fl oz + Induce 0.125% v/v……	17.5b	8.5	1.5b	51.2ab	49.9	18.8	63.3	1.9b
AMS 21619A 3.6 fl oz + Folicur 4 fl oz + Induce 0.125% v/v……	19.0b	7.8	1.5b	45.9ab	52.2	16.4	60.0	2.1ab
BAS 505H 6.4 fl oz + Induce 0.125% v/v……	26.5ab	11.8	2.8ab	44.5ab	50.7	24.0	72.7	2.0ab
TrigoCor 1448 variable….	19.8b	9.0	1.5b	55.2a	51.1	19.0	84.0	2.2ab
TrigoCor 1448 variable + Folicur 4 fl oz + Induce 0.125% v/v……	22.8ab	8.8	1.5b	47.5ab	51.5	20.8	76.7	2.0ab
CGA 64250 13.7 fl oz + Induce 0.125% v/v……	33.3a	11.0	3.5a	40.5b	50.3	16.9	82.7	3.2a
Non-Treated……	32.0a	11.5	3.8a	42.8b	51.1	25.9	76.6	2.1ab

@: Inc = FHB incidence in plots; Sev = Average severity of FHB for diseased spikes; Plot sev = Average FHB severity across plot.
* = Visually "scabby" kernels.
** = Seed colonized by *Fusarium* spp.
+ = Vomitoxin
#Means followed by a common letter are not significantly different P=0.05, Student- Newman-Keuls; ns=no significant differences.

MULTIPLE INFECTION EVENTS AND SPLIT TIMING OF FOLICUR FUNGICIDE APPLICATIONS FOR CONTROL OF FHB IN HARD RED SPRING WHEAT, DURUM WHEAT, AND SPRING BARLEY, 2002

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ABSTRACT

Studies at North Dakota State University and at other research locations have indicated that wheat is most vulnerable to infection by the Fusarium head blight fungus (*Fusarium graminearum)* during anthesis, while spring barley cultivars are most susceptible to infection after the grain head fully emerges from the leaf sheath. However, if environmental conditions are very suitable for disease development over a long time span, multiple infections may occur up to soft or mid-dough stage, and not just at the most susceptible stage of the crop. Despite the possibility of multiple infection events, fungicide applications to durum and barley to control this disease have generally been applied once and targeted to the single-most critical infection periods. Cost of spray applications and time restraints of producers often prohibit multiple applications. Information was needed on the effect of multiple infection events on the level of FHB and on the effect of multiple applications of split rate fungicides in controlling multiple infection events.

A study was established in a controlled greenhouse environment in which spring wheat, durum wheat, and spring barley were exposed to multiple infection events and treated with either a single full rate (4 fl oz) or multiple, reduced rate applications of Folicur (tebuconazole) fungicide. Inoculations and/or fungicide applications were applied at single or multiple growth stages: Feekes growth stage 10.3 (head half emerged); Feekes 10.5 (head fully emerged but not flowering); Feekes 10.51 (early flowering in wheat); Feekes 10.54 (kernel watery ripe). Ten ml of a dilution of *Fusarium graminearum* spores (10,000 spores/ml) were atomized onto grain heads at the appropriate growth stage. For fungicide treatments, Folicur was applied approximately four hours before inoculation, using a track sprayer equipped with XR8001 flat fan nozzles oriented forward and backward at 60^0 from the vertical. FHB incidence, head severity and field severity were determined at the soft dough stage of kernel development.

Multiple infection events resulted in higher FHB field severities than did a single inoculation event at the most susceptible growth stage. However, split applications of reduced rates of Folicur across multiple growth stages generally did not significantly improve disease control over a single treatment of the full label rate at the most susceptible growth stage. For all three crops, the least satisfactory control of FHB among fungicide treatments tested was when a single application of the full label rate of Folicur was applied late, at Feekes 10.54 (kernel watery ripe).

EVALUATION OF FOLIAR FUNGICIDES AND BIOPROTECTANTS FOR CONTROL OF FUSARIUM HEAD BLIGHT OF WINTER WHEAT IN NEW YORK IN 2002

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OBJECTIVES

To quantify the ability of promising fungicides and bioprotectants, applied to flowering wheat spikes, to control Fusarium head blight (FHB) and to reduce deoxynivalenol (DON) contamination of the harvested grain.

To assess the efficacy of the bioprotectant TrigoCor 1448 to act synergistically with foliar fungicides in control of FHB and reduction of DON contamination.

INTRODUCTION

Efforts are being made through the USWBSI to provide safe, affordable and efficacious fungicides and biological protectants for the integrated management of FHB of wheat and barley. This study provided a New York site for the Uniform Fungicide and Biocontrol Tests in 2002. In addition to uniform core treatments, we assessed additional biocontrol agents at two locations. The reduction of DON contamination of the harvested grain to acceptable levels remains of critical importance in the management of this disease. We were especially interested in assessing the ability of *Bacillus subtilis* isolate, TrigoCor 1448, to enhance the reduction of DON when applied to flowering wheat spikes in mixture with fungicides, based on initially promising results with the combination of TrigoCor 1448 with Folicur 3.6F (Stockwell *et al*., 2001).

MATERIALS AND METHODS

Uniform Fungicide/Bioprotectant Field Trial – Musgrave Farm, Aurora, NY

Twelve treatments were included in the uniform fungicide/bioprotectant trial conducted at Aurora, NY. Treatments were replicated four times and arranged in a randomized block design. In addition to AMS 21619A, AMS 21619A plus Folicur, Folicur, BAS 500, TrigoCor 1448 and the USDA/Peoria Yeast which were included as core treatments tested at all locations, this trial included the commercial *Bacillus subtilis* bioprotectant product, Serenade (AgraQuest; Davis, CA) and an experimental, endophytic *Streptomyces* EN27 (courtesy Justin Coombs, Cornell University). Commercial products were applied at labeled rates. In this same trial, TrigoCor 1448 was combined in treatments with AMS 21619, BAS 500, or Folicur to determine if the combination would give enhanced FHB control over either bioprotectant or fungicide alone. TrigoCor 1448 was grown for 5 days in nutrient broth with

yeast extract, NBYE, (2-4 X 10^8 cfu/ml) and applied undiluted as whole broth. Yeast cells were supplied as a paste by Dr. Shisler and were suspended in distilled water. Corn grain infested with *G. zeae* was scattered in the alleys between the plots one month prior to anthesis. Treatments were applied to wheat at anthesis with a backpack type sprayer at 40 psi, 18-20 gpa using a nozzle arrangement that allowed angled spraying of the heads. After the heads had dried, they were inoculated with *G. zeae* at a rate of 2.7 x 10^{10} macroconidia per acre. The plots were rated visually for the incidence and severity of Fusarium head blight. Test weight, yield, % Fusarium damaged kernels (fdk), % seed infection (on SNAWS selective medium) and DON were determined from the harvested grain. Seed from each plot were sent to Michigan State University for DON analysis.

B*ioprotectant Trial* - M*cGowan Field, Ithaca, NY*
Five treatments were included in a biocontrol trial conducted at Ithaca, NY on Caledonia soft white winter wheat. Treatments were replicated 6 times and arranged in a randomized block design. Wheat heads were sprayed with the treatments on 7 June, 2002. After the heads had dried, they were inoculated with *G. zeae* at a rate of 2.7 x 10^{10} macroconidia per acre. The plot was wetted for 15 min each afternoon with a fine mist from overhead irrigation. The plots were rated visually for the incidence and severity of Fusarium head blight. Test weight, yield, % *Fusarium* damaged kernels (fdk), % seed infection (on SNAWS selective medium) and DON were determined from the harvested grain. Seed from each plot were sent to Michigan State University for DON analysis.

RESULTS AND DISCUSSION

U*niform Fungicide*/B*ioprotectant Field Trial* – M*usgrave Farm, Aurora, NY*
Of all the fungicides and bioprotectants tested, only AMS 21619A showed great promise for control of FHB under the epidemic conditions experienced in New York in 2002 (Table 1). None of the treatments reduced DON contamination to levels acceptable to the grain trade, though AMS 21619A reduced DON significantly. The performance of any of the three synthetic fungicides was not increased in combination with the bioprotectant TrigoCor 1448.

B*ioprotectant Trial* - M*cGowan Field, Ithaca, NY*
None of the biocontrol treatments or the fungicide Folicur controlled Fusarium head blight or reduced DON contamination in the harvested grain (Table 2).

CONCLUSIONS

If results from other test locations (summary report in this volume by D. Hershman) are similar to those in New York, extensive evaluation of the foliar fungicide AMS 21619A for its potential in the integrated management of Fusarium head blight of wheat and barley will be warranted.

REFERENCE

Stockwell, C.A., Bergstrom, G.C., and Luz, W.C. da. 2001. Biological control of Fusarium head blight with *Bacillus subtilis* TrigoCor 1448:2001 field results. Pages 91-95 in: Proc. 2001 National Fusarium Head Blight Forum, Holiday Inn Cincinnati-Airport, Erlanger, KY, December 8-10, 2001.

***Table* 1**. Effect of foliar treatment with fungicides and bioprotectants at anthesis on scab incidence, *Fusarium*-damaged kernels, yield, test weight, and DON contamination in Caledonia winter wheat in Aurora, NY in 2002.

Treatment and amount	Scab (spike) incidence on 21 Jun (%)	*Fusarium* damaged kernels (%)	Test weight @ 13.5% moisture (lb/bu)	Yield @ 13.5% moisture (bu/A)	DON ppm
Nontreated	38.2	15.1	50.9	62.5	31.0
AMS 21619A (5.7 fl oz/A) + Induce (0.125% v/v)	14.0	9.9	58.0	76.1	8.0
AMS 21619A (5.7 fl oz/A) + Folicur 3.6F (4 fl oz/A) + Induce (0.125% v/v)	21.1	10.8	57.6	73.5	10.0
AMS 21619A (5.7 fl oz/A) + Induce (0.125% v/v) + TrigoCor 1448 (2.1×10^{10} cfu/A)	23.4	10.8	57.5	76.6	12.0
BAS 500 50W G (0.4 lb/A) + Induce (0.125% v/v)	37.6	12.1	55.3	69.9	20.5
BAS 500 (0.1 lb a.i./A) + Induce (0.125% v/v) + TrigoCor 1448 (2.1×10^{10} cfu/A)	32.1	14.1	54.7	70.4	21.0
Folicur 3.6F (4 fl oz/A) + Induce (0.125% v/v)	32.0	12.8	52.1	67.8	29.5
Folicur 3.6F (4 fl oz/A) + Induce (0.125% v/v) + TrigoCor 1448 (2.1×10^{10} cfu/A)	32.8	14.9	53.8	68.8	25.5
OH 182.9 Yeast (2.2×10^{9} cfu/A)	37.7	17.6	52.7	63.9	33.5
Serenade (6 lb/A)	43.7	18.9	51.0	59.2	35.0
EN 27 Streptomyces (3.8×10^{9} cfu/A)	35.9	16.1	50.7	69.0	36.6
TrigoCor 1448 (2.1×10^{10} cfu/A)	43.2	13.6	52.0	61.0	33.0
LSD (P=0.05)	8.6	0.4	2.5	NS	8.2
CV (%)	38.2	10.4	3.3	12.8	23.1

***Table* 2.** Effect of foliar treatment with bioprotectants at anthesis on scab incidence, *Fusarium*-damaged kernels, yield, test weight, and DON contamination in Caledonia winter wheat in Ithaca, NY in 2002.

Treatment and amount	**Scab (spike) incidence on 24 Jun (%)**	***Fusarium* damaged kernels (%)**	**Test weight @ 13.5% moisture (lb/bu)**	**Yield @13.5% moisture (bu/A)**	**DON ppm**
Nontreated	29.9	12.1	53.5	72.1	28.0
Folicur 3.6F (4 fl oz/A) + Induce (0.125% v/v)	29.7	9.9	53.9	72.1	30.7
OH 182.9 Yeast (2.2 X 10^9 cfu/A)	28.8	12.0	53.2	68.9	31.0
Serenade (6 lb/A)	38.2	12.5	51.3	65.4	35.0
TrigoCor 1448 (2.1 x 10^{10} cfu/A)	31.5	11.8	53.2	70.8	28.3
LSD (*P*=0.05)	6.2	0.1	1.6	NS	NS
CV (%)	16.5	15.4	2.5	12.8	17.8

HISTORY AND ACCOMPLISHMENTS OF THE USWBSI UNIFORM FUNGICIDE AND BIOLOGICAL CONTROL TRIALS, 1998-2002

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ABSTRACT

The devastating Fusarium head blight (FHB) epidemics in the US in the early 1990s resulted in intensive individual and regional efforts to evaluate fungicides for control of this disease. These early evaluations did not use the same treatments and procedures, and this made comparisons among locations difficult or impossible. A cooperative effort was needed to assure that tests of chemical and biological control agents (BCAs) would provide useful information on efficacy and yield parameters each year.

A group of researchers met at the first National Fusarium Head Blight Forum in 1997 in St. Paul and established the Fungicide Technology Network. This group developed a set of five uniform fungicide treatments to be tested on three classes of wheat and spring barley in seven states (IN, KY, MO, MN, ND, OH, SD) during the growing 1998 season. At the 1998 National FHB Forum in East Lansing, Michigan, the Fungicide Technology Network became part of the Chemical and Biocontrol research area of the USWBSI. At that meeting, plant pathologists from 14 states (AR, IL, IN, KY, MD, MI, MN, MS, NY, NC, ND, OH, SD and VA) agreed to cooperate in a uniform trial with a total of seven treatments. In succeeding years, protocols for applying treatments and recording data were improved and standardized. Each year, selection of the uniform treatments was decided by the Chemical and Biocontrol Committee, with new treatments being tested for at least two years. In 2001, the first BCAs were included in the uniform treatments.

During its first five years, the Uniform Fungicide and Biocontrol Trials have evaluated ten fungicides provided by six crop protection companies and BCAs from EMBRAPA/Cornell University and the USDA/Ohio State University. Reductions in FHB field severity across locations have averaged about 50% and have been as high as 78% with the best fungicide treatment. Most of the tested treatments have been eliminated from further consideration because of poor efficacy, tendency to increase DON levels, and/or termination by the crop protection company. Folicur and AMS 21619A from Bayer have had the most consistent efficacy against FHB, controlled other important diseases, and generally increased yield and test weight. Data generated in the Uniform Trials were instrumental for justifying Section 18 registrations for Folicur in several states and likely will be important for any future registrations. Furthermore, a team of experienced collaborators has been established across the US that uses common protocols for evaluating fungicides and BCAs across multiple environments and grain classes, and that readily shares data and ideas for improving the evaluations.

ND UNIFORM WHEAT FUNGICIDE AND BIOLOGICAL AGENT TRIALS, 2002

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OBJECTIVE

To evaluate registered and experimental fungicides and biological agents for control of Fusarium head blight (FHB) in hard red spring and durum wheat at multiple locations in ND.

INTRODUCTION

Uniform fungicide trials on wheat in ND in recent years (McMullen *et al*. 2000, 2001) have shown statistically significant reductions in Fusarium head blight (FHB) field severity with some registered and experimental fungicides. Similar results have been observed in the national uniform trials (Milus *et al*. 2001). Biological agents tested (from Cornell University and the USDA at Peoria, Illinois) were less successful in reducing FHB severity (Milus *et al*. 2001). In 2001, treatments containing the experimental fungicides AMS 21619 or BAS 505 resulted in the lowest FHB field severity and lowest DON levels among treatments in both the ND trials and the national uniform trial summary. Experiments in 2002 were designed to further test the efficacy of these two experimental fungicides, applied alone or in combination, and to further evaluate the biological agents, applied alone or in combination with a fungicide. Tests in ND were established across two wheat classes and four locations to enhance evaluation across multiple environments and crops.

MATERIALS AND METHODS

A uniform set of four fungicide treatments and three biological agent treatments were evaluated on spring wheat and four fungicide treatments and one biological agent were evaluated on durum wheat in ND in 2002 (Tables 1 and 2). Treatments for each wheat class were tested across three locations and three cultivars: Oxen spring wheat at Fargo, Russ spring wheat and Munich durum at Carrington, Ingot spring wheat and Plaza durum wheat at Langdon, and Ben durum at Minot. Artificial inoculum in the form of inoculated grain was dispersed in plots at Fargo and Langdon, wheat straw was distributed at Carrington, and infections at Minot were from natural inoculum. 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. Spray was delivered in 18- 20 gpa at 40 psi. All treatments were applied between 6:00 and 8:00 am. Treatments included Folicur (tebuconazole) fungicide, AMS 21619A (experimental fungicide from Bayer Crop Science), BAS 505 (experimental

fungicide from BASF), a yeast biological (*OH 182.9 - Cryptococcus nodaensis*) from Dr. Dave Schilser with the USDA, Peoria, a bacterial biological agent (*TrigoCor 1448 - Bacillus subtilis*) from Dr. Gary Bergstrom, Cornell University, a combination treatment of TrigoCor and Folicur, and a combination treatment of AMS 21619 and Folicur (Table 1). TrigoCor was not tested on durum wheat at Carrington and Langdon. Fusarium head blight incidence and head severity and leaf disease ratings were taken at soft dough kernel stage. Plots were harvested with small plot combines. DON (vomitoxin) data was determined by the NDSU Toxicology Lab using gas chromatography and electron capture techniques. Plots were in a randomized complete block design and data were statistically analyzed across locations using ANOVA.

RESULTS AND DISCUSSION

Hard red spring wheat: All fungicide treatments significantly reduced Fusarium head blight field severity over the untreated check (47-59%), while treatments with the biological agents did not (Table 1). DON levels were not significantly reduced by the fungicide or biological treatments. All fungicide treatments significantly reduced leaf rust severity at Fargo and Langdon. Leaf rust ratings were a part of overall leaf disease ratings at Carrington, where leaf rust was much more severe than at Fargo or Langdon. All fungicide treatments significantly reduced leaf diseases on spring wheat, while the biological treatments did not (Table 1). Yields were significantly increased by fungicide treatments, from 18 to 28%. Test weights were increased by fungicide treatments, but not significantly.

Durum wheat: At the Minot site, visible Fusarium head blight (FHB) symptoms were too low to rate, due to drought and heat stress at that site. However, harvested grain at Minot was tested for DON and treatments ranged from 0.7 (AMS treatment) to 2.3 ppm (untreated). The AMS 21619A and BAS 505 fungicide treatments resulted in the lowest FHB field severities, but differences among treatments were not significant (Table 2). DON levels were significantly reduced by fungicide treatments containing AMS 21619A or BAS 505. Leaf spots were significantly reduced by all fungicide treatments but not by the OH 182.9 biological treatment. Yields and test weights were significantly improved by all fungicide treatments (19% to 32% yield increase and 1 to 1.8 lb test weight increase), but not by the biological (Table 2). Heat stress in July during the time of flowering and grain development may have made differences among treatments less significant in 2002 than in previous years.

ACKNOWLEDGMENTS

The funding for this project was provided by the US Wheat and Barley Scab Initiative. Fungicides were provided by BASF and Bayer CropScience. Biological agents were provided by Dr. Gary Bergstrom, Cornell University, and Dr. Dave Schisler, USDA, Peoria, Illinois.

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Table 1. Spring wheat: Effect of fungicides and biological agents on Fusarium head blight (FHB), DON, leaf rust, fungal leaf diseases, yield and test wt. across Fargo, Carrington, and Langdon , ND, 2002.

Treatment and rate/acre[1]		FHB FS[2] %	DON[3] ppm	Leaf rust[4] % on flag	Leaf spot[5] % on flag	Yield bu/a	Test Wt. lbs/bu
Untreated check		17	7.5	5.6	55	39	56.5
Folicur 3.6 F	4 fl oz	7	6.5	0.4	26	46	57.8
AMS 21619A 480 SC	5.7 fl oz	9	5.3	1.5	22	49	58.0
BAS 505 50 WG	6.4 oz	7	5.5	1.5	21	50	58.8
OH 182.9 (*Cryptococcus nodaensis*)		15	6.6	3.5	51	41	56.8
TrigoCor 1448 (*Bacillus subtilis*)		14	7	4.5	45	42	57.0
TrigoCor 1448 + Folicur	4 fl oz	12	6.7	0.2	21	46	57.4
AMS 21619A 3.6 fl oz + Folicur	4 fl oz	8	6.3	0	15	48	57.6
LSD P = 0.05		6	NS	1.8	19	6	NS

[1] All fungicide treatments had 0.125% Induce added; AMS 21619A an experimental fungicide from Bayer; BAS 505 an experimental fungicide from BASF; OH 182.9 an experimental yeast from the USDA, Peoria; and TrigoCor 1448 an experimental bacterium from Cornell University
[2] FHB FS = Fusarium head blight field severity; field severity = incidence x head severity
[3] DON levels were not available from Langdon at time of report
[4] Leaf rust reported only at Fargo and Langdon
[5] Leaf spot diseases primarily tan spot and Septoria leaf spot complex at Fargo and Langdon, but leaf spot readings at Carrington included leaf rust, which was severe at that site

Table 2. Durum wheat: Effect of fungicides and a biological agent on Fusarium head blight (FHB), DON, fungal leaf diseases, yield and test wt. across Carrington, Langdon, and Minot, ND, 2002.

Treatment and rate/acre[1]		FHB FS[2] %	DON[3] ppm	Leaf spot[4] % on flag	Yield[5] bu/a	Test Wt.[5] lbs/bu
Untreated check		36	2.3	43	37	59.5
Folicur 3.6 F	4 fl oz	24	1.9	12	45	60.5
AMS 21619A 480 SC	5.7 fl oz	19	0.7	13	46	61.0
BAS 505 50 WG	6.4 oz	21	0.9	12	49	61.3
OH 182.9 (*Cryptococcus nodaensis*)		32	2.6	35	39	60.0
AMS 21619A 3.6 fl oz + Folicur	4 fl oz	23	0.8	12	49	60.7
LSD P = 0.05		NS	1.3	14	5	1.1

[1] All fungicide treatments had 0.125% Induce added; AMS 21619A an experimental fungicide from Bayer; BAS 505 an experimental fungicide from BASF; OH 182.9 an experimental yeast from the USDA, Peoria; TrigoCor 1448 was NOT tested on durums at Carrington and Langdon
[2] FHB FS = Fusarium head blight field severity; field severity = incidence x head severity; ratings only from Carrington and Langdon as Minot did not have enough visible FHB in 2002 due to drought and heat
[3] DON levels were available from Carrington and Minot at time of this report; significance at P = 0.1 confidence level
[4] Leaf spot diseases primarily tan spot and Septoria leaf spot complex
[5] Yield and test weight data from Carrington and Minot only

NEW AND EFFECTIVE FUNGICIDES AGAINST THE FHB IN WHEAT

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OBJECTIVES

To describe the antifusarium effect and efficacy of several novel fungicides including the four years experiences with the AMS 21619.

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 because the rate used in Hungary were significantly lower than that of suggested in Western Europe. A part of the results was made public last year (Mesterházy and Bartók 2001). 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. 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 when epidemics occurred.

MATERIALS AND METHODS

The methodology the methods were the same as published last year (Mesterházy and Bartók 2001). FHB % means disease severity, e. g. the ratio of spikelets showing infection. In all years three cultivars with differing resistance were used and they were inoculated by two *Fusarium graminearum* and two *F. culmorum* isolates at full flowering, one day after the fungicide treatment. From 2001 we modified the duration of covering the head of groups by polyethylene bags from 24 hrs to 48 hrs to allow better disease development under dry conditions. FHB was rated five times, mean infection severity was calculated together with AUDPC, but here only arithmetical means are given as the two parameters originating from the same data show a relationship above 0.998. Leaf rust was rated as ACI, average coefficient of infection, where the coverage of the whole leaf system as a % was multiplied by 1 at S, 0.8 at MS, 0.6 at MR, 0.4 at R and 0.2 at VR reaction type.

Every year FHB severity, FDK, and relative yield loss were rated. Deoxynivalenol was measured in 1998 for the AMS 21619, however in 1999 and 2000 the experimental fungicides were not measured, as the formulations tested were not the products yet for commercial use. For this reason no DON data are listed for 1999-2001. In the tables only the averages are given across isolates and cultivars, e. g. the mean of 12 epidemic situations. Active ingredients of the fungicides used for a L product: Folicur Solo: 250 g tebuconazole, Folicur Top: 125 g tebuconazole, Falcon 465 EC: 167 g tebuconazole, spiroxamine 250 g + triadimenole 43, Kolfugo Super carbendazime 200, Caramba SL metconazole 60, Juwel:

Kresoxym-methyl 125 + epoxyconazole 125, Granit SC: bromuconazole 200, Tango: epoxyconazole 125 + tridemorph 375, Flamenco: fluquinconazole 100, Stratego: trifloxystrobin 125 + propiconazole 125EC, Sphera: trifloxystrobin 188 + cyproconazole 080 EC.

RESULTS AND DISCUSSION

Table 1 shows the 1998 data. As some lower effective fungicides were mentioned last year, we present here only the more effective ones to see the performance of the new experimental fungicide. The AMS 21619 was the most effective, 30-50 % better than the second best fungicide.

In 1999 (Table 2) a wider set of fungicides were tested. Last year we presented only those that had also DON analysis. Here the whole set is printed to see also products that are maybe less known in US. In this year we added the carbendazime to 0.8 L/ha Falcon rate (1 L/ha) and this mixture was equivalent with the lower rate (0.8 L/ha) for AMS 21619. Lower rates of this experimental fungicide produced less control, the situation is the same we hade with the different tebuconazole containing fungicides, too. The epidemic severity measured by the *Fusarium* check or Folicur Solo was about the same, and the best fungicides were significantly better than this. However the leaf rust epidemic showed that this new product has only medium or lower protection ability. From the spraying on about three weeks controlled leaf rust well, but thereafter the infection by rust increased rapidly. Other fungicides like kept their activity against rust until the end of the vegetation period allowing lower than 10 % infection. For this reason the task was to find another fungicide that does not decrease the antifusarium effect, but increases the efficacy against rust.

For this reason tebuconazole was chosen as partner fungicide in the 2000 trials. The whether was dryer and warmer, so the infection severity was lower than in 1999 or 1998 that were favorable for disease development. The AMS concentration was lower and tebuconazole was also half of the concentration of Folicur Solo, being equivalent with Folicur Top. The results showed that the new mixtures at 0.8 and 1 L/ha rate were about as effective as Folicur Solo itself, however slightly lower within the LSD 5 %. All kept FDK lower than 0.5 %.

In the 2001 trials therefore this new combination was tested at two rates (0.8 and 1 L/ha) and we applied as check the 0.8 L/ha rate of AMS 21619. The results clearly show that the new combination is as effective as the AMS 21619, but controls leaf rust as good as Folicur Solo does. All three AMS fungicides were more effective than Folicur Solo even the disease development was better than in 2000, but the humidity period was longer. These combinations were also better than our carbendazime-tebuconazole version. It is remarkable that Caramba at 1.2 L/ha performs better than at 1 L/ha. An increase of the rate to 1.5 L/ha may provide further improvement. The result support the data of El-Allaf *et al*. (2001), Hart *et al*. (2001), Hershman *et al*. (2001), McMullen *et al*. (2001), Milus *et al*. (2001), however this positive efficiency could be demonstrated also at higher epidemic severity. This means that it will be effective also under more severe epidemic conditions than at mostly moderately infected field trials.

Summary. The data show that AMS 21619 or JAU 6476 combined with tebuconazole provided more powerful control of FHB and was effective also against leaf rust. At susceptible cultivars a higher dosage (1 L/ha, or somewhat higher) can give hope that food safety could be secured better until more resistant cultivars will grow on the Great Plain, in Hungary or elsewhere. In Hungary the standard antifusarium fungicide is Falcon at 0.8 L/ha. It is clear that any of the new combinations decreases at least 50 % the infection severity in comparison to Falcon 0.8 L/ha. Therefore a change of fungicide should come in the near future. It is important that the chances of the moderately susceptible or moderately resistant cultivars will provide higher safety when sprayed with these new fungicides even they have susceptibility to rust. Novel products are developed also elsewhere, their test will also be necessary to identify other valuable products. This is necessary to change fungicides not allowing the selection of fungicide resistant strains in the fungal populations.

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Table 1. Summary of fungicide tests against FHB in wheat, 1998

Fungicide and		Traits		
rate L/ha	FHB	Yield loss	FDK	DON
	severity %	%	%	ppm
AMS 21619 250 EC 1.0	5.07	11.08	8.33	2.82
Folicur Top 1.0+Kolf.S 1.5	7.88	16.00	15.53	4.19
Fol. Solo 1.0	8.13	20.87	19.73	3.79
Falcon 0.8	9.85	20.57	28.08	6.24
Falcon 1.0	11.63	18.43	25.86	5.72
Fus.kontr.	41.55	50.36	58.56	11.37
Mean	8.41	13.73	15.61	3.41
LSD 5 %	0.71	2.89	3.33	2.07

Table 2. Fungicides against Fusarium head blight of wheat. Summary of general means for 1999.

Fungicide		Original data		
rate L/ha	FHB %	Yield loss %	Kernel inf. %	Leaf rust ACI
Falc.0.8+Kolf.1.5	12.19	23.43	14.36	0.4
AMS 21619 250EC 0.8	13.19	26.00	15.86	31.2
Folicur Solo 1.0	13.42	27.14	22.37	1.6
AMS 21619 250EC 0.6	14.50	28.25	18.24	34.5
Falcon 0.8	14.99	28.61	20.47	1.8
Folicur Top	17.38	29.03	19.91	3.4
AMS 21619 250EC 0.4	19.00	37.05	26.13	42.1
Caramba 1.0	20.12	36.17	25.42	7.6
Falcon 0.6	20.68	39.69	37.86	7.9
Juwel 1.0	23.75	38.97	32.69	9.5
Granit 1.0	23.81	38.90	30.46	27.0
Alert 1.0	24.84	36.32	27.92	34.2
Kolfugo Super 1.5	25.13	39.93	31.31	58.2
Tango 0.8	26.75	39.96	28.44	4.6
Flamenco 1.0	33.58	48.79	43.40	14.2
Fusarium check	41.97	56.11	58.79	64.0
Mean	21.58	35.90	28.35	21.39
LSD 5 %	0.91	2.93	3.21	5.97

Table 3. Fungicides against FHB in wheat. Summary, 2000.

Fungicide		Parameters	
rate L/ha	FHB %	Yield loss %	Kernel inf.%
Folicur Solo 1.0	1.06	6.78	0.08
Falcon 0.8+Kolf.1	1.45	8.74	0.45
AMS 21619 250EC 0.8	1.59	9.28	0.42
AMS 216191 125EC+HWG 125, 1.0	1.79	9.44	0.42
Falcon 1.0	1.93	9.70	0.86
Caramba, 1.2	2.54	8.53	0.68
Falcon 0.8	2.96	10.26	1.19
Juwel, 1.0	3.20	14.96	1.61
Kolfugo, 1.5	4.57	14.82	3.45
Fusarium check.	8.67	22.96	7.70
Flamenco, 1.5	9.10	24.06	10.24
Mean	2.43	8.72	1.69
LSD 5 %	0.50	1.06	1.10

HWG = tebuconazole

Table 4. Fungicide tests against Fusarium head blight in wheat, summary for 2001.

Treatment	Overall means			
	FHB %	FDK %	Yield loss %	Leaf rust
AMS 21619 125 EC + HWG 125EC 1.0	0.60	5.61	3.9	3.00
AMS 21619 250EC 0.8	0.92	6.59	3.5	32.52
AMS 21619 125 EC + HWG 125EC 0.8	0.99	7.44	1.1	2.56
Folicur Solo 1.0	1.14	9.08	4.8	1.74
Falcon 0.8+Kolf.S.1.5	1.39	13.80	6.5	3.59
Caramba 1.2	2.28	12.94	7.7	6.70
Falcon 0.8	2.91	14.91	12.3	5.37
Stratego 1.0	3.08	18.81	8.8	21.78
Sfera 1.0	3.40	16.41	16.2	6.63
Kolfugo S 1.5	5.71	22.56	17.0	58.15
Fusarium check.	12.02	38.54	17.2	74.07
Mean	2.65	14.69	100.00	17.66
LSD 5 %	0.49	2.05	2.97	3.49

HWG = tebuconazole

UNIFORM BARLEY FUNGICIDE AND BIOLOGICAL AGENT TRIALS, FARGO, ND, 2002

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OBJECTIVE

To evaluate registered and experimental fungicides and biological agents for control of Fusarium head blight (FHB) in spring barley at Fargo, ND.

INTRODUCTION

Uniform fungicide trials on spring barley in ND in recent years have shown inconsistent results in reduction of Fusarium head blight (FHB) field severity and DON levels (McMullen *et al*., 2000 and 2001). In 2000, fungicides tested reduced FHB field severity by 45 to 66.7%, but differences among treatments were not significant. In 2001, all fungicide treatments significantly reduced FHB field severity, with the experimental fungicide, AMS 21619, giving the greatest reduction (70.5%). DON levels, however, were not statistically reduced by treatments in either year. Biological agents were not consistently tested on barley across locations. An experiment in 2002 at Fargo, ND further tested experimental fungicides, applied alone or in combination, and evaluated biological agents, applied alone or in combination with a fungicide, for efficacy in controlling FHB in spring barley. Treatments were the same as those in the uniform trials for wheat.

MATERIALS AND METHODS

A uniform set of four fungicide treatments, two biological agent treatments, and a biological + fungicide treatment were evaluated on six row 'Robust' spring barley at Fargo, ND in 2002 (Table 1). Plots were planted on May 3, 2002 into wheat stubble that had been chiseled twice prior to planting. Plants emerged on May 16, but were frosted several times in late May. Two weeks before head emergence in early July, artificial inoculum in the form of inoculated corn grain was dispersed uniformly in the plots, approximately 100 g per 162 square foot plot. Natural rainfall was augmented by mist irrigation starting on July 3 and continuing until July 19.

All treatments were applied at early head emergence (Feekes 10.5) with a CO_2 backpack type sprayer, equipped with XR8001 nozzles mounted at a 60^0 angle forward and backward toward the grain heads. Spray was delivered in 18- 20 gpa at 40 psi. All treatments were applied between 6:00 and 8:00 am. Treatments were: Folicur (tebuconazole) fungicide; AMS 21619A (Bayer CropScience experimental fungicide); BAS 505 (BASF experimental fungicide); a yeast biological (*OH 182.9 - Cryptococcus nodaensis*) from Dr. Dave Schisler, USDA, Peoria; a bacterial biological agent (*TrigoCor 1448 - Bacillus subtilis*) from Dr. Gary Bergstrom, Cornell University; a combination treatment of TrigoCor and Folicur; and a combination treatment of AMS 21619 and Folicur (Table 1). FHB ratings and leaf disease

ratings were taken at soft dough kernel stage. Plots were harvested with a small plot combine. DON (vomitoxin) was determined by the NDSU Toxicology Lab using gas chromatography and electron capture. Plots were in a randomized complete block design and data were statistically analyzed across locations using ANOVA.

RESULTS AND DISCUSSION

All fungicide and biological treatments significantly reduced FHB head severity and field severity over the untreated check (Table 1). DON levels were significantly reduced by the AMS 21619A, BAS 505, the TrigoCor + Folicur and the AMS 21619A + Folicur treatments. Yields were significantly increased by most fungicide treatments, but not by the biological treatments. Test weights were significantly increased by only two treatments, the Folicur alone and the AMS 21619A + Folicur treatment. Although FHB levels were fairly high in this experiment, late season heat stress and low natural precipitation at this location may have resulted in poor grain fill, low yields and low test weights, and concomitant smaller differences among treatments.

ACKNOWLEDGEMENTS

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Table 1. Effect of fungicides and biological agents on Fusarium head blight (FHB), DON, yield and test weight in 'Robust' spring barley, Fargo, ND, 2002.

Treatment and rate/acre[1]		FHB I[2] %	FHB HS[2] %	FHB FS[2] %	DON ppm	Yield bu/a	Test wt lbs/bu
Untreated check		97	25.0	24	4.3	50	43.9
Folicur 3.6 F	4 fl oz	89	8.7	8	3.6	55	44.5
AMS 21619A 480 SC	5.7 fl oz	94	7.3	7	2.9	54	43.9
BAS 505 50 WG	6.4 oz	93	6.7	6	2.7	55	44.1
OH 182.9 (*Cryptococcus nodaensis*)		92	8.8	8	3.3	50	43.9
TrigoCor 1448 (*Bacillus subtilis*)		93	7.9	7	3.9	49	43.3
TrigoCor 1448 + Folicur	4 fl oz	94	6.9	6	2.9	52	43.8
AMS 21619A 3.6 fl oz + Folicur	4 fl oz	90	7.9	7	3.1	57	44.5
LSD P = 0.05		8	5.6	6	1.2	5	0.6

[1] All fungicide treatments had 0.125% Induce added; AMS 21619A an experimental fungicide from Bayer; BAS 505 an experimental fungicide from BASF; OH 182.9 an experimental yeast from the USDA, Peoria; and TrigoCor 1448 an experimental bacterium from Cornell University

[2] FHB I = Incidence (% tillers showing symptoms); FHB HS = % of kernels showing symptoms; FHB FS = Fusarium head blight field severity; field severity = incidence x head severity

EFFICACY OF FUNGICIDES AND BIOCONTROLS AGAINST FHB ON WHEAT IN ARKANSAS IN 2002

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INTRODUCTION

Identifying fungicides and biocontrols 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 and Biocontrol 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.

METHODS

The susceptible wheat cultivar 'Hazen' was planted at the University Farm at Fayetteville on 8 October 2001. 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 transmitting barley yellow dwarf. Individual plots were 7 rows by 13 ft. Plots were fertilized with 80 lb nitrogen from ammonium nitrate that was applied in equal splits on 28 February and 12 March. Ryegrass and broadleaf weeds were controlled with recommended herbicides. Infested corn kernel inoculum was applied to the plots on April 1 and 9 at a total rate of 12 kernels / sq ft. The mist system operated for eight 10-minute periods between midnight and 8:00 am for eight nights between 30 April and 8 May. TrigoCor 1448 was grown in broth culture and OH 182.9 was suspended from frozen paste according to directions supplied with the biologicals. To determine the concentration of viable cells of each biological agent, the suspension of each biological was assayed by dilution plating on TSA medium immediately before application to the plots. Fungicides and biocontrols were applied in a randomized complete block design with six replications in the late afternoon on 2 May when 50% of the main stems had begun to flower. Applications were at 20 gal / acre except for one AMS 21619A treatment that was applied at 10 gal / acre. On 23 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 14 June, and grain was passed once through a seed cleaner before test weight and percentage of scabby grain were measured. Grain samples were sent to Pat Hart's laboratory for DON analysis.

RESULTS AND DISCUSSION

Except for low levels of barley yellow dwarf from spring infection in some plots, FHB was the only significant disease. Sixteen days of rain totaling 11.3 inches during April and May provided very favorable conditions for sporulation on the corn inoculum, infection, and FHB development. Fusarium head blight was severe by the end of the season, as indicated by

the high levels of scabby grain (Table 1). Compared to the non-treated check, all fungicides significantly reduced plot severity and increased test weight, but the two biologicals did not. However, there were no significant differences among the fungicides. Plots treated with fungicides had numerically greater yields that the non-treated check or plots treated with biologicals, but differences were not significant at the 5% level of confidence because of variability among plots of the same treatment. Poor performance of the biologicals did not appear to be due to low viability of cells in the suspension applied to the plots. AMS 21619A applied at 10 gal / acre appeared to have greater efficacy than at 20 gal / acre, but the differences were not statistically significant.

Table 1. Efficacy of fungicides and biocontrols against FHB on wheat in Arkansas.

Product and rate per acre	Plot severity (%)	Incidence of infected heads	Infected head severity (%)	Scabby grain (%)	Yield (bu/A)	Test wt. (lbs/bu)
AMS 21619A 480SC 5.7 fl. oz. + 0.125% Induce*	7.1	0.29	25.7	51	72.4	51.9
AMS 21619A 480SC 5.7 fl. oz.+ 0.1% Kinetic	7.9	0.34	20.6	60	64.9	49.9
AMS 21619A 480SC 5.7 fl. oz. + 0.125% Induce	8.5	0.44	19.4	52	68.9	50.4
BAS 505F 50W G 6.4 oz. + 0.125% Induce	8.6	0.35	23.5	58	63.1	50.5
AMS 21619A 480SC 3.6 fl. oz. + Folicur 3.6F 4 fl. oz. + 0.125% Induce	8.6	0.39	21.7	52	58.0	50.8
TrigoCor1448 ($5x10^{13}$ cfu) + Folicur 3.6F 4 fl. oz. + 0.125% Induce	9.3	0.38	24.4	67	63.7	50.1
AMS 21619A 480SC 5.7 fl. oz. + Succeed 1.17 L/ha	10.9	0.42	27.2	62	61.2	49.6
Folicur 3.6F 4 fl. oz. + 0.125% Induce	11.0	0.48	22.2	65	65.1	49.6
OH 182.9 ($1.1x10^{14}$ cfu)	15.4	0.52	29.8	72	54.8	47.6
TrigoCor1448 ($5x10^{13}$ cfu)	15.7	0.53	30.3	67	57.7	48.3
Non-treated check	17.3	0.51	33.0	75	55.5	46.7
LSD (P=0.05)	5.4	0.14	NS	10.0	NS	2.7
% cv	42.3	27.6	31.0	14.0	14.0	4.6

*Applied in 10 gal/acre

PRACTICAL ASPECTS OF GROUND APPLICATION OF FOLIAR FUNGICIDES

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ABSTRACT

While remaining at the forefront of intensive wheat management, Opti-Crop is also an industry leader in providing state-of-the-art consulting services to corn and soybean growers.

Presently, our staff of over 25 Opti-Crop consultants – most with CCA certification – manage over 200,000 acres of corn, soybeans and wheat in Kentucky as well as parts of Indiana, Illinois, Tennessee, Kansas, Oklahoma, South Dakota and North Dakota. Opti-Crop also has divisions that manage over 150,000 acres in Australia, plus consulting operations in Russia, Romania and Bolivia.

Ground application of foliar fungicides is a very important component of our intensive crop management program. Our company custom applies over 1,000,000 acres of chemicals and fertilizer annually, so logistics and timing are always a challenge. We strive to educate and train our personnel on the latest application technology by conducting field days and training sessions.

Selection of the appropriate fungicide, rates and specific adjuvants has a major impact on product effectiveness. Correct water volumes and product application timings are also crucial. We have 8 replicated research sites across the Midwest and Northern Plains, so we have the luxury of being able to apply different products at different rates and timings to determine the relative differences and economics of the individual treatments.

EFFICACY OF FUNGICIDES IN CONTROLLING BARLEY FUSARIM HEAD BLIGHT IN LINES WITH PARTIAL RESISTANCE

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ABSTRACT

Research to test the efficacy of fungicides in controlling Fusarium head blight (FHB) and deoxynivalenol (DON) levels in barley was previously conducted using cultivars (i.e. Robust, Foster, and Stander) that are susceptible to FHB. Results indicate that fungicides had little to no effect in reducing DON concentration to levels acceptable to the malting and brewing industry. Minimal information is available on the efficacy of fungicides in controlling FHB and DON levels on genotypes with partial FHB resistance. The objective of this study is to determine if the integrated use of fungicides and barley cultivars with partial resistance to FHB will control FHB severity and accumulation of DON. Experiments were conducted in the field in North Dakota since 2000 and included genotypes resistant, partially resistant, and susceptible to FHB. Fungicides used were Folicur in 2000, 2001, and 2002; and AMS21619 in 2001 and 2002. Folicur did not significantly reduce FHB severity or DON accumulation in resistant, moderately resistant, or susceptible genotypes. However, genotypes sprayed with Folicur generally had greater yield due to control of septoria speckled leaf blotch (SSLB), incited by *Septoria passerinii*. Yield gains due to control of SSLB tended to be sufficient to cover the cost of Folicur and its application on cultivars developed and released by upper Midwest barley breeding programs. Preliminary data indicates that efficacy of AMS21619 was slightly better than Folicur in reducing FHB and DON.

AUTOMATED CONTROL OF A WATERING SYSTEM FOR FUSARIUM HEAD BLIGHT RESEARCH

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OBJECTIVES

Use an automated water application control system to create a favorable growth environment for Fusarium head blight in the uniform fungicide trial plots. Evaluate the effectiveness of the watering system by 1) monitoring the microclimate in the plots and 2) measuring the FHB field severity levels in the watered control plots and surrounding dryland plots.

INTRODUCTION

To properly evaluate the effectiveness of fungicides, a favorable microclimate for the growth of FHB must be provided either by nature or artificially. Keeping the grain heads "wet" and/or maintaining a high humidity during the crucial FHB formation period is important for the evaluation of fungicides. Many researchers participating in the uniform fungicide trials use some type of watering system but some do not, relying on natural climatic conditions to provide the environment for the growth of FHB (McMullen, 2001).

A search of the literature reveals no set protocol for the operation of the watering systems during the FHB infection period. Most researchers haven't included a description of the watering system operation in their reports or research papers. Warnes (1995) used a misting system with a windbreak around the research plots. He ran the misting system for 20 minutes on even hours from 6 am to 6 p.m. and 10 minutes on even hours from 6 p.m. to midnight. The intended precipitation amount was 0.3 inches per day. Nelson (2000) ran his misting system for a half-hour at 7 p.m., 11 p.m. and 5 am. Neither researcher explained how they developed the watering protocol.

Research by Francl (2001) provided a guide for when and how often to operate a watering system. He is developing a model that uses weather data to predict when the weather conditions are right for FHB infection. He says, "Details of the interactions among environmental factors, infection, spore survival, etc. are not yet fully understood. *As a general guide, infection is indicated for wet periods longer than nine hours, but this may be substituted for by a high relative humidity and an average temperature above 60°F."* These guidelines could be used to operate the watering system using a feedback control system.

For statistical verification of the effectiveness of fungicides, the water application pattern should be uniform on all plots. The means the frequency, duration and amount of applied water should be equal for all plots. If too much water is applied, the fungicide could be washed off, loose its effectiveness and the fungus would overwhelm the plots. If too little water is applied, the fungus will not grow at an equal rate in all plots. A balance must be

struck that mimics natural conditions that favor the growth of FHB. The watering system must apply the amount of water that maintains the proper microclimate to grow the fungus without interfering with the effectiveness of the fungicide.

MICROSPRINKLER WATERING SYSTEM

During the 2000 growing season, a watering system which used microsprinklers was designed and installed in the FHB uniform fungicide trial plots (Scherer, *et al*., 2000). The same watering system was used during the 2001 and 2002 growing seasons. The research field covered about 1.8 acres. About 1.3 acres were planted to one variety of wheat and the remaining area planted to barley.

The watering system has three zones each 70 feet wide by 360 feet long. Within each zone, laterals are spaced 10 feet apart to match the plot width. The microsprinklers are spaced 15 feet apart along the laterals. Each lateral has 25 microsprinklers and the total for all three zones is 528 microsprinkler heads. Two zones had seven laterals and the other zone (a combination of wheat and barley) had eight laterals. Each zone has its own control valve and filter. The duration and frequency of the watering system was controlled by a programmed datalogger but could be operated manually.

AUTOMATED CONTROL AND REMOTE MONITORING SYSTEM

The automated control system comprised two sensor stations. One was located in a control plot in zone 1 and the other in a control plot in zone 2. They were installed on June 28 when the flag leaf was just starting to show. Each sensor station had a very accurate relative humidity/temperature sensor and a leaf wetness sensor placed at the same elevation as the flag leaf on the wheat. The sensor stations were connected to the programmable datalogger that controlled the watering system and recorded the data. The critical infection period started on July 1. Relative humidity and temperature was read continuously from each sensor and an average value recorded every 10 minutes. The leaf wetness sensors were read continuously to record the “wetness duration” during the critical infection period. An automated recording rain gage was place in a watered plot and another was placed in an adjacent non-watered plot to record both watering and rainfall events.

In addition to the sprinkler control sensor system, remote microclimate monitoring stations were located in four watered control plots and two adjacent dryland plots. In the watered area, one station was located near the beginning of the sprinkler laterals, one near the end of the laterals and two were located halfway between. In the dryland area, stations were placed at one-third and two-thirds the lateral length. Each remote monitoring station had three self-contained dataloggers to measure relative humidity, wet bulb temperature and dry bulb temperature (HOBO Pro temperature/RH meters). The three dataloggers at each station were mounted on a single support pole at 15, 45 and 75 cm (6, 18 and 30 inches) above ground surface.

They were installed in the plots on June 24 when the wheat was approximately 20 cm (8 inches) tall. They were programmed to record data every 10 minutes. The data were downloaded once per week until July 24 when the wheat had passed the infection stage. A North

Dakota Agricultural Weather Network (NDAWN) weather station is located about 3000 feet from the research site and weather data for the area is recorded on an hourly basis. These data will be used to obtain stratified data of the climatic variables in and above the small grain canopy.

Control Algorithm

Based on recommendations from Dr. Francl and Dr. McMullen, the control system was programmed to begin watering at 5 p.m. each day if the relative humidity was below 92%. Each zone was watered for a total of 30 minutes. The first watering cycle ended at 6:30 pm. At 9 p.m., the relative humidity was checked and if it was below 92%, the watering system was activated and each zone was misted for 15 minutes. This was repeated every hour on the hour until 8 am in the morning. This assured at least 9 hours of wet conditions each day. The dry period during the day allowed the FHB spores to dry and move with the wind to ensure infection. The watering system was manually tested on June 28 when the wheat heads were just beginning to emerge. On July 1, the watering system was turned on and automatic control began. The watering system was under automatic control until July 19 when the system was shut off.

RESULTS AND DISCUSSION

Throughout the control period (July 1 to July 19), the watering system successfully maintained the relative humidity in the plots above 92% from 9 pm to 9 am except on July 5 and 6. On these two days, the wind speed stayed between 20 to 30 miles per hour and the air temperature between 79 to 93Ú F the entire time. Even under these conditions, the relative humidity was maintained at slightly over 80 percent.

The readings from the leaf wetness sensors show that the grain heads were wet about 73% of the time and dry about 27% of the time. By comparison, the wheat heads in the dryland plots were wet and dry 10% and 90% of the time, respectively. We did not have a leaf wetness sensor in the dryland plots, so these data were estimated using rainfall and relative humidity readings from the NDAWN station.

Remote Monitoring Sensors

The remote sensors measured the stratification of temperature and relative humidity in both the watered and dryland plots. One way to determine the wetness of the plots is to measure the amount of time the relative humidity was at a certain level during the critical infection

Sensor Location	Percent time the RH was greater than 92% in the watered plots (average of 4 stations)	Percent of time the RH was greater than 92% in the dryland plots (average of 2 stations)
6 inches above ground	80%	47%
18 inches above ground	65%	42%
30 inches above ground	45%	30%

period. The effectiveness of the watering protocol can be verified by examining the relative humidity data from the four remote monitors in the watered plots and compare that with the relative humidity data from the dryland plots. These results are shown in the following table.

The relative humidity was above 92% almost 80 percent of the time for the bottom sensors compared to 48% of the time for the dryland sensors. The difference decreased at the sensor stations higher in the canopy indicating there was a stratification effect induced by the watering schedule. It is interesting to note that the top sensor, which is at head height, is above 92% relative humidity about 50% of the time in the watered plots and 35% in the dryland plots.

FHB Infection Rates

The objective of this project was to make sure the all the plots had an equal chance for infection and that the microclimate was conducive to the growth of FHB. The level of infection in each plot was measured by taking 30 wheat heads and using a standardized scale to rate the severity of infection. The untreated checks in the watered plots had FHB field severity that ranged from 12 to 36 percent with an average of 30%. These levels provided a sufficient infection rate to evaluate fungicide treatments without an overwhelming amount of FHB. The field severity levels in inoculated dryland plots in adjacent research areas south of the watered plots (planted with the same variety of wheat) was about 2%.

DISCUSSION

Although the watering system and watering protocol successfully created the microclimate for the growth of FHB, limitations need to be addressed. The dryland plots, (part of the fungicide trials where two remote monitor stations were located) were not inoculated with FHB like the misted plots. We were not able to evaluate the growth of FHB in ***inoculated*** watered plots compared to ***inoculated*** dryland plots within the confines of this study. We did not have a leaf wetness sensor in the dryland plots and therefore had to infer the time of head wetness. We did not measure the amount of the time the sprinkler system was on and therefore could not pick out the periods when natural conditions were favorable for the growth of FHB.

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USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT 1: DISCOVERY AND SCALE-UP OF A FREEZE-DRYING PROTOCOL FOR BIOMASS OF ANTAGONIST *CRYPTOCOCCUS NODAENSIS* OH 182.9 (NRRL Y-30216)

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OBJECTIVES

To 1) identify cryoprotectant compounds and quantities of these that would enhance the shelf-life of freeze-dried biomass of OH 182.9 and 2) evaluate the propensity of a superior cryoprotectant compound to enhance shelf-life and maintain efficacy of OH 182.9 inoculum produced using precommercial, 100-L fermentor environments.

INTRODUCTION

Fusarium head blight (FHB), primarily incited by *Gibberella zeae*, can be a devastating disease of wheat and barley in humid and semi-humid regions of the world. In previous research, we have demonstrated the potential of several biological control agents to significantly reduce the severity of FHB in greenhouse and field environments (Schisler *et al*., 2002). A critical step in producing a commercially available biocontrol product is devising procedures for stabilizing biomass of the biological agent while maintaining product efficacy. A product comprised of frozen biomass of our yeast antagonist *Cryptococcus nodaensis* OH 182.9 was developed and tested at over 15 field sites as part of the U.S. Wheat and Barley Scab Initiative in the 2001 field season (Schisler *et al*., 2001, Milus *et al*, 2001). Though this product significantly reduced FHB, the development of a dried biocontrol product would have potential advantages of convenience, ease of handling, favorable economics, and consumer acceptance. However, dehydration of antagonist biomass can adversely affect its viability and efficacy.

MATERIALS AND METHODS

Eight cryoprotectant compounds (Fig. 1) were added separately at 25mM to semi-defined complete liquid medium (SDCL, Slininger *et al*., 1994), and shake-flask cultures of OH 182.9 initiated. Flasks were maintained at 250 rpm and 25°C for 96 h. Two milliliter aliquots of colonized broth were placed in 5 ml vials, freeze-dried for 48 h in a 6-L tray freeze-dryer, and stoppered under vacuum at a final temperature of 4°C. Colony forming units per milliliter (CFU/ml) were determined prior to freeze-drying and for rehydrated freeze-dried products stored at 24°C for 0, 8 and 37 days.

The effect of 1 mM to 100 mM concentrations of melezitose (a trisaccharide composed of two molecules of glucose and 1 molecule of fructose) on OH 182.9 survival and stability after freeze-drying was studied by adding melezitose and/or 10% (w/v) skim milk to washed biomass from 48 h shake-flask cultures (Fig. 2). The CFU/ml were determined prior to freeze-drying and after product storage at 24°C for 0, 6, 13, and 21 days.

Yeast antagonist OH 182.9 was then produced in a B Braun D-100 fermentor charged with 80 L of SDCL medium. To initiate a production run, cells from a log-growth stage SDCL culture served as a 5% seed inoculum for the D-100 fermentor. Reactor medium pH, temperature, dissolved O_2, antifoam dose, and agitation rate were monitored and/or maintained to insure near identical production runs. After completion of biomass production at approximately 48 h, colonized reactor broth was concentrated into a paste using a Sharples 12-V tubular bowl centrifuge. The paste was resuspended using buffer containing 25 mM melezitose and 1% skim milk. The cell suspension was then freeze-dried in a 24-L tray freeze dryer for 48 h and vacuum sealed in mylarfoil bags. The CFU/ml were determined prior to freeze-drying and after product storage at 4°C for 0, 3, 10, 14, 21, 28, 35 and 42 days (Fig. 3). The effect of the freeze-dried product, freshly produced OH 182.9 cells, and cryoprotectants alone on FHB severity was determined in greenhouse bioassays after 0, 10 and 28 days storage (data not shown).

RESULTS AND DISCUSSION

Melezitose is characterized, for the first time, as an effective cryoprotectant (Fig. 1). Melezitose and turanose were the most effective in enhancing the survival of freeze-dried biomass of FHB antagonist *C. nodaensis* OH 182.9 compared to six other cryoprotectants found to be effective when drying biomass of other microorganisms.

Melezitose was effective in extending the shelf-life OH 182.9 at 100 mM and 50 mM concentrations but was not at concentrations of 10 mM and lower (Fig. 2). Amending biomass of OH 182.9 with 10% skim milk was effective in combination with melezitose or alone in extending OH 182.9 shelf-life.

The precommercial process of producing OH 182.9 biomass in a 100-L fermentor, separating cells from broth using a tubular bowl centrifuge, resuspending the biomass in a solution containing 25 mM melezitose and 1% skim milk, and freeze-drying the product in a 24-L tray freeze-drier produced a product that lost more than a log unit of CFU's during processing and freeze-drying but then maintained nearly constant CFU's over the next five weeks (Fig. 3).

Though cell survival of the precommercial product was satisfactory after freeze-drying (Fig. 3), the biocontrol efficacy of this product was less than that of similar concentrations of freshly produced of OH 182.9 cells in greenhouse bioassays with high disease pressure (data not shown). A portion of the failure of the freeze-dried product to control disease appears to be due to 25 mM melezitose and 1% skim milk enhancing disease (data not shown). Alternative drying methodologies such as air, fluidized bed or spray-drying may be required to produce a dried OH 182.9 biocontrol product that maintains biocontrol efficacy.

ACKNOWLEDGMENTS

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

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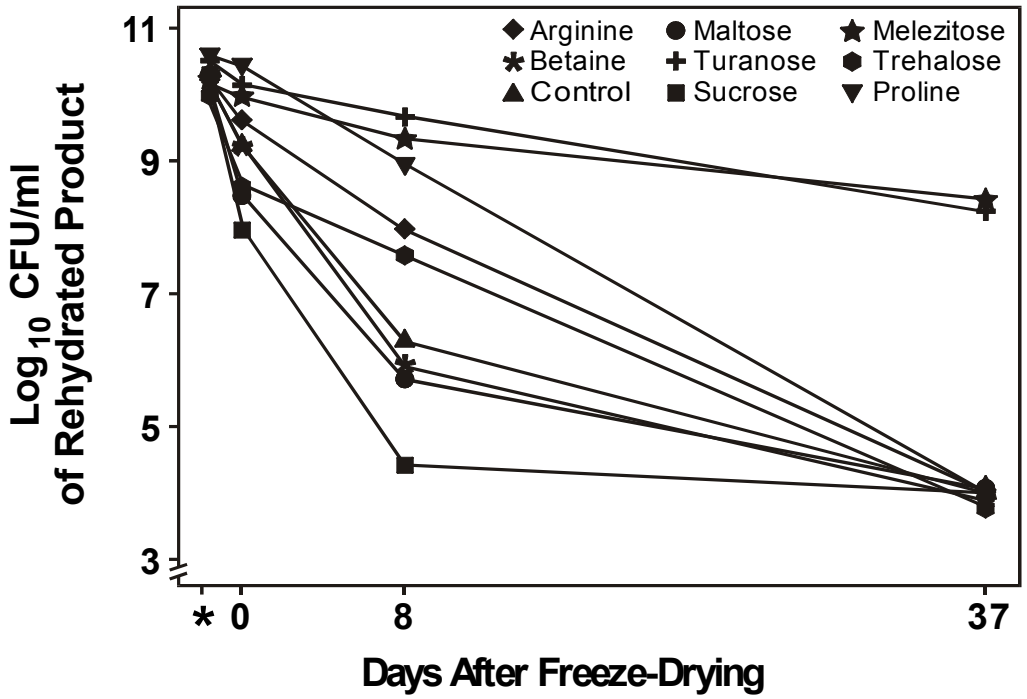
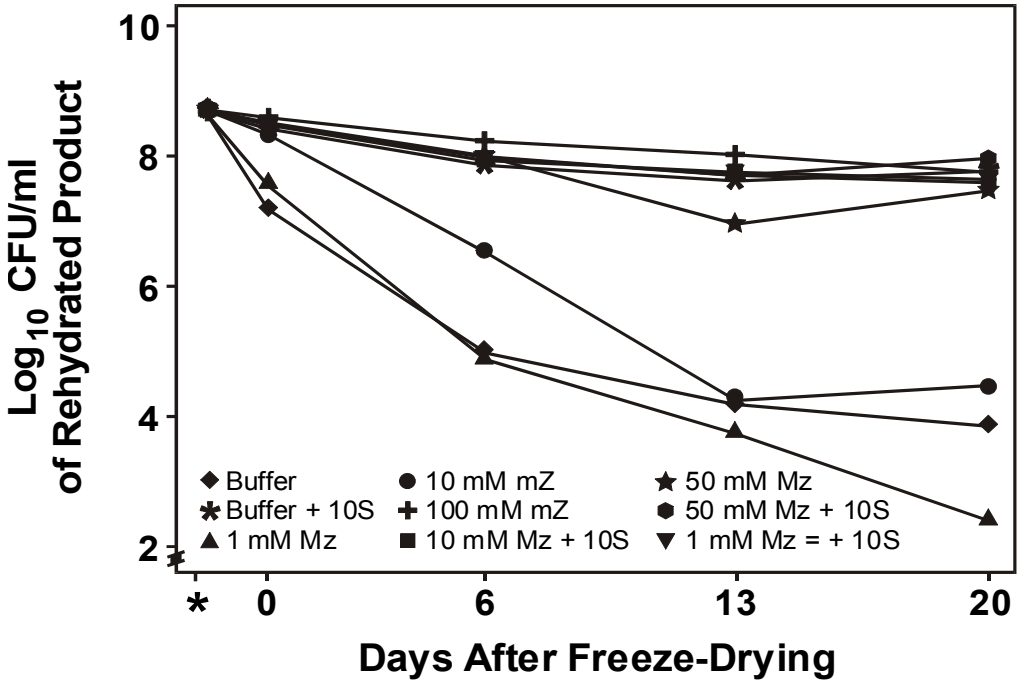


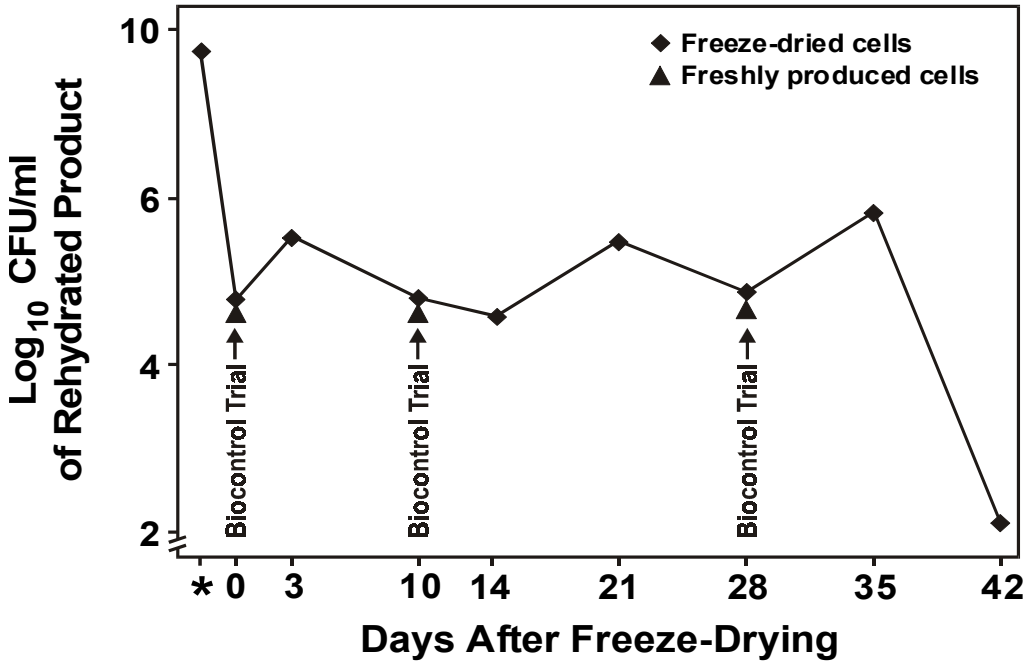
*Fresh CFU/ml count just prior to initiating a two-day freeze-drying process.

Figure 1. Influence of cryoprotectants added to liquid production medium in shake-flasks on the survival of freeze-dried biomass of FHB antagonist *Cryptococcus nodaensis* OH 182.9 stored at 24°C.



*Fresh CFU/ml count just prior to initiating a two-day freeze-drying process.

Figure 2. Influence of adding various concentrations of melezitose (Mz) and 10% skim milk (10S) to shake-flask-produced, washed biomass of OH 182.9 on the viability of freeze-dried cells stored at 24°C.



*Fresh CFU/ml count just prior to initiating a two-day freeze-drying process.

Figure 3. Survival of freeze-dried biomass of FHB antagonist Cryptococcus nodaensis OH 182.9 amended with 25 mM melezitose and 1% skim milk after production in a 100 L fermentor and storage at 4°C.

USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT 2: 2002 FIELD TESTS OF ANTAGONIST AND ANTAGONIST/ FUNGICIDE MIXTURES

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OBJECTIVE

Determine the effect of FHB antagonists, antagonist mixtures, and mixtures of antagonists and fungicides on FHB symptom development in field tests conducted in Illinois and Ohio on two cultivars of winter wheat.

INTRODUCTION

Fusarium head blight (FHB) is an important disease of wheat and barley in humid and semihumid regions of the world (McMullen *et al*., 1997). Research on optimizing methods for selectively isolating, mass producing and utilizing microbial antagonists effective against FHB was initiated in 1997 at the NCAUR in Peoria, IL, in conjunction with The Ohio State University. Several biological control agents remain under consideration for commercial development (Schisler *et al*., 2002). In addition to biological control, promising possibilities for reducing Fusarium head blight include fungicides and resistant cultivars. Combining these control measures may provide levels of control superior to that obtained when employing these control measures individually. Disease control measures utilized in various combinations in field tests conducted in Peoria, Illinois and Wooster, Ohio during the 2002 field season included biocontrol agents, a moderately resistant wheat cultivar, and fungicides.

MATERIALS AND METHODS

A naturally occurring fungicide-tolerant (FT) variant of superior yeast antagonist *Cryptococcus nodaensis* OH 182.9 (wild type (WT)) was selected from cultures grown in one-fifth strength Tryptic soy broth amended with 50 ppm of the fungicide BAS 505 50DF. Inoculum of OH 182.9 WT, OH 182.9 FT and *Bacillus subtilis* OH 131.1 was produced using a semidefined liquid culture medium (SDCL) with a carbon:nitrogen ratio of 11 and total carbon loading of 15 g carbon/liter (Schisler *et al*., 2002). The soft red winter wheat cultivars Pioneer 2545 (susceptible) and Freedom (moderately resistant) were used in both locations. Biomass was harvested from Fernbach shake flasks and applied at the beginning of wheat flowering (Schisler *et al*., 2002). 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 fungicides BAS 505 50DF and Folicur 3.6F were applied at recom-

mended rates singly and in combination with microbial treatments (Tables 1 and 2). Controls were untreated plants and plants treated with buffer/wetting agent only. Corn kernels colonized by *Gibberella zeae* (Schisler *et al*., 2002) 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 to promote FHB development. Heads were scored for disease incidence (presence or absence of disease symptoms) and severity using a 0-100% scale approximately three weeks after inoculation. Heads were then allowed to dry and threshed. 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*=4 in Peoria; *n*=5 in Wooster).

RESULTS AND DISCUSSION

In Peoria, IL, most single and combination treatments reduced FHB symptoms versus at least one control on both susceptible cultivar Pioneer 2545 and moderately resistant cultivar Freedom (Table 1). A combination of yeast OH 182.9 FT and BAS 505 reduced disease severity by 70% compared to the untreated Freedom control. Combined biological control agent or biocontrol agent and fungicide treatments did not synergistically interact to reduce disease to a greater extent than the component parts of the combinations.

In Wooster, OH, on Pioneer 2545, most treatments reduced disease severity compared to the untreated control with the most effective treatments of BAS 505, OH182.9FT+BAS 505, OH182.9FT + Folicur and OH131.1+Folicur reducing disease severity by as much as 64% (Table 2). Treatments did not differ when tested on cultivar Freedom.

Across both locations, the lowest levels of FHB symptom development were found when two and sometimes three of the available control measures of antagonists, fungicides and the moderately resistant cultivar were combined. While methodologies for drying biomass require further development before fresh and dried preparations of OH 182.9 achieve equivalent efficacy, these results indicate that biocontrol products could play a key role in the integrated control of FHB.

ACKNOWLEDGMENTS

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Table 1. 2002 field trial results at Peoria, Illinois: Influence of *Cryptococcus nodaensis* OH 182.9, *Bacillus subtilis* OH 131.1, BAS 505 50DF, Folicur 3.6F and combinations thereof on FHB disease severity and incidence on two cultivars of winter wheat[1]

	Wheat Cultivar			
	Freedom		Pioneer 2545	
Treatment	% Disease Severity	% Incidence	% Disease Severity	% Incidence
Untreated control	4.1	19.6	1.6	7.5
Buffer/tween[2]	3.0	15.0	4.1	15.0
BAS 505[3]	1.4	7.1	1.3	3.8
Folicur[3]	1.6	9.6	3.5	12.1
OH 182.9 WT[4,5]	1.7	9.2	2.5	9.2
OH 182.9 FT[4]	3.0	14.6	0.8	4.2
OH 182.9 WT + BAS 505	1.8	10.8	0.8	2.5
OH 182.9 WT + Folicur	4.0	20.4	3.1	11.2
OH 182.9 FT + BAS 505	1.2	6.2	1.0	3.8
OH 182.9 FT + Folicur	2.2	11.2	2.8	8.3
OH 131.1[5]	2.6	12.9	2.3	9.2
OH 131.1 + BAS 505	1.8	9.6	1.0	3.3
OH 131.1 + Folicur	3.3	16.7	2.5	7.9
OH 182.9WT+OH 131.1	2.4	12.9	2.8	11.2
OH 182.9FT+OH 131.1	2.2	11.2	2.7	10.8
OH 182.9FT+OH 131.1+BAS	3.2	13.8	1.0	3.8
OH 182.9FT+OH 131.1+Fol	3.7	17.9	3.8	12.9
LSD $_{(0.05)}$	1.3	6.0	1.5	4.8

[1]Within a column, the LSD value represents the critical value for separating treatment means at the P#0.05 level. Disease severity values are arc sine transformed.
[2]Weak PO4 buffer (Schisler et al., 2002) and 0.036% Tween 80.
[3]Applied at recommended label rates.
[4]WT = wild type of strain, FT = Fungicide tolerant natural variant of strain
[5]OH 182.9 WT and FT CFU/ml ~ 5 x 10^7, OH 131.1 CFU/ml ~ 1 x 10^8

Table 2. 2002 field trial results at Wooster, Ohio: Influence of *Cryptococcus nodaensis* OH 182.9, *Bacillus subtilis* OH 131.1, BAS 505 50DF, Folicur 3.6F and combinations thereof on FHB disease severity and incidence on two cultivars of winter wheat[1]

	Wheat Cultivar			
	Freedom		Pioneer 2545	
Treatment	% Disease Severity	% Incidence	% Disease Severity	% Incidence
Untreated control	2.6	25.0	20.4	63.8
Buffer/tween[2]	3.7	30.7	16.8	57.9
BAS 505[3]	3.0	25.0	7.4	32.1
Folicur[3]	2.5	25.3	12.9	48.3
OH 182.9 WT[4,5]	2.5	23.7	21.4	65.4
OH 182.9 FT[4]	2.8	28.0	12.1	46.7
OH 182.9 WT + BAS 505	2.1	21.3	12.4	46.7
OH 182.9 WT + Folicur	2.7	22.7	13.4	50.8
OH 182.9 FT + BAS 505	2.2	23.0	8.6	30.4
OH 182.9 FT + Folicur	2.0	18.7	9.7	39.2
OH 131.1[5]	2.3	22.0	14.9	52.5
OH 131.1 + BAS 505	3.3	23.3	12.7	46.7
OH 131.1 + Folicur	2.5	22.7	9.5	42.5
OH 182.9WT + OH 131.1	3.4	26.0	13.2	51.2
OH 182.9FT + OH 131.1	2.3	23.0	15.4	55.0
OH 182.9FT + OH 131.1 + BAS	2.5	22.0	11.3	42.1
OH 182.9FT + OH 131.1 + Fol	2.4	22.3	13.1	52.9
LSD $_{(0.05)}$	NSD	NSD	2.8	8.8

[1]Within a column, the LSD value represents the critical value for separating treatment means at the P#0.05 level. Disease severity values are arc sine transformed.
[2]Weak PO4 buffer (Schisler et al., 2002) and 0.036% Tween 80.
[3]Applied at recommended label rates.
[4]WT = wild type of strain, FT = Fungicide tolerant natural variant of strain
[5]OH 182.9 WT and FT CFU/ml ~ 5 x 10^7, OH 131.1 CFU/ml ~ 1 x 10^8

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 and biological control products that are effective in minimizing the damage from Fusarium head blight 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 and biological 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, new experimental fungicides and biological control agents. Missouri has participated in the Uniform Fungicide Trial since 1998 (Sweets, 2000). Results from the 2002 trial are presented in this report.

MATERIALS AND METHODS

Seven fungicide or biological control treatments and an untreated control were evaluated on 'Elkhart' and 'Pioneer variety 2540' soft red winter wheats at the Bradford Research Center, near Columbia, MO. 'Elkhart' and 'Pioneer variety 2540' were drilled directly into soybean stubble on 12 Oct 01. 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 pre-plant 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 14 May 02. Plots were rated for foliage diseases on 28 May 02. Ratings were done as estimates of the percentage of leaf area covered with Septoria leaf blotch or leaf rust on each of 10 flag leaves randomly collected from each plot. Fusarium head blight incidence and head severity measurements were taken 30 May 02. For harvest the plots were end trimmed and individual plot lengths measured. Plots were harvested on 25 June 02 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 2002 season was warm and dry early; cool and wet as the wheat was flowering and heading; and then hot and dry as the crop matured. Septoria leaf spot and leaf rust did not begin to develop until late in the season. When foliage disease ratings were made, the level of leaf rust was very low across the trial so only Septoria leaf blotch ratings were recorded. Fusarium head blight was also in very low levels throughout the plot at the time Fusarium head blight incidence and severity ratings were made. However, the number of heads showing symptoms of Fusarium head blight seemed to increase as the crop matured. At harvest most plots had noticeable amounts of shriveled, lightweight kernels or tombstone kernels. Barley yellow dwarf was prevalent throughout the trial. Low temperatures in May caused head damage across the plot area. Hail on May 12 damaged plants and heads across the plot area.

The yield of the untreated control was significantly lower than the yields for the seven fungicide and biological control treatments on Pioneer variety 2540. There were no statistically significant differences in yield between the seven treatments and the untreated control on Elkhart. Septoria leaf blotch ratings were significantly higher for the untreated control than the seven fungicide and biological control treatments on both Pioneer variety 2540 and Elkhart. Septoria leaf blotch ratings were significantly lower with TrigoCor 1448 + Folicur 3.6F + Induce on Pioneer variety 2540 and with TrigoCor 1448 + Folicur 3.6F + Induce and Folicur 3.6F + Induce on Elkhart. Although there were no statistically significant differences between the untreated control and any of the seven treatments for percent of Fusarium head blight incidence, percent average head severity, percent field severity or percent of scabby kernels on Pioneer variety 2540, the untreated control was at the high end of the range for each of these. The AMS 21619A 480SC + Folicur 3.6F + Induce treatment tended to be at the low end of the range for percent Fusarium head blight incidence, percent average head severity and percent field severity. The two AMS 21619A treatments had significantly lower levels of DON than the untreated control and the other five treatments. On Elkhart there were statistically significant differences between treatments for percent Fusarium head blight incidence, percent average head severity, percent field severity, percent scabby kernels and DON levels. The two AMS 21619A treatments had consistently low ratings for all of these variables with the combination of AMS 21619A 480SC + Folicur 3.6F + Induce performing slightly better than the AMS 21619A 480SC + Induce. The OH189.2 biological

control agent had the most variation in results. The OH189 treatment had low percent of Fusarium head blight incidence, moderate percent of average head severity and percent of field severity but high percent of scabby kernels and DON levels compared to the other treatments. The untreated control for Elkhart had the highest percent of Fusarium head blight incidence, percent average head severity and percent of field severity and among the highest percent of scabby kernels and DON levels.

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Table 1. Elkhart

Treatment and Rate/A	Yield[1] bu/A	Test Wt. (lb/bu)	SLB Rating[2]	% FHB[3]	% Ave. Head Sev.[4]	% Field Sev.[5]	% Scab Kernels[6]	DON ppm
Untreated control	42.8	61.0	3.62	18.33	12.05	2.12	10.7	2.23
Folicur 3.6F 4.0 floz + Induce 0.125% v/v	42.3	61.6	0.17	8.33	5.08	0.68	7.9	1.68
AMS 21619A 480SC 5.7 floz + Induce 0.125% v/v	45.8	61.8	0.27	1.67	1.17	0.12	7.0	1.18
BAS 505F 50W G 6.4 oz + Induce 0.125% v/v	45.2	61.4	0.52	3.33	1.17	0.23	9.1	1.77
OH 182.9~5 x 10e8 cfu/ml	44.1	61.0	0.18	1.67	2.33	0.23	11.8	2.28
TrigoCor1448~7.5 x 10^12^cfu/A	42.7	60.6	0.53	3.33	2.33	0.23	11.0	1.90
TrigoCor1448~7.5 x 10^12^cfu/A + Folicur 3.6F 4.0 floz + Induce 0.125% v/v	44.4	60.4	0.17	8.33	6.83	0.68	9.6	1.53
AMS 21619A 480SC 3.6 floz + Folicur 3.6F 4.0 floz + Induce 0.125% v/v	45.0	62.1	0.23	0.00	0.00	0.00	7.8	1.10
LSD (P=0.05)[7]	NS	NS	0.86	6.81	4.35	0.62	2.9	0.36

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

[2]SLB rating or Septoria leaf blotch rating based on the average % of flag leaf showing symptoms 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)	SLB Rating[2]	% FHB[3]	% Ave. Head Sev.[4]	% Field Sev.[5]	% Scab Kernels[6]	DON ppm
Untreated control	51.8	60.2	4.40	10.00	4.88	0.84	12.7	2.17
Folicur 3.6F 4.0 fl oz + Induce 0.125% v/v	56.8	60.6	1.12	6.67	4.50	0.45	10.8	1.62
AMS 21619A 480SC 5.7 fl oz + Induce 0.125% v/v	59.4	61.0	1.07	5.00	7.00	0.70	11.5	1.08
BAS 505F 50W G 6.4 oz + Induce 0.125% v/v	60.9	61.1	0.97	6.67	3.60	0.88	9.5	1.72
OH 182.9~5 x 10e8 cfu/ml	54.1	60.5	1.25	3.33	2.33	0.23	10.4	1.72
TrigoCor1448~7.5 x 10^12^cfu/A	53.5	60.4	1.33	5.00	1.55	0.46	11.9	2.05
TrigoCor1448~7.5 x 10^12^cfu/A + Folicur 3.6F 4.0 fl oz + Induce 0.125% v/v	56.1	60.6	0.80	5.00	2.77	0.44	9.8	1.63
AMS 21619A 480SC 3.6 fl oz + Folicur 3.6F 4.0 fl oz + Induce 0.125% v/v	57.6	60.8	1.18	1.67	2.00	0.20	11.0	1.17
LSD (P=0.05)[7]	4.6	NS	0.67	NS	NS	NS	NS	0.26

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

[2] SLB rating or Septoria leaf blotch rating based on the average % of flag leaf showing symptoms 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.

REPORT ON INDUCED RESISTANCE AND FIELD BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT BY *LYSOBACTER ENZYMOGENES* STRAIN C3

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ABSTRACT

The bacterial biocontrol agent *Lysobacter enzymogenes* strain C3 was previously reported to be effective in field tests against a number of fungal pathogens in turfgrass and against rust in common bean. C3, when applied as a chitin broth culture, also inhibited leaf rust (*Puccinia triticina*), spot blotch (*Bipolaris sorokiniana*), and Fusarium head blight (FHB) (*Fusarium graminearum*) on wheat in laboratory and greenhouse experiments. Chitinolysis was one mechanism by which C3 suppressed a number of pathogens. Induced resistance involving a heat stable elicitor also is a mechanism in the control of *Bipolaris sorokiniana* by C3. One objective in this study was to determine if induced resistance could be involved in the control of FHB by C3. Another objective was to assess the potential for using C3 to control FHB under field conditions. Induced resistance was investigated in greenhouse experiments in which chitin broth culture of C3 was compared with a culture heated to 70°C for 20 minutes, and with a distilled water control. The heat treatment was intended to kill C3 cells and inactivate lytic enzymes excreted into the broth, but leave the elicitor intact. All treatments were sprayed onto wheat heads 1 day prior to inoculation with pathogen conidia. Both C3 treatments significantly reduced scab infection as compared to the distilled water check, suggesting that FHB inhibition could be due to induced resistance. A field test was conducted at South Dakota State University in collaboration with Yue Jin to evaluate the interaction of C3 and spring wheat genotypes in the control of FHB. Three treatments (C3 chitin broth culture, Folicur, and water) were applied at anthesis to four cultivars (Alsen, Ingot, Russ, and Norm) that differ in susceptibility to FHB. Plots were inoculated with suspensions of pathogen conidia and misted at night to favor development. Disease severity in three of the four cultivars was reduced by Folicur. C3 significantly reduced FHB severity in 'Russ' (39% infected spikelets) as compared to the control (48% infected spikelets), but had no effect on disease development in the other cultivars. The highest levels of FHB occurred in 'Russ', and thus, lack of C3 efficacy in the other cultivars could be explained in part by low disease development. Differential C3 activity on different cultivars also is a possible explanation. C3 colonized wheat heads and increased in numbers to the same extent on all of the cultivars. This suggests that C3-cultivar interactions may be related to induced resistance rather than antagonism.

INFLUENCE OF CROP ROTATION AND COVER CROP ON FUSARIUM HEAD BLIGHT OF WHEAT

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INTRODUCTION

Fusarium head blight (FHB) is a devastating fungal disease affecting the wheat industry worldwide (Bai and Shaner, 1994, Gilbert and Tekauz, 2002 and McMullen *et al*., 1997). FHB reduces yield and grade and may also contaminate the grain with fungal toxins (vomitoxins) which make grain unfit as food and feed (Gilbert and Tekauz, 2002). Host plant resistance has been considered as the most economical and environmentally-friendly means of disease management. Wheat varieties with sufficient resistance to FHB are not available for cultivation. Fungicide application is the common practice for the control of FHB. Resistance to some fungicides in the populations of FHB pathogen has been reported (Zhou *et al*, 1994). Of concern too, is the fact that chemicals have long-term environmental consequences. Therefore, alternative disease management options are needed to meet the immediate need of wheat growers against FHB. The FHB pathogen overwinters mainly on wheat stubble. We often recommend manipulations of cultivation practices, including crop rotation with non-host crops for disease management, as a good rotation would allow enough time for infested residue to decompose before the next cereal crop is seeded. There is little information available on long-term research data and the benefits of crop rotation with non-host crops on FHB disease management. FHB disease initiation starts with landing of ascospores originating from infected crop residue left on soil. Therefore, a cover-crop would likely act as a barrier to ascospore dispersal onto wheat heads. With these objectives in mind, two long-term experiments were initiated to determine the effect of crop rotation and cover crop on FHB disease management in wheat.

MATERIALS AND METHODS

Effect of crop rotation

The experiment was conducted at Carman Field Research Station, Carman, MB where FHB is known to be endemic. It was initiated in 2001 with the establishment of four foundation crops: canola, wheat, oats and peas on four plots of 10 X 60 M (main plot). The four main plots were separated by a 30 M strip of fall rye. In 2002, the main plots were divided into four sub-plots (10 X 15 M) where canola, wheat, oats and peas were grown. The crops included were canola Liberty Link variety 2663 (transgenic for regular herbicide), oats Riel, peas Carnival and spring wheat CDC Teal. The crops in the rotation were seeded under zero tillage conditions on the stubbles of previous years' foundation crops. In 2002, in the center of the 30 X 60 M barrier strip of fall rye planted in 2001, canola, wheat, oats and peas were seeded as foundation crops in 10 X 60 M plot for 2003, creating 10 M-wide barrier strips between the main plots to avoid inter-plot interference. There are 16 crop rotations in

this trial to be conducted over the years, allowing us to obtain two identical replicates for statistical analysis. The crops in the rotation were treated with appropriate herbicides for weed management. At maturity, the crops were combined and harvested and the stubbles were spread back into plots.

To estimate daily release of ascospores and macroconidia of *Fusarium graminearum*, one rotorod spore sampler (Aerobiology, Nepean, ON) was set up in the center of each main plot. Two other traps were placed outside of the crop plots to determine the background inoculum. The rods were changed every 24 hrs. The rods were stored in the cold room until further analysis.

Three 1-meter row wheat head samples were collected from each wheat plot in the rotation, and the samples were frozen until disease assessment. Percent disease incidence and disease severity were determined. FHB disease index was calculated as: % incidence X % severity/ 100. Before combining the harvest, three samples of six 2.5-meter rows were also hand harvested for yield, FDK and DON analysis.

Effect of cover crop on FHB

This experiment was conducted at the Point Field Research Station, Winnipeg, Manitoba following a randomized complete block design with four replications. The treatments were i) no *Fusarium* inoculum and no cover crop, ii) *Fusarium* inoculum but no cover crop, iii) no *Fusarium* inoculum but cover crop and iv) *Fusarium* inoculum and cover crop.

Trifolium pratense L. (red clover) was established as the cover crop treatment in plots about three weeks before wheat seeding. After seeding clover, all the plots including non-cover crop treatment plots were harrowed once. Usual agronomic practices were performed as and when necessary for crop management. *Fusarium* inoculum treatment plots were inoculated about two weeks before anthesis of wheat by spreading 100g *Fusarium*-infested corn inocula/M^2 (corn spawn). The plots were irrigated with a boom sprayer in the evening for three days after inoculation to provide high humidity for perithecia development. In each plot, two rotorod spore samplers were set up at two levels, one just above the height of cover crop and the other at wheat head height. Four other spore traps were also placed at two said heights outside of the plots to monitor background inoculum levels.

Data on dry matter of wheat, cover crop and weeds were recoded four times during the period of spore trapping. Wheat yield and %FDK were also recorded.

RESULTS AND DISCUSSION

Crop rotation and Fusarium head blight

Overall disease incidence and severity of FHB on wheat was low in 2002. However, results indicated that percent disease incidence on wheat was the highest in pea-wheat crop sequence (19.25%) followed by canola-wheat (11.66%), wheat-wheat (10.33%) and oats-wheat (8.18%), while the percent disease severity was the highest in canola-wheat (43.09%) followed by oats-wheat (22.71%), wheat-wheat (18.35%), and peas-wheat (12.64%) crop rotations (Table 1). The FHB disease index on wheat was in the order canola-wheat (5.02), peas-wheat (2.43), wheat-wheat (1.89), and oats-wheat (1.85) crop se-

quences. *Fusarium* damaged kernel (FDK) or tombstone analysis yielded similar results. The FDK on canola-wheat, peas-wheat, wheat-wheat and oats-wheat were 6.84%, 6.76%, 5.07 and 4.85%, respectively (Table 1). One would not expect more FHB disease when wheat was followed by non-host crops such as canola or peas. Our data corroborate results of other crop rotation trials in Brandon, Manitoba where FHB was also higher when wheat was followed by canola (personal communications with Debbie McLaren, AAFC, Brandon). We also observed higher FHB disease incidence and severity in a wheat plot few blocks away from this experimental plot where canola was grown in the preceding year (personal observation). In Manitoba, *Fusarium graminearum* is the dominant species associated with FHB. It is likely that canola and peas are better substrates to harbor and induce perithecium or ascospore production of *F. graminearum*, and this warrants investigation. Furthermore, canola and peas have a closed canopy and leaf defoliation that likely provided an advantageous environment for the establishment of the pathogen. These results are contrary to accepted theory that a three-year crop rotation with non-hosts including canola, pulses and forage legumes will reduce the risk of spreading and increasing the disease. However, Dill-Macky and Jones (2000) reported that FHB disease incidence was the greatest in corn-wheat rotation followed by wheat-wheat and the least in soybean-wheat rotation. Our one-year data is not enough to enable us to draw any conclusion on the benefit of the crop rotation with non-host crops like canola and peas.

As expected, yield was reduced when the same crop (i.e. wheat-wheat, or canola-canola) was planted in two consecutive years (Table 2). Wheat yield was significantly higher when wheat followed another crop. Bourgeois and Entz (1996) have studied the effect of crop rotation on wheat yields in Manitoba. They found 11% and 8% higher yield of wheat when wheat was grown after peas and canola, respectively. A similar report has been posted on the web by Manitoba Management Plus Program (source- Manitoba Crop Insurance Corporation) (www. mmpp.com/Crop_rotation_page.htm).

Cover crop and Fusarium head blight

Results of this experiment are being tabulated and analyzed at the present time. Yield, FDK and data on spore trapping will be presented at the meeting.

ACKNOWLEDGEMENTS

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Table 1. Fusarium head blight (FHB) disease incidence, severity and index on wheat following different foundation crops.

Foundation crop in 2001	Crop in 2002	%Incidence	%Severity	FHB index	%FDK
Canola	Wheat	11.66	43.09	5.02	6.84
Wheat	Wheat	10.33	18.35	1.89	5.07
Oats	Wheat	8.18	22.71	1.85	4.85
Peas	Wheat	19.25	12.64	2.43	6.76

Three samples of one meter row were randomly chosen from each wheat plot. Data are the mean of three samples. The samples included 67-120 wheat heads. One hundred grams of seed was used to determine percent FDK.

Table 2. Yield of canola, wheat, oats and peas following different foundation crops.

Foundation crops in 2001	Yield ton/ha			
	Crops in 2002			
	Canola	Wheat	Oats	Peas
Canola	**1.36**	1.47	3.19	2.47
Wheat	2.20	**1.01**	3.06	2.63
Oats	2.01	1.30	**2.39**	2.67
Peas	1.77	1.44	2.75	**1.59**

Figures are the mean of three samples from each plot, and the sample area was 1.22 X 2.5M (six rows of 2.5 meter long).

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 initially tested, with promising results. Errors found in the NEXRAD precipitation estimates analyzed during the first and second years of this project with Michigan precipitation data were less pronounced than previous studies, with 96.3% of the precipitation-hours across the state of Michigan during the 1999 and 2000 growing seasons correctly classified and an overall mean bias and mean absolute precipitation differences of -1.6mm and 2.3mm respectively. An initial validation of simulated leaf wetness duration in 6 wheat field sites in Lower Michigan at head height during June and July of the 2002 growing season resulted in mean differences of -0.2 hours and mean absolute differences of 3.4 hours over 116 separate events associated with dew, precipitation, or both. Mean differences and absolute differences for events associated with precipitation only or with precipitation and dew were +1.5 hours and 3.7 hours, respectively, indicating a slight tendency for overprediction. In an effort to better parameterize the wetness duration simulation including evaporation rates of dew and total intercepted precipitation, a field study began in April, 2002 with greenhouse flats planted with spring wheat in individual 10cm pots. Following heading, the flats were monitored with a weighing lysimeter over time, providing estimates of plant evapotranspiration, dewfall, and interception of precipitation. Preliminary results from these data suggest a total nightly dewfall ranging from 0.0-0.3mm. To study rainfall interception, wheat heads at the flowering stage were cut and collected from extra plants in the flats and mounted on 30 cm long, 0.1mm diameter steel wires. The heads and steel 'stems' were in turn mounted on a heavy steel wire frame which held the mounted wheat heads and wire in a fashion similar to that grown in the field. Rainfall interception totals on the order of 0.1mm to 0.3mm were recorded for 11 events. The canopy interception was observed to be associated with the drop diameter of precipitation, with less canopy interception occurring with large droplet diameters and vice versa.

A SECOND GENETIC MAP OF *GIBBERELLA ZEAE*

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ABSTRACT

We recently reported the construction of a genetic map of *Gibberella zeae* made by crossing nitrate non-utilizing (*nit*) mutants of strains R-5470 (lineage 6 from Japan) and Z-3639 (lineage 7 from Kansas). This genetic map is based on 1048 AFLP markers that have been assigned to nine linkage groups. The map contains numerous loci with distorted segregation ratios and two possible chromosome rearrangements between the parental strains. The high degree of polymorphism and high marker density in this linkage map make it very useful for gene mapping studies. It has been used to map several genes related to trichothecene toxin biosynthesis and can also be used for QTL analysis. However, the segregation distortion in this wide cross may limit certain uses. Therefore, we constructed a second genetic map by making a narrow cross between two lineage 7 strains (Z-3639 and PH-1 from Michigan). The Z-3639 strain had a deletion in the *MAT*2 gene, which made it heterothallic. This avoided the segregation distortion associated with *nit* markers. In addition to AFLP markers, we also mapped some nuclear genes using RFLP-PCR. Segregation in the cross is normal, but marker polymorphism is low so more AFLP primer pairs will be needed to saturate the map. Loci common to the two genetic maps will allow identification of the linkage groups and elucidation of the segregation distortion and putative chromosome rearrangements in the original map.

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WHAT PART DOES PROGRAMMED CELL DEATH PLAY IN FUSARIUM HEAD BLIGHT?

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ABSTRACT

Deoxynivalenol (DON) is a strong inhibitor of protein synthesis and also induces programmed cell death (PCD) in animal cells (where it is termed "apoptosis") (Yang *et al*., 2000). Results of genetic manipulation of toxin production by the FHB pathogen, *Fusarium graminearum*, indicate that DON or other trichothecene toxins contribute to pathogen virulence in diseased wheat spikes (Desjardins *et al*., 1996.). DON is known to be toxic to plant cells but the processes leading to cell death have been little investigated. In studying the effects of DON in detached leaves of barley (Bushnell *et al*., 2002), we obtained preliminary results that support the hypothesis that DON induces PCD: 1) DON induced a gradual dissolution of chloroplasts (with concomitant loss of carotenoid and chlorophyll pigments) extending over three to five days before cells collapsed. Mitochondria likewise became degenerate. The tissues also suffered significant electrolyte loss over the 3-5 day period. Thus, cells underwent an ordered sequence of autolytic events leading to death, typical of PCD. Furthermore, the bleached tissues resulting from loss of chlorophyll pigments mimicked lesions of FHB in host spikes; 2) Like DON, cycloheximide and chloramphenicol, well known inhibitors of protein synthesis, induced gradual loss of chloroplast pigmentation and of electrolytes preceding cell collapse in the detached leaf tissues. Cycloheximide and several other inhibitors of protein synthesis have been reported to induce PCD in animal cells (Kochi & Collier 1993); 3) Ca ++ ions, known to be essential for PCD in plant cells (Groover & Jones, 1999), markedly accelerated DON-induced loss of both chloroplast pigments and electrolytes from leaf tissues. Together, these results indicate that DON induces PCD in leaf tissues and, therefore, may do likewise in FHB-infected spike tissues. We are following up these experiments by applying treatments to DON-treated leaves that are known to enhance or inhibit PCD. Further, we will extend these treatments to FHB- infected tissues to obtain cytological and physiological evidence for a possible role of PCD in FHB pathogenesis.

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INFLUENCE OF IRRIGATION FOLLOWING DISEASE ASSESSMENT ON DEOXYNIVALENOL ACCUMULATION IN *FUSARIUM*-INFECTED WHEAT

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ABSTRACT

A field trial was established in 2002 to evaluate the effect of moisture on deoxynivalenol (DON) accumulation in *Fusarium*-infected wheat. The trial was a split-split plot design with four replicates. Main plots were irrigation levels, subplots were wheat cultivar and sub-subplots were inoculation concentrations. Plots of the spring wheat cultivars, Wheaton (susceptible), Pioneer 2375 (moderately resistant) and Alsen (resistant), were inoculated with *Fusarium graminearum* at anthesis (Zadoks growth stage [GS] 61). Two inoculum concentrations (25,000 macroconidia/ml and 100,000 macroconidia/ml) were used to generate different Fusarium head blight (FHB) severities. Mist-irrigation (3.8 mm/day) was applied uniformly to all plots from anthesis until 15 days after inoculation (DAI) (GS 83). Then, the different irrigation treatments were imposed. Half of the plots continued receiving irrigation at the initial rate until harvest (35 DAI) and half received no irrigation. FHB severity was determined 16 DAI as a percentage of symptomatic spikelets for 20 spikes per plot. Sixty heads per subplot in each of two severity categories (FHB < 50%, FHB ≥ 50%) were tagged 16 DAI. Tagged heads (10/severity category) were harvested for each variety at GS 83 (early dough), GS 87 (hard dough), GS 91 (caryopsis hard) and GS 94 (harvest ripe). Kernels were dissected from collected spikes and assayed for DON concentration using gas chromatography / mass spectrometry. FHB severities among inoculation concentration treatments were significantly different ($P < 0.001$). Mean FHB severities were 30% and 70% for Wheaton; 29% and 50% for Pioneer 2375; and 26% and 51% for Alsen at the low and high inoculum treatments, respectively. Data from this experiment should provide an insight into aspects of DON accumulation in wheat. Sequential sampling following disease assessment may help characterize the timing of DON accumulation during an epidemic. The influence of irrigation treatments could aid in the prediction of the DON concentration in grain based on post anthesis weather variables.

SPATIAL PATTERNS OF FUSARIUM HEAD BLIGHT IN NEW YORK WHEAT FIELDS IN 2002

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ABSTRACT

Fusarium head blight (FHB), caused by the fungus *Gibberella zeae*, is a disease of worldwide occurrence that severely reduces the yield, quality, and marketability of wheat. The spatial pattern of FHB incidence was studied in 60 arbitrarily selected winter wheat fields in central and western New York at kernel soft dough stages in June 2002. The fields varied in wheat cultivar, preceding crop, presence of corn residue, and intensity of FHB epidemic. Incidence of FHB was randomly distributed among 60 sampling quadrats in 55 of the 60 fields. Fields with random FHB ranged from trace to 23% in average incidence of FHB and followed bean, corn, oat, pea, sorghum, and soybean. The five fields with aggregated FHB ranged from trace to 27 % in average incidence of FHB and followed bean, corn, oat, and pea. Mean incidence of FHB was not significantly different between fields with and without corn residue, though incidence of FHB and aggregation was highest in two fields sown into standing corn residue without tillage. For eight fields that had corn residue from a corn crop 2 or more years before wheat, there was no evidence of aggregation among all quadrats in a field or among quadrats with corn stubble; also there was no difference in the mean incidence of FHB between quadrats with and without corn residue. Spatial patterns do not supply direct proof of inoculum source, but they suggest likely origins of inoculum that can be confirmed by other observations and experimentation. Based on the predominantly random patterns of FHB in 2002, we suggest that FHB epidemics in rotational wheat fields of New York may be initiated by deposition of spores from diffuse atmospheric inoculum. Over-wintered corn residues are the most prevalent and likely regional source of atmospheric inoculum for FHB in New York.

INFLUENCE OF CORN RESIDUE AND CULTIVAR SUSCEPTIBILITY ON THE ACCURACY OF FUSARIUM HEAD BLIGHT RISK ASSESSMENT MODELS

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OBJECTIVES

To evaluate the performance of forecasting models for Fusarium head blight of wheat in the United States

INTRODUCTION

Disease forecasting models for Fusarium head blight (FHB) of wheat were developed by a cooperative effort among researchers in OH, PA, ND, IN, SD, MO, and KS (De Wolf *et al*. 2000). These forecasting models were based on logistic regression analysis of 50 location-years of disease observations and predict the probability of a FHB epidemic based on environmental variables. Members of the cooperative effort are currently evaluating two models for delivering FHB forecasts at a regional level. For convenience, we will refer to these models as Model 1 and Model 2.

Model 1 uses weather variables observed during a 7-day period prior to flowering. More specifically, these models use duration of time (h) that temperature is between 15 and 30°C and the duration (h) of precipitation (De Wolf *et al*. 2001). This model correctly predicted 70% of 50 cases used to develop the models. Model 1 correctly predicted 78% of non-epidemic years (FHB severity greater than 10%), but correctly classified only 56% of the epidemic years.

Model 2 uses environmental variables observed during the 7-days period prior to flowering and a 10-day period beginning at flower initiation (De Wolf *et al*. 2001). Variables used by this model are the duration (h) of temperature between 15 and 30°C for the 7-day period prior to flowering, and the duration (h) in which temperature is between 15 and 30°C and relative humidity is greater than 90% during the flowering-time period. Model 2 correctly classified 84% of the 50 cases used to develop the model with near equal accuracy for both epidemic and non-epidemic cases.

MATERIALS AND METHODS

Researchers in PA, OH, ND, SD, and IN provided crop growth stage and disease observations from replicated research plots, and this information was combined with hourly measurements of temperature, relative humidity, and precipitation. The presence or absence of corn residue within the plots was noted at each location. The total data set consisted of 23 location years not used in model development.

Models 1 and 2 were evaluated for prediction accuracy with the new data, and model accuracy was compared with previous estimates. Model errors were evaluated for trends that should facilitate application of present models and development of the next generation of forecasting models.

RESULTS AND DISCUSSION

The total number of cases provided for this project from each state included three cases from IN, four from ND, six from OH and five from both PA and SD. Disease severity at these sites ranged from 0 to 74%. Nine of the 23 cases were considered to be epidemics when converted to the binary scale used by the models (FHB severity greater than 10% = 1). Seven of 23 cases had significant levels of corn residue within the plots.

Model 1 correctly classified 15 of the total 23 validation cases correctly (Table 1). All eight errors made by Model 1 were false negatives (incorrectly predicting low disease). In comparison, Model 2 correctly predicted 17 of the 23 cases. Five of the six errors made by Model 2 were false negatives. These prediction accuracies were lower than previous estimates of model accuracy (De Wolf *et al*. 2001). The high rate of false negative errors was of particular concern. However, nearly all of these errors were associated with sites that had high levels of corn residue, or the highly susceptible spring wheat cultivar 'Norm'.

Corn residue

When the models were evaluated with sites with little or no corn residue, Model 1 correctly predicted 11 of the 16 cases, and Model 2 correctly classified 13 of the 16 cases (Table 1). In contrast, both Model 1 and 2 correctly classified only four out of the seven sites that had high levels of corn residue within the plots. The reduction in model accuracy in association with corn residue may, in part, be explained by the high levels of inoculum often associated with this type of residue (Francl *et al*. 1999).

Cultivar susceptibility

Cultivar susceptibility also appeared to affect model accuracy. In this analysis, the highly susceptible cultivar Norm was associated with three of the five errors made by Model 1 for the low residue data set. All three errors were false negative predictions (incorrectly predicting low disease). Similarly, two of the three errors made by Model 2 for the same data set involved Norm, and both errors were false negative predictions. The number of errors that correspond to Norm suggest that highly susceptible cultivars may have an increased likelihood of severe disease that is not considered by the prediction models.

Verification of the prediction accuracy of Models 1 and 2 supports continued deployment in disease forecasting efforts. However, these results indicate that the models may be less accurate when wheat is produced in fields with high levels of corn-residue, or when highly susceptible cultivars are grown. Future modeling efforts will attempt to incorporate potential inoculum source and cultivar susceptibility into the forecast models.

***Table* 1.** Prediction accuracy of FHB forecasting models for 23 location-years not used in model development.

	Model Prediction Accuracy (%)	
Data set	**Model 1**	**Model 2**
Full data set (n=23)	65	74
Location years with low level of corn residue (n=16)	69	81
Location years with high level of corn residue (n=7)	57	57

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EFFECT OF CEREAL RESIDUE BURNING ON THE INCIDENCE AND STRATIFIED DISTRIBUTION OF *FUSARIUM GRAMINEARUM* AND *COCHLIOBOLUS SATIVUS* IN WHEAT AND BARLEY PLANTS

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ABSTRACT

The effect of residue burning on the stratified incidence of *Fusarium graminearum*, *Cochliobolus sativus*, and other pathogens was studied in barley and wheat planted at three locations in Minnesota in 2001. Cereal residues were burned 1-5 days after planting using a propane-powered flame thrower. Subcrown internodes, crowns, nodes, and kernels were excised from 30 plants collected from each plot at maturity. Tissue segments were surface-sterilized, plated onto half strength PDA (pH=5.5), incubated at 20-24°C under flourescent lights (12:12 light:dark) for 6-7 days. The observed colonization of tissues showed that regardless of the host, *F. graminearum* was mostly associated with kernels, whereas *C. sativus* was mostly associated with crowns and the first node. In contrast, *Pyrenophora teres* in barley was mostly associated with the third node. Burning significantly reduced cereal residues ($P<0.01$), and also significantly reduced the survival of *F. graminearum* and *C. sativus* ($P<0.01$). The overall incidence of *F. graminearum* was significantly less ($P=0.05$) in wheat plants collected from burned plots (3.3%) in comparison with those collected from the non-burned plots (5.3%). The effect of residue burning on the incidence of *C. sativus* and *P. teres* was not significant. Our data shows that *F. graminearum*, *C. sativus* and *P. teres* preferentially colonize certain plant parts and that residue burning may provide an option in the management of cereal diseases such as Fusarium head blight.

IDENTIFICATION OF ENVIRONMENTAL VARIABLES THAT AFFECT PERITHECIAL DEVELOPMENT OF *GIBBERELLA ZEAE*

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ABSTRACT

Perithecial development of *Gibberella zeae* and the severity of Fusarium head blight are dependent upon favorable environmental conditions. Information about the conditions favorable to *G. zeae* perithecial development could be useful in predicting wheat head blight epidemics. The development of *G. zeae* perithecia on corn stalk residue was monitored in replicated plots in wheat fields near Wooster, OH (2000) and State College, PA (2001-02). Environmental variables including temperature, relative humidity and rainfall were recorded directly within the plots with an automated datalogger. The moisture levels of the stalks were monitored with electrical resistance sensors, and the duration of stalk wetness (DSW) was recorded. Observations of perithecial development were made every five to seven days, and paired with environmental variables to identify those variables associated with perithecial development. The rate of perithecial production was the greatest in 2000 and the lowest in 2001. An extended period of DSW was associated with an increase in perithecial development at all locations over in the three years of this study. During this increase in perithecial development, the 2000 and 2002 years had 14 and 18 days respectively with average temperatures greater than 15°C. In comparison, only eight days with average temperature greater than 15°C occurred in 2001. Both the 2000 and 2002 locations received a more than 100 mm of rain during the period of rapid perithecial increase. However, only 60 mm of rain were recorded in 2001. In 2002, a decrease in the rate of perithecial production was associated with a six-day period of average temperature less than 7°C but the rate increased again when temperatures increased to greater than 15°C. These results suggest that the number of perithecia produced and their rate of development are influenced by temperature and moisture in a wheat field environment. In the future, information relating weather conditions with critical periods for perithecial developmental may improve the accuracy of wheat head blight forecasting systems.

RELATIONSHIP OF TEMPERATURE AND MOISTURE TO *GIBBERELLA ZEAE* PERITHECIAL DEVELOPMENT IN A CONTROLLED ENVIRONMENT

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OBJECTIVES

To examine the relationship between temperature and moisture on *Gibberella zeae* development in a controlled environment on corn stalk residue.

INTRODUCTION

Fusarium graminearum (*G. zeae*) survives in association with debris of corn, wheat, barley and many cultivated grasses (Parry, 1995). These residues are major sources of inoculum for epidemics of Fusarium head scab of wheat in North America (Francl *et al*. 1999). A better understanding of factors affecting survival and reproduction of *G. zeae* on these residues is important to the development of new management strategies for head scab of wheat and barley.

Past research has shown that temperature and osmotic water potential are two important factors in stimulating growth, reproduction, and sporulation of *G. zeae* (Sung, 1981; Tschanz, 1976). This research, however, has only examined temperature and moisture independently, and has not used crop residues as a substrate. Our objectives were to further examine the effects of temperature and moisture on the development of *G. zeae*, and examine these two important factors together using crop residues.

MATERIALS AND METHODS

Inoculation of stalks – Corn stalks collected near State College, PA once the plants had reached physiological maturity. These stalks were cut into ~30 cm sections, disinfested twice and placed into cold storage (-10°C) until inoculation. At the time of inoculation, the stalks were removed from the cold storage, placed into stainless steel trays, covered with aluminum foil, and disinfested for a third time. A 3mm^2 section of *G. zeae* infested carnation leaf was placed on the stalks, and stalks were incubated for approximately 14 days at 25°C in continuous darkness.

Calibration of Sensors with Stalks – After the stalks were infested, six stalks were arbitrarily selected and paired with an electrical resistance sensor. The relationship between electrical resistance and water content was individually calibrated for each stalk. These calibrations were done by wetting the stalks and taking repeated measurements of electrical resistance and stalk water content as the stalks dried. Regression analysis was used to

develop a calibration curve for each stalk-sensor combination. Sensors were used to monitor and adjust moisture levels within a humidity chamber.

Controlled Environment Chambers – A three-compartment humidity chamber was used to control the three moisture treatments of dry (< 40% RH), moderate (40-80% RH) and wet (>80% RH). A layer of disinfested sand, two stalk-sensor pairs, six infested stalks, and a temperature/humidity probe were placed into each compartment of the humidity chamber. The temperature for each run was held constant at 15, 25, or 30°C. The number of perithecia at previously selected points on the six infested stalks were counted every 5 days for a 20-day period. Developmental stages of the perithecia were also recorded at this time. All treatments were repeated at least 3 times and analysis of variance used to identify differences among treatments.

RESULTS AND DISCUSSION

After 11 to 16 days of incubation, the number of perithicia produced at 15 or 25°C was significantly greater (p = 0.01) than the number produced at 30°C, but there was no significant difference detected between the 15 and 25°C treatments. The number of perithecia was significantly (p = 0.01) increased at the high moisture level compared to the low moisture level treatments. There were no perithecia produced with treatments that included 30°C or the low moisture level (Figure 1). The results indicate the perithecial development of *G. zeae* maybe limited by extended periods of residue dryness or temperatures above 30°C.

In these experiments, perithecia with ascospores were produced at 15°C and 25°C (Figure 2). These results do not agree with previous reports that suggest that perithecial development was limited or did not occur at 15°C (Tschanz 1976). Further experiments are underway to further evaluate temperatures that may limit *G. zeae* perithecial production.

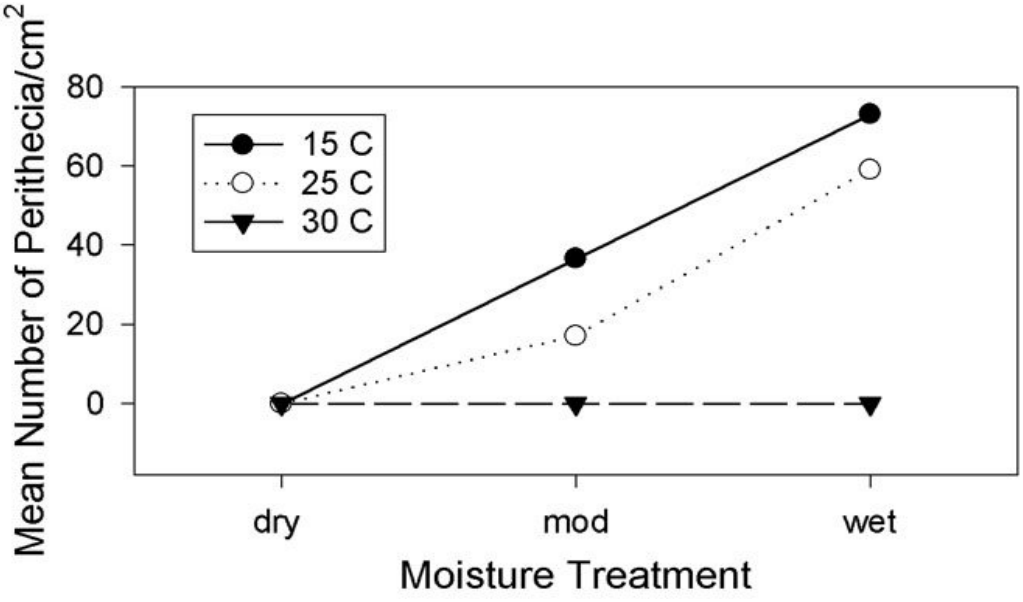
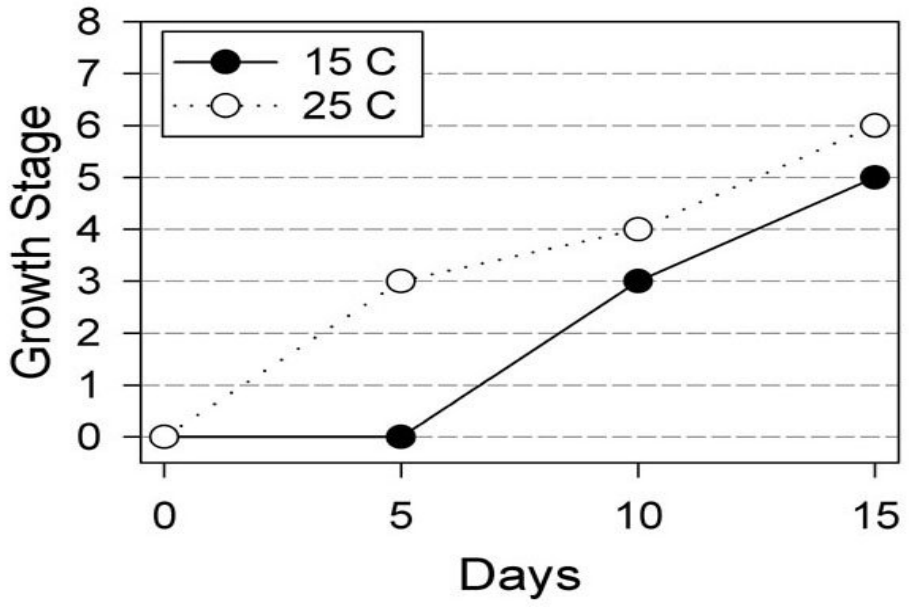


***Figure* 1.** Mean number of *Gibberella zeae* perithecia produced on infested sections of corn stalk incubated at different combinations of temperature and moisture level



Perithecia growth stages: 2 = perithecia just pigmented; 4 = perithecia beginning to form; 6 = asci formed but spore development incomplete; 8 = ascospore development complete

***Figure* 2.** Developmental stages of *Gibberella zeae* perithecia produced at 15 or 25°C at similar moisture levels

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INCIDENCE-SEVERITY RELATIONSHIPS FOR FUSARIUM HEAD BLIGHT ON WHEAT

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ABSTRACT

The relationship between incidence (proportion of plant units diseased) and severity (the amount of plant tissue affected by disease) is a valuable tool that is useful for making disease surveys, assessments, evaluating host resistance, and for determining action thresholds for management decisions. The assessment of severity is tedious and time consuming and may be prone to bias and large experimental error. Therefore, the existence of a quantifiable relationship between incidence and severity greatly facilitates evaluation of disease intensity for estimates of crop damage. These benefits arise because incidence is determined easily, with more accuracy and precision than severity, and with lower cost. Thus, the intent of this study was to determine the relationship between incidence (*I*; percent heads infected) and severity (*S*; percent infected spikelets within infected head) of Fusarium head blight, and determine if severity could be predicted reliably from incidence data. Disease assessment for both incidence and severity were made visually at several sample sites (ranged from 45 to 100 sites per field) in artificially and naturally inoculated research plots and production fields over four years. At each sample site, at least 20 heads were evaluated for incidence and severity. Incidence of infected heads and the average percentage of spikelets with disease on each date for each field in each year were analyzed using linear regression analysis. Ten different, but interrelated, models were fitted to the data and models were compared based on R^2, mean square error, and residual plots. Mean disease incidence and severity varied among data sets, ranging from 28.0 to 75.4% for incidence, and from 9.1 to 28.2% for severity.

The best fitting model was CLL(S) = α + ß CLL(I), in which CLL is the complementary log - log transformation. R^2 values ranged from 0.69 to 0.91. Although there was considerable variability of S for a given I in some years, there was a highly significant relationship between S and I in each year, and the functional relationship was very consistent between years.

SPATIAL ASPECTS OF FUSARIUM HEAD BLIGHT EPIDEMICS ON WHEAT IN OHIO

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OBJECTIVE

To quantify the spatial pattern of Fusarium head blight incidence in wheat fields.

INTRODUCTION

Fusarium head blight of wheat (*Triticum aestivum* L.) caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*) is a limiting factor in wheat and barley production. It reduces wheat yield in many production regions of North America (Bai and Shaner 1994; Parry *et al*.1995; McMullen *et al*. 1997). When environmental conditions are favorable, the disease can cause yield losses up to $1billion (McMullen *et al*. 1997). The analysis of spatial patterns of plant diseases is an important component of epidemiology. Disease pattern is a useful ecological characteristic that helps define a population such as diseased wheat heads (Campbell, and Madden, 1990; Madden, *et al.*, 1995).

Despite the economic importance of Fusarium head blight, there is little information showing the spatial patterns (dispersion) of infected heads and the changes in patterns over time as disease incidence increases. This information would be useful for better understanding the spatio-temporal dynamics of Fusarium head blight. Additionally, data may help us determine efficient sampling procedures that result in precise estimates of mean disease intensity and help determine the proper statistical analysis for comparing treatments.

MATERIALS AND METHODS

Disease Assessments.
Epidemics of Fusarium head blight of wheat were monitored in four fields in 2001 and in six fields in 2002. In each field, three transects with 15 sample points per transect, spaced at 1-m intervals, for a total of $N = 45$ sample points per field, or 10 transects with 10 sample points per transect, spaced at 1-m intervals, for a total of $N = 100$ sample points per field were established. Each sample point was marked with a flag that remained in the field throughout the assessment period. At each sample point, the incidence of scab was recorded for a 1-ft sub-transect across the plant rows.

DATA ANALYSES

Heterogeneity Analyses: Distribution and indices.
The beta-binomial and the binomial distributions were fitted to data on the incidence of diseased heads per transect for each individual field assessment using the computer program BBD, Version 1.2 (Madden and Hughes, 1994). The beta-binomial has two param-

eters, *p*, which is the expected probability of disease (a measure of disease incidence), and θ, a measure of the variation (heterogenity or aggregation) in disease incidence per sample unit. Values of θ greater than 0 indicate aggregation. The binomial has a single parameter representing the probability of disease. A good fit to the binomial distribution is suggestive of a random spatial pattern of disease incidence, while a good fit to the beta-binomial is suggestive of an aggregated (overdispersed) spatial pattern of disease incidence. Standard X^2 goodness-of-fit tests were calculated for each distribution to determine the most appropriate distribution.

For each field and assessment date, the index of dispersion, *D,* was also calculated. *D* is the ratio of the observed variance of incidence among the sampling units to the expected binomial (i.e., random) variance (Madden and Hughes, 1995).

The effect of disease aggregation is to inflate or increase the observed variance above the expected binomial variance. Therefore, values of $D > 1$ suggest spatial aggregation. *D* has a X^2 distribution under the null hypothesis of randomness. A large test statistic and small significance level (<0.05) indicate that one should reject the null hypothesis of randomness (=binomial) in favor of aggregation (overdispersion). Moreover, the so-called C(á) test, which is more specific than the test of *D*, was used to test for overdispersion. Here, the alternative hypothesis is not just overdispersion, but overdispersion described by the beta-binomial.

RESULTS AND CONCLUSIONS

Mean disease incidence per field, an estimate of the expected probability of a head being diseased (*p*), ranged from 0.018 to 0.693, with a median among fields of 0.024 in 2001(Table 1), and from 0.137 to 0.687,with a median of 0.250 in 2002 (Table 2). As anticipated, *p* increased over time within all fields.

The program BBD successfully calculated maximum likelihood estimates of *p* and θ for all the data sets in both years. Where there was a sufficient number of disease classes for the test to be preformed, the frequency distribution of diseased heads could be described by the beta-binomial distribution in over 75% in 2001 and over 60%of the data sets in 2002, and by the binomial distribution in 58% and 40% of the data sets in 2001 and 2002, respectively.

The values of θ ranged from 0.00 to 0.073, with a median of 0.011 in 2001, and from 0.00 to 0.039, with a median of 0.019 in 2002. Estimated θ in over 90% of data sets were greater than 0 (Tables 1 and 2) indicating overdispersion.

The index of dispersion *D*, ranged from 0.88 to 4.50, with a median of 2.22, and from 0.89 to 2.80, with a median of 1.83 in 2001 and 2002, respectively. *D* and θ were both positively correlated with the estimated parameter *p.*

The X^2 test for *D* (Madden and Hughes, 1995), and the C(α) test both had indicated significant heterogeneity in more than 90% of the data sets (Tables 1 and 2).

In conclusion, it was found that heads of wheat infected with scab were aggregated within the wheat fields. Moreover, the degree of aggregation was moderate and increased over time as incidence increased.

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Table 1. Statistics for describing the spatial pattern of the incidence of Fusarium head blight in four wheat fields in Ohio in 2001.

Field	Disease assessment date	Estimated beta-binomial parameters[a]				C(α) test[b]	
		p	se(p)	θ	se(θ)	*z*	P(z)
F 1 (Wooster)	06/11	0.071	0.0092	0.053	0.0179	8.54	<0.001
	06/14	0.081	0.0088	0.039	0.0105	8.32	<0.001
	06/18	0.204	0.0110	0.018	0.0077	6.21	<0.001
	06/21	0.304	0.0097	0.021	0.0089	2.86	0.002
	06/25	0.587	0.0171	0.047	0.0113	14.91	<0.001
F 2 (Wooster)	06/11	0.018	0.0033	0.011	0.0090	3.01	<0.001
	06/14	0.030	0.0047	0.011	0.0084	2.12	0.017
	06/18	0.276	0.0147	0.031	0.0108	6.64	<0.001
	06/21	0.635	0.0162	0.047	0.0108	12.01	<0.001
	06/25	0.693	0.0113	0.013	0.0058	4.75	<0.001
F 3 (Hoytville)	06/26	0.047	0.0145	0.000	-[c]	-1.30	1.000
F 4 (Hoytiville)	06/26	0.623	0.0143	0.073	0.0134	23.20	<0.001

[a] *p*, expected probability of a leaf being diseased, estimated as the mean incidence; θ, aggregation parameter; se(θ), standard error of designated estimated parameter.

[b] *z*, standard normal statistic of the C(α) test; P(*z*): significance level of *z*.

[c] se not defined when θ = 0.

Table 2. Statistics for describing the spatial pattern of the incidence of Fusarium head blight in six wheat fields in Ohio in 2002.

Field	Disease Assessment date	Estimated beta-binomial parameters[a]				C(α) test[b]	
		p	se(*p*)	θ	se(θ)	z	P(z)
F 1 (Wooster)	06/10	0.419	0.0121	0.007	0.0058	1.69	0.045
	06/12	0.523	0.0143	0.017	0.0079	3.99	<0.001
	06/14	0.590	0.0155	0.025	0.0097	5.91	<0.001
	06/17	0.656	0.0165	0.036	0.0120	8.32	<0.001
	06/19	0.687	0.0152	0.039	0.0106	6.83	<0.001
F 2 (Wooster)	06/26	0.320	0.0121	0.000	-c	-0.85	1.000
F 3 (Wooster	06/26	0.363	0.0080	0.008	0.0040	2.65	0.004
F 4 (Wooster)	06/15	0.137	0.0072	0.029	0.0066	8.89	<0.001
	06/18	0.176	0.0082	0.031	0.0069	9.85	<0.001
	06/21	0.253	0.0072	0.008	0.0040	2.66	0.004
	06/24	0.342	0.0084	0.012	0.0045	4.02	<0.001
	06/28	0.426	0.0108	0.029	0.0071	9.97	<0.001
F 5 (Hoytville)	06/27	0.285	0.0078	0.010	0.0043	3.44	<0.001
F 6 (Hoytiville)	06/27	0.265	0.0085	0.017	0.0054	6.07	<0.001

[a] *p*, expected probability of a head being diseased, estimated as the mean incidence; θ, aggregation parameter; se(*), standard error of designated estimated parameter.

[b] *z*, standard normal statistic of the C(α) test; P(z), significance level of z.

[c] se not defined when θ = 0.

EFFECT OF WHEAT FLORAL STRUCTURE EXTRACTS AND ENDOGENOUS COMPOUNDS ON THE GROWTH OF *FUSARIUM GRAMINEARUM*

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INTRODUCTION

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum,* has become a wide spread problem in the United States with the increased use of reduced tillage practices (2). Infection of the florets causes sterility, poor seed fill, reduced seed quality and contamination of grain with mycotoxins (2). Since FHB severity levels are higher when wet weather coincides with wheat anthesis in the field and when anthers, rather than emasculated spikelets, are inoculated in the greenhouse, it is presumed that anthers are the common route of entry into the plant (2,7). These findings led to the theory that there may be compounds in anthers that stimulate growth of *F. graminearum*. The compounds thought to be responsible for stimulation were choline acetate and glycinebetaine (4,7-9). *F. graminearum* has been shown to possess separate constitutive high-affinity transport system that is specific for both choline and betaine, indicating that choline and betaine may be specifically utilized by *F. graminearum* (5,6). These compounds may play a role in pathogenesis of *F. graminearum* or reaction of wheat cultivars to the pathogen.

OBJECTIVES

The first objective of this study was to examine the effect of choline and betaine on spore germination and hyphal elongation of *F. graminearum*. The second objective was to determine the relationship between fungal hyphal growth and extracts of different floral structures from nine wheat genotypes with varying reactions to *F. graminearum*.

MATERIALS AND METHODS

Rate of hyphal radial growth of three *F. graminearum* isolates was measured on water agar and 2% dextrose agar amended with 10nM to 1000µM stock solutions of choline chloride or betaine hydrochloride in two separate experiments. The control was unamended water agar and dextrose agar.

Ascospore or macroconidia germination was evaluated on glass slides covered with a 10µM, 100µM, and 1000µM layer of choline, betaine, or an equal molar mixture amended agar. Unamended agar was the control.

Nine genotypes were selected based on differences in mean FHB severity and incidence from the 1999 Uniform Winter Wheat FHB Screening Nursery (1), (Table 1). Mean incidence and severity were based on seven field locations across six states in the United States and

one nursery in Ontario, Canada. Plants were grown in the greenhouse and spikes were collected when one floret had extruded anthers. Extracts from anthers, paleas or lemmas were combined with water agar and rate of hyphal growth of two *F. graminearum* isolates were measured.

The percentage of increased growth compared to the unamended control was calculated for the floral part extracts. Analysis of variance (ANOVA) was conducted using the general linear model in MINITAB software package for rate of hyphal extension, percentage of germinated spores and percentage of increased growth compared to the unamended contol.

RESULTS

The three *F. graminearum* isolates had significantly different ($P = 0.05$) growth rates, but growth rate of an isolate was constant across repeats of experiments, with an average radial growth rate of 0.35 - 0.64 mm/hr on water agar and 0.35 - 0.54 mm/hr on dextrose agar. Choline had a significant ($P = 0.05$), but relatively small, effect on radial growth on water agar, but not on dextrose agar at concentrations from 10nM to 1000nM compared to the unamended control by 72 hours after plating in the first experiment. However, this small effect was not observed in the second experiment at concentrations ranging from 10µM to 1000µM.

Betaine had a significant ($P = 0.05$), but relatively small, effect on radial growth on water agar, but betaine did not affect growth on dextrose agar at concentrations from 10nM to 1000nM compared to the unamended control by 72 hours after plateing in the first experiment. In the second experiment, betaine did not significantly affect radial growth on the 10µM to 100µM amended agar, although there was inhibition of hyphal growth of all isolates at the 1000µM concentration compared to the unamended control of both agars in the second experiment.

Likewise, the equal molar mixture of choline and betaine significantly ($P = 0.05$), although only slightly, affected the radial growth in concentrations ranging from 10nM to 1000nM in the first experiment. In the second experiment, the equal molar concentrations of choline and betaine significantly ($P = 0.0001$) inhibited hyphal growth at the 1000µM concentration compared to the unamended control of both agars, but had little effect on hyphal growth with concentrations ranging from 10µM to 100µM.

Ascospores and macroconidia germinated readily on unamended water and dextrose agar with 99% germination 24 hours after plating. Germination of ascospores and macroconidia were not significantly ($P = 0.05$) affected by 10, 100, and 1000µM concentrations of choline, betaine, or an equal molar mixture when compared to the unamended control plates over a 24 hour period (data not presented).

Hyphal growth was not significantly affected ($P = 0.05$) by anther, palea, or lemma extracts from the resistant or susceptible genotypes when compared to the unamended control (data not presented), or when growth rate was expressed as a percentage of the control.

CONCLUSIONS

Macroconidial germination has not been shown to be enhanced by choline or betaine (3,7). This study agrees with these findings and also shows that ascospore germination was unaffected. Germination of ascospores or macroconidia appears to be unaffected by the presence of choline or betaine.

Various *in vitro* studies have found that choline, betaine and equal molar mixtures of concentrations ranging from 0.1µM to 1mM stimulated, inhibited, decreased hyphal branching, increased hyphal extension and had no affect on specific growth rate of hyphea of *F. graminearum* (3,7,9,11). In the current study, radial growth was found to be enhanced by low concentrations of choline on water agar, which agrees with previous findings (10), but this enhancement is not believed to be biologically significant. Regardless of the different isolates of *F. graminearum* or different protocols used, results of this study are in agreement with the findings of previous studies (3,11) in that levels of choline or betaine in floral parts probably have little if any stimulatory effect on growth of *F. graminearum*.

Results of this study did not show a correlation between FHB resistance reaction of nine wheat genotypes and rate of radial growth of two *F. graminearum* isolates on agar amended with anther, palea or lemma extracts. These findings are in partial agreement with previous findings (3). Therefore, endogenous compounds of wheat floral structures are not thought to be important in resistance reactions of wheat genotypes.

Our results indicate that endogenous compounds in wheat floral structures do not enhance their colonization by *F. graminearum* and that putative compounds in floral structures have no substantial role in resistance to *F. graminearum*.

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A PHENOLOGY-BASED PREDICTIVE MODEL FOR FUSARIUM HEAD BLIGHT OF WHEAT

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OBJECTIVES

Our goal was to establish a model to account for anther extrusion period that could be used to calculate probabilities of Fusarium head blight incidence as the window of opportunity for infection advances from beginning of anther extrusion to complete anther fall. The model combines several elements of meteorology, biology of *G. zeae* and wheat phenology.

INTRODUCTION

Fusarium head blight (FHB), incited by a fungus (*Gibberella zeae* (Schwein.) Petch), is an important disease affecting wheat. Fusarium head blight fungus survives in crop debris and windborne or splashed spores infects the heads during flowering. Humid weather and moderate temperatures are favorable for infection (Sutton, 1982). Fusarium head blight can be devastating to yields if a large proportion of plants are infected. The fungus also can produce harmful mycotoxins which depreciate grain value (McMullen *et al*., 1997).

Despite the absence of reliable data for comparing FHB intensity amongst different years in southern Brazil, it is generally accepted that the disease has been more severe in the last decade. It is believed that a combination of 'El Niño' years and of abundant sources of inoculum are the cause for such high intensity of FHB in the wheat fields in southern part of Brazil (Fernandes, 1997).

The relative narrow susceptible phase of wheat and the strong dependence on climatic requirements for infection success makes the pathosystem suitable for modeling. A realistic approach should account for availability of susceptible tissue besides weather driven pathogen dynamics.

Process-based models of crop growth and development are exciting tools emerging from the on-going information technology. Models can improve our understanding of the complex processes underlying wheat production including Fusarium head blight management. Their analytical power can help deal with difficult tasks such as predicting the incidence of Fusarium head blight on wheat.

MATERIAL AND METHODS

Brief Model Description

Model Framework. To develop a wheat simulation model into an Object Oriented environment we started with a small generic crop model. This model is available at

www.icasanet.org/modular. The model contains three main modules: Soil, Plant and Weather (Jones, *et al*., 2001). The model originally written in FORTRAN was converted to JAVA and followed the principles of Object Oriented approach. The modular structure was used to depict classes and provide them with the right data behavior. One of key features of a modular approach is that models should relate to the real world components or processes.

Wheat Simulation Model. Wheat simulation, a process oriented model which is based on daily time-steps considers 1 m^2 area of wheat crop. It simulates the dynamics of wheat biomass through inputs of historical records of weather data, cultivar coefficients, and soil properties. The wheat simulation model includes growth, phenology and water balance routines.

The plant growth module computes crop growth and development based on daily values of maximum and minimum temperatures, radiation and the daily value of two soil water stress factors, deficit and surplus. This module also simulates leaf area index (LAI), which is used in the soil water module to compute evapotranspiration.

Crop development is simulated based on thermal time required to reach specific growth stages. The model also accounts for simulating the dynamics of heading emergence including extrusion of anthers (flowering). State variables and simulated processes allow accounting for incidence of Fusarium head blight.

The water budget in the model includes precipitation, irrigation, runoff, water infiltration in the soil profile, crop transpiration, and evaporation. Crop evapotranspiration is determined from leaf area index.

Fusarium head blight Simulation Model. A module was developed to simulate head infection through inputs of local weather data. The first anthers were empirically set to be extruded on day five after heading emergence. Flowering dynamics was handled as a cohort of heads exhibiting anthers resulting from simulation and assumed to be a potential infection site.

Predictive modeling tries to match the rules (models) for guessing (predicting) the Fusarium head blight incidence from weather variables. Stepwise multiple regression procedures were used to determine the prediction rules. The weather variables examined were solar radiation, maximum temperature, minimum temperature and precipitation.

M*odel Inputs*

Input data such as location, soil, crop and management files are required to run the model. An advanced user-friendly interface allows users to easily manipulate input files, create simulations, execute single and batch run simulations and produce text and graphical reports. The data base was implemented using PostgreSql and Interbase for remote and local access, respectively.

RESULTS AND DISCUSSION

The model predicted reasonable well the phenological stages of the wheat cultivar BR23, especially at the flowering stage, except at very early or very late sowing dates. In general, the date for heading stage (50% heads emerged) was predicted within an interval of two-three days around the observed date.

Findings from field experiments revealed that daily number of anthers per head varied significantly. In general, in a single head flowering lasts from five to eight days. As a contrast, in a group of heads the course of anther extrusion last from 14 to 18 days. The peak of number of extruded anthers was observed at six to eight days after the beginning of flowering.

Growth chamber experiments showed that anther extrusion was responsive to temperature. Rate of extrusion increased proportionally to temperature increments (Vargas *et al*., 2001). This conceptual model was translated to the predictive model.

The model attempts to predict the probability of Fusarium head blight based on the weather variables occurring around flowering. The weather variables inserted in the model are rain greater than 1mm and maximum temperature. An ascospore cloud is formed every day rain is greater than 1 mm. The ascospore maturation rate is reduced at temperatures lower than 20°C. Daily ascospore cloud values are summed in simple 4-day moving periods.

If anthers are present infection occurs during a rain event greater than 1 mm. The proportion of infected heads depends on the time course of anther extrusion and the size of the ascospore cloud.

In the field, the level of Fusarium head blight varied among experiments. The disease intensity was dependent on weather conditions during the flowering stage. Sowing date could alter flowering date of a cultivar in a particular year causing great differences in disease levels. As a consequence, fields with distinct sowing date can have a different level of disease (Figure 1). Thus, to predict Fusarium head blight incidence the simulator first needs to be very accurate in predicting growth stages of wheat. Any slight deviation from the target (susceptibility window) may cause a considerable error in predicting Fusarium head blight incidence. Further studies on wheat phenology are being planned. Hopefully, as more data becomes available it will be possible to improve the model performance.

The Fusarium head blight predictive model predicts the probability of disease occurrence; it does not predict level of disease severity. The predictive Fusarium head blight model predicted moderate to high levels of incidence for a majority of simulated wheat fields with sowing dates in the period of 1998 to 2002, at Passo Fundo, RS, Brazil. This moderate to high incidence was probably due to the high frequency of rainy days during the flowering stage of wheat.

In the year 2002, for example, a “El Niño” event occurred. In southern Brazil a “El Niño” year means precipitation above normal at spring time coinciding with heading stage of wheat. Thus, diseases such as tan spot, glume blotch and Fusarium head blight are usually severe

in “El Niño” years. As a consequence during such years wheat yields are degraded (Cunha, *et al*., 2001). Besides, rainfall around harvest time may contribute to a lower test weight of wheat which penalizes profits.

Plans for the future

“As is” the predictive model is a convenient tool for researchers, teachers and students to use in the study of wheat development and incidence of Fusarium head blight. The model was developed using up to date technology for Web deployment. Therefore, it can be shared over the Web with a wide variety of potential users.

So far, this predictive Fusarium head blight model has been developed and tested using the Brazilian wheat cultivar BR23 and historic weather data from Passo Fundo, RS, Brazil. Thus, model outputs should be interpreted cautiously avoiding extrapolation to other cultivars and regions before further testing and validation. Nevertheless, the model is suitable for general research and educational purposes. Hopefully, as more data becomes available, it can be easily modified to accommodate different cultivars and regions.

In the meantime, aiming to reduce the error in estimating the “window of susceptibility“ model is being modified so that the user can enter the date(s) for any growth stage(s) before flowering. Finally, the modular structure adopted in the model construction should facilitate adding new components, as they become available, to expand model capability.

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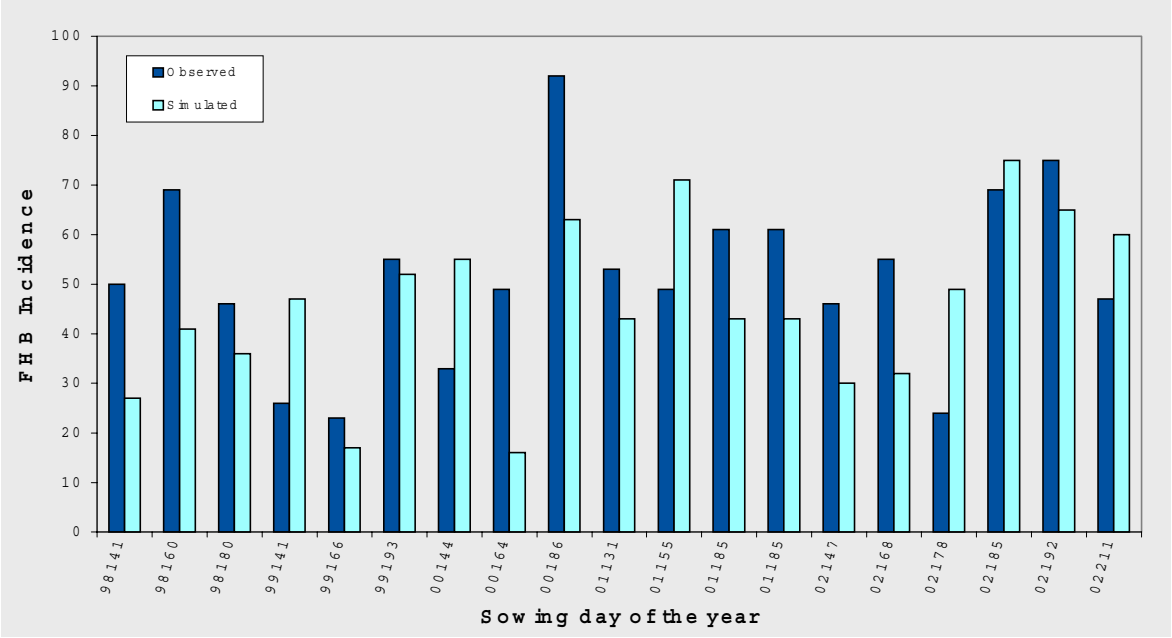

Figure 1. Simulated and observed FHB incidence for the wheat cultivar BR 23 at Passo Fundo, RS, Brazil.

AFLP-ASSISTED GENETIC CHARACTERIZATION OF *FUSARIUM GRAMINEARUM* ISOLATES FROM CANADA

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INTRODUCTION AND OBJECTIVES

Fusarium graminearum (Teleomorph - *Gibberella zeae*) is one of the most important plant pathogens, which causes fusarium head blight (FHB) in economically important cereals such as wheat, barley and corn. FHB has caused high losses in yield and grain quality, thus affecting every aspect of the grain industry (Gilbert *et al*., 2001. Mycopathologia 153: 209 – 215). Hence, to combat the disease, chemical and biological control, and breeding for resistance have been the methods of control. But, in the process of developing the various control measures, we should also bear in mind the natural ability of the pathogen to evolve over these control strategies. For example in China, resistance of *F. graminearum* to benzimidazole and cabendiazime fungicides has been reported (Zhou *et al*. 1994. Journal of Nanjing Agricultural University 17(3): 33 - 41). Most of our current resistance to FHB has been traced back to only a few sources (Van Ginkel *et al*., 1996. Plant Dis. 80: 863 – 867) and hence, it is possible that *F. graminearum* could adapt to resistant varieties. Also, this adaptation of the pathogen could extend to chemical and biological control. A better understanding of the genetic structure would shed more light on the biology of the pathogen, which would be the key in controlling the disease. DNA-based molecular genetic characterization of the pathogen with molecular marker techniques is better suited for such studies. Among the various marker techniques available, AFLP fingerprinting is more accurate and produces a larger number of polymorphic bands with high reproducibility, than the other techniques such as the slow and laborious RFLP and the less reproducible RAPD. The objectives of this study were to determine: 1. the genetic diversity of the *F. graminearum* isolates; 2. the geographical and host specificity of the isolates; 3. the correlation between genetic structure and toxin production.

MATERIALS AND METHODS

Fifteen isolates of *F. graminearum*, isolated from different geographical locations (Alberta, Manitoba, Ontario and Saskatchewan) and hosts (barley, corn, weed and wheat), were used for the AFLP analysis. The origin, vegetative compatibility group, aggressiveness and levels of toxin production of the isolates are presented in Table 1. The mycelia grown in potato dextrose broth were frozen and ground in liquid nitrogen, and the genomic DNA isolated with the help of the CTAB method. The standard protocol, (Vos *et al*., 1995. Nucleic Acid Res. 23: 4407 – 4414), with a few modifications, was used for the AFLP analysis. The genomic DNA was cut with *Eco RI* and Mse I restriction enzymes, ligated with E*co RI* and *Mse I* adapters, preamplified and amplified with five set of selective primers during the AFLP analysis (Table 2). The polymorphic bands were viewed with the help of silver nitrate stain-

ing (Promega, Madison, WI). The scored polymorphic bands were analyzed using unweighted pair group mean analysis (UPGMA), in SAHN program of NTSYS- pc 2.1 software package (version 2.1; Exeter Software, Setauket, NY), which was used for the cluster analysis and the construction of the dendrogram.

RESULTS AND DISCUSSION

The AFLP analysis of the 15 isolates of *F. graminearum* yielded 105 polymorphic bands from the five primer sets used. Two isolates, FG8 and FG14, both isolated from corn in Ontario and belonging to the same VCG-E (Table 1), showed great similarity and the least genetic distance (Fig 1). FG7, an isolate from corn in Ontario, with significant aggressiveness, produced four toxins, namely DON, 3-ADON, 15-ADON and NIV, and was seen as a distinct sub-branch in the dendrogram (Fig 1). The geographic location and the hosts from which the isolates were obtained seem to have had a mild influence on the clustering of the isolates, as they seem to form small clusters based on either their originating geographic location or host (Fig 1). Among the isolates that were all isolated from wheat, FG2 isolated from winter wheat was distinct from the other two isolates, FG1 and FG4, isolated from red spring wheat, which shared higher genetic similarity (Fig 1). It is interesting to note that isolates FG1 and FG4 came from Alberta and Manitoba (Table 1), respectively. Among the isolates from Ontario that formed small clusters in the dendrogram, isolates FG5 and FG10, isolated from winter wheat, were distinct from the other isolates from corn and barley (Fig 1 and Table 1). Isolate FG13, which was isolated from a weed in Saskatchewan, formed a distinct sub-branch and thus was distinct from the isolates from Ontario in the other branch of the cluster (Fig 1).

AFLP analysis showed genotypic diversity between the *F. graminearum* isolates with reference to their vegetative compatibility groups, and this supported tan earlier work (Bowden and Leslie, 1992. Exp. Mycol. 16: 308 – 315), which showed genotypic diversity among G. zeae isolates based on their VCG. The branching of the isolates in the tree was more influenced by the VCG and the levels of toxin production of the isolates (Fig 1). This was clearly seen in the analysis, as isolates FG8 and FG14 belonging to the same VCG- E showed the least genetic distance, and isolate FG7 with the highest levels of all the four toxins, formed a distinct branch in the large upper cluster of the tree (Fig 1). The analysis showed a weak host or geographic specificity among the isolates, as observed by the weak clustering of the isolates in the tree based on their geographic location or the host from which they were isolated. This supported an earlier work (Van Eeuwijk *et al*., 1995. Theor. Appl. Genet. 90: 221 – 228), which showed non-specificity of resistance in wheat with European strains of *F. culmorum, F. graminearum and F. nivale.* The low geographic specificity of the data, as indicated by isolates FG1 and FG4 isolated from wheat in Alberta and Manitoba, respectively clustering together, seems to suggest the movement of the pathogen to new areas. The distinct branching of FG2 (from winter wheat) from the cluster of FG1 and FG4 (from spring wheat), seems to suggest that they produce and release spores at different times in a season to coincide with the availability of the susceptible growth stage of the respective host (winter or spring wheat) for infection and colonization.

It would be interesting to find the lineage of these 15 isolates from Canada that come from different geographical locations and different hosts. The *F. graminearum* clade includes seven distinct lineages (O'Donnell 2000. Proc. Natl. Acad. Sci. 97: 7905 – 7910), with the isolates from the USA falling within lineage 7. Therefore, it would be useful to find the lineage of the Canadian isolates and to check whether they shared any similarity with their US counterparts. Isolates of *F. graminearum* that produce the FHB toxins belong either to the DON chemotype or the NIV chemotype (Marasas *et al*. 1984. The Pennsylvania State University Press, University Park, PA.). Most of the isolates from the USA belong to the DON chemotype (Anne Desjardins - Presentation at the CPS Annual Meeting, Waterton, Alberta, 2002). They produce small amounts of 3ADON and 15ADON. The NIV chemotype produce DON at only less than one percent of NIV (Anne Desjardins- personal communication). But two of our isolates, FG7 and FG10, produced significantly very high levels of DON when compared to NIV, especially FG7, which produced 249 ppm of DON and 1 ppm of NIV. It would be very interesting to genetically characterize these isolates, which would throw more light on the biochemistry of toxin production and also help us to understand whether the pathogen is going through a process of evolution to become a more aggressive form!

ACKNOWLEDGEMENTS

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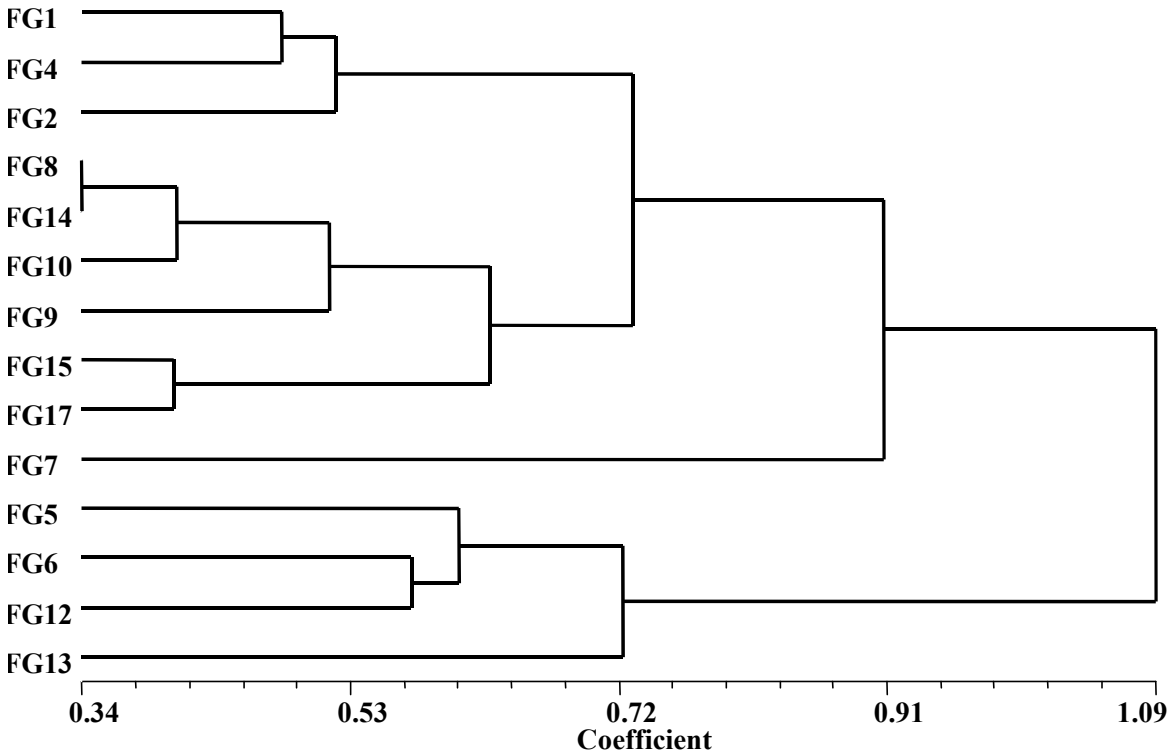

Figure 1. Dendrogram from UPGMA of AFLP data of *F. graminearum*

***Table* 1.** Origin, VCG, aggressiveness and toxin production of *F. graminearum* isolates.

DAOM #	Host	Location[$]	VCG[†]	Aggressiveness		T1*	T2*	T3*	T4*
				SFI[‡]	Spray[£]				
213295 (FG1)	Wheat	AB	J	37	64.9	0.2	0	0.5	0
177409 (FG2)	Wheat	ON	C	17.2	56.4	88.3	8.6	0	0
192132 (FG4)	Wheat	MB	H	32.8	69	2.3	0	5.9	0
177406 (FG5)	Wheat	ON	A	33.2	56.1	10	0	13.1	0
178149 (FG6)	Barley	ON	L	33.4	56.2	27.8	0	24.7	0
170785 (FG7)	Corn	ON	K	31.5	43.6	249	11.7	3.1	1
180378 (FG8)	Corn	ON	E	30.5	45.9	20.5	0	16.2	0
180379 (FG9)	Corn	ON	F	36.3	60.2	80.9	2.8	0.4	0
177408 (FG10)	Wheat	ON	B	29	53.4	53.1	0	44.6	0.3
180377 (FG12)	Corn	ON	M	26.8	39.1	35.3	0	26.9	0
213384 (FG13)	Weed	SK	I	24.8	51	12.1	0	15.4	0
180376 (FG14)	Corn	ON	E	36.9	67.6	21.7	0	17.3	0
192130 (FG15)	Wheat	MB	D	32.2	52.2	6	0	11.6	0

$Location: AB- Alberta; MB- Manitoba; ON- Ontario; SK- Saskatchewan

†Vegetative Compatibility Group

‡Single Floret Inoculation with macroconidia (10µl of 50,000spores/ml)

£Spray inoculation with macroconidia (2 – 3 ml of 50,000 spores/ml)

*T1-DON- Deoxynivalenol; T2- 3ADON- 3-acetyl DON; T3- 15ADON- 15-acetylDON; T4- NIV- Nivalenol (in ppm)

(Modified from Gilbert et al., 2001. Mycopathologia 153: 209)

Table 2. List of selective primers used for the AFLP analysis.

Primer Set	*Eco*R I end	*Mse* I end
1	AC	A
2	AC	T
3	AA	T
4	AA	AT
5	TG	TT

ASSESSMENT OF THE DIFFERENTIAL ABILITY OF *FUSARIUM* STRAINS TO SPREAD ON WHEAT AND RICE

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ABSTRACT

In order to understand gene function related to pathogenicity we are evaluating the abilities of different strains of *Fusarium* to spread on the hosts and conducting genomic analysis of these interactions. Several strains selected from each of eight known phylogenetically distinct lineages of *Fusarium graminearum* were tested for their ability to spread on Norm, a susceptible cultivar of wheat, after inoculation of a single central floret. Similar studies were also conducted using strains belonging to other *Fusarium* species namely, *F. cerealis, F. pseudograminearum, F. culmorum* and *F. lunulosoporum*. All these strains were found to differ significantly in both their ability to spread within the wheat head as well as the type and amount of mycotoxins they produce. A few of the *F. graminearum* strains were also tested for their ability to infect rice panicles. These strains caused necrosis in rice, but mycotoxin production was not detected in infected rice florets. Symptom expression, the presence of fungus in each spikelet, as determined by culturing, and mycotoxin concentrations were recorded from inoculated wheat heads and rice panicles 14 days after inoculation. Based on these pathogenicity tests one highly aggressive and one less aggressive strain were chosen for studies conducted with the aim of understanding these variations at the genomic level. cDNA libraries were created by subtractive hybridization to compare mRNA populations from wheat heads inoculated with the two strains in order to identify genes specific to each interaction. Marked differences in the transcript profile of these two interactions was revealed during the initial infection phase.

DEVELOPMENT OF *GIBBERELLA ZEAE* ON WHEAT TISSUE

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease of cereal grains worldwide. In the United States this disease is mostly attributed to infections by the fungus *Gibberella zeae* (anamorph *Fusarium graminearum*). The disease cycle of FHB is a valuable resource when considering control of *G. zeae*. The development of perithecia on wheat residues and the inoculum produced by perithecia have important impact on disease in reduced tillage systems. Our objective is to characterize the colonization of vegetative tissue and the subsequent development of perithecia. All plant tissue and cell types are susceptible to ramification by hyphae of *G. zeae.* However, the colonization of tissues adjacent to cells supporting perithecium formation is especially significant to the development of perithecia. Chlorenchyma tissue of the internodes and parenchyma tissue of the stem nodes are tissues found to directly underlie cells that support perithecium development. Perithecia form through stomates above chlorenchyma of the stem internode and from epidermal cells above the parenchyma of the stem node region. We are also interested in determining whether head infections proceed down the stem or if stem tissue is colonized from independent stem infections. Development of strategies for limiting infection of vegetative tissue is contingent upon understanding the mode of infection. The results of this study will give insights into the disease cycle as well as an understanding of infection pathways.

THE DONCAST MODEL: USING WEATHER VARIABLES PRE- AND POST-HEADING TO PREDICT DEOXYNIVALENOL CONTENT IN WINTER WHEAT

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ABSTRACT

Accurate predictions of deoxynivalenol (DON) concentrations in mature wheat grain are needed at heading for decisions on whether a fungicide application is necessary to control fusarium head blight, *Fusarium graminearum* Scwabe. Our model, now named "DONcast", was developed using weather and DON data from 399 farm fields across southern Ontario, Canada, from 1996 to 2000 (Hooker *et al*., 2002). A web site was launched in 2000 for providing DON predictions (in ìg g^{-1} of mature wheat grain) to growers across Ontario http://www.ownweb.ca/models/public/fusarium/default.cfm?location=none. From 2000 to 2002, DONcast was validated on 121 wheat fields on private farms across Ontario. All parameters of the first DONcast model were reviewed and other variables were considered with the addition of both weather and DON data from 2000 and 2002. DONcast was refined further by considering agronomic influences such as wheat variety, previous crop, and tillage system from all 520 fields between 1996 and 2002. In the refined DONcast model, weather was still important between 7 days before heading and 10 days after heading. In the first period 4 to 7 days before heading, DON generally increased with the number of days with >5 mm of rain, and decreased with the number of days of <10°C. In the second period 3 to 6 days after heading, DON increased with the number of days of rain >3 mm, and decreased with days >32°C. In the third period 7 to 10 days after heading, DON increased with the number of days with >3 mm of rain. Using multiple regression procedures, the refined model accounted for lower concentrations of DON when cool temperatures (mean daily temperatures < 15°C) occurred between 3 and 10 days after heading. Wheat variety susceptibility coefficients from inoculated misting trials were also included in the refined model, along with a variable for the presence of host crop residue on the soil surface at wheat planting (Schaafsma *et al*. 2000). While only one equation is used in each case to forecast DON, the equation is different depending on the situation. In fields where the previous crop was not wheat or corn, the refined model explains 78% of the variation in DON using the equation if rain occurred between 3 and 6 days after heading. If no rain occurred between 3 and 6 days after heading, then another equation is used, which explains 63% of the variation in DON. In fields where the previous crop was corn or wheat, another prediction equation explains 86% of the variability in DON. Using the refined DONcast model, DON concentrations of < 1 ìg g^{-1} were predicted correctly on 46 of 52 fields in 2001 and on all 34 fields surveyed around weather stations in 2002. Concentrations of > 1 ìg g^{-1} were predicted correctly on 9 of 14 fields in 2001, and on 5 of 11 fields in 2002. Accurate predictions of < 1.0 ìg g^{-1} suggests that control strategies may not be warranted, while predictions of 1 to 2 ìg g^{-1} suggests that control strategies may be warranted to improve the grade and marketability of wheat.

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FUSARIUM HEAD SCAB RISK FORECASTING FOR OHIO, 2002

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ABSTRACT

During the 2002 wheat growing season, head scab risk assessment models were used to predict the risk of Fusarium head scab in Ohio. This was the second year 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 14 weather stations located in Ohio, Indiana and Michigan 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 inclusive of the 7 day pre-anthesis plus 10 additional days post anthesis (Model II). 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) due to cool weather that slowed plant development in May. Precipitation events became more frequent during late April and throughout May across the state with most locations reporting up to 32 hours of measurable precipitation during the 7 days prior to anthesis. 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. Of 42 location-anthesis date scab risk probabilities calculated, 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. Only one location (Ft. Wayne, IN) had a moderately high risk prediction for Model I and Model II and another site (Oxford, OH) had a moderately high risk prediction for Model II. 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. 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 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 average incidence of head scab was 4.1% with a range of 0% to 48.6%. Over 75% of the surveyed fields had scab incidence levels below 5%, and only 4% of the surveyed fields had incidence levels above 15.1%. Results of the Scab Risk Assessment Models indicated that they generally predicted the risk of scab correctly for the majority of locations in the state.

PRACTICAL APPLICATION OF FUSARIUM HEAD BLIGHT RISK PREDICTIONS

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Fusarium head blight (FHB), primarily caused by the residue-borne fungus *Fusarium graminearum*, continues to be an important economic problem in the more humid, temperate regions of the US and Canada (Bai and Shaner 1994, McMullen *et al*. 1997). Control of this disease has been difficult, but progress has been made by scientists funded by the U.S. Wheat and Barley Scab Initiative (USWBSI) toward the management of FHB. An important objective of the USWBSI has been the development of adequate disease forecasting systems for FHB.

The most significant purpose of a head scab forecasting system would be to function as an early warning system. If accurate disease predictions could be made prior to floret infection at anthesis, then growers could use preventative disease control options, such as chemical or biological agents, to avert disease and yield loss. Secondly, a timely disease warning would also provide valuable time for farmers, grain handlers and food processors to deal with the prognosis of disease and the potential for mycotoxin contaminated grain by establishing the necessary infrastructure to appropriately test for, and manage, damaged grain.

Last year in a presentation at the 2001 National Fusrium Head Blight Forum, Dr. Len Francl reviewed the various types of forecasting systems available for small grain diseases (Francl 2001). He discussed two FHB forecasting systems that are now being tested. In Ontario, Canada, Hooker *et al* (2002) have developed a model that utilizes weather data pre anthesis and weather forecasts post anthesis to predict deoxynivalenol levels in harvested grain. In Ohio, De Wolf *et al*, (2001) have developed a disease forecasting system based on risk assessment. Generally, risk assessment models estimate the probability (i.e., risk) of an undesirable event occurring at a given location and time (Teng and Yang, 1993). FHB appears well suited for risk assessment modeling because of the severity of epidemics, compound losses from mycotoxin contamination and yield loss, and the relatively narrow time periods of pathogen sporulation, inoculum dispersal, and host infection (De Wolf *et al*. 1999, Francl *et al*. 1999).

Our main objectives were to develop relatively simple models using readily accessible weather variables that would be applicable over a large geographic area including spring and winter wheat areas. Secondly, to meet the immediate need of farmers we needed to develop a forecasting system in as short a time as possible. To meet this goal we used historic disease data and weather records for model development. Dr. De Wolf presented a description of the initial FHB risk models at the National Fusarium Head Blight Forum meeting in 2000 (De Wolf *et al* 2000). We have been testing the models in Ohio during 2001 and 2002 (Lipps and Mills, 2002) and other states (ND, SD, MO, MI and PA) have tested them during 2002.

The risk predictions models were developed using historic disease and weather data obtained from cooperators in ND, OH, MO and KS (De Wolf *et al*, 2000, De Wolf *et al*, 2001). Logistic regression models were developed from hourly weather, crop growth and disease level observations from 50 site-years representing three wheat production regions in the US. Correlation analysis identified combinations of temperature, relative humidity and rainfall across time periods 7 days prior to and 10 days after anthesis as significant independent variables. Of several logistic regression models developed the following two models were adopted for further testing because of their relatively high prediction accuracies.

Model I predicts the probability of head scab based on the weather that occurs prior to anthesis. This is the time when fungal inoculum develops. Model I utilizes the duration of precipitation in hours and the number of hours when the air temperature is between 15 and 30°C for 7 days prior to flowering. Cross validation prediction accuracy for this model was 78% for determining when disease will not be severe (severity $\leq$ 10%). Its accuracy for predicting when an epidemic will occur (severity $\geq$ 10%) was 56%.

Model II predicts the probability of scab based on the weather that occurs 7 days before and 10 days after anthesis. This model addresses the time when the fungus is developing spores, when infection occurs and when disease develops. Model II utilizes the number of hours when the air temperature is between 15 and 30°C for 7 days prior to flowering and the number of hours when the relative humidity is 90% or above and the air temperature is between 15 and 30°C for 10 days after flowering. Cross validation prediction accuracy for this model was 83% in determining when disease will be severe (severity $\geq$10%).

In order to make the FHB risk forecasting models more user-friendly, Dr. De Wolf and Mr. Mills developed a Microsoft Excel workbook that contains the various logistic equations. Probabilities are automatically calculated when the appropriate weather data is entered and the anthesis date is designated. The Excel file not only calculates the scab risk probability values, but also graphs the weather variable coordinates and plots them in relation to a risk threshold curve (logistic regression equation where predicted FHB severity is $\geq$ 10%). The distance of weather variable coordinates from the threshold curve defines the relative risk probability for the weather station location.

There is considerable flexibility for using FHB risk models, especially for processing weather data and presenting the prediction information to the growers. In Ohio during the 2002 season, hourly weather data from 14 weather stations were used to make risk probability calculations for three anthesis dates (early, mid and late anthesis) for each weather station location. Actual risk probabilities (as a percentage) were not presented directly to the public, but numerical probabilities were classified into 'Risk Levels' (low, moderately low, moderately high, and high) based on logistic regression thresholds in order to help growers better interpret the risk of scab in their area. To facilitate the timeliness of reporting information during the critical period of scab development, a web page (www.oardc.ohio-state.edu/ohiofieldcropdisease/) was used to deliver scab risk assessments.

Michigan and Pennsylvania, took a similar approaches to providing public access to FHB risk forecasts and managing the problem of obtaining accurate anthesis date information. Both reported prediction results on a web site (URL for MI was http://www.cips.msu.edu/

cips/headblight/index.htm and for PA was http://www.wheatscab.psu.edu/) and used weather data from multiple locations (18 location in MI and 33 locations in PA). Additionally, both provided daily risk probabilities for each of the weather locations and presented these as contour maps of the state with risk probabilities as color-coded areas between contour lines. This map-based presentation style required the farmer to choose the appropriate anthesis date for his fields to obtain the FHB risk probability map for that date.

Validation of the FHB risk assessment models in the field is a problem because of the large area, and consequently large number of fields, for which predictions are made. In Ohio, thirty County Extension Agents assessed the incidence of head scab in 159 fields by counting the number of diseased and non-diseased heads per foot of row in 10 locations per field. The FHB risk models predicted low to moderately low scab risk for most of the state in 2002. The FHB survey indicated that over 75% of the fields had incidence levels below 5% (average for all fields = 4.1%). In Michigan, risk assessment Model II predicted low to moderately low scab risk for 17 of the 18 weather station locations. A survey of fields in southern Michigan indicated that FHB incidence was low to moderately low and ranged from 0 to 25% in individual fields. Of 50 grain samples submitted from fields throughout the state all but one had DON levels between 0 and 0.5 ppm. Risk assessment models were not developed to predict DON levels in grain. The low DON levels detected in MI were probably due to the low level of disease and the dry conditions during the grain filling period.

Disease forecasting systems are never 100% accurate because they are mathematical predictions representing a multitude of variables that determine disease progress. Each of the variables can have a wide range of values. Problems that limit the accuracy of FHB forecasting models include variables associated with the pathogen (variation in inoculum levels in the field due to differences in residue management systems, variation in crop rotation sequences among fields, wind and rain splash dispersal gradients, pathogen species composition); host (anthesis date differences, susceptibility level, duration of anther retention, head height); and weather (variation in rain and RH duration across an area, temperature variation due to topography). Although models are designed to be robust, they will not accurately describe all possible situations. Risk predictions may be improved by using weather data from many sites, obtaining more accurate anthesis dates for an area, averaging risk probabilities over a multiple-day anthesis periods for locations and monitoring inoculum levels. Currently, cooperative research is being conducted in ND, SD, IN, OH and PA to develop a database of weather, crop development and pathogen inoculum level information to further validate and improve FHB risk assessment models.

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EPIDEMIOLOGICAL STUDIES ON FUSARIUM HEAD BLIGHT OF WHEAT IN SOUTH DAKOTA FOR 2002

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INTRODUCTION AND OBJECTIVES

South Dakota State University is part of a multi-state collaborative project studying epidemiology of Fusarium head blight (FHB) on wheat under different environments throughout the upper mid-west. The ultimate goal is to develop a disease risk advisory/forecast system. Primary objectives include: 1) monitoring inoculum dynamics and disease development in relation to specific environmental parameters; and 2) to evaluate currently proposed forecast models.

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. Environmental conditions are thought to influence the FHB disease cycle, but it is not certain which factors are critical, and which are most predictive of epidemics. By collecting disease and environmental data for multiple plantings of susceptible wheat across several locations, we might better characterize both epidemic and non-epidemic conditions for FHB development.

MATERIALS AND METHODS

Spring wheat (cv. 'Norm') susceptible to FHB was planted into strips 1.4m by 45m using a 7-row grain drill. Two adjacent strips were planted on each of three planting dates (26 April, 6 May, and 22 May, 2002), referred to as planting date (PD) 1, 2, and 3, respectively. Multiple dates were initially intended to ensure that susceptible host stage and pathogen inoculum would be present concurrently. Each planting was divided into three replicate plots. Each plot was further divided into two subplots, one sampled and one unsampled. The unsampled subplot was used to assess final disease levels 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). A wash of the cyclone unit was performed daily to ensure uniform sampling. The sample and wash were plated on Komada's medium for spore enumeration (Komada, 1975). Counts were reported as colony forming units (CFU) per day. Inoculum on wheat spikes was estimated by washing spikes using protocols de-

scribed by Francl *et al*. (1998), with some modification (sampled spikes were not covered prior to sampling). 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 were described and counted after incubation. Colonies were reported as CFU per spike per day.

Disease incidence and severity data were collected from each replicate within each planting date three to four times between late anthesis (Zadoks 67) and soft dough stage (Zadoks 85). In each replicate, 150 spikes from primary tillers were visually rated for FHB. Severity of FHB for each spike was rated on a 0-9 scale roughly based on percent of the spike visually blighted (0 to 90+%). Incidence rate was calculated by: number of infected spikes divided by total spikes counted per replicate. Severity was calculated for infected spikes by: (sum of spike severity ratings) divided by the number of infected spikes per replicate.

Data from the 2002 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*, 2000). 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

Major environmental parameters for each planting date are summarized in Table 1. Generally, dry conditions with warm temperatures were experienced throughout the growing season in 2002. A short period of three to four days of wet weather was experienced just prior to flowering of PD 1, but was followed by very warm, dry conditions for several days.

Table 1. Environmental conditions over susceptible periods in each planting date.

PD	Time period (susceptible)	Avg. air temp (^{o}C)	[a]Avg. e_a (kPa)	Precip. (mm) / duration (hrs)	15^{o}C < T < 30^{o}C (hours, max=168)	RH > 90% (hours)	[b]T*RH (hours)
1	DOY 177-183	25.7	2.00	0 / 0	107	10.5	7.5
2	DOY 180-186	26.4	2.15	0 / 0	108	9.5	9.5
3	DOY 190-196	20.5	1.75	2.0 / 2	123	46	27.5

a. vapor pressure of the air
b. hours temp is between 15^{o}C and 30^{o}C **and** RH > 90%

Inoculum level estimates for 2002 are presented in Table 2, and disease levels are presented in Table 3. Inoculum was considered to be moderate as estimated by both the Burkard spore trap and by the spike-wash method. Disease incidence was much higher than expected, ranging from 10 to 45% of head affected by FHB. Severity however was very low in all cases, ranging from 1 to 8% blight on infected spikes, on average.

Table 2. Inoculum level estimates over susceptible periods in each planting date.

PD	Time period (susceptible)	Burkard Spore Trap (cfu / day)	[a]Spike-wash (cfu / spike)
1	DOY 177-183	335	75
2	DOY 180-186	321	105
3	DOY 190-196	215	122

a. average of 3 reps, 10 spikes per rep.

Table 3. Final disease ratings.

	Plant Date 1		Plant Date 2		Plant Date 3	
	Incidence %	Severity %	Incidence %	Severity %	Incidence %	Severity %
Rep 1	44.7	8.2	43.3	6.1	21.3	2.4
Rep 2	46.7	7.2	38.7	5.0	14.7	1.6
Rep 3	26.7	7.3	37.3	5.6	10.7	1.2
PD Mean	39.3	7.6	39.8	5.6	15.6	1.7
	Overall:	Disease Incidence = 20%		Disease Severity = 5%		

The high incidence levels, coupled with the moderate levels of airborne and spike-borne spores suggest that inoculum levels were present at a level conducive to disease, however environmental conditions experienced during anthesis and after were not considered to be conducive to FHB development beyond the initial infections.

The results of model validation of Ohio models I and II are given in Table 4. FHB disease index for all plantings is given as an indicator of overall disease level and is the product of incidence and severity for each planting date. The probability of an epidemic occurring based on the two models is also given. Based on this set of validation runs, Ohio model I was consistent across plantings in relative rank and appear to correlate well to FHB incidence, however, it suggests that an epidemic is likely for PD 1, which had only 2.5% disease. Ohio model II suggests that no epidemic levels would be reached, which corresponds to the final disease estimates. It is believed that the parameter for precipitation (hours of precipitation duration) incorporated into Ohio I does not account for precipitation patterns of the Great Plains, which typically receive large quantities of precipitation in relatively short periods of time.

Table 4. Validation of Risk Assessment / Disease Prediction Models (Ohio I and II)

Planting Date	FHB Index (Inc*Sev, %)	FHB Incidence (% infected spikes)	Ohio Model I (risk probability)[a]	Ohio Model II (risk probability)[b]
1	2.5	39	0.53	0.08
2	2.2	40	0.31	0.14
3	0.3	16	0.19	0.33

a. epidemic threshold = 0.5
b. epidemic threshold = 0.44

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FHB INOCULUM DISTRIBUTION ON WHEAT PLANTS WITHIN THE CANOPY

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ABSTRACT

Fusarium head blight (FHB) of wheat is a potentially devastating disease in many of the wheat growing regions of the U.S. and Canada. The primary inoculum for this disease is generally considered to be ascospores of the fungus *Gibberella zeae* (ana: *Fusarium graminearum*). Perithecia of the fungus develop on infected crop residues, especially corn stalk pieces, remaining on field surfaces. The perithecia forcibly eject ascospores, but their fate is not certain. A large proportion of ascospores may not be able to contact susceptible host tissues because the infection window is quite narrow. Instead, these spores may land on non-susceptible tissues (leaf, stem, etc.). These spores may germinate and reproduce epiphytically. A study was initiated in the 2002 field season to investigate the types (conidia or ascospores) and distribution of spores of the FHB pathogen. Wheat plants were collected from 10 sites (3 groups per site) around the state and subsequently dissected and processed to enumerate conidia (of *F. graminearum*) and ascospores (of *G. zeae*) on individual leaves at specific leaf positions on the plants, as well as on the spikes. Ascospores and conidia were recovered at levels from 0 to 1500 spores per leaf. Relative ratios of ascospores to conidia varied greatly from 7:1 down to 1:4. Generally, ascospores outnumbered conidia at all leaf positions across most locations, with some notable exceptions. The results of the sampling show a distinct bimodal distribution pattern for ascospore counts with higher concentrations (50 to 200% greater) at the upper-most leaf position and the lower-most leaf position within the canopy than at the center leaf position. It is also noted that conidial distribution among leaves varied widely across locations. In some locations, few conidia were identified, while at other locations, all leaves were found to hold large numbers (up to 1500 spores) per leaf. This suggests that the fungus may undergo epiphytic growth and reproduction, resulting in increased inoculum load within the canopy of a wheat crop.

SOUTH DAKOTA FUSARIUM HEAD BLIGHT RISK ADVISORY FOR 2002

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ABSTRACT

In 2002, the small grains pathology project at South Dakota State University launched a web-delivered, weather-based risk advisory for Fusarium head blight (FHB) in northeastern South Dakota. A thirteen-county area comprising the majority of the spring wheat region in the state was selected for intensive inoculum, disease and environment monitoring. This area was selected for a FHB risk advisory to be issued on a county by county basis. Advisory information was to be posted to the internet every one to two days detailing potential risk of disease to wheat crops in each of the 13 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. An advisory of 'high-risk" was issued for the entire thirteen county region for a three-day period near the end of June, but was downgraded as weather conditions became unfavorable for disease development. Disease levels were low in nearly all counties, with levels approaching 5% incidence for small sections of two counties in extreme north and northeast SD. Following the 2002 season, much of the environmental and disease data from the past three years were incorporated into a model development phase resulting in several linear models for the prediction of inoculum, infection and disease.

INCIDENCE OF *FUSARIUM GRAMINEARUM* AND *COCHLIOBOLUS SATIVUS* IN WHEAT AND BARLEY CULTIVARS AT THREE LOCATIONS IN MINNESOTA

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ABSTRACT

Wheat and barley entries in the 2002 Red River Valley on Farm Yield Trials grown at Perley, East Grand Forks and Humboldt, MN, were assayed for the colonization of kernels by *Fusarium graminearum* and *Cochliobolus sativus*. The trial consisted of 24 wheat and 8 barley lines-grown in commercial fields in a randomized complete block design with two replications. At maturity, 100 spikes per plot were arbitrarily collected and threshed. Kernels (200-400 per treatment) were surface sterilized, plated onto half strength PDA (pH=5.5) and incubated at 20-24ºC, under fluorescent lights (12:12 light:dark) for 5-6 days. The incidence of kernels colonized by *F. graminearum* was highest at Humboldt (18.4%, wheat; 22.2%, barley). The incidence of *C. sativus* colonized kernels was highest in wheat at Perley (30.6%), and in barley at East Grand Forks (23.1%). Ranking of wheat cultivars for kernel colonization by *F. graminearum* and *C. sativus* was significantly affected by the interaction of cultivar by location, however at all locations, the wheat cultivars Alsen and Gunner had low levels of *F. graminearum* and Dandy, Norpro, Pioneer 2375, Oxen and AC Vista were more highly colonized. Oxen and Gunner generally had low levels of kernel colonized by *C. sativus*, while AC Vista and MN97803 showed higher kernel colonization across locations. The six-rowed barley lines MN109, MN110 and Lacey generally had greater kernel colonization by *F. graminearum* than Robust, Drummond, Foster and Legacy. The incidence of *C. sativus* colonized kernels was similar in all barley entries except Conlon. Kernels of Conlon, a two-rowed barley, had the lowest incidence of *F. graminearum* but the highest incidence of *C. sativus*. The data suggests that the colonization of wheat kernels by *F. graminearum* and *C. sativus* may be influenced by differences in inoculum availability in a particular location, and site-specific environmental conditions.

AIRBORNE POPULATIONS OF *GIBBERELLA ZEAE*: SPATIAL AND TEMPORAL DYNAMICS OF SPORE DEPOSITION IN A LOCALIZED FUSARIUM HEAD BLIGHT EPIDEMIC

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ABSTRACT

Viable propagules of *Gibberella zeae* (anamorph *Fusarium graminearum)* were collected from the air over two wheat fields (spaced 0.5 km apart) in Aurora, New York in May/June 2002. Corn kernels inoculated with a clonal isolate of *G. zeae* were placed in one of the fields. Petri plates with *Fusarium* selective medium were suspended 30 cm above the wheat canopy. Fields were sampled a total of 20 days before, during, and after wheat anthesis. Ninety six plates were exposed continuously during each day (sunrise to sunset) and another 96 plates were exposed continuously during each night (sunset to sunrise). Significantly more colonies were collected during the night than during the day. Seven major deposition events were apparent during the sampling period, and three of these were coincident with rainfall. Three major deposition events occurred during flowering; the largest occurred two days after anther extrusion. The field bearing the clonal source of *G. zeae* was exposed to more colonies than the other field. DNA fingerprinting analyses are being conducted to assess the genetic diversity of airborne populations of the pathogen and contributions from local and regional sources of inoculum.

DEVELOPMENT OF FUSARIUM HEAD BLIGHT IN INDIANA, 2002

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ABSTRACT

We are participating in a multi-state cooperative study on the epidemiology of Fusarium head blight (FHB) of wheat. We monitored weather, inoculum production, and disease development in order to obtain data that can be used to quantify the effects of weather on inoculum production and disease development. We planted 3 winter wheat cultivars at 3 dates at the Purdue Agronomy Research Center. Corn residue in the plots served as a source of inoculum. An automated Campbell station recorded weather data. The dates of flowering initiation among treatments ranged from 22 to 30 May. There were several days of unusually warm weather during the third week of April, and then several weeks of cooler than normal weather. During the 2 wk prior to 22 May, rain fell on 8 days, but mean daily temperature was above 15 °C on only 16 May. Daily mean temperatures began rising after 26 May, but by then there was little rainfall. Daily airborne spore concentrations estimated from a Burkard sampler ranged from 0 to 164 cfu·10 m^{-3} d^{-1}. A second sampler was located in a field 1.6 km away, but also with corn residue on the surface. There was close agreement between the numbers of spores collected each day at the 2 sites (r=0.95). Daily variation in number of airborne spores was large. On only one occasion, 26 and 27 May, were there 2 consecutive days with high counts. Each day we also collected heads at both sites for direct assay of spores of *G. zeae*. Spores recovered per head ranged from 0 to 750 d^{-1}. The higher values occurred later in the season, when wheat was in the grain filling stage. Numbers of spores recovered from heads at the 2 sites were in general agreement (r=0.75) except for 5-7 June, when substantially more spores were recovered at the main site. Based on estimates of the volume of air intercepted by a wheat head during 24 h, the Burkard samplers and the head washing assays gave similar estimates of the number of spores that impact a head each day, although correlations between daily values were low. Detailed assessment of incidence and severity of FHB were made at 2- to 3-day intervals in the 2nd planting of cultivar Elkhart. Incidence and severity both increased linearly from 5 June, when symptoms first appeared, through 21 June. Incidence increased from 3 to 16% (0.8% per day) and severity increased from 30 to 83% (3.5% per day). We assessed incidence and severity in all plots on 21 June. Among the cultivar-planting date treatments, mean FHB incidence ranged from 1.4 to 9.2%. The effects of cultivar, planting date and their interaction were all highly significant. For the various cultivar-planting date combinations, there was a significant correlation between incidence of FHB and the number of spores detected with the Burkard sampler on the 4th or 5th day after beginning of anthesis (r=0.81 and r=0.91, respectively). The correlation between incidence and the sum of spore densities on these 2 days was 0.97. We used data from this study to evaluate 2 weather-based forecast models developed by DeWolf *et al*. These models predicted a low probability of a “severe” epidemic, defined as an incidence of greater than 10%, for all cultivar-planting date combinations, consistent with what we observed.

COMPARISON OF SPRAY, POINT INOCULATION METHODS, AND FDK TO FACILITATE EARLY GENERATION SELECTION FOR FUSARIUM HEAD BLIGHT RESISTANCE IN WINTER WHEAT

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INTRODUCTION

Fusarium head blight (FHB) caused by *Fusarium graminearum* (Schwabe) is an important disease of wheat (*Triticum aestivum* L.) in Canada, and worldwide (Sutton, 1982). Different types of wheat resistance to FHB have been reported, but in the breeding programs Type 1, or resistance to initial infection, and Type 2, or resistance to spread of symptoms within the head are used most often (Schroeder and Christensen, 1963, Mesterhazy, 1995). The most frequently used source of FHB Type 2 resistance worldwide is Sumai 3.

Several authors reported that Type 1 and Type 2 resistance varied independently among cultivars (Schroeder and Christensen, 1963; McKendry *et al*., 2001; Desmeules *et al*., 2001), and that selection of genotypes with resistance to FHB depends on the inoculation technique used (Engle *et al*., 2001; Bockus *et al*., 2001). Shaner and Buechley (2001) proposed the expression of severity as the number of spikelets blighted, not the proportion of spikelets blighted, in order to avoid the influence of spike size on the degree of Type 2 resistance. Van Saford *et al*., (1999), and Hall *et al*., (2000) reported that some genotypes have a lower kernel infection rate then expected from their spikelets infection rate, and also the opposite.

In order to increase the comprehensive resistant level to FHB, different resistance types to FHB should be pyramided into improved varieties (Xu *et al*., 2001).

Even though spray and point inoculation methods are used most frequently as the ways to estimate wheat resistance or susceptibility to FHB, these two methods are not often directly compared using segregating populations with known type of FHB resistance.

The objectives of this study are:

-to directly compare FHB severity after spray and point inoculation method using a segregating population with type 2 FHB resistance,
-to compare Type 1 and Type 2 resistance with Type 4, or resistance to kernel infection,
-to compare expression of FHB severity as the number of spikelets infected with expression of FHB severity as the proportion of spikelets infected, in order to examine the influence of spike size on the degree of FHB resistance.

MATERIALS AND METHODS

The influence of different methods of inoculation on number or proportion of FHB infected spikelets, and percent of Fusarium damage kernels (FDK), were studied using an F_3 population carrying Type 2 FHB resistance. The progeny (n=85) was derived from a cross of resistant (WEKO60DH3 - a Sumai 3 derivative) and susceptible (AC RON) parents. The segregating generations, and parents, were planted on October 20, 2000, in 2-m long single rows, spaced 17.8 cm apart, at Ridgetown, Ontario.

In order to obtain uniform growth stage at the time of inoculation, individual heads, rather than whole plants or plots, were inoculated at 50 % of anthesis (Zadoks growth stage 60-69) (Zadoks, 1974). The heads were spray inoculated with 2 mL of the suspension sprayed onto individual heads, and point inoculated with 10 µl of suspension injected into single florets. The suspension of macroconidia, including three isolates of *F. graminearum,* was produced in liquid shake culture using modified Bilay's medium, and used at a concentration of 50,000 spores/mL.

Between 10 to 20 plants from each progeny and the parents were inoculated using both methods of inoculation. Clear plastic bags were placed over the inoculated heads, and left for 48 hr to maintain humidity. The plots were misted daily with an overhead mister that delivered about 7.5 mm of water each day. The plots were fertilized and maintained using provincial recommendations.

The number of diseased spikelets and the total number of spikelets were recorded for each inoculated wheat head. The spikelet infection rate was calculated as the number of diseased spikelets, or percentage of diseased spikelets of the total number of spikelets. The average infection rate from each row was calculated. The inoculated heads were hand harvested separately. Heads from each row, with the same inoculation method, were threshed together using a single head thresher, retaining all light kernels.

The number of healthy and *Fusarium* damaged kernels were counted, and percentage of FDK was calculated for each line and parents, after both methods of inoculation. In order to avoid the influence of inoculation method on % of FDK, % of FDK after both methods of inoculation was also averaged for each line, and their parents. FDK were identified as shriveled kernels, with chalky, pink or white color. The F_3 progeny was assigned to phenotypic classes on the basis of their position in the distribution of the proportion of FHB infected spikelets, and % of FDK.

For the proportion of FHB infected spikelets the following classes were used: 1=0-2.5, 2=2.51-5, 3=5.01-7.5, 4=7.51-10, 5=10.01-12.5, 6=12.51-15, 7=15.01-17.5, 8=17.51-20, 9=20.01-22.5, 10=22.51-25, 11=25.01-27.5, 12=27.51-30, 13=30.01-32.5, 14=32.51-35, 15=35.01-37.5, 16>37.51.

For % of FDK, there were the following classes: 1=0-2, 2=2.01-4, 3=4.01-6, 4=6.01-8, 5=8.01-10, 6=10.01-12, 7=12.01-14, 8=14.01-16, 9=16.01-18, 10=18.01-20, 11=20.01-22, 12>22.01.

Data management and all statistical procedures were completed using SAS v. 6.0. (SAS Institute Inc, 2001).

RESULTS AND DISCUSSION

Transgressive segregants, with higher levels of resistance than the parents, were found using visual symptoms and % FDK after both methods of inoculation (Fig. 1-2). Minimum, maximum, and mean values for % of infected spikelets after point inoculation were 3.7, 32.7, and 11.4, and lower than after spray inoculation where they were 4.8, 42.0, and 15.4, respectively. This result was expected because this population carrying Type 2 resistance from Sumai-3. Overall correlation between % of diseased spikelets after spray and point inoculation was positive, but low ($r=0.38$, $P<0.001$).

Values for minimum, maximum, and mean percent of FDK after point inoculation were 0, 31.0, and 9.0, and these were higher than % FDK after spray inoculation (0, 20.6, and 6.2, respectively). AC RON, a FHB susceptible cultivar, had lower scores than WEKO609H3 for % FDK after spray inoculation with *F. graminearum* (Fig. 2 B). Correlation between % FDK and % FHB infected spikelets after the spray inoculation method was significant ($r=0.28$, $P<0.05$), while correlation between % FDK and % FHB infected spikelets after the point inoculation method was not. When % FDK after both methods of inoculation was averaged for each line, and correlated with % FHB infected spikelets, the results showed again that % FDK correlated weakly with % FHB infected spikelets after the spray inoculation method ($r=0.24$, $P<0.05$), but not after the point inoculation method in this population (even while carrying Type 2 resistance). According to our results, % FDK can be estimated more accurately after spray, than after point inoculation method. It was unexpected that there was no significant correlation between % FDK after spray, and % FDK after point inoculation method in this population. When lines were ranked, according to % FDK after different methods of inoculation, just 2 of the 10 top lines were the same.

The number of infected spikelets correlated well with proportion of infected spikelet ($r=0.82$, 0.87, $P<0.001$), after point (Fig. 3A), and spray (Fig. 3B), inoculation method, respectively, even when several outliers were also identified (Fig. 3). There was a good segregation for total number of spikelets within the spike in this population, and ranged from 15 to 22. When lines were ranked, according to proportion of FHB infected spikelets, or number of FHB infected spikelets, 7 of 10 lines with lowest % of FHB infected spikelets were the same, confirming that there is no influence of spike size on degree of FHB resistance. We concluded that visual estimate of the proportion of FHB infected spikelets can be recommended for selection in early generations, rather than the more time and labor intensive counting of FHB infected spikelets.

This study showed that there is an advantage of using both methods of inoculation, because progeny lines with higher levels of Type 1, Type 2, and Type 4 were identified, and they would not have been identified if only one of the methods was used. When these Types of resistance are identified in an early segregating generation, they should be pyramided sooner in the improved FHB varieties.

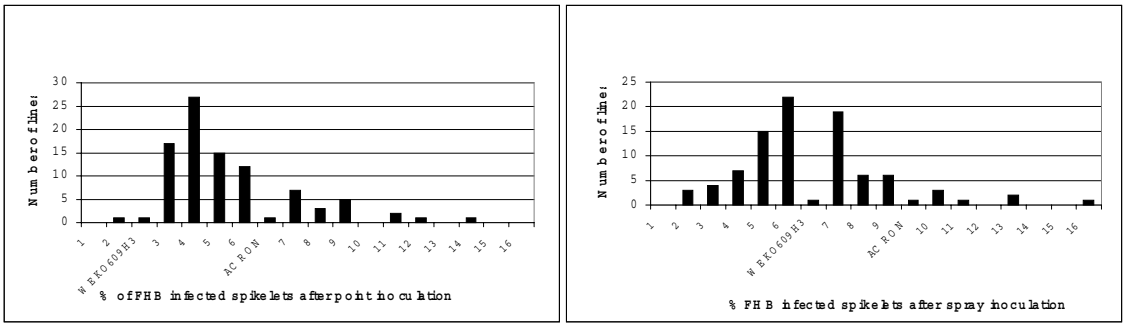

Figure 1. Frequency distribution of proportion of infected spikelets in F_3 generation after point (A), and spray (B) inoculation with *F. graminearum*.

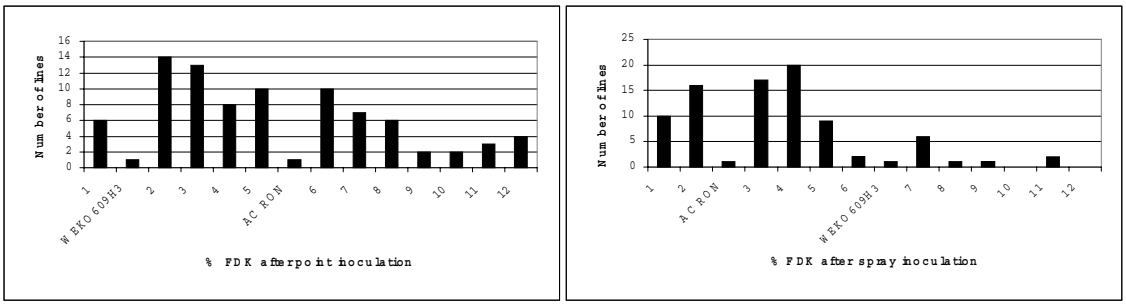

Figure 2. Frequency distribution of % FDK in F_3 generation after point (A), and spray (B) inoculation with *F. graminearum*.

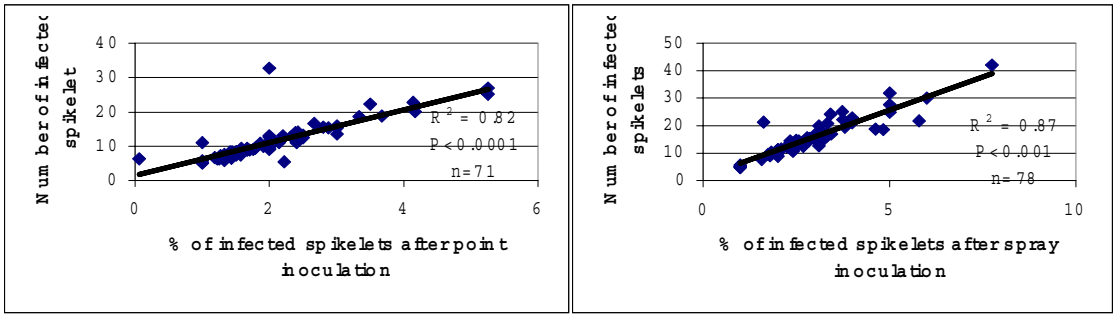

Figure 3. Relationship between number and proportion of infected spikelets in F_3 generation after point (A), and spray (B) inoculation with *F. graminearum*.

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REMI MUTAGENESIS IN THE WHEAT SCAB FUNGUS *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. In the past decade, wheat head blight (scab), primarily caused by *F. graminearum* in North America, has emerged as a major threat in wheat production. 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 7000 hygromycin-resistant transformants have been generated by transforming pCB1003 or pCX12 into *F. graminearum PH-1*. A corn-silk infection assay was devised to screen for mutants with reduced virulence. Many of the REMI pathogenicity mutants identified in corn-silk assays were dramatically reduced in their ability to infect and colonize flowering wheat heads. Genetic analysis and plasmid rescue are underway to identify and characterize genes disrupted in these mutants.

THE *FUSARIUM GRAMINEARUM* GENOMICS PROJECT

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ABSTRACT

*Fusarium graminearum (*teleomorph *Gibberella zeae)* is a broad host range plant pathogen that infects many crop plants worldwide. We have taken a genomics approach to better understand pathogenicity in this fungus. We have generated a collection of ESTs derived from cDNA libraries generated from cultures grown under several culture conditions and from infected wheat plants. Sequences were initially assembled into contigs and singletons, based on sequence comparisons, and a putative single gene set was identified. These sequences were compared to a yeast protein sequence reference set and to the GenBank non-redundant database using BLASTX. These results can be observed on the web (see link from www.scabusa.org). Based on presumptive gene function identified by this process, we were able to compare patterns of gene expression among cDNA libraries. Homologues of some known fungal virulence and pathogenicity factors and developmentally important genes were identified by this analysis. Funding for the complete genome sequence has been obtained. A discussion of the availability of tools for genomics and their potential uses will be presented.

COMPARATIVE VIRULENCE OF ISOLATES OF *FUSARIUM* SPECIES CAUSING HEAD BLIGHT IN WHEAT

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ABSTRACT

Fusarium head blight (FHB) is an important disease of wheat in Canada. To supplement the development of FHB-resistant cultivars, the virulence of *Fusarium* isolates representing eight pathogenic species was investigated. Six wheat genotypes were artificially inoculated with 12 isolates of *Fusarium graminearum* and six isolates each of *F. acuminatum, F. avenacium, F. crookwellense, F. culmorum, F. equiseti, F. poae*, and *F. sporotrichiodes*. The pathogens were isolated from naturally infected wheat, barley, and oat heads collected from cross Canada from 1965 to 2001. A single spore culture was established for each isolate, from which spore suspension was produced. Inoculation was performed by spraying spores over spikes at the 50% anthesis stage. Symptoms of FHB were rated as disease severity using a 0-9 scale at 4, 7, 14, 21, and 28 days after inoculation; and as percent infected spikelets after 21 days. All isolates caused visible infections to the six wheat genotypes but only those of *F. graminearum, F. crookwellense,* and *F. culmorum* resulted in severe disease development and were considered highly pathogenic. Significant differences ($P < 0.05$) were also observed among isolates and from genotype x isolate interactions for the three highly pathogenic species. However, the genotype x isolate interactions were low (< 15%) compared to differences between isolates or genotypes and did not suggest the occurrence of pathogenic races. The presesence of different virulence among isolates suggests that screening for resistance to FHB require a mixture of several isolates of these pathogens to be included. Wheat genotypes differed significantly ($P < 0.001$) in susceptibility, and responses of the genotypes to isolates of the highly pathogenic species were generally similar. AC Foremost, CIMMYT11, and Quantum were the most susceptible; FHB37 and HY664 were intermediate; and Sumai 3 was resistant. These results indicate that selection for resistance to one species in wheat may also confer resistance to the others.

POPULATION GENETIC DIFFERENTIATION AND LINEAGE COMPOSITION AMONG *GIBBERELLA ZEAE* (*FUSARIUM GRAMINEARUM*) IN NORTH AND SOUTH AMERICA

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ABSTRACT

Gibberella zeae (*Fusarium graminearum*) causes Fusarium head blight (FHB) of wheat and barley, and has been responsible for severe economic losses worldwide. Sequence analyses of *G. zeae* have been interpreted to mean that populations of *G. zeae* are composed of eight potential phylogenetic lineages, with a phylogeographic structure among these lineages. We used AFLP polymorphisms to compare populations of *G. zeae* from the United States, Mexico, Brazil, and Uruguay. We have also examined populations of *G. zeae* isolated from sorghum seed in Uruguay. Populations of *G. zeae* causing FHB in the United States include only a single phylogenetic lineage (Lineage 7). Subpopulations from throughout the United States have high genotypic diversity, do not deviate from expectations of random mating, and are interconnected by extensive gene-flow. South American populations of *G. zeae* from both wheat and from sorghum include a minority component of isolates that cluster with other phylogenetic lineages (Lineages 1, 2, and 6), but are dominated by genotypically diverse populations of isolates from Lineage 7. Populations of *G. zeae* causing FHB on wheat from two locations in Mexico are dominated by isolates from Lineage 3. Population genetic comparisons of Lineage 7 isolates from North and South America indicate that while intercontinental gene flow may occur, the amount of gene flow between the continents is much less than that which occurs within each continent.

METABOLISM OF TRICHOTHECENES BY WHEAT

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ABSTRACT

Feeding experiments were conducted to determine whether wheat could metabolize exogenously added trichothecenes. Middle spikelets of a moderately resistant wheat cultivar Su8060 were fed in triplicate, on ten sets of plants, with a fixed amount of deoxynivalenol (DON, 4000 ng). One day after adding DON, the treated spike on one set of plants and adjacent tissue was removed for toxin analysis using GC-MS. The remaining nine sets of plants were fed with DON again. This daily process of toxin addition and sampling was continued until all spikes were harvested for toxin analysis. The same procedure was carried out with 15-acetyldeoxynivalenol (15ADON, 1000ng), 3-acetyldeoxynivalenol (3ADON, 500ng) or a combination of the three toxins (4000ng of DON, 1000ng of 15ADON and 500ng of 3ADON). The results showed that, when fed DON alone, DON was found in both the fed spikelets and adjacent fragments of rachis. Additionally in DON treatments, both 15ADON and 3ADON were recovered but only from the fed spikelets. When fed with 15ADON, both DON and 15ADON were recovered from both the fed spikelets and adjacent fragment of rachis. When fed with 3ADON, both DON and 3ADON were detected only in the fed spikelets. In all cases, change in the amount of toxins followed the same pattern: reaching the highest cumulative level on the fifth or sixth day of the experiment, and then decreasing to a lower level on the seventh or eighth day despite continued toxin addition. Toxin levels increased from the ninth day until end of this experiment. When fed with the combination of DON, 15ADON and 3ADON in the ratio of 8:2:1, the relative amounts of the three toxins recovered from the fed spikelets varied significantly, from 24:3:1 to 80:7:1. Nivalenol was not detected in any treatment. We hypothesize that the resistant wheat plant metabolizes trichothecenes into different forms as well as other uncharacterized metabolic products. (This study is supported by National Natural Science Foundation of China, 30170601and 39870471)

YEAST STRAINS ALLOWING PHENOTYPIC DETECTION OF ESTROGENIC ACTIVITY: DEVELOPMENT OF A SENSITIVE AND INEXPENSIVE YEAST BIOASSAY FOR ZEARALENONE

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ABSTRACT

Zearalenone (ZON) is a non-steroidal estrogenic mycotoxin produced by plant pathogenic species of *Fusarium*. As a consequence of infection with *F. culmorum* and *F. graminearum*, ZON can be found in cereals and derived food products. Since ZON is suspected to cause human disease such as premature puberty syndrome as well as numerous cases of hyper-estrogenism in farm animals, several countries have established monitoring programs and guidelines for ZON levels in grain intended for human consumption and animal feed. Austria, for instance, has set guideline levels of 60 µg/kg for wheat intended for human consumption and 50 µg/kg for whole feed for breeding pigs. In epidemic situations much higher levels have been found, for instance average levels of more than 500 µg/kg wheat have been measured in Northern Germany in 1998. In Northern Iran highly contaminated wheat has been reported for 1996 (35/35 samples positive, average ZON level of 3,4 mg/kg).

We have developed a low-cost method for monitoring of ZON contamination in grain based on a sensitive yeast growth bioassay. The indicator *Saccharomyces cerevisiae* strain YZRM7 is unable to grow, unless an engineered pyrimidine biosynthetic gene is activated by the expressed human estrogen receptor in the presence of exogenous estrogenic substances. The deletion of the genes encoding ATP-binding cassette (ABC) transporters Pdr5p and Snq2p increases net ZON uptake synergistically. Less than 1 µg ZON per liter medium is sufficient to allow growth of the indicator strain. To prevent interference with pyrimidines potentially present in biological samples, we have also disrupted the genes *FUR1* and *URK1*, blocking the pyrimidine salvage pathway. The bioassay strain YZRM7 allows qualitative detection and quantification of total estrogenic activity in cereal extracts without requiring further clean up steps. The high sensitivity makes this assay suitable for low cost monitoring of contamination of maize and small grain cereals with estrogenic *Fusarium* mycotoxins.

We have furthermore constructed yeast strains allowing phenotypic detection of the estrogenic activity of ZON by engineered *ADE2* and *MEL1* genes. Together with a positive selection marker (*URA3* with estrogen responsive elements in the promoter), such easily screenable markers are valuable tools for cloning ZON degradation genes by expression of cDNA libraries in yeast.

DIAGNOSTIC VOMITOXIN (DON) SERVICES IN 2002/2003 SAMPLES

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OBJECTIVES

To provide analytical services for deoxynivalenol testing for researchers investigating mitigation of Fusarium head blight in wheat and barley.

INTRODUCTION

Deoxynivalenol (DON or vomitoxin) concentrations in cereal grains are an indicator of Fusarium head blight (FHB). Researchers evaluating techniques to reduce the adverse effects of in grains have used DON to determine the resistance in cultivars. Mycotoxin testing, specifically DON testing, is an important part of the cooperative efforts to reduce FHB. In 2002, the U.S. Wheat and Barley Scab Initiative provided grants to four regional DON testing laboratories in Michigan, Minnesota, and North Dakota to analyze for DON in wheat and barley cultivars.

MATERIALS AND METHODS

The four laboratories involved in DON testing are listed below. The analytical methods used by the laboratories include ELISA, gas chromatography with electron capture detection, and gas chromatography with mass spectrometry analysis. The sample preparation method used for the gas chromatography analysis was developed by Tacke and Casper (1996).

The individual laboratories conduct their own intralab quality control on appropriate control pools throughout the analysis period for DON testing to ensure quality of the analysis. The intralab quality control data spans the time from the beginning of DON testing in the labs through the end of October in 2002. Additionally, a collaborative quality assurance program, using wheat, was conducted among the laboratories (P. Hart, coordinator). Each laboratory was requested to perform analyses from a divided sample on two successive days within a 30 day period. The collected data were summarized and sent back to the laboratories. These check samples allowed each laboratory to evaluate the accuracy and precision of their system. Data from three check sample tests are included (May through October 2002).

Data (May through October 2002) are also included from a larger collaborative quality assurance program conducted by North Dakota State University, Department of Cereal Sciences. All four laboratories participate in this program, which uses malt and barley as check samples. These data are included for additional matrix quality assurance information.

Laboratories *:*

Patrick Hart, Ph.D., Department of Botany & Plant Pathology, Michigan State University, East Lansing, MI 48824; *Phone: 517-353-9428, FAX: 517-353-5598, e-mail: hart@msu.edu*
Method: water extraction and DON quantitation with the Neogen Veratox test (*ELISA*)
Sample types: wheat

Yanhong Dong, Ph.D., Department of Plant Pathology, University of Minnesota, St. Paul, MS 55108; *Phone: 612-625-2751, FAX: 612-625-9728*; *e-mail: dongx001@umn.edu*
Method: acetonitrile and water extraction, silylation and quantitation by gas chromatography/ mass spectrometry (*GC/MS*)
Sample types: wheat, barley, (*bulk, single head, single spikelet, single kernel, and small fragment*)

Paul Schwarz, Ph.D., Department of Cereal Science, North Dakota State University, Fargo, ND, 58105; *Phone: 701-231-7732, FAX: 701-231-7723, e-mail: Paul.Schwarz@ndsu.nodak.edu*
Method: acetonitrile and water extraction, silylation and quantitation by gas chromatography/ electron capture detection (*GC/ECD*)
Sample types: barley, malt, single kernel

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Method: acetonitrile and water extraction, silylation and quantitation by GC/ECD
Sample types: wheat, barley

RESULTS AND DISCUSSION

Table 1 summarizes the participating number of collaborators (*principal investigators*), *number of states, and estimated number of DON samples to be analyzed in the grant year of May 2002 through April 2003. Approximately 24,500 cereal grain samples submitted by about 57 principal investigators in 14 different states investigating FHB will be analyzed for DON by the four laboratories in 2002/2003.*

The intralab coefficient of variation for the four laboratories varies from 6 to 16 % on the grain control pools that were analyzed with the samples during 2002 (*Table 2*). *The interlab proficiency check samples for FHB testing show that the four laboratories are determining similar results, in a wheat matrix, using different analytical methods* (*Table 3*).

Additional interlab check samples were analyzed in malt and barley matrices by the same four laboratories using the same methodology (*Table 4*). *The data in Table 4 represent the time frame from May through October 2002, and are part of a two-year check sample program involving a number of participants. The z-value is given for each laboratory by month.* [*Note that the z-values were calculated as the lab's individual value minus the sample mean divided by the sample standard deviation. A smaller z-value represents less spread of actual results and higher accuracy and precision.*] *The data in Table 4 show that the*

z-values for the different laboratories are fairly small (close to zero) and no major differences were observed in analytical values at lower DON concentrations.

The repeatability of results on successive days reflects the precision of the analysis and was good for the interlab FHB check samples for those cooperating laboratories. Also, the intralab coefficients of variations on the control pools were fairly low for the participating diagnostic lab. These results indicated that the variation in analyses over several days is low and no major differences in analytical values of check samples occurred between the four DON diagnostic centers.

Table 1. *Estimated DON analyses by laboratories in 2002 through 2003*

DON Laboratory	Number of Collaborators	Number of States	Estimated Number of Samples Tested in 2002
MI: P. Hart	20	9	3,000
MN: Y. Dong	9	3	10,000
ND: P. Schwarz	4	3	7,500
ND: B. Tacke	25	7	4,000

Table 2. *Intralab quality control data for DON testing through October 2002*

DON Laboratory	Grain	Number	Mean (ppm)	Standard Deviation	Coefficient of Variation (%)
MI: P. Hart	Wheat	122	0.9	0.1	12
MN: Y. Dong	Wheat	30	7.2	0.9	13
ND: P. Schwarz	Barley	31	13.8	2.0	15
	Barley	18	39.7	4.9	12
	Barley	9	2.1	0.3	13
ND: B. Tacke	Wheat	104	1.8	0.1	7
	Barley	104	3.1	0.2	6
	Corn	104	4.7	0.5	11

Table 3. *Interlab proficiency check samples for DON testing for FHB*

DON Laboratory	Grain	DON (ppm)					
		Test 1		Test 2		Test 3	
MI: P. Hart	Wheat	1.0	1.0	4.0	4.4	<0.5	<0.5
MN: Y. Dong	Wheat	0.77		3.8		0.3	
ND: P. Schwarz	Wheat	0.6	0.4	3.0	3.2	0.3	
ND: B. Tacke	Wheat	0.9	1.0	2.8	2.6	0.4	0.4
Mean ± std.dev.		0.8 ± 0.2		3.4 ±0.7		0.4 ± 0.1	

Table 4. Interlab check samples for DON in barley (bar) and malt from May through October 2002 by four laboratories (part of a larger check sample program, North Dakota State University, Department of Cereal Sciences)

Lab	DON (ppm)											
	May		June		July		August		September		October	
	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt
MI: P. Hart	5.00	0.60	2.40	0.60	1.70	<0.5	3.20	0.60	4.40	<0.5	4.30	0.70
MN: Y. Dong	5.35	0.26	2.57	0.31	1.43	0.13	4.02	0.40	4.58	0.12	18.47	0.39
NDSU: P.Schwarz	4.60	0.20	2.20	0.30	1.50	0.15	2.80	0.20	4.00	0.10	20.30	0.50
NDSU: B. Tacke	5.50	0.40	2.40	0.40	1.60	<0.2	3.70	0.40	5.00	0.20	17.30	0.40
Sample MEAN	5.11	0.37	2.39	0.40	1.56	0.14	3.43	0.40	4.5	0.14	15.09	0.50
Sample Std. Dev.	0.40	0.18	0.15	0.14	0.12	0.01	0.54	0.16	0.41	0.05	7.30	0.14
Z-values by Lab	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt	Bar.	Malt
MI: P. Hart	-0.28	1.32	0.05	1.42	1.21		-0.43	1.22	-0.23		-1.48	1.41
MN: Y. Dong	0.59	-0.59	1.17	-0.66	-1.08	-0.71	1.10	0.0	0.20	-0.38	0.46	-0.75
NDSU: P.Schwarz	-1.28	-0.93	-1.27	-0.74	-0.49	0.71	-1.17	-1.22	-1.19	-0.76	0.71	0.02
NDSU: B. Tacke	0.97	0.20	0.05	0.0	0.36		0.50	0.0	1.22	1.13	0.30	-0.68

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HUMAN SUSCEPTIBILITY TO TRICHOTHECENE MYCOTOXINS

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ABSTRACT

The trichothecene deoxynivalenol (DON), also given the colloquial name vomitoxin, has occurred with alarming frequency in wheat, corn and barley produced Michigan and Midwest. A major concern is that, because of the paucity of information on human toxicity, action levels might be set artificially low, thereby reducing the marketability of Michigan wheat containing trace levels of DON but posing no risk. Based on studies in the mouse model, we believe that the most critical step for toxicity induction by DON and other trichothecenes are their action on leukocytes (white blood cells) either by activation of cellular hormones known as cytokines or by the induction of programmed cell death (apoptosis). If human leukocyte cytokine dysregulation and/or apoptosis induction are indeed targeted by the same levels of DON and other 8-ketotrichothecenes in mice as in the mouse, then the risk of low ppm levels of DON to humans will be extremely small when one considers the diversity of the human diet and the actual potential level of DON exposure in human tissues. Two types of models are being used to test this hypothesis- cloned and primary cells.

DON and other 8-ketotrichothecenes induce, in the U937 human macrophage clonal model the production of three critical proinflammatory mediators, namely, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and the chemokine, interleukin-8 (IL-8). Interestingly, the higher trichothecene concentrations markedly reduced proliferation and were cytotoxic. The key signals for cytokine upregulation are likely to involve ribosomal binding DON ribosome binding Ġ protein kinase R/Hck kinase Ġ MAPKinases Ġ cytokine upregulation. DON also affected a cloned human T lymphocyte model (Jurkat cells). Although DON stimulated IL-2 production, the four other 8-ketotrichothecenes did not stimulate production of this cytokine. DON and 15-acetyl DON at 60 to 500 ng/ml and 3-acetyl DON at 600 to 5000 ng/ml could induce IL-8 production, whereas NIV and FX were not stimulatory. Again, the higher trichothecene concentrations markedly impaired proliferation and were cytotoxic. conditions optimized for the primary culture of human leukocytes and conducted preliminary experiments on the effects of DON.

Two primary leukocyte culture approaches have been evaluated. The first involved direct culturing of human blood obtained from volunteers. Using the first approach, we have observed that DON will directly induce IL-6. Of particular importance was the finding that some donors were much more sensitive to DON-induced IL-6 than others in terms of minimum effective DON concentration and magnitude of response. Furthermore, the doses required for these effects in primary cells appear to be lower than for cloned cell models suggesting that human primary cells are slightly more sensitive to DON and potentially other trichothecenes. Analogous results were found for IL-8 but sensitive donors did not correspond to IL-6 responders. Rather, the low IL-6 responders were high IL-8 responders. The

second type of primary culture involved leukocytes obtained from processed Red Cross blood. Similar variability and sensitivity was observed in these cells. However, since we did not have control of these samples from initial blood draw and know nothing about the donors, it will be difficult to reproduce or interpret findings from with Red Cross materials. Thus, we will focus all future efforts on human blood culture.

It will be important to ascertain whether blood cells from specific individuals are more or less sensitive to the toxin and whether these effects are consistent over repeated blood collections. If so, it will suggest that toxin-susceptible and resistant individuals may exists among the human population, possibly because of genetic polymorphisms related to toxin metabolism or cellular target interaction. If responses are variable among the same individual, it will be possibly indicative that a hormonal or environmental factor (eg diet, medication) differentially affects an individual response to DON. Either type of information will be critical for conducting accurate risk assessments for DON and other 8-ketotrichothecenes.

USING NEAR INFRARED TRANSMITTANCE AS A SCREENING TOOL FOR DON IN BARLEY

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ABSTRACT

Near infrared transmittance (NIT) spectroscopy has been investigated for rapid estimation of deoxynivalenol (DON) in whole kernel barley (and wheat). Barley samples, spectra, and reference analysis data provided by study participants from the USA, Austria, and France. All spectra (570 – 1100 nm) collected using the FOSS Infratec 1241 Grain Analyzer with the added color module, and reference analysis using HPLC or GC methods. Calibrations tested included partial least squares (PLS) and artificial neural network (ANN) for DON (ppb, ppm) and log (DON ppb). Independent validation data showed best performance using the ANN calibration log (DON ppb) across locations (N 257, Slope 0.79, Correlation 0.88, SED 0.3024, and Bias –0.0013). Based on current findings, it appears NIT can be used as a screening tool to measure DON in barley. It is recommended to use a limit of 3.5 (3.2 ppm), values above 3.5 indicate the sample might be infected and should be set aside for further testing (HPLC, GC). DON calibration development work will continue, by expanding calibration databases to include additional growing seasons, locations, and by addressing issues associated with sampling and laboratory errors, to improve accuracy and precision of DON analysis using NIT.

STORAGE OF SCABBY WHEAT: *FUSARIUM* GOES AWAY, DON DOESN'T

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ABSTRACT

Some 60 years ago, R. G. Shands at the University of Wisconsin reported that scabby barley stored for five years (1931-1936) retained its emetic activity when fed to pigs. He proposed that the effect was due to some "toxic principle", then unknown, and not to the presence of the live fungus since the barley remained toxic although *Fusarium* cultures could no longer be recovered from it (Phytopathology 27:749-762). Today we would recognize that his "toxic principle" affecting the pigs was likely DON. Shands did actual feeding experiments with grain extracts to demonstrate the toxicity of his five-year-old barley samples. The widespread outbreak of FHB in the northern spring grain region in 1993 was perhaps the worst since 1928, the one that had prompted Shands' interest. We had evaluated a large number of scabby grain samples from the 1993 crop in eastern North Dakota and northwestern Minnesota. Many of these grain samples had DON levels in excess of 10 ppm, some as high as 50 ppm. Some grain samples from this survey had been retained in storage since 1993. For the present study, we chose 50 of the 1993 wheat samples to reanalyze for DON in 2001. As originally analyzed, the grain samples had contained from <0.5 ppm to 18 ppm. The same procedure for extraction and analysis by GC-MS was used in 1993 and in 2001. The level of DON found in the 2001 analysis of these samples was about 73% of that found in 1993. That ratio of the two analyses was remarkably consistent for most of the samples ($R^2 = 0.90$). The correlation of DON to presence of tombstone kernels in grain was nearly the same for ine 1993 as the 2001 analyses ($R^2 = 0.60$, 0.62, respectively). When cultured in 1994, 65% of the kernels in 1993 grain samples had given colonies of *Fusarium graminearum*. Despite the substantial amount of DON remaining in this grain in 2001, not a single culture of *F. graminearum* could be recovered when the 660 representative scabby kernels from these samples were plated out on media suitable for recovery of *Fusarium*. Our results show that the commonly-held assumption that DON is long-lasting in grain is correct for wheat as well as barley. The wheat samples tested represented multiple locations in the region and several cultivars; neither site not cultivar showed any particular deviation from the overall relationship of 1993 to 2001 DON. (This poster was presented at the 2002 Annual Meeting of the Canadian Phytopathological Society, Waterton Lakes, Alberta, June 2002.)

VARIATION FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN *TRITICUM DICOCCOIDES*

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ABSTRACT

Head blight of wheat (FHB, scab) caused by *Fusarium* spp. is a severe fungal disease problem worldwide. Apart from yield and grain quality losses, the contamination of the harvest with toxic fungal metabolites, known as mycotoxins, is of serious impact. In spite of the fact that several sources for resistance against FHB have been found and utilized in hexaploid wheat, virtually no resistant tetraploid wheat cultivar has been identified so far.

Wild emmer wheat, *Triticum dicoccoides*, previously identified as a rich source for disease resistance genes to several pathogens, was tested for resistance to FHB. Single point inoculations were applied to evaluate a set of 151 *T. dicoccoides* genotypes, originating from 16 habitats in Israel and one habitat in Turkey, for resistance to fungal spread (Type II resistance) in replicated greenhouse experiments. A considerable level of diversity was found among the tested genotypes, the broad sense heritability for Type II FHB resistance was 0.71. Among the eight *T. dicoccoides* lines with the lowest relative infection rates, five originated from the Mt. Gerizim population, and three from the Mt. Hermon population. These two habitats are characterized by a relatively cool and semi-wet climate. Hence, it may be possible that *Fusarium* occurrence in these habitats was responsible for natural selection in favor of resistance.

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DESIGNATING TYPES OF SCAB RESISTANCE: A DISCUSSION

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At a meeting of the Germplasm Introduction and Enhancement Group of the U.S. Wheat and Barley Scab Initiative, held Sept. 12-13, 2002 in St. Paul, MN, the status of terminology for types of scab resistance was reviewed and discussed. The two principal types of resistance described by Schroeder & Christensen (1963), resistance to initial infection and resistance to spread of infection in the head (usually designated types 1 and 2, respectively) have been widely used. However, several additional types have been postulated without agreement among laboratories on either their definitions or in the sequence of numbering (or lettering) to be used. Several other factors contribute to confusion among designated types of resistance (Bushnell 2000). These include:(1) differences among laboratories in the way disease development, toxin accumulation, and kernel yield and quality are measured; (2) the need to deduce the amount of some postulated types of resistance from two measured qualities as, for example, disease severity and yield reduction must be measured to determine tolerance, or toxin concentration and yield loss must be measured to deduce insensitivity to toxin; (3) differences in objectives among laboratories; e.g. a focus on mechanisms of resistance can lead to postulated types of resistance that are not feasible to measure routinely in breeding for resistance; (4) uncertainty about the role of trichothecene toxins in pathogenesis; and (5) limited available information on the physiology and (in most cases) the genetics of resistance.

Lively and candid discussion by the group led to the following results: About half the participants favored continued use of “type 1” to designate resistance to initial infection and “type 2” for resistance to spread in the head. The remaining participants did not favor use of type 1 or type 2 alone to designate the type of resistance. This subgroup recommended that each worker describe both what was measured and the inferred type of resistance in words instead of depending only on use of “type 1” and “type 2”. For resistances other than types 1 and 2, the group was nearly unanimous that it is premature to codify them into a standardized list. Too little is known about them, methods for measuring them are not standardized, and there is lack of agreement among workers on how to designate them. Postulations of resistance mechanisms are valuable as a basis for experimental investigation, but should not be designated by number (or letter) until they are well established and until practical, uniform methods of measuring them are available. The group hopes these conclusions will lead to further discussion by the larger FHB research community.

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INHERITANCE OF FUSARIUM HEAD BLIGHT RESISTANCE (TYPE II) IN NEW WHEAT GERMPLASM CJ 9306 AND CJ 9403

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ABSTRACT

Fusarium head blight (FHB or scab) caused by *Fusarium graminearum* is a worldwide serious disease in wheat. Exploitation and genetic studies of elite resistance sources can speed up the development of resistant cultivars. Two new resistance sources CJ 9306 and CJ 9403 developed in a recurrent selection program of Nanjing Agricultural University, China were crossed to two susceptible cultivars. Experiments with P_1, P_2, F_1, F_2, BC_1 and BC_2 generations for four crosses and with $F_{6:7}$ RILs for one cross were carried out in greenhouse to evaluate FHB resistance to fungal spread within a spike. Single-floret inoculation was conducted at heading and flowering stages, and the inoculated plants were subsequently misted for three days. The number and percentage of scabby spikelets (NSS and PSS) on the 25th day after inoculation were scored. The frequency distribution in F_2s and BC_1s showed continuous with two major peaks and one minor peak between them, indicating that scab resistance in wheat should be a qualitative-quantitative trait. A high level of resistance in CJ 9306 was mainly attributed to co-presence of two genes. The major gene expressed at a moderate to resistant level and was the prerequisite for the expression of the second or minor gene that enhanced the resistance to a high level. In CJ 9403 there might be three major genes and two to three minor genes governing the resistance. The fittest genetic model varied depending on specific crosses. A four-parameter model with additive × dominance interaction provided the most complete and precise elaboration in the two crosses with CJ 9306. A simple additive-dominance model was best fitted for the data from Veery/CJ 9403 and NSS in Norm/CJ9403. For PSS in Norm/CJ 9403, a five-parameter model with additive × additive and dominance × dominance effects seemed to be more adequate than others. The additive effects always significantly increased the resistance and played a major role in the inheritance of scab resistance. The estimates of broad-sense and narrow-sense heritabilities were 60%-86% and 32%-65%, respectively. As new improved germplasm of scab resistance, CJ 9306 not only has a high level of Type II resistance as well as a feature of simpler inheritance, but also possesses well-improved agronomic traits. Therefore, it should be a good choice for breeding scab resistant cultivars. CJ 9403 could be directly applied in production in adapted areas and breeding programs because of its excellent agronomic traits and high yielding potential even if its resistance is a little lower.

SCREENING WINTER AND FACULTATIVE WHEATS FOR FUSARIUM HEAD BLIGHT INFECTION

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ABSTRACT

Severe epidemics of Fusarium head blight (FHB) occur regularly in the Southern Cone region of South America, especially in Argentina, Brazil, Paraguay and Uruguay. Historically the National Wheat Programs have identified sources of resistance to FHB in the spring wheats, including Frontana, Alvarez 110, Encruzilhada, Klein Atlas etc. In the recent years, these have been combined with the Chinese germplasm derived from Sumai#3 and Chuan Mai#18 to achieve higher level of resistance in the wheat programs.

In order to broaden the spectrum of resistance to include winter and facultative wheats, CIMMYT's regional program, based in INIA La Estanzuela, Uruguay, screens local and introduced germplasm under naturally occurring and artificially inoculated conditions. The level of naturally occurring FHB infection during 2001 was extremely severe, which allowed excellent evaluation of the introduced germplasm (Table 1).

***Table* 1.** Classification of winter and facultative wheat germplasm for FHB, 2001.

Source	Total entries	Scab classification*				
		R	MR	MS	S	VS
4th WONIR	112		2	3	29	77
10th FAW WON	62		6	8	18	30
Georgia	86		6	8	45	27
Louisiana**	14	1	3	4	5	1
Kansas	118		2	20	81	15
Oklahoma	30		2	2	17	9
Texas	20		2	3	5	10
Mexico (winter)	19			8	5	6
TOTAL	**461**	**1**	**23**	**56**	**205**	**175**
%	100	0.2	5.0	12.1	44.5	38.0

*R = Resistant; MR = Mod. Resistant; MS = Mod. Susceptible; S = Susceptible; VS = Very susceptible
** Collection of parent lines from the crossing block

The evaluation of the CIMMYT international nurseries and winter wheat germplasm from various collaborators in the US, confirms the presence of large variability in the FHB resistance. In spite of the fact that FHB infection was uniform throughout the cycle, later heading germplasm tended to show lower levels of infection. The lines from this group will need to be checked through artificial inoculations. Other lines, early or intermediate for their heading, selected for lower level of FHB infection are presented in Table 2.

While these results confirm the resistance of some parent lines (Shou Chou), they also demonstrate that other lines such as CIMM1FHB#5, Coker 960208 and ND 2928 are, in fact, moderately susceptible under Uruguayan field conditions. Two lines (X950412-F-7 and Pioneer 26R61) were rated at par with local check *INIA Tijereta,* considered to be moderately resistant and will need to be confirmed in future evaluations. Several other lines (Bezostaja, Irneria/Mukkab hib., Star/Bwd, OK 98637 and X950446-F-1), rated moderately resistant to moderately susceptible, represent germplasm with very wide genetic backgrounds which can be useful in the breeding programs.

***Table* 2.** Fusarium head blight reaction of selected facultative and winter wheat lines

Entry	Heading	FHB		Origin
		(1-5/1-5)	Reaction	
I. TIJERETA (Local check)	**E***	**22**	**MR****	**Uruguay**
ND2928	E	24	MS	Louisiana
CIMM1FHB#5	E	25	MSS	Louisiana
SHOU CHOU	E	11	R	Louisiana
OK97508	E	24	MS	Oklahoma
TX98V6610	E	23	MS	Texas
I. TORCAZA (Local check)	**I**	**32**	**MS**	**Uruguay**
BEZOSTAJA	I	22	MRMS	10FAWWON
STAR/BWD	I	22	MRMS	10FAWWON
IRNERIA/MUKKAB HIB.	I	22	MRMS	10FAWWON
SULTAN	I	23	MS	10FAWWON
93435-1-10	I	13	MS	Georgia
UGA 931463E27	I	23	MS	Georgia
PIONEER 26R61	I	12	MR	Louisiana
APD99-5627	I	23	MS	Louisiana
COKER960208	I	14	MS	Louisiana
LA422	I	13	MS	Louisiana
9388D22-1-3	I	23	MS	Louisiana
X950337-II-2	I	23	MS	Kansas
X950446-F-1	I	22	MRMS	Kansas
X950412-F-7	I	11	RMR	Kansas
OK98637	I	22	MRMS	Oklahoma
OK95571	I	14	MS	Oklahoma
I. GORRION (Local check)	**L**	**45**	**S**	**Uruguay**

** E= Early (150d), I= Intermediate (160d), L= Late (170d)*

Field screening of facultative and winter wheat germplasm under naturally occurring epidemics of FHB at a hot spot site such as La Estanzuela, Uruguay, provides an excellent opportunity to identify new sources of resistance. These, in addition, can also be screened for foliar blights and leaf rust diseases. CIMMYT, in collaboration with the National Agriculture Research Institute, INIA, is trying to incorporate these and other sources of FHB resistance into locally adapted wheats. The preliminary results are very encouraging.

TYPES I, II AND FIELD RESISTANCE TO FUSARIUM HEAD BLIGHT IN WINTER AND SPRING WHEAT GERMPLASM

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OBJECTIVE

The objective of this research was to evaluate and determine the relationship among Type I, II and field resistance in spring and winter wheat germplasm that we had previously identified as having potentially useful levels of resistance to scab.

INTRODUCTION

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), also known as scab, is a devastating disease of wheat and barley in warm and humid regions of the world. Host plant resistance has long been 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, often in unadapted types. The identification of different sources of resistance in winter wheat through a systematic evaluation of accessions maintained in the National Small Grains Collection at Aberdeen, ID has been identified as a key objective of the US Wheat and Barley Scab Initiative's (USWBSI) germplasm research area. As such, approximately 4600 winter wheat accessions have been evaluated at Missouri. Additionally, spring and winter wheat germplasm has been introduced into the United States through a collaborative effort established between the USWBSI and CIMMYT. Both initiatives have resulted in the identification and introduction of wheat germplasm with high levels of Type II resistance. Less is known, however, about the Type I resistance in these lines, how the two types of resistance are correlated, and whether those types of resistance relate to field resistance.

MATERIALS AND METHODS

Germplasm: Germplasm was selected for evaluation from two sources. Winter wheat germplasm was acquired from the National Small Grains Collection in Aberdeen, ID and was kindly provided by Dr. Harold Bockelman. Germplasm was selected that had functional levels of Type II resistance in each of 3 successive cycles of greenhouse evaluation. Winter wheat germplasm included was from China, South Korea, Japan and Italy and included land races, cultivated genotypes and cultivars. Spring wheat germplasm included most genotypes introduced into Missouri in 2000 through the CIMMYT/USWBSI collaboration. Lines included were from the CIMMYT breeding program and included advanced breeding lines and wide crosses. Genotypes also included lines introduced from China and from Romania. Of the Romanian wheat introduced, 6 had a winter wheat growth habit.

Greenhouse Evaluations: Vernalized seedlings were arranged in a split-plot design with genotype as the main plot and type of resistance as the sub-plot. For each accession, 10 plants per treatment were planted and the experiment was replicated six times. For evaluation of Type II resistance, plants were inoculated at first anthesis with 10µL of a macroconidial suspension of *Fusarium*

graminearum concentrated to 50,000 macroconidia/mL. Inoculum was placed in a single central floret at first anthesis using an Oxford 8100™ repeat dispensing syringe. For all inoculations, a single isolate was used which had been previously determined to be aggressive on the resistant cultivar, Ernie. Plants were incubated in a mist chamber (100% relative humidity) for 72 h post-inoculation to promote disease development and then returned to the greenhouse bench. Ratings for Type II resistance (disease spread in the spike) were made at 21 d after inoculation. A Fusarium head blight index (FHBI) was also determined at 21 d post-inoculation as the ratio of diseased spikelets to total spikelets in the inoculated head.

For evaluation of Type I resistance, heads were again inoculated with a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was sprayed directly on the head at full anthesis using a Pulmo-Aide nebulizer as the power source and an atomizer (Model 163, DeVilbiss Sunrise Medical, Somerset, PA 15501-0635, USA). Inoculum was delivered to each head, spraying one side and then the other. Plants were incubated in a mist chamber as described above. At 10 d post-inoculation heads were rated for symptoms of Fusarium head blight. Total spikelets in the head were recorded followed by the number of spikelets in the head showing disease. Incidence was determined as the total number of spikelets on the inoculated head showing disease symptoms. The Type I FHBI rating for each head was determined as the number of spikelets with disease divided by the total number of spikelets on the head. Ratings were taken again at 21 d post-inoculation to determine the scab index for the head. The 21-d rating (total number of infected spikelets/total spikelets in the inoculated head) provided an estimate of severity on the inoculated head.

Field Evaluations: The field scab index was determined from unreplicated spray inoculations or winter wheat germplasm. Individual rows were inoculated at 75% anthesis with a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia /mL using a CO_2 backpack spray system. Plants were maintained under overhead mist irrigation throughout the inoculation period (approximately 2 wk). Twenty heads from each row were evaluated for symptoms of scab 18-21 d post-inoculation. Infected spikelets were counted on each head. Incidence was determined as the number of heads with visible symptoms of disease. Severity was determined as the ratio of diseased spikelets to total spikelets in the inoculated heads. The field scab index was calculated as incidence*severity.

RESULTS AND DISCUSSION

Data presented in Table 1 are those from winter wheat germplasm screened with high levels of Type I, II and field resistance. Superior lines included land races and cultivated lines from China, South Korea and Italy. Of 45 lines evaluated, 12 lines had good levels of Types I and II resistance, coupled with good field resistance. Data for spring wheats with good levels of resistance are given in Table 2. Of 57 wheat genotypes introduced through the CIMMYT collaboration in 2000, 23 genotypes had excellent levels of Type II resistance (< 10%) while 15 had good Type I resistance (<40%). Nine genotypes combined good levels of both Type I and Type II resistance. Four of these lines were introduced from Romania, while two were introduced from China. Type I and Type II resistances were not highly correlated. Complete data for all lines evaluated will be available at the Scab Forum.

Table 1. Type 1 (greenhouse spray), II (greenhouse point) and field scab resistance for winter wheat germplasm with putative genes for scab resistance. Values that are bolded do not differ significantly within a column.

Name	Designation		Origin	Heading date	Mean spikelets by accession	Greenhouse Point Inoculation		Greenhouse Spray Technique				Field Scab Index
						21 d	21 d	10 d	10 d	21 d	21 d	
				Julian	No. spikelets	Spread	Index	Incidence	Index	Incidence	Index	%
c4-3-3	CHOW	CULTIVATED	China	319	15.1	3.7	0.24	**6.7**	**0.44**	9.1	**0.59**	**9**
c5-1-1	Kuang tu erh hsiao mai	LANDRACE	China	327	14.3	2.1	0.15	8.6	**0.62**	8.9	**0.64**	36
c12-2-3	COLOGNA VENETO	LANDRACE	Italy	324	12.8	**2.0**	0.16	**6.8**	**0.55**	7.7	**0.62**	48
70-1-2	CItr 9428	LANDRACE	China	318	13.9	**1.8**	**0.13**	**6.5**	**0.46**	**7.1**	**0.51**	76
71-2-3	CItr 9429	LANDRACE	China	316	14.1	3.0	0.22	7.7	**0.57**	8.9	**0.66**	**14**
77-1-2	CItr 9445	LANDRACE	China	322	13.1	**1.0**	**0.08**	**7.0**	**0.57**	7.7	**0.62**	28
92-2-1	CItr 9490	LANDRACE	China	317	12.8	2.3	0.18	7.2	**0.57**	8.1	**0.64**	**20**
93-2-1	CItr 9506	LANDRACE	China	318	13.1	**1.9**	0.16	7.8	**0.59**	8.8	**0.66**	**15**
94-2-2	CItr 9507	LANDRACE	China	323	16.0	**1.8**	**0.11**	9.0	**0.58**	10.8	0.70	36
102-3-1	CItr 9521	LANDRACE	China	319	13.0	2.7	0.21	**7.0**	**0.54**	7.7	**0.60**	45
122-2-2	A1	CULTIVATED	China	315	10.3	**1.7**	0.18	**5.9**	**0.56**	**5.7**	**0.54**	**15**
124-2-2	A11	CULTIVATED	China	321	11.5	**1.0**	**0.09**	**5.9**	**0.52**	**6.6**	**0.57**	34
126-4-3	A23	CULTIVATED	China	310	10.2	2.6	0.26	**5.5**	**0.53**	**6.0**	**0.61**	**12**
147-3-4	B22	CULTIVATED	China	312	11.3	4.7	0.42	**6.1**	**0.53**	**7.2**	**0.62**	35
151-2-4	B36	CULTIVATED	China	317	13.3	3.6	0.27	**6.0**	**0.45**	7.7	**0.58**	**20**
202-2-1	D3A	CULTIVATED	China	320	15.8	4.8	0.31	10.4	**0.65**	12.4	0.78	32
244-2-4	D127A	CULTIVATED	China	318	15.1	4.9	0.32	9.2	**0.61**	9.7	**0.64**	30
355-2-1	COLORBEN 4	CULTIVAR	Italy	320	12.5	**1.5**	**0.12**	8.5	0.68	8.9	0.71	29
419-2-4	QUADERNA	CULTIVAR	Italy	311	10.1	**1.7**	0.17	7.3	0.72	**7.5**	0.75	39
433-1-2	NORIN 50	CULTIVAR	Japan	314	13.1	3.1	0.23	**6.8**	**0.50**	7.7	**0.58**	36
451-1-2	SEU SEUN 6	CULTIVAR	Korea, S	313	9.6	**1.3**	0.14	**4.6**	**0.47**	**5.1**	**0.53**	**27**
497-2-1	NORIN 96	CULTIVAR	Japan	312	11.3	**1.9**	0.17	7.4	**0.65**	8.3	0.73	32
568-1-4	TRENTO	CULTIVAR	Italy	322	17.3	2.3	0.14	11.7	**0.67**	13.1	0.75	**17**
687-2-2	CAMPOFIORITO	CULTIVAR	Italy	324	14.6	**1.8**	**0.13**	7.6	**0.50**	8.5	**0.56**	**26**
816-3-4	LING HAI MAO YANG MO	CULTIVATED	China	291	11.2	**1.2**	**0.12**	**6.0**	**0.54**	**6.6**	**0.59**	**10**
829-1-2	XIN DONG NO. 2	CULTIVATED	China	354	19.2	**1.2**	**0.06**	11.2	**0.58**	12.4	**0.64**	38
829-4-1	XIN DONG NO. 2	CULTIVATED	China	333	18.8	**1.6**	**0.09**	10.6	**0.56**	11.3	**0.60**	38
870-4-2	WAN SHUI BAI	CULTIVATED	China	316	12.4	**1.1**	**0.09**	**5.4**	**0.46**	**5.9**	**0.50**	**18**
877-1-2	YANG LA ZI	LANDRACE	China	320	15.8	**1.6**	**0.11**	8.5	**0.51**	9.8	**0.59**	**23**
937-2-3	84-5418	CULTIVATED	China	309	9.4	**2.0**	0.23	**4.1**	**0.44**	**4.6**	**0.49**	32
Ernie	Resistant Check (early)	Cultivar	USA	315	10.8	2.1	0.20	**6.4**	**0.60**	**6.9**	**0.65**	**14**
MO 94-317	Susceptible Check	Cultiar	USA	321	13.3	10.3	0.79	9.9	0.74	12.0	0.89	76
Sumai 3	Resistant Check (late)		China	339	18.0	**0.7**	**0.04**	9.5	**0.52**	10.3	**0.56**	-
Ning 7840	Resistant Check (late)		China	350	20.5	**0.9**	**0.04**	10.0	**0.48**	10.6	**0.51**	-
Average				320	13.4	2.7	0.21	7.7	0.57	8.6	0.64	30
LSD at 0.05				3.1	1.6	1.3	0.09	2.9	0.23	2.9	0.19	18
c.v.%				3.9	11.0	41.4	37.6	33.3	30.7	29.2	26.6	

Table 2. Types 1 (incidence) and II (point) resistance to Fusarium head blight in selected germplasm introduced from CIMMYT in 2000.

	Greenhouse spray (incidence)				Greenhouse point	
CIMMYT 2000 - Pedigree/Designation	10 Day # diseased spikelets	21 Day # diseased spikelets	10 day FHBI	21 day FHBI	21 day # diseased spikelets	21 day FHBI
SHA3/CBRD	7.2	7.6	0.50	0.52	1.1	0.07
NG8675/CBRD	4.0	5.5	0.22	0.29	1.4	0.07
NS73/PCI//B143.241.2/3/NING8647	13.4	17.1	0.61	0.77	1.7	0.08
MIAN YANG81-5//PC B084.985/JIANZIMAI	13.1	14.5	0.58	0.64	1.6	0.07
MIAN YANG81-5//PC B084.985/JIANZIMAI	8.0	8.8	0.37	0.41	1.0	0.05
PC B084.985/JIANZIMAI//8744	12.0	14.9	0.54	0.67	2.1	0.10
SHANGAI	13.4	14.2	0.68	0.72	0.7	0.04
SHANGAI	11.0	11.9	0.64	0.70	1.0	0.06
GOV/AZ//MUS/3/DODO/4/BOW	6.0	7.6	0.31	0.39	3.6	0.18
RECURRENT SELECTION 1	19.6	20.9	0.91	0.97	2.3	0.11
SODAT/SUM3//NING820/3/NING8626	8.7	10.6	0.47	0.58	1.6	0.09
BCN//DOY1/AE.SQUARROSA (447)	7.5	7.7	0.37	0.38	3.9	0.18
MAYOOR/5/CS/TH.CU//GLEN/3/ALD/PVN/4/CS/LE.RA//2*CS/3/CNO79	2.8	5.3	0.21	0.40	3.2	0.23
BUC//RUFF/AE.SQ/3/MAIZ	4.5	7.6	0.36	0.60	6.3	0.51
FUNDULEA 201 R (Winter)	5.8	6.0	0.34	0.35	0.7	0.04
FUNDULEA 183 P5 (Winter)	10.6	11.7	0.55	0.61	0.7	0.04
FUNDULEA 143-T3-103 (Winter)	5.9	7.7	0.30	0.39	1.2	0.06
TURDA 95 (Winter)	5.0	5.7	0.27	0.31	0.7	0.04
TURDA 195 (Winter)	11.8	14.6	0.57	0.70	1.0	0.05
TURDA 2317-90 (Spring)	6.5	7.4	0.36	0.41	0.5	0.03
NING 896013	1.6	5.1	0.10	0.32	2.0	0.14
NING 894037	13.8	16.6	0.73	0.87	0.9	0.05
MUTANT AT 1	13.7	17.8	0.60	0.78	1.2	0.06
MUTANT AT 2	15.6	17.8	0.68	0.77	1.2	0.06
YANGMAI 9	8.9	10.1	0.43	0.48	1.5	0.07
EMAI 6	5.1	7.9	0.38	0.59	1.7	0.12
ZHONGHUA 1	10.9	12.9	0.57	0.68	1.4	0.07
SUMAI 2	11.5	11.8	0.49	0.50	1.2	0.05
85004/MEXICO 354	5.1	6.0	0.26	0.30	1.6	0.08
Ernie (resistant check)	3.4	3.6	0.26	0.28	2.0	0.17
Sumai 3 (resistant check)	9.5	10.3	0.52	0.56	0.7	0.04
MO 94-317 (susceptible check)	8.7	10.7	0.64	0.79	10.3	0.68

RESISTANCE IN HEXAPLOID WHEAT TO FUSARIUM HEAD BLIGHT

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When Fusarium head blight (FHB) re-emerged as a major disease of wheat in North America about 15 years ago, it was evident that most cultivars were susceptible. Small grain breeders and pathologists began working with Sumai 3 and related resistant lines from China as sources of resistance. Although there seem to be no races of *Fusarium graminearum* that have adapted to a particular source of resistance, numerous examples from other pathogens suggest that we should be cautious about reliance on one source of resistance. Moreover, Sumai 3 is not completely resistant. The discovery of other resistance genes may allow us to create genotypes with a greater level of resistance than any that are currently known. To further these objectives, the USWBSI created a Germplasm Introduction and Enhancement (GIE) research area "to identify new sources of FHB resistance and to facilitate the utilization of resistant germplasm".

ACCOMPLISHMENTS

Participants in the USWBSI, as well as workers in other areas of the world, have identified many wheat lines resistant to FHB (e.g., 2,4,6,9). Snijders hypothesized 3 main pools of resistant germplasm (12). The GRIN database includes 34 lines that have FHB index values of 2 or 3. McKendry and Bestgen identified 17 lines from the CIMMYT/USWBSI collaborative germplasm effort that had 1 spikelet or less blighted following point inoculation (McKendry, personal comm.). No line of common wheat is completely resistant, but several have a high degree of resistance.

SCREENING TECHNIQUES

Most germplasm screening efforts employ point inoculation, in which an aqueous suspension of conidia is placed in a single floret at the middle or near the tip of the spike. Point inoculation is designed to detect resistance to spread of the fungus throughout the spike, based on spread of symptoms, referred to as Type II resistance. Resistance to initial infection (Type I resistance) may be as important as Type II resistance. If weather is favorable for infection throughout anthesis and early grain fill, there may be multiple primary infection events, leading to severe head blight without the need for spread of the pathogen through the rachis. Workers evaluate germplasm for Type I resistance by spraying heads at full flowering with a spore suspension.

Mesterhazy proposed 5 types of active resistance mechanisms in wheat to *Fusarium* infection, based on his own and previous work (6). This classification of resistance types (now referred to as Types I to V) has had a major influence on how researchers view resistance and its genetic control. As more has been learned about the interaction between *Fusarium* and wheat, there are questions about how some of these types of resistance are defined and measured. Participants at a recent workshop of the GIE group agreed that Type I and

Type II resistance are reasonably straightforward, as first defined (8). The other 3 types of resistance (III-V) pose problems.

Type III resistance (resistance to kernel infection) is a valid concept, but operationally it poses problems. What is the appropriate way to measure it? Point inoculation is not suitable. If a line has a high degree of Type II resistance, then the kernels from a head on which a single floret was inoculated will show a low frequency of infection and damage because the fungus never reached them or reached them too late to cause visible damage. For evaluation of Type III resistance, every kernel evaluated should be exposed to infection. Spray inoculation might be more suitable, but a line with resistance that impedes progress of the fungus from the stamen to the ovary could mask whatever resistance or susceptibility kernels might have to invasion. There is a more fundamental question about this kind of resistance. Does it refer to the ability of the fungus to penetrate a kernel or to the degree to which the fungus ramifies the grain? If Type III resistance is meant to measure differences in the amount of mycelium in grain, then a test that measures fungal biomass in kernels should be used.

Mesterhazy defined Type V resistance (active resistance mechanism “e”) as “resistance to toxins in ears by decomposing them” and cited Miller *et al*. 1985 (7) and Snijders and Perkowski 1989 (sic) (13) as sources (The citation of the Snijders and Perkowski paper is incorrect in Mesterhazy’s paper. The correct citation is given in the reference list below). Miller at al. suggest 2 reasons for low DON levels in grain: the plant has factors that prevent formation of toxin, or factors that promote degradation of toxin. Accurate measurement of resistance to toxin accumulation poses difficulties. If lines have Type I or Type II resistance, or resistance to invasion of kernels from other tissues in the head, then they would have lower levels of DON in kernels compared to lines that lacked these forms of resistance. Detection of resistance to toxin accumulation requires that grain from different wheat lines has not only been equally exposed to infection, but that fungal biomass in the grain can be measured and related to DON content (7).

Correlation analysis of DON content versus fungal biomass in kernels may reveal that some lines have less DON than would be expected. Such lines should be investigated for presence of genes that act to influence the accumulation of DON. It would be desirable to combine these genes with genes for other types of resistance. However, selection for resistance to DON accumulation against a background of resistance that reduces the frequency or extent of kernel infection would be difficult. Genes for resistance to DON accumulation would be ideal candidates for marker-assisted selection.

The concept of tolerance (Type IV, or “d” in Mesterhazy’s list of active defense mechanisms) has traditionally been applied to foliar or root diseases, in which grain is not directly infected, but its mass and quality are reduced by the stress of infection of vegetative organs. A tolerant cultivar sustains less yield reduction for a given severity of disease than an intolerant cultivar. What does tolerance mean for a pathogen that infects reproductive organs? If grain is relatively sound despite severe head blight symptoms, the plant may have a resistance mechanism that interferes with invasion of the grain. Mesterhazy used yield relative to uninoculated controls as a measure of tolerance, i.e. if a group of lines had equivalent head blight severity scores, but differed substantially in relative yield, he considered the line(s)

with higher yield to be tolerant. Without direct evidence of comparable timing and extent of kernel invasion (fungal biomass per kernel), conclusions about tolerance in FHB must remain tentative.

INHERITANCE OF RESISTANCE

Discovery and phenotypic characterization of resistant lines are only the first steps in using germplasm. Resistance must be incorporated into cultivars adapted for each region where FHB is a threat. Breeders can use germplasm without knowing the genetic basis of resistance, but the work is more efficient if they have this knowledge.

Original accessions are often heterogeneous for resistance and it is necessary to reselect from this germplasm to obtain lines that consistently express resistance. Once this has been done, genetic studies can be undertaken.

Most studies published so far have evaluated Type II resistance in response to point inoculation. Two or more genes condition resistance in Sumai 3 or its derivative, Ning 7840. A major QTL has been mapped to 3BS (1,14). Results from a number of studies with various wheat lines indicate QTLs for FHB resistance may reside on most chromosomes of the wheat genome (see 14). Many of these genes show additive action. This suggests that as new genes are identified in other sources of resistance, they will act additively, or in more complicated manners, with the genes already in hand, to give higher levels of resistance. Even moderately susceptible lines have contributed useful genes for resistance (1,3,11).

FUTURE DIRECTIONS OF THE GIE PROGRAM

If Types I and II resistance are inadequate to protect the crop, resistance to kernel invasion or to DON accumulation could provide another layer of protection. The germplasm program of the USWBSI should give more attention to discovery of germplasm with these other forms of resistance. With respect to Type I and Type II resistance, emphasis should shift to genetic characterization of the germplasm already in hand. We need to know more about inheritance of resistance in various sources, the uniqueness of their genes, and how genes interact to affect the resistance phenotype.

We may be approaching the limit of Type II resistance. Several lines show only slight necrosis in the inoculated floret. It will be difficult to detect higher levels of Type II resistance when such sources are combined. It may be more useful to combine other types of resistance with a high degree of Type II resistance. Evaluation of the same wheat lines by both point and spray inoculation suggests that different genes control Type I and Type II resistance. If yet other genes control resistance to kernel infection and toxin accumulation, then it should be possible to combine all of these into a single cultivar. For reasons outlined above, phenotypic selection would not work. This is clearly a project that requires marker-assisted selection technology. First we need to carefully characterize, phenotypically and genetically, resistance other than Type II, and then find reliable markers for these genes.

To accomplish this efficiently within the USWBSI, I propose creation of a coordinated program analogous to programs in other research areas. Many accessions with Type II resistance have been identified in hexaploid wheat. The most resistant accessions, particularly

any that lack the major QTL on 3BS (see e.g., 5), need to be thoroughly studied genetically. We also need to identify sources of Type I resistance and sources of resistance to kernel invasion and DON accumulation. For this, we need to develop reliable methods of phenotypic screening. These will likely be more complicated and costly than the point inoculation technique currently used to identify Type II resistance.

ACKNOWLEDGEMENTS

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NOVEL SOURCE OF TYPE II RESISTANCE TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Sources of resistance in wheat (*Triticum aestivum*) to Fusarium head blight (FHB) of wheat are limited despite extensive screening of germplasm since Arthur first reported this disease in 1891. Wheatgrass has been demonstrated to be an important resistance source for wheat leaf rust, stem rust, and powdery mildew diseases. Here we report the excellent resistance to FHB of *Lophopyrum elongatum* (2n = 2X =14, genome EE).

A series of Chinese Spring- *L. elongatum* substitution lines from 1E(1A) to 7E(7D) except 4E(4D) and 5E(5A) (provided by J. Dvorak, Department of Agronomy and Range Science, University of California, Davis, CA), were evaluated for Type II resistance to *Fusarium graminearum* in a greenhouse, February-April 2002. The recipient parent Chinese Spring was also included in the experiment. In a completely randomized design, 12 – 24 plants per line were evaluated for disease severity (DS), the percentage of diseased spikelets in inoculated spikes. The mean DS of Chinese Spring was 41%. The mean DSs of the substitution lines ranged from 5% - 74%. Pairwise comparisons of means showed that three lines had significantly higher DSs than Chinese Spring. They are 3E(3D), 2E(2D), and 6E(6A) with respective DSs of 74%, 71%, and 62%. Three lines had DSs that were significantly lower than Chinese Spring. They are 7E(7A), 7E(7B), and 7E(7D), with respective DSs of 5%, 5%, and 6%. The disease did not spread beyond the inoculated spike in all tested plants in these three lines. Our data shows that the 7E chromosome of *L. elongatum* conditions Type II FHB resistance. Chinese Spring itself has resistance to FHB. The resistance of Chinese Spring may be located on chromosomes 2D, 3D, and 6A, because when these chromosomes were replaced with their respective homoeologous *L. elongatum* chromosome, these substitution lines were more susceptible to *Fusarium graminearum* than Chinese Spring.

The experiment is being repeated in the greenhouse, October-December, 2002. Results to date are consistent with our results in the test of February-April, 2002.

EVALUATION OF THE NATIONAL SMALL GRAINS COLLECTION OF BARLEY FOR RESISTANCE TO FUSARIUM HEAD BLIGHT AND DEOXYNIVALENOL ACCUMULATION

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INTRODUCTION

Barley is a major crop in the Red River Valley of Minnesota, North Dakota and Manitoba. Production peaked in the 1980s and decreased to its lowest level in 30 years in 1999. Many factors have contributed to barley's decline in the region, including Fusarium head blight (FHB) (McMullen, *et al*).

Grain affected by FHB, caused primarily by *Fusarium graminearum*, has reduced quality and may be contaminated with unacceptable levels of deoxynivalenol (DON) (Salas, *et al.*). With the advent of the FHB epidemics of the 1990s, some brewing companies imposed strict limits on the levels of DON present in grain going to the malt houses. Barley exceeding the DON specifications is relegated to feed grade with a corresponding drop in price. The risk involved with potential grade reduction is a primary factor in declining barley hectarage. This has lead to increased awareness of the problem and a need for barley varieties that are resistant to FHB and to toxin accumulation.

In 1998, Busch Agricultural Resources, Inc. (BARI) scientists began a study to identify sources of resistance to FHB and resulting DON accumulation. This effort concentrated on screening the entire 6-rowed spring barley collection held by the National Small Grains Collection. Public and private barley breeders may be able to utilize accessions identified as having high levels of resistance to disease and toxin accumulation to improve malting barley.

MATERIALS AND METHODS

Initially 7,475 accessions were received from the USDA-ARS, National Small Grains Collection, Aberdeen, Idaho. Field evaluation sites included Casselton, ND and Crookston, MN in 1998, Park River and Osnabrock, ND in 1999 and 2000, and Park River in 2001. Accessions were planted mechanically as single, non-replicated rows. In July 1998 accessions with few or no visible symptoms were selected and then hand harvested in August. In 1999, 2000 and 2001 all accessions were hand harvested regardless of visible symptoms. In 2000 percent FHB was determined in July by counting number of infected kernels vs. total kernels on 10 heads. In all years harvested material was transported to Fort Collins, CO and threshed. Grain samples were submitted to the Barley DON Diagnostic Laboratory located in the Department of Cereal and Food Sciences at North Dakota State University for DON analysis (Tacke and Casper). Selections were made for further testing based on both percent FHB and ppm DON.

Selected accessions were planted in the greenhouse in Fort Collins, CO during the winters of 1999/2000 and 2000/2001. Heads were inoculated at anthesis with an isolate of *F. graminearum* collected from Midwest-grown barley. Inoculations were carried out late in the day and plants placed in clear plastic chambers equipped with a humidifier for approximately 36 hours. Heads were rated for percent FHB by counting the total number of kernels and the number of visibly infected kernels at 14- and 21-days post inoculation. In the 2000/2001 season, greenhouse samples also were submitted for DON testing.

RESULTS AND DISCUSSION

A number of the 7,475 rows planted in 1998 were winter, hulless, black, hooded, dwarf, 2-rowed or other types of barley and were discarded. Because all the barley at the Crookston nursery appeared to be free of disease, selections were made and harvested from plants at the Casselton nursery. The same accessions were harvested at Crookston. Based on visual selection, the top 98 accessions and 2 checks were submitted for DON analysis from both locations. DON levels ranged from non-detectable to more than 40 ppm across locations.

Eighty-two accessions were advanced to the 1999 field screening. Toxin levels were generally lower than the previous year and ranged from 0.2 to 11.0 ppm (avg. 2.9 ppm) at Park River and non detectable to 12.5 ppm (avg. 1.9 ppm) at Osnabrock.

Fifty-six accessions were selected for testing in the greenhouse in 1999/2000. An additional 13 accessions that had been discarded in 1998 but were identified as resistant by North Dakota State University (NDSU) in 1999 were returned to the study. Twelve accessions did not develop any symptoms of FHB. In all, 9 of the 56 accessions were eliminated from further testing based on high DON, high FHB or very poor agronomic traits. All of the NDSU selections that were returned to the study were carried over to the next field season.

In 2000 sixty accessions were planted at Park River and Osnabrock. Toxin analysis and disease ratings were possible on 48 accessions. Toxin levels averaged 0.3 ppm DON at Park River but averaged 1.6 ppm at Osnabrock. None of the 12 accessions that had 0% FHB in the greenhouse remained completely free of disease. However, these and other accessions low in disease continued to perform well.

A total of 47 accessions were tested in the greenhouse in 2000/2001, 15 from previous years' tests and 32 selected by NDSU in 2000. Disease averaged 18% and was high (25 to 95%) in many accessions. Toxin levels ranged from non-detectable to 13.6 ppm with a mean of 2.1 ppm. All accessions were carried over for another year of field screening.

DON level for 2001 averaged 2.4 ppm. Levels ranged from 0.6 ppm to 3.3 ppm for the 15 accessions remaining in the study.

We have selected 15 accessions to recommend for inclusion in breeding for resistance (Table 1). Several of these accessions selected were already reported to have resistance and have been used in various breeding programs over the years (including the 2-rowed types, Svanhals and Svansota). Steffenson and Scholz also selected a number of acces-

sions in their studies (Steffenson and Schulz). As a result of these studies, several new accessions can be added to the list of resistant germplasm. The geographic sources of these accessions cover four continents. However, at least 2 accessions from the US have Chevron in their background. Another US selection has a Svanhals parent. The full diversity of the most resistant accessions needs to be assessed through molecular genetics. This can further focus breeding efforts on numerous sources of resistance.

Table 1. Accessions from the National Small Grains Collection selected for resistance to Fusarium head blight and Deoxynivalenol.

ACCESSION	CIho	PI	ORIGIN	PEDIGREE
Mammoth Winter	CIho 220		Ukraine	
Wisconsin Pedigree	CIho 835		USA	
Abyssinninan Intermediate	CIho 2414		Ethiopia	Selection from PI25674
Hietpas 3	CIho 6611		USA	Selection from Oderbrucker
Seed Stocks 1148-1	CIho 6613		USA	
Markhinstz	CIho 7279	PI149782	Russia	
Iowa 5286	CIho 9539		USA	Manchuria, CI4471/Chevron
ELS 6402-302	CIho 12904	PI298751	Ethiopia	
1948D		PI371317	Switzerland	
UNA 8392		PI477854	Peru	
Cross	CIho 2492		Sweden	
Svansota	CIho 1907		USA	No.456/Svanhals
Svanhals	CIho 2274		Sweden	Selection from PI5474
Peatland	CIho 2613		USA	Selection from same landrace as Chevron
Chevron	CIho 1111		Switzerland	Selection from landrace

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FUSARIUM HEAD BLIGHT TYPE II RESISTANCE OF A SPRING WHEAT POPULATION DERIVED FROM A HUNGARIAN WINTER WHEAT

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused mainly by *Fusarium graminearum*, has plagued farmers in the spring wheat region for nearly a decade, causing substantial loss. Disease management by crop rotation, tillage, or fungicides has been marginally successful at best. The best long term solution to the FHB problem in this region is by incorporation of resistance into adapted cultivars. Several cultivars resistant or moderately resistant to FHB have been released in the region over the past several years, and many more advanced lines are being tested. The resistance to FHB in nearly all such adapted spring wheats has come from Chinese germplasm sources such as Sumai 3 and its derivatives. Other sources of resistance need to be explored. In the 1980's, Akos Mesterhazy in Hungary identified non-Chinese lines which showed resistance to FHB. He produced several advanced resistant lines by intercrossing these sources. One of us (RWS) obtained several of his lines in 1996 and crosses were made to adapted spring wheats. A population derived from crossing Mesterhazy's line 'Ringo Sztarr/Nobeoka Bozu' by the ND cultivar 'Grandin' was advanced by single seed descent to F-6, selecting only for spring habit. We grew 182 lines from this population in the greenhouse in two randomized replicates. At anthesis, ten spikes per replicate were inoculated by single spikelet inoculation and then given 3 days of intermittent mist treatment. At 3.5 weeks post-anthesis, FHB symptom development on each spike was scored on a 0-100% severity scale. FHB severity scores of the 182 lines ranged from 8% to 85%. Based on FHB severity, 14 of the 182 lines were resistant as or better than our standard resistant check line 'ND2710' and other best Sumai 3 derived lines. We conclude that lines derived from other resistance sources may be as resistant to FHB as those from presently used Chinese germplasm. This can serve to diversify the germplasm base for FHB resistance.

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PROPOSED CHROMOSOMAL LOCATION OF FHB RESISTANCE GENES IN ADDITIONAL SETS OF DURUM DISOMIC SUBSTITUTION LINES DERIVED FROM DIFFERENT *T. DICOCCOIDES* ACCESSIONS

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ABSTRACT

Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, has been a serious disease problem on spring wheat in North Dakota and surrounding states for nearly a decade. North Dakota is the principal durum growing state in the USA and durum has been especially hard hit by FHB. Development of cultivars with FHB resistance has been much slower for durum than for the spring bread wheats, in part because the best known sources of FHB resistance are in hexaploid backgrounds and effective transfer to the tetraploid durum seems to be difficult. In the 1980's, USDA geneticist L.R. Joppa had produced a set of durum disomic chromosome substitution lines derived from a wild emmer (*Triticum dicoccoides*, TDIC) selection "Israel A", identified for high grain protein levels. We recently reported (Crop Sci. 42:637-642) the finding of FHB resistance on chromosome 3A in the durum disomic substitution line from this series. Other researchers have found molecular markers for this gene. In searching for potential sources of FHB resistance, we previously had screened 290 accessions of TDIC from the USDA world collection, and we had identified several lines with useful levels of FHB resistance. Two of these accessions were used to produce new sets of chromosome substitution lines in 'Langdon' durum following the method used for the original TDIC chromosome substitution series. The purpose of the present study was to determine which chromosomes held the resistance loci in these FHB resistant TDIC accessions. Each substitution line was grown in replicated trials in the greenhouse and inoculated at anthesis with *Fusarium graminearum* by the single spikelet method. FHB response was determined visually 3.5 weeks after inoculation. LDN(DIC) substitution lines representing five different chromosomes (1A, 3A, 5B, 7A, 7B) had significantly less FHB than the Langdon checks. The other LDN(DIC) lines showed intermediate responses, not significantly different from the Langdon durum parent. The five TDIC chromosomes substituted in the lines with significantly reduced FHB are proposed as sites of FHB resistance genes in these two accessions.

WILD EMMER, *TRITICUM DICOCCOIDES*, AS A SOURCE OF FHB RESISTANCE FOR TETRAPLOID AND HEXAPLOID WHEATS

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ABSTRACT

Wild emmer, *Triticum turgidum* L. var. *dicoccoides* (a.k.a '*T. dicoccoides*') (TDIC), is wild tetraploid wheat found throughout the Middle East. Because it shares the AB genome with modern durum (*T. turgidum* L. var. *durum*), it readily crosses with it and also crosses with hexaploid wheat - with some care as to choice of parent. TDIC has long been known as a source of novel disease resistance including genes for resistance to stem rust, stripe rust, leaf rust and powdery mildew, among others. Our research with TDIC as a source of FHB resistance began as two separate lines of inquiry which have since come together. One area of study was the evaluation of a set of disomic chromosome substitution lines developed by Leonard Joppa in the 1980's to study a gene for high grain protein. In each of these lines, one chromosome pair from TDIC replaces the corresponding pair in a durum background. We tested this set of substitution lines for FHB. The entire story of this aspect of the work was recently reported in Crop Science (42:637-642). We found a major FHB resistance gene on 3A and a major gene on 2A that appears epistatic to FHB resistance. Somewhat less strong resistance was present on 1A and 6B. Another research group at NDSU has identified molecular markers for the 3A QTL. Research on the 2A epistatic locus is currently underway. Concurrently, we began screening the USDA world collection of TDIC for FHB. Between 1995 and 1997 we tested 449 TDIC collections. Of these, 33 (7.3%) showed levels of FHB substantially lower than durum check lines. About half have held up as moderately to highly resistant upon repeated testing. In direct crosses between these TDIC selections and durum, the FHB resistance appears in the offsprings but along with many undesirable traits. From that point the two lines of research joined together. Two TDIC accessions from among those confirmed as having FHB resistance were selected upon which USDA cytogeneticist Leonard Joppa would base new sets of durum disomic substitution lines. An abstract elsewhere in this proceeding describes that process and the results. From the new series of substitution lines those which showed FHB scores significantly lower than the Langdon durum background parent were those with TDIC chromosomes 1A, 3A, 5B, 7A, and 7B; however none of these by itself is likely to confer adequate resistance to FHB. In a diallele study on the original disomic lines, we found that the strong resistance gene on 3A showed positive combining ability with those on 1A and 6B. In a field trial in 2002 we also confirmed that the FHB resistance on 3A will effectively reduce FHB in a hexaploid wheat background. Several of the chromosomes in these substitution lines are not among those previously recognized to bear FHB resistance genes in hexaploid wheat.

EFFICIENCY AND EFFICACY OF MARKER ASSISTED SELECTION OVER PHENOTYPIC SELECTION FOR FHB RESISTANCE IN DURUM WHEAT

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ABSTRACT

We are studying the efficiency of Marker Assisted Selection (MAS) for Type II Fusarium Head Blight (FHB) resistance in two durum wheat populations derived from a Chinese bread wheat source 'Sumai 3'. This study is based on the hypothesis that for a trait such as FHB, the use of molecular markers for MAS would reduce the time involved in selection along with a reduction in cost. The first population consisted of 1,814 $F_{2:4}$ lines that were developed from crossing a cultivar Ben to Sumai3/Sceptre//D88816 line. The second population consisted of 320 $F_{2:5}$ that were derived from backcrossing cultivar Lebsock to the line Lebsock//Sumai3/Lebsock. These two populations were screened for FHB resistance in the greenhouse in spring 2002 by inoculating the heads with *Fusarium graminearum* and later scoring the diseased heads. Screening for the resistance QTL located on the chromosome 3BS was done using the microsatellite locus *Xgwm533*. In the greenhouse evaluation, 1,124 lines in the first population and 180 lines from the second population were found resistant with scores of less than 21%. Microsatellite marker identified the resistant QTL in 524 lines from population I and131 lines from population II. Apart from the lines that were found to be resistant in the presence of marker and susceptible in its absence, some lines had the marker but were susceptible and some did not have the marker and still were resistant to the disease. Lines representing these four groups will be evaluated in summer 2003 in a replicated scab nursery and the efficacy of both the selection methods will be calculated. In the present study the molecular data showed that using MAS the population size could have been reduced from 1,814 lines in population I to 524 and 131 from 320 lines in population II, thus saving a significant amount of greenhouse space, resources and time in screening. We calculated the efficiency of each selection process so far and found that, with MAS it took us 44 working days to screen the two populations with an approximate cost of $1.43 per data point and with phenotypic selection in the greenhouse; it took 141 days with an approximate cost of $0.99 per data point. In terms of time involved, MAS was found to be 3.2 times quicker saving 97 days. With the use of high throughput non-denaturing gel system, the efficiency of MAS in terms of time and labor will be higher at much reduced cost. In our next step of study, we plan to advance the agronomically desirable lines by repeated backcrossing to cultivars Ben and Lebsock. These lines will then be further analyzed for their FHB resistant phenotype and agronomic performance.

PUTATIVE SOURCES OF FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT IDENTIFIED FROM THE USDA SMALL GRAINS COLLECTION

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INTRODUCTION

The use of host resistance will likely be one of the major components in managing Fusarium head blight (FHB) of wheat. Germplasm improvement and varietal development for FHB resistance will depend upon continued efforts in discovery and characterization of diverse resistant sources. Since 1998, we have evaluated 4,400 accessions of spring wheat from the USDA small grains collection. This report summarizes putative sources of resistance that underwent three consecutive years of field evaluations in replicated trials.

MATERIALS AND METHODS

Spring wheat germplasm from the USDA collection (Aberdeen, ID) were first evaluated in a preliminary screening nursery (PSN). This is a non-replicated nursery with entries planted into rows (ca. one meter in length). ND 2710 and BacUp were used as resistant checks and Sonalika and Wheaton as susceptible checks with a check-to-entry ration of 1:28. The nursery was inoculated with infected corn grain and conidial suspension. Details in nursery management, inoculation, and data collection were as described previously (Zhang *et al*. 2000; 2001). Accessions or plants within an accession with a low FHB index (incidence*severity) and/or low percentage of Fusarium damage 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 and arranged into split-plot design, with maturity as the main plot and genotype as the subplot. Maturity groups were determined based on days between planting and flowering: early (≤55), intermediate (55-65), and late (≥66).

RESULTS AND DISCUSSION

In each of the three evaluation years, high disease pressure was generated by artificial inoculation and mist-irrigation. FHB indices on the susceptible checks (Wheaton and Sonalika) consistently exceeded 80%.

Table 1 lists selections with low FHB indices (≤ 40%) and low FDK (≤ 40%). This group of materials generally exhibited stable low FHB reaction over years. Selections with low FHB indices (≤ 40%) but high FDK (>40%) or high FHB indices but low FDK (≤40%) are given in Table 2. The first group of materials from Table 2, namely Sin Chunaga, Norin 61 and several other lines originated primarily from Japan, consistently showed lower disease indices, but high visual FDK ratings. Kernels rated as FDK in this group were mostly bleached, but remained plump. A recent study on Fusarium infection of seed harvested from the 2002 field

FHB screening nursery suggested that discoloration (bleaching) of plump kernels might not be due to fungal infection (Zhang and Jin, unpublished). Although FHB indices of second group in Table 2 were high, lines in this group generally had low FDK scores and might contribute useful resistance/tolerance genes in breeding.

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Zhang, X., Y. Jin, J. Rudd, and H. Bockelman. 2001. Evaluation of USDA Spring Wheat Germplasm for Fusarium Head Blight Resistance. Pages 220-224 *In:* 2001 National Fusarium Head Blight Forum Proceedings. Dec. 8-10, 2001, Erlanger, KY.

Table 1. Spring wheat germplasm selections with low Fusarium head blight indices and low percentage of damaged kernels.

		FHB index (%)				FDK (%)		
Accession	ID	2000	2001	2002	**mean**	2000	2001	**mean**
PI 382161	Tokai 66	10.5	5.9	12.8	**9.7**	16.0	12.0	**14.0**
PI 382154	Nyu Bai	13.7	9.4	11.9	**11.7**	15.0	15.3	**15.2**
PI 382153	Nobeoka Bozu	17.0	9.8	12.2	**13.0**	13.8	15.7	**14.7**
	ND 2710 (CK)	14.2	10.2	14.7	**13.0**	22.8	19.5	**21.2**
	Sumai 3 (CK)	15.0	17.0	15.8	**15.9**	28.3	25.0	**26.7**
PI 182568	Norin 43	21.5	16.1	28.0	**21.9**	46.7	30.0	**38.3**
PI 462151	Shu Chou W. 3	18.5	29.8	18.9	**22.4**	18.8	20.0	**19.4**
Citr 12002	Renacimiento	25.5	21.2	22.2	**23.0**	41.7	36.7	**39.2**
PI 345731	Tezanos P.P.	20.2	19.3	30.0	**23.2**	20.0	23.0	**21.5**
PI 519790	274-1-118	19.8	28.9	24.8	**24.5**	40.0	35.3	**37.7**
PI 434987	Estazuela Young	22.2	23.4	31.2	**25.6**	58.0	15.5	**36.8**
CItr 5103	274	14.5	27.2	35.1	**25.6**	19.0	23.3	**21.2**
PI 81791	Sapporo H.K.J.	24.4	40.0	15.9	**26.8**	21.7	17.7	**19.7**
PI 596533	**BacUp (CK)**	35.0	16.5	30.2	**27.2**	31.9	17.0	**24.5**
PI 192660	Prodigio Italiano	39.0	20.2	24.1	**27.8**	22.5	18.7	**20.6**
PI 185380	Prodigio Italiano	35.7	25.0	26.2	**28.9**	27.5	16.0	**21.8**
PI 285933	Chudoskaja	36.2	30.5	22.5	**29.7**	26.7	21.7	**24.2**
PI 382167	16-52-9	10.9	28.5	49.8	**29.7**	23.3	18.0	**20.7**
PI 351256	Japon 2	21.2	36.2	38.3	**31.9**	41.7	18.7	**30.2**
CItr 12021	Centenario	32.2	31.8	34.0	**32.6**	41.7	25.0	**33.3**
PI 163429	PI 163429	27.0	31.8	40.2	**33.0**	30.0	28.7	**29.3**
PI 351221	Newthatch Sel.	34.0	35.2	30.0	**33.1**	20.0	20.0	**20.0**
PI 382144	Encruzilhad	29.6	37.3	35.6	**34.2**	45.0	33.3	**39.2**
PI 294975	Artemowska	24.5	67.0	16.8	**36.1**	20.0	20.0	**20.0**
Citr 13136	Rio Negro	42.8	35.2	32.5	**36.8**	50.0	23.7	**36.8**
PI 264927	220	31.9	48.3	30.5	**36.9**	16.7	20.7	**18.7**
PI 104131	Excelsior	36.3	34.2	44.5	**38.3**	21.7	14.0	**17.8**
Citr 17427	16-52-2	34.5	43.5	39.9	**39.3**	33.3	24.0	**28.7**
PI 83729	Magyagovar 81	51.0	49.8	18.2	**39.7**	46.0	22.7	**34.3**
PI 469271	**Wheaton (CK)**	87.6	88.8	83.5	**86.6**	93.3	83.7	**88.5**

***Table* 2.** Spring wheat germplasm selections with low Fusarium head blight indices and high percentage of damaged kernels or vice versa.

		FHB index (%)				FDK (%)		
Accession	ID	2000	2001	2002	**mean**	2000	2001	**mean**
	ND 2710 (CK)	14.2	10.2	14.7	**13.0**	22.8	19.5	**21.2**
	Sumai3 (CK)	15.0	17.0	15.8	**15.9**	28.3	25.0	**26.7**
PI596533	**BacUp (CK)**	35.0	16.5	30.2	**27.2**	31.9	17.0	**24.5**
PI469271	**Wheaton (CK)**	87.6	88.8	83.5	**86.6**	93.3	83.7	**88.5**
PI478282	**Sonalika (CK)**	87.1	84.3	87.8	**86.4**	76.4	84.0	**80.2**
PI382140	Abura	15.1	17.7	17.5	**16.8**	38.3	47.3	**42.8**
PI182561	Sin Chunaga	22.7	22.0	22.0	**22.2**	86.7	76.7	**81.7**
PI182586	Norin 43	30.0	20.7	26.7	**25.8**	50.0	56.7	**53.3**
PI197128	Shinchunaga	17.0	36.7	27.8	**27.2**	80.0	76.7	**78.3**
PI182583	Chuko	19.1	39.8	23.2	**27.3**	78.8	75.0	**76.9**
PI411132	Gogatsu-Komugi	28.3	24.2	34.2	**28.9**	77.5	66.7	**72.1**
PI351816	Froment Du Japon	32.0	29.0	33.0	**31.3**	70.0	33.3	**51.7**
PI182591	Norin 61	37.0	29.2	31.0	**32.4**	66.7	41.0	**53.8**
PI192634	Trintecinco	53.7	23.1	49.2	**42.0**	41.7	34.7	**38.2**
PI351743	CLUJ49-926	40.2	65.0	21.5	**42.2**	26.0	30.0	**28.0**
PI185843	Surpresa	57.7	39.7	30.2	**42.5**	42.5	23.3	**32.9**
PI362437	III/14-B	35.5	52.3	43.0	**43.6**	33.3	22.3	**27.8**
PI264998	628	43.7	47.8	40.5	**44.0**	30.0	26.7	**28.3**
PI264940	111a	55.3	47.7	30.0	**44.3**	41.7	16.0	**28.8**
PI168727	Bahiense	36.8	28.2	68.5	**44.5**	25.0	19.7	**22.3**
Citr2492	Manchurian	57.0	23.0	56.0	**45.3**	25.0	23.5	**24.3**
PI344467	Oncativo Inta	48.0	45.3	48.5	**47.3**	38.8	31.7	**35.2**
PI256958	Academia 48	42.4	59.0	46.2	**49.2**	20.0	26.7	**23.3**
PI163439	PI163439	59.0	38.6	51.0	**49.5**	40.0	27.7	**33.8**
PI132856	Mentana	48.3	41.8	60.3	**50.1**	36.7	29.7	**33.2**
PI351993	Z.88.54	54.2	57.2	39.5	**50.3**	30.0	25.3	**27.7**
PI168716	Klein Condor	54.8	64.7	32.2	**50.6**	35.0	31.0	**33.0**
PI349534	533B	54.3	67.3	34.0	**51.9**	26.7	20.0	**23.3**
PI351476	Vaulion	55.8	75.3	35.4	**55.5**	25.0	40.0	**32.5**
PI184512	H 51	76.7	37.7	52.5	**55.6**	33.3	19.0	**26.2**
PI344465	Laureano Alv.L.	48.3	62.5	61.2	**57.3**	36.7	30.0	**33.3**
PI192219	Hatvani	48.8	79.3	44.4	**57.5**	36.7	25.3	**31.0**
CItr11215	Belgrade 4	39.6	84.3	54.3	**59.4**	35.0	29.0	**32.0**
PI344454	Buck Austral	64.5	81.5	34.6	**60.2**	28.8	30.0	**29.4**
PI351187	Taillens Velu Sel.	46.5	79.5	58.1	**61.4**	26.7	34.0	**30.3**
PI113949	Stepnjachka	63.0	70.8	50.7	**61.5**	38.3	24.7	**31.5**
PI519798	PF 79782	35.9	67.0	82.5	**61.8**	27.5	24.0	**25.8**
PI225160	Mentana	39.0	68.0	81.2	**62.7**	30.0	27.5	**28.8**
PI584934	Westphalen	62.5	69.3	58.0	**63.3**	41.3	31.7	**36.5**
PI362043	Arnautde Toam.	59.6	61.3	79.5	**66.8**	23.3	33.3	**28.3**
PI352000	Z.89.37	52.2	82.2	68.5	**67.6**	26.7	43.3	**35.0**
PI192229	Gran Com.Ung.	57.7	77.8	74.5	**70.0**	31.7	40.0	**35.8**
PI113948	Kooperatorka	77.7	88.3	61.7	**75.9**	26.3	43.3	**34.8**

THE DEVELOPMENT OF SCAB (*FUSARIUM GRAMINEARUM*) RESISTANT VARIETIES OF WHEAT

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OBJECTIVE

The primary objective was to identify and develop elite winter wheat varieties that are tolerant to Fusarium head blight (FHB, scab). The second objective was to field screen the elite hard winter wheat lines including those in the Regional Germplasm Observation Nursery (RGON).

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 resistance. 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 in the Regional Germplasm Observation Nursery (RGON).

MATERIALS AND METHODS

Sources of FHB resistant germplasm originating from our biotechnology efforts, spring and soft wheat germplasm, and exotic germplasm, were collected for crossing into our elite lines. F2 and F3 seed produced from these crosses was screened for FHB resistance, in the field in 2002.

All solutions of inoculum used in the greenhouse and field, were created by combining 6 isolates of *Giberella zeae* and 5 isolates of *Fusarium graminearum* to create a 70000 conidia/mL solution. We screened the germplasm for FHB tolerance, in the greenhouse to allow for better parent identification. Nine replicates of each line were screened in the greenhouse using a randomized complete block design. One spikelet per head was injected with 0.1 mL of a 70000 conidia/mL solution. The plants were then misted for 72 hrs at 98% humidity. Concerns about induced resistance in response to injury, led us to adjust this method. In later studies, the replication number was increased from nine to twelve and 2 mL of 70000 conidia/mL, was sprayed onto the entire head and sealed it in a 16 x 9.5 cm^2 snack

size Ziploc bag for 72 hrs. This procedure avoids false negatives, due to potential induced resistance from awns being cut or injection.

In the field, twenty six 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. Inoculation was carried out in two ways; naturally occurring *F. graminearum* infected corn stalks were spread in the field in fall; and 70000 conidia/mL of inoculum, was sprayed 4 times, at a rate of 50 mL per plot, using a CO^2 powered back pack sprayer, in 2002. This was followed by mist irrigation, using a modified misting system similar to that employed by Zhang *et al*. (1999) to mist the plots for 2 minutes at 30 min intervals. This began 1 week before the plots were inoculated and continued until the first readings were taken. Bordering the scab nursery with forage triticale provided an excellent buffer and greatly reduced wind in the misting nursery.

FHB was rated by counting the number of infected spikelets on 30 individual heads (Shaner and Buechley, 2001). Plot severity or FHB index was calculated by the averaging the 30 FHB ratings. Intensity was calculated by taking a count of the infected heads and dividing it by 30 the total # of heads scored. 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

Of the eight hundred lines that were screened in 2002, sixty have had extensive FHB screening in at least 3 mutually exclusive trials, including an independent determination using different isolates, by South Dakota State University. Fig 1, shows nineteen lines that have consistently shown significant FHB resistance relative to a FHB susceptible variety “Wahoo”. These lines will be screened again in the field in a replicated trial in 2003. Of the RGON lines, 42% show promise and will be screened in a replicated trail in 2003. The grain, from one of our three most advanced nurseries showed no correlation between levels of DON and the # of FHB infected florets per head (Fig 2).

CONCLUSION

FHB tolerance in winter wheat breeding nurseries was generally high. F2 and F3 seed produced from the FHB crosses was screened in the field in 2002. Additional seed will be produced from the new crosses that have been made for future planting in the field. As soon as we have recovered bulks with the level of agronomic performance required to survive our winters, are resistant to stem rust *(P. graminis* Pers. : Pers. f. sp. *tritici* Eriks & E. Henn), and yield well, head row selection for elite line identification, will begin.

In the 2002-2003 cycle, the most FHB tolerant transgenics will be crossed to varieties having some FHB tolerance, and to Wesley, a very widely grown, but FHB susceptible line. 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.

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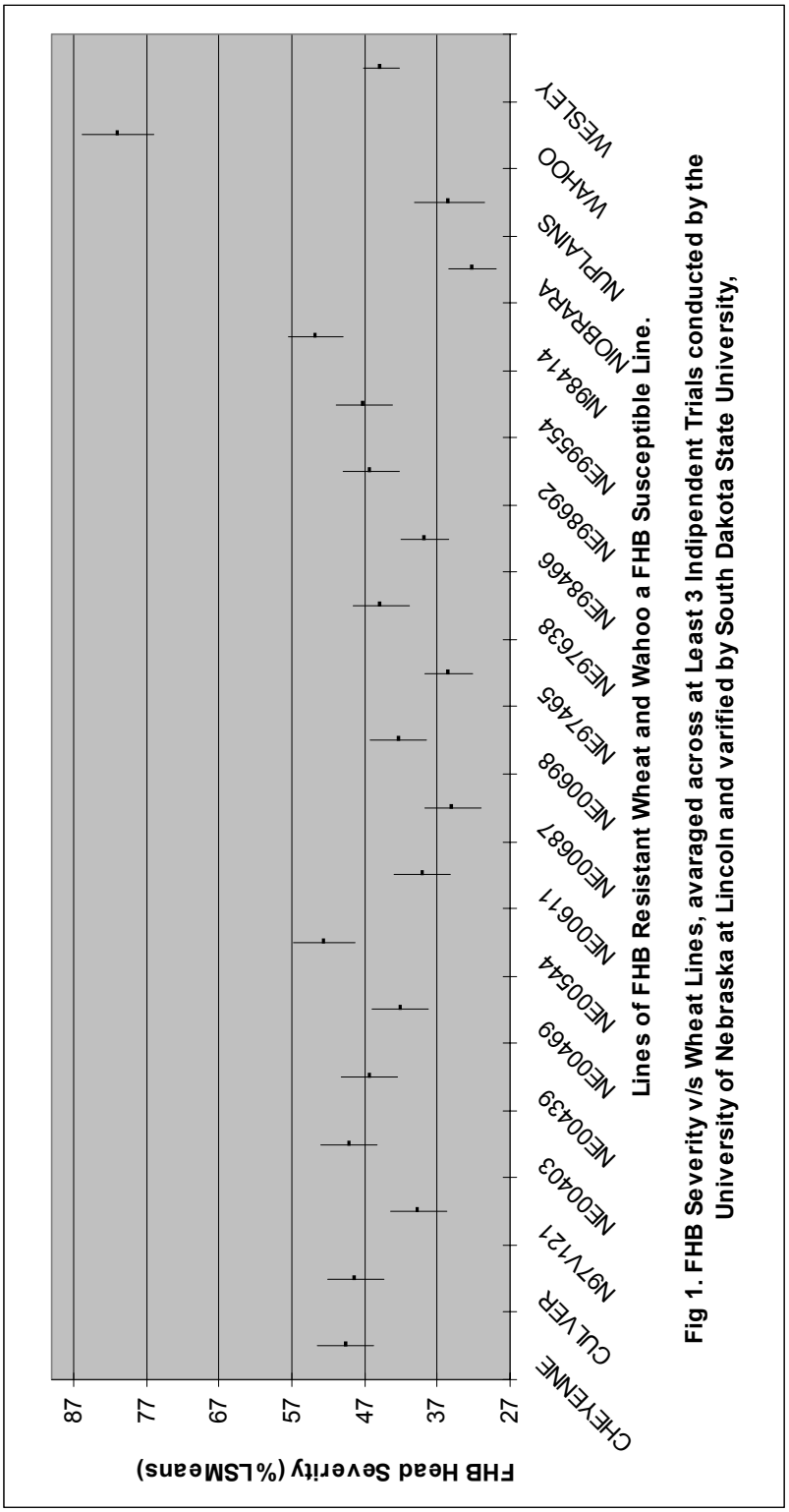

Fig 1. FHB Severity v/s Wheat Lines, avaraged across at Least 3 Indipendent Trials conducted by the University of Nebraska at Lincoln and varified by South Dakota State University,

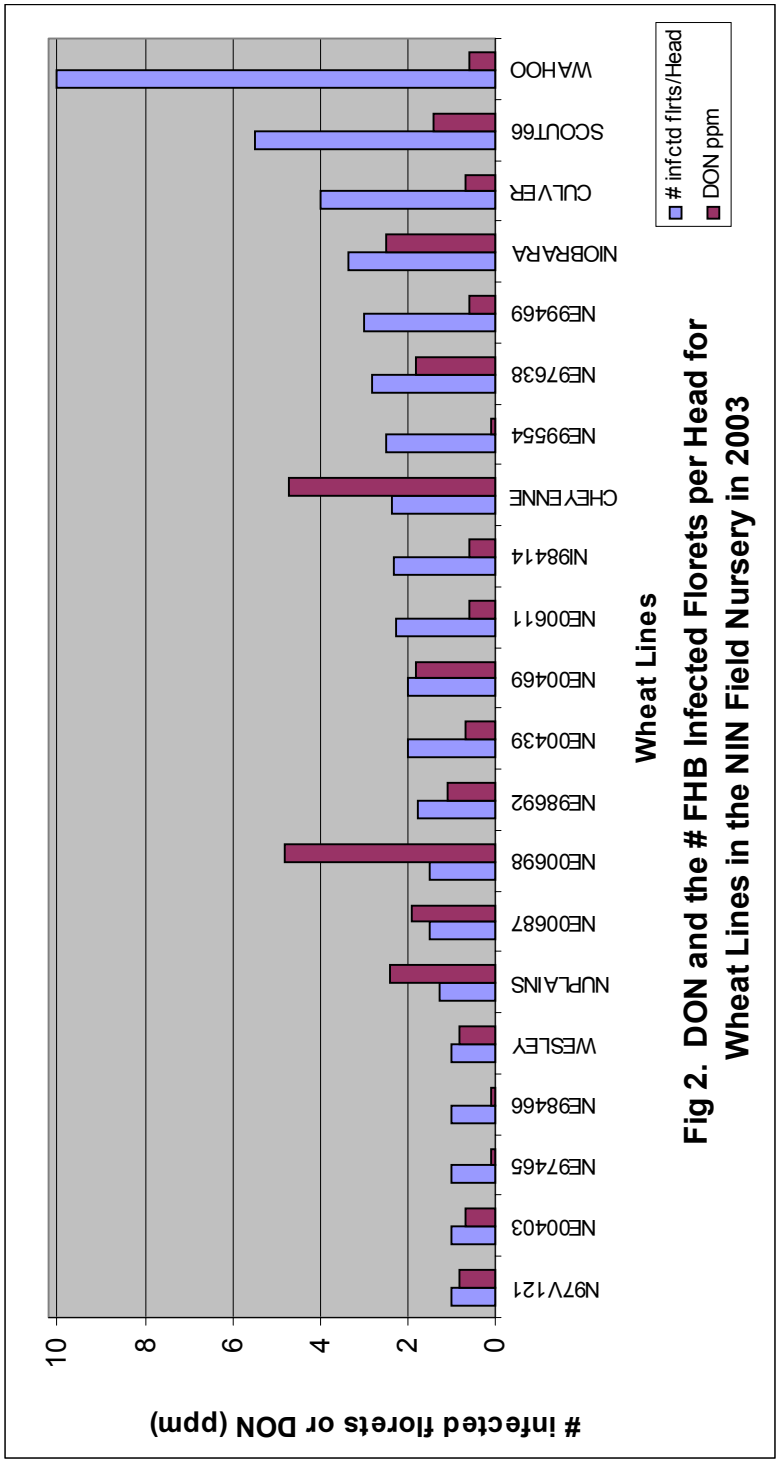

Fig 2. DON and the # FHB Infected Florets per Head for Wheat Lines in the NIN Field Nursery in 2003

NUMBER OF LOCATION-YEARS NEEDED TO DETERMINE THE REACTION OF WINTER WHEAT CULTIVARS TO FUSARIUM HEAD BLIGHT

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ABSTRACT

Fusarium head blight (FHB) is a serious disease of wheat and barley that is best controlled by host resistance. In KSU Extension publications, the reaction to FHB for winter wheat cultivars is reported to producers using a 1-9 scale where 1-3 = resistant, 4-6 = intermediate, and 7-9 = susceptible. This research sought to determine how many location-years are needed to accurately determine the reaction of a cultivar to FHB. Twenty-nine different winter wheat cultivars were screened 2-12 times in 12 field nurseries over a 3-yr period (n=133). Experimental design for each location-year was a randomized complete block with four replications. Corn grains, colonized by *Fusarium graminearum* and spread on the soil surface, followed by sprinkler irrigation were used to produce the epidemic. FHB index (% diseased spikelets) was determined for each cultivar between four and six times for each experiment and averaged. To compare data across location-years, linear regression was used to fit a 1-to-9-scale model to the data for each location-year. To produce the model, an index value of zero was assigned a scale value of 1 and the highest index value in a location-year was assigned a scale value of 9. The model was then used to calculate scale values for all other cultivars in that location-year. A mean scale value was calculated for each cultivar (n=2-12 location-years) and an overall standard deviation for all cultivar-location-years (n=133) was calculated using the departure from the mean for each scale value for each cultivar-location-year. To estimate the number of location-years needed to determine the reaction of a cultivar to FHB, the formula x-bar +/- t(alpha/2) * s / sqroot(n) was used to calculate 95% confidence intervals. In this formula, x-bar is the observed mean scale value (1-9 scale) of a cultivar, t(alpha/2) is the t-value corresponding to the desired alpha level (0.05) divided by 2, s is the standard deviation among location years, and n is the sample size (number of location years). If an observed mean scale value is within +/- 0.5 units of the correct value, it will be rounded to the correct scale value. Required standard deviations to produce a mean within +/- 0.5 units were calculated for samples of n=2-20 (not shown). If an observed mean scale value is within +/- 1.5 units, it will be rounded to a scale value that is +/- 1 unit from the correct scale value. To achieve a mean scale value within +/- 1.5 units, required standard deviations for samples of n=2, 3, 4, and 5 are 0.167, 0.604, 0.943 and 1.208, respectively. In our data, the overall standard deviation for departure from the mean for all cultivar-location-years (n=133) was 1.05. Based upon the overall standard deviation, 20 location-years would be needed to have a mean scale value that would have a 95% chance of being within +/- 0.5 units of the correct mean and, therefore, rounded to the correct scale value. Based upon the overall standard deviation (1.05), 5 location-years would be needed to have a mean scale value that would have at least a 95% chance of being within +/- 1.5 units and, therefore, rounded to +/- 1 unit of the correct scale value. For most purposes, +/- 1 unit is sufficient accuracy for producers; therefore, we recommend that reactions of winter wheat cultivars that are reported to producers be based upon data from at least five location years.

IDENTIFICATION OF DNA MARKERS FOR FUSARIUM HEAD BLIGHT RESISTANCE OF WHEAT LINE HUAPEI 57-2

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ABSTRACT

Fusarium head blight or scab greatly affects grain yield and quality of wheat (*Triticum aestivum* L.). Because it is a trait of low heritability and costly to evaluate, marker assisted selection is particularly attractive for breeding programs. To identify DNA markers for Fusarium head blight resistance, a population of 163 recombinant inbred lines was developed by single seed descent from the cross between the resistant line 'Huapei 57-2' and the moderately susceptible cultivar 'Patterson'. All lines including parents were evaluated in one field experiment and two greenhouse tests for resistance to spread of disease (Type II resistance). Based on phenotypic data, extreme lines were selected to initiate bulked segregant analysis using microsatellites. Markers suggesting association with a putative quantitative trait locus (QTL) were then tested on the entire population to confirm the linkage. A major QTL was identified on the chromosome 3BS in a region well known from previous studies. Additional QTLs were also found on chromosomes 3A, 3BL and 5B.

COORDINATED FUSARIUM HEAD BLIGHT SCREENING NURSERY FOR WHEAT BREEDING PROGRAMS IN WESTERN CANADA

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ABSTRACT

Fusarium head blight (FHB) continues to be a serious disease of wheat in western Canada and in particular, the eastern prairies. Screening for FHB resistance has been difficult for breeders working outside of the eastern prairie region. As a result, breeders and pathologists entered into a collaborative agreement to establish a common FHB screening nursery at Carman, Manitoba. This region is known to provide an environment that is more conducive to FHB development. The nursery was established in 2001, and lines were evaluated in 2001 and 2002. Advanced lines that are in the final stages of testing for cultivar registration were evaluated in replicated rows, while earlier generation breeding lines were evaluated in non-replicated rows. In 2001 approximately 6000 1 m row plots were grown in the nursery. In 2002 the number of plots was increased to approximately 9900. Five checks were placed every 50 plots within the nursery. In 2001 the checks were AC Morse, AC Vista, CDC Teal, FHB 37 and Glenlea. The same checks were used in 2002 with the exception that CDC Teal was replaced by AC Cora to provide a check with an intermediate FHB reaction. In 2001 FHB infected corn inoculum was applied to plots two to three weeks prior to anthesis. Date of heading and anthesis were recorded for each plot. A macroconidial suspension of *F. graminearum* was applied to plots at 50% anthesis and again three to four days later. After each macroconidial inoculation, plots were irrigated with a mist irrigation system to maintain high humidity. Eighteen to 21 days after inoculation each plot was visually rated for incidence (% of spikes infected) and severity (% area of spike infected) of FHB infection and an FHB index was calculated. In 2002 corn inoculum was not applied to the plots but all other inoculation and evaluation protocols were similar to 2001. In 2001 conditions in the nursery were highly conducive to FHB development. The mean FHB index on susceptible checks ranged from 28 to 41. The resistant check had an FHB index of 5. This provided a good distinction between susceptible and resistant lines. In 2002, weather conditions were drier and cooler than in 2001 and FHB levels were lower in the nursery, overall. The mean FHB index for the susceptible checks ranged from 11 to 21. The intermediate check produced a mean FHB index of 6, while the mean FHB index of the resistant check was 0.3. Therefore, there were still clear distinctions between resistant and susceptible lines. However, disease levels were higher in groups of lines that were inoculated earlier in the season, when conditions were warmer and more humid, than those inoculated later in the season. The variability noted in the nursery indicated that there may be more escapes in the 2002 nursery and that lines with intermediate reactions may be difficult to separate from resistant lines. This emphasizes the need for multi-year testing to fully characterize FHB reaction. In general this nursery is providing useful information to plant breeders and will facilitate development of FHB resistant cultivars.

TIMING OF INOCULATIONS OF DRYLAND WHEAT PLOTS AND THE EFFECT ON FUSARIUM HEAD BLIGHT (FHB) SEVERITY AND MYCOTOXINACCUMULATION DUE TO *FUSARIUM GRAMINEARUM* INFECTION

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ABSTRACT

Eight wheat (*Triticum aestivum*) genotypes were assessed for reaction to FHB in a dryland inoculation study. The test was a randomized complete split-split block design. Main-plots were timings of inoculation (TOI), sprayed at anthesis (SAA), 3 days post-anthesis (DPA), and 6 DPA. Sub-plots consisted of two spray-inoculation treatments, inoculated once or twice, and sub-sub plots were the eight wheat genotypes. Significant differences were found among TOI treatments for FHB severity (7.1 % SAA, 7.7 % sprayed 3 DPA, and 0.6 % sprayed 6 DPA with $l.s.d._{0.05}$ = 2.42) and deoxynivalenol (DON) accumulation (3.0 ppm SAA and 3 DPA, and 0.3 ppm sprayed 6 DPA with $l.s.d._{0.05}$ = 0.60). Average DON ppm accumulations in grain of wheat genotypes were 0.2 for BacUp, 0.2 for ND2710, 0.2 for Ingot, 0.2 for Forge, 0.8 for Oxen, 1.4 for Parshall, 5.3 for Norm, and 8.6 for Wheaton ($l.s.d._{0.05}$ = 1.07). Our data demonstrate that dryland inoculations of wheat can be useful for screening germplasm for reaction to FHB.

VARIETY DEVELOPMENT AND UNIFORM NURSERIES: FHB RESISTANCE IN BARLEY

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ABSTRACT

Acknowledgements: This report is an overview of research progress made on FHB resistance by researchers of cooperating barley improvement programs in the upper Midwest.

Development of barley (*Hordeum vulgare*) cultivars having resistance to Fusarium head blight (FHB), incited primarily by *Fusarium graminearum*, is the main goal of barley improvement programs in the upper Midwest. Unlike many other disease problems, strategies for control of FHB did not exist prior to 1993 when epidemics began to occur annually. Initial goals included: 1) identification of accessions resistant to FHB, 2) establishment of screening procedures, 3) determination of inheritance patterns for FHB resistance, and 4) development of more focused breeding schemes. Nearly ten years later, what have we accomplished? Or more important, have we developed strategies for control of FHB?

FHB screening nurseries were established in North Dakota (ND) and Minnesota using prepared inoculum and mist irrigation systems to enhance disease development. Cooperative nurseries were developed in Eastern China where natural inoculum and favorable weather often cause high levels of FHB. Greenhouse tests using various inoculation procedures were conducted. Laboratory testing of cultivars and breeding lines for deoxynivalenol (DON) content helped determine the effectiveness of genetic and cultural controls of FHB. We have learned that the FHB problem on barley in the upper Midwest has both regional and international aspects. Midwest spring barley cultivars, both two- and six-rowed, have a unique genetic system for control of maturity and plant height. FHB is a problem in barley growing areas where these adaptation genes are used: the Canadian Prairies, Central Mexico, and Uruguay. Thus, the cooperative regional FHB nursery (MinnDak) was expanded in 2002 to include a Canadian cooperator and a test site at Brandon, Manitoba, and renamed the North American Barley Scab Evaluation Nursery (NABSEN). Agreements to have the ICARDA/CIMMYT barley program in Mexico as a full participant in the NABSEN nursery for 2003 season are in place. Contacts have been made regarding a participant in Uruguay. Data from the MinnDak and NABSEN nurseries suggest that progress in development of FHB resistant cultivars has been slow. Differential heading dates or photoperiod responses across sites contribute to the variable data obtained on FHB incidence and DON values. Expanded cooperation on FHB testing should improve our understanding of these problems.

Barley accessions from southern Germany and eastern China were identified as having the high levels of FHB resistance. Some resistance is also present in current Midwest barley cultivars. Cultivars from Brazil (PFC88209) and Mexico (Atahualpa) are being used as sources of FHB resistance. Evaluations of mapping populations have found QTL for FHB

resistance on all seven barley chromosomes. The largest ones were consistently located on chromosome 2H near loci that control spike type (*vrs1*), spike length (*lin1*), plant height (*hcm1*), and heading date (*Eam6*) in Midwest barley cultivars. Most FHB resistant accessions differ from Midwest cultivars in the alleles present at these four loci. Since at least three QTL for FHB resistance on chromosome 2H may be involved, a major breeding problem exists. This linkage group help explain why many FHB resistant selections have two-rowed spikes and are tall and late. A further complication is the lack of recurrent parents having good resistance to leaf spot diseases incited by *Cochliobolus sativus*, *Pyrenophora teres*, and *Septoria passerinii*.

Utilization of the two-rowed cultivar Conlon as a malting barley has provided barley growers in ND with some relief from FHB epidemics. Conlon often has slightly lower FHB readings and significantly lower DON values than other malting barleys recommended in ND. Yet, much higher levels of FHB resistance are needed to keep malting barley as major crop in the upper Midwest. Progress is being made in developing breeding lines with more FHB resistance, accessions as Shenmai 3 with more resistance to FHB are being identified among early-heading two-rowed lines from eastern China, alternative genes for control of plant height and maturity are being investigated, alternative breeding strategies are being evaluated, and results from marker assisted selection experiments are positive. None of these studies, however, offers a quick, easy solution to the FHB problem in barley.

A *FUSARIUM* RESISTANCE GENE AND AN AWN PROMOTOR ARE ASSOCIATED ON CHROMOSOME 5A OF SPRING WHEAT

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ABSTRACT

Fusarium head blight (FHB) has been a serious concern in the spring wheat region of the northern Great Plains for nearly a decade. Most spring wheat cultivar are moderately to highly susceptible to FHB, and no lines are completely immune to infection. Farmers in the region continue to grow susceptible cultivars, in part because most of the lines released as having resistance to FHB have other more severe defects. One North Dakota line, 'ND2710', derived from the Chinese wheat Sumai 3, has shown high resistance to FHB in many environments. Our objective was to determine the chromosome(s) where the FHB resistance gene(s) in ND2710 reside using the set of 21 monosomic lines developed by S.S. Maan in the 1960's and based on the hard red spring wheat cultivar 'Chris'. For the present investigation, the entire set of 21 Chris monosomic plants as female were crossed to ND2710. The monosomic F1's were identified and 20 or more monosomic F1-derived F-2's were grown and advanced to F2:5 by single seed descent. Seed from the F5 lines was planted in a field FHB testing nursery in 1999. Plots of the parents and a non-monosomic Chris X ND2710 check population also were present. In this FHB nursery, disease was produced by inoculation with *Gibberella zeae* and regular mist irrigation. At 3.5 weeks post anthesis, spikes were cut and frozen for later scoring. Individual spikes were scored using a 0 - 100% scale for FHB severity and plot means calculated. On average there were 24 spikes per plot and 45 plots per monosomic cross. In 2000, there were 15 spikes scored per plot and 50 plots per monosomic cross. The control cross Chris (tip awned spikes) X ND2710 (awned spikes) produced F1's with all tip awned spikes, as did 19 of the 21 monosomic F1's. In subsequent generations these displayed segregation for awns. Crosses of monosomic 2A and monosomic 5A produced disomic and monosomic F1's which had awned spikes. Subsequent generations also had awned spikes. In 1999 and 2000, the Chris/ND2710 check population had a mean FHB severity score of 40%, just midway between the scores of the parents Chris (62%) and ND2710 (22%). This is typical of FHB scores in such resistant by susceptible crosses. Several of the monosomic crosses, however, had FHB severity scores significantly lower; in particular, ChrisM5A/ND2710 was nearly as low as the resistant parent ND2710. When the co-occurrence of awn type and FHB score was tested, they were found to be associated and not independent. We suggest that a FHB resistance gene is associated with an awn promotor in chromosome 5A of ND2710. The type of association between these two remains to be determined but we propose that Chris mono 5A has a T 2A 4A translocation chromosome and ND 2710 has an awn promoter on 5A. Chromosome banding is presently being used to verify the translocation. (This poster was presented at the 2001 ASA Annual Meeting, Charlotte NC Nov. 2001)

A HISTORICAL ANALYSIS OF THE UNIFORM REGIONAL SCAB NURSERY FOR SPRING WHEAT PARENTS

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OBJECTIVES

We sought to evaluate progress that has been made by spring wheat breeding programs seeking to enrich their germplasm pool for resistance to Fusarium head blight. Fusarium head blight resistance data of spring wheat germplasm representing several breeding programs in the Upper Midwest and Canada is available through annual reports of the Uniform Regional Scab Nursery for Spring Wheat Parents (URSN). We sought to use the data in these reports to monitor progress in scab resistance enhancement within spring wheat germplasm during the last seven years, during which time breeding programs in the spring wheat region have focused intensively on achieving this goal.

INTRODUCTION

The severe epidemics of Fusarium head blight, or scab, in the 1990's have caused economic losses that measured in the billions of dollars in the spring wheat region encompassing North Dakota, Minnesota, and South Dakota (McMullen, 1997; Nganje, 2001). As a result of these epidemics, concerted efforts to develop scab-resistant wheat varieties were accelerated in several regions of the U.S. and continue today. A component of these efforts was the implementation of regional nurseries specifically devoted to assessing new wheat germplasm emerging from breeding programs for scab resistance at multiple locations. For the northern spring wheat region, Dr. Robert Busch established the Uniform Regional Scab Nursery for Spring Wheat Parents (URSN) in 1995. It has since been conducted annually, with support from the U.S. Wheat and Barley Scab Initiative.

The benefits of the URSN and similar nurseries for other market classes of wheat are twofold. First, these nurseries provide a vehicle for obtaining multi-site scab resistance data, which is important given the large environmental effect on scab development and severity (Groth *et al*., 1999). Second, these nurseries provide a means of germplasm exchange among wheat breeding programs. An additional benefit of these nurseries is that they provide a record of progress in enhancing scab resistance in wheat germplasm over time. The goal of this paper is to provide a historical review of progress in developing scab-resistant germplasm in the northern spring wheat region, based on several years of URSN data.

MATERIALS AND METHODS

Data on scab resistance were obtained for the years 1996 through 2001. Although 1995 was the first year that of the URSN was run, consistent protocols for rating scab (disease

index = incidence x severity) and kernel damage (tombstones or vsk) started in 1996. Thus, the analysis was restricted to data for the years 1996-2001. Entry lists and complete data for each year of the URSN are available at gopher://greengenes.cit.cornell.edu:70/11/.Performance/.hrswregional/Uniform%20Regional%20Scab%20Nursery/. For purposes of analysis, BacUp and ND 2710 were designated as resistant checks because they were grown in each of these years. For each of these years, the grand mean for disease index across entries (excluding checks, durums, and plant introductions) was calculated. A standardized disease index for each year was obtained by dividing each grand mean by the disease index grand mean for BacUp and ND 2710 in the same year. This same procedure was also completed for tombstone frequency.

RESULTS

Since 1995, the URSN has been grown at six different locations in Minnesota, North Dakota, South Dakota, as well as Canada (attempts to grow the nursery further south in Iowa were attempted for a few years, but were not successful and thus were not continued). The number of entries in each year has increased relatively steadily during this period, and is now averaging over 40 per year. These entries include common wheat, durum wheat and more recently, plant introductions. The entries have come from public breeding programs in the abovementioned states and Canada, as well as from private breeding enterprises. Several resistant and susceptible check varieties were included in each nursery. General information on the URSN from 1996-2001 is summarize in Table 1.

Table 1. Summary statistics for the URSN common wheat entries, 1996-2001.

Year	Number of Locations	Number of Entries[1]	Disease Index	Disease Range	Mean Tombstone	Tombstone Range
1996	6	31	38	12-73	24	7-59
1997	6	31	38	20-65	29	15-45
1998	6	25	40	21-62	25	13-40
1999	7	30	42	24-63	28	16-42
2000	7	26	25	11-39	21	12-32
2001	7	29	32	16-55	27	14-47

[1]Excludes checks, durum entries and unimproved introductions.

URSN entries were categorized by their resistance source(s) by examination of their pedigrees (Table 2). The number of “native” resistance sources (i.e. no identifiable scab resistance source in their pedigree) dropped from 17 of 31 entries in 1996 to only 5 of 25 entries in 1998. This corresponded with an increase in the number of entries with parentage tracing to Sumai 3 and its derivatives. In recent years, entries with South American and European sources of scab resistance were entered in the nursery. Including composite crosses, the number of entries with multiple resistance sources (excluding native sources) in their pedigree have been limited, averaging three per year. The majority of entries in 1995-1997 containing a defined resistance source were 1/4 to 1/2 by pedigree of that resistance source. By comparison, the 2002 nursery contained only one entry that is 1/4 Sumai 3 by pedigree, with all other entries 1/8 or less.

Table 2. Fusarium head blight Scab resistance source of entries in the URSN common wheat entries, 1995-2002.

		Resistance Source[1]				
Year	No. Entries	"Native"	Sumai 3	Nyu Bay	S. Amer.	Europe
1995	28	16	11	1	0	0
1996	31	17	13	1	0	0
1997	31	10	17	0	4	0
1998	25	5	18	2	0	0
1999	30	9	18	3	0	0
2000	26	5	20	1	0	0
2001	29	4	21	2	2	3
2002	27	5	17	2	0	2

[1]"Native" sources include those present in the germplasm prior to 1990 and not containing an identifiable scab resistance source in its pedigree; Sumai 3 includes its derivatives, Ning 8331 and Ning 7840.

Evaluating historical trends in scab resistance and related traits within the URSN over time provides a means of assessing progress by spring wheat breeding programs attempting to enrich for scab resistance in their germplasm. We assessed this progress by calculating standardized disease indices and tombstone frequencies, relative to the resistant checks BacUp and ND 2710. The results of this analysis suggest that the relative scab resistance of the germplasm entries has increased substantially since 1996. In 1996, the disease index of entries was approximately 216% of the mean of BacUp and ND 2710. However, between 1997 and 1999, this decreased significantly, with relative disease levels dropping to 128% of the resistant checks by 1999 (Table 3). This trend reversed somewhat in 2000 and 2001 however, with the relative disease index of entries rising to approximately 153% of the resistant check means in 2001. The standardized tombstone frequencies of entries also exhibited consistently lower values between 1997 and 2001, relative to 1996 (Table 3).

Table 3. Standardized scab disease indices and tombstone frequencies for URSN common wheat entries, 1996-2001.

Year	Standardized Disease Index[1]	Standardized Tombstone[1]
1996	216	228
1997	151	123
1998	129	138
1999	128	144
2000	149	138
2001	153	143

[1]values are the % of the mean of BacUp and ND 2710.

DISCUSSION

The major benefit that the URSN provides to wheat breeders is that it permits germplasm to be evaluated in multiple locations under different environmental conditions. Given the large effect that the environment has on expression of scab resistance, evaluation of a genotype in multiple environments ensures that useful data are obtained. However, the data obtained by the URSN is also useful for assessing overall progress by several different breeding programs each seeking to develop resistance to the same disease. The results of our analysis suggest that the different breeding programs that, as a whole, contribute contributing entries to the URSN for evaluation, as a whole, have made substantial progress in enhancing scab resistance in their germplasm over the last 6 years. The challenge that remains is to determine whether the plateau of resistance that has been obtained to date, principally by deploying Sumai 3-derived resistance, can be reduced further by incorporating new sources of resistance. Entries in the nursery the past few years also have improved agronomic qualities and resistance to rust diseases as the proportion of the pedigree, by resistance source, has dropped to less than 1/8 for the majority of entries. Incorporating additional novel sources of resistance and combining it with the Sumai 3 resistance is necessary to provide higher levels of resistance and genetic diversity.

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GENES WITH MAJOR EFFECTS ON FHB RESISTANCE PROMISE EASY MARKER APPLICATION

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OBJECTIVES

To determine the inheritance of resistance to FHB in three resistant bread wheat lines, and describe the implication of this finding.

INTRODUCTION

Chromosomes and genes with major and minor effects - Many chromosomes have been implied to carry genes conferring resistance to Fusarium head blight (FHB). However, some contribute more to overall resistance than others. Likewise many genes have been reported, but there is evidence that gene effects may vary, with some genes having a stronger effect on phenotype. Molecular analyses also indicate that many different genes are available in genetic stocks (Anonymous, 2001).

The inheritance of FHB resistance in some of the key progenitors, such as Frontana, Sumai 3 and Ning7840, used by wheat breeding programs around the world is likely to be controlled by two or three genes with major effects (Ban and Suenaga, 2000; Singh *et al*., 1995a; van Ginkel *et al*., 1996). The latter study also found evidence that the four genes were distinct, providing promise that pyramiding these genes may result in increased resistance (Singh and van Ginkel, 1997).

In this study we determined the mode of inheritance of FHB resistance in three wheats considered to be genetically distinct based on their pedigrees.

MATERIALS AND METHODS

Genetic materials - We used the following three lines expressing similar FHB resistance but of distinct parentage (see Table 1).

Table 1. Parental lines used in the inheritance study.

G O V /A Z //M U S /3 /D O D O /4 /B O W
C A T B IR D
B A U /M IL A N

Some of these lines, such as Gov/Az//Mus/3/Dodo/4/Bow and Catbird-derived lines, have been successfully used by participating breeders the U.S. Wheat and Barley Scab Initiative in crosses to locally adapted germplasm (Bacon *et al*., 2000).

Random F2-derived F8 progenies of crosses among the three resistant parents were used in this inheritance study.

Inoculation methodology - Inoculation was carried out at anthesis, using the so-called 'cotton method' to detect Type II resistance (Gilchrist *et al*., 2002, this Proceedings). Evaluation of infection was carried out by counting the total number of spikelets per spike and the number of spikelets infected; an infection percentage was then obtained.

RESULTS

The distributions of the 200 F8 lines per cross displayed discrete classes in each case and X^2 analyses confirmed a relatively simple gene inheritance. The data confirmed a preliminary analysis in 2001 (van Ginkel *et al*., 2001).

1. In the crosses of BAU/MILAN with GOV/AZ//MUS/3/DODO/4/BOW and BAU/MILAN with CATBIRD, two major genes segregated.

2. In the cross of CATBIRD and GOV/AZ//MUS/3/DODO/4/BOW, four genes of major effect segregated.

3. A total of four loci were involved.

DISCUSSION

With some luck and perseverance, FHB may become a textbook example of the application of markers to breeding. Why? Two points in favor need to be considered.

1. It is clear from the literature that while many chromosomes have been implicated and many genes have been described as contributing to FHB resistance, a few key genes often explain most of the variation observed. Their gene action is frequently observed to be additive. This study of just three parents found up to four genes controlling resistance, confirming that genes for scab are not uncommon in wheat. Genes with such major effects should be targeted for use in the application of markers in wheat breeding.

2. The evaluation of germplasm for FHB response is cumbersome, requiring several rounds of inoculation, a minimum of 5-10 inoculated spikes per plot, and replication at each site and across years, to show consistency in resistance response. Frequently, germplasm that is resistant one year may no longer be so the following year. Only after 3-4 years of testing can resistance patterns be confirmed, and confident statements made about resistance/susceptibility. The reason lies in the significant interaction between infection processes and climatic factors. This is not very encouraging for a breeding program hoping to make rapid progress.

Simple genetic resistance (point 1) is confirmed through a time-consuming and extremely laborious process (point 2). Hence, developing and then linking phenotypic data with molecular data is a protracted undertaking requiring great precision. However, unlike the traditional breeding process where progeny from every cross must be screened for the disease, markers promise a quick test for the presence/absence of desired alleles with major effects on resistance.

Environment-neutral marker systems will provide significant savings in time and costs. The liberated funds could be spent more effectively on marker development than on perpetual resistance screening of segregating populations in the field or greenhouse.

Clearly there is no lack of genetic diversity for FHB. It is also clear that some of these genes have major effects, particularly with regards to Type II resistance. It should not be difficult to develop markers for such major genes, and they would have ready application in any wheat-breeding program.

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SOURCES OF COMBINED RESISTANCE TO FUSARIUM HEAD BLIGHT, STRIPE RUST, AND BYD IN TRITICALE

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OBJECTIVES

1) To identify triticale lines that possess high levels of FHB resistance under Mexican conditions and thus could be used as resistance sources in breeding programs, and 2) to find out whether the lines also carry resistance to yellow rust and BYD.

INTRODUCTION

Fusarium head blight (FHB, caused by *Fusarium graminearum*) infects small-grain cereals during flowering and grain filling in temperate and humid weather conditions. It causes considerable yield losses and contaminates grain with mycotoxins, which are harmful to both human and animal health. In general, triticale has been reported to be resistant to many pathogens, but under Mexican conditions, it has shown susceptibility to different *Fusarium* species (*F. nivale, F. graminearum, F. avenaceum, F. culmorum, F. poae*, and *F. equiseti*) that cause fusarium head blight (FHB), as well as to yellow rust (*Puccinia striiformis*) and barley yellow dwarf (BYD), two other serious diseases affecting triticale crops today. In the past few years, changes in the pattern of yellow rust races that attack triticale have been observed in high altitude locations in different parts of the world, including Mexico. Though not a problem every year, BYD has the potential to cause substantial losses when present. For this reason, combined resistance to FHB, stripe rust, and BYD is considered to be highly useful in environments where all three can be present at the same time.

The objectives of this study were 1) to identify triticale lines that possess high levels of FHB resistance under Mexican conditions and thus could be used as resistance sources in breeding programs, and 2) to find out whether these lines also carry resistance to yellow rust and BYD.

MATERIALS AND METHODS

Adequate levels of genetic variation for FHB resistance have been found in primary triticales, which can be used as basic breeding materials through a pre-breeding scheme (Dorman and Oettler, 1993).

The CIMMYT triticale program routinely evaluates advanced hexaploid triticale lines for FHB resistance under natural infection in two hot-spot locations in Mexico: Toluca (state of Mexico) and Patzcuaro (state of Michoacan). Breeders observed signs of FHB resistance in the test triticale lines and pre-selected them for use in this study.

Twenty-six advanced hexaploid triticale lines were planted in small plots under artificial inoculation in Toluca, located in the central highlands of Mexico. During three cycles (2000, 2001, and 2002), the triticale lines were inoculated, evaluated, and characterized for different types of *Fusarium* resistance: penetration (Type I), spread (Type II), toxin content (Type III), and grain filling (Type IV) (Gilchrist *et al*., 1997; Gilchrist, 2001). The methodology and procedures used for inoculating and evaluating the lines have been described by Gilchrist *et al*. (1997). Two triticale varieties, IAPAR 23 and BR 2, reported as FHB resistant by Capan *et al*. (1987), were included as checks.

The test triticale lines and the resistant checks were also planted in Patzcuaro in 2001 and 2002 to confirm the results obtained in Toluca. To that end, two FHB readings under natural infection were conducted at the milk grain stage.

Toxin analysis of triticale grain was carried out in CIMMYT's toxin laboratory following the FluroQuant Romer procedures. Barley yellow dwarf was evaluated using a scale 1 to 9 (Bertschinger, 1994).

RESULTS

Fusarium head blight symptoms are more difficult to observe in triticale than in wheat. Mainly due to the gray-green color of triticale, it is very easy to confuse FHB symptoms with other diseases, and only for a few days is it possible to observe the infected spikelets, which show premature darkening of the straw color. However, if the spikes are carefully evaluated at the correct time and appropriate check cultivars are used for comparison, valuable information can be collected that will allow the identification of triticale lines with superior resistance to FHB.

Sixteen of the twenty-six test lines consistently showed resistant reactions to FHB during the three cycles in Toluca. Results of characterizing the lines for different types of FHB resistance and of evaluating them for resistance to yellow rust and BYD are shown in Table 1. It should be noted that some lines showing FHB resistance were also resistant to stripe rust in the year 2001. A new stripe rust race was detected in 2002, and the damage increased in some of the lines that had shown a resistant reaction the year before. Glume damage was also observed in some lines.

Barley yellow dwarf is not common in Toluca in summer, but a dry period at the beginning of the cycle in 2002 caused a considerable natural increase in the number of aphid vectors of MAV and PAV strains of the BYD virus. This in turn raised the level of BYD infection and provided an ideal testing ground for the disease.

DISCUSSION

In studies by Maier and Oettler (1993), triticale appeared to have higher levels of FHB resistance than the resistant wheat cultivar Frontana. In the evaluations carried out in Toluca and Patzcuaro, a great majority of the lines showed a superior level of FHB resistance. As for the check lines IAPAR 23 and BR2, only the latter proved to have intermediate resistance in both Toluca and Patzcuaro; IAPAR 23 was susceptible in Toluca and the second

and third years in Patzcuaro. This is an indication that FHB resistance expressed in one location is not necessarily effective in all places where FHB is a problem.

CONCLUSION

The combined resistance to FHB, stripe rust, and BYD that we found in triticale has good potential as a source of resistance in improvement programs that apply a plant breeding strategy in which different types of resistance are combined in a single genotype.

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Table 1. Characterization of advanced triticale hexaploid lines for different types of fusarium head blight (FHB) resistance: I (penetration), II (spread), III (toxin content), IV (grain losses and grain filling) under artificial inoculation during three cycles (2000, 2001, 2002) in Toluca and % FHB infection during two cycle (2001,2002) under natural conditions in Patzcuaro (Michoacan). Also evaluated in Toluca was resistance to yellow rust (in 2001, 2002) and BYD (in 2002).

Crosses	TOLUCA																			PATZCUAR	
	Type I damage %			Type II damage %			Type III Toxin (ppm)		Type IV losses %			Type V Grain (1-5)			Stripe rust Leaf damage		glumes damage	BYD	% FHB infection	
	2000	2001	2002	2000	2001	2002	2000	2001	2000	2001	2002	2000	2001	2002	2001	2002	2002	2002	2001	2002
150./2WALRUS 1//ERIZO 6/NIMIR 4	4.25	4	0.85	5.03	6.46	5.41	0.68	0.23	6.69	13.01	1.48	1	1	2	10MS-MR	60 MS	1	1	3-	3
150./2WALRUS 1//ERIZO 6/NIMIR4 /4/GNU/ASAD//ARDI/3/MANATI 1	1.79	3.45	0.7	6.4	6.79	4.42	0.49	0.71	2.76	8.64	1.01	2	2	3	10MS-MR	20 MR	0	1	5	3
804/BAT/4/ERIZO 11/3/BGL/JLO// YOGUI 1/5/JIL96/6/GAUR 3/ANOAS 2/ / BANT-1	2.69	3.49	0.43	4.95	11.09	N.A.	0.42	0.27	9.63	3.27	5.24	1	1	1	TS	10MR	2	2	5	5
ANOAS-1/2*BULL 1-1//ERIZO 11/YOGUI 3/3/ANOAS 5/STIER 13	1.68	5.47	2.56	5.05	7.49	5	0.96	0.47	6.22	10.74	6.47	2	**3**	**2**	TR	10MR	2	5	3	5
ANOAS 5/STIER 13//ANOAS 5/ FARAS 1	1.65	4.13	1.13	6.27	8.7	5.07	0.88	0.43	4	13.88	3.11	2	**1**	**1**	0	TR	0	6	5	3
ARDI 1/TOPO 1419//ERIZO/ 9/3/SUSI 2	3.78	0.81	0.5	4.18	6.47	1.97	0.17	0.24	7.98	3.97	1.99	1	**1**	**1***	0	20MR	0	5	40	3
ERIZO 10/BULL 1-1/5/TAPIR/YOGUI 1//2*MUSX/3/ERIZO 7/4/FARAS 1	2.49	1.13	0.39	5.95	5.68	4.37	0.53	0.23	5.2	8.41	4.51	2	**2**	**1**	10MS	40MR	0	1	3	3
FAHAD 4/FARAS 1	3.15	2.67	0.23	5.66	5.95	7.08	0.59	0.4	7.19	6.76	6.13	1	**1**	**2**	0	20MR	1	5	5	5
HIPO 2/ASAD/4/2*BGL/ CIN//MUSX/3/TESMO 8	3.81	2.6	0.5	5.71	5.51	4.7	0.89	0.29	3.46	11.91	4.69	2	**2**		10MS	TR	2	6	10	25
KER 6/FARAS 1//BULL 2	2.06	3.94	0.22	5.96	6.78	5.25	0.61	0.3	5.49	13.15	5.91	2	**2**	**3**	0	40MR	1	7	60	30
KER 6/FARAS 1//BULL 2/ 4/TAPIR/ YOGUI 1//2*MUSX/ 3/BAGAL 2	1.18	2.14	0.21	4.82	7.02	8.45	0.74	0.3	1.84	2.94	4.63	1	**1**	**2**	0	TR	0	4	3	3
KISSA_7/POLLMER_4//ERIZO_10/BULL_1-1	1.34	2.28	0.5	5.93	10.11	4.25	0.73	0.29	4.6	4.09	4.26	2	**1**	**3**	0	40MR	0	5	50	
LIRON_2-1/3/MUSX/LYNX//STIER_12-3	3.65	3.86	0.41	5.77	7.71	4.33	0.22	0.22	2.29	11.65	3.97	2	**1**	**3**	0	40MR	1	1	10	30
MAH 17486.3/3/HARE 132/CIVET//STIER 28/4/CAAL	2.78	2.19	1.01	5.81	9.52	4.82	x	0.28	3.33	6.93	1.2	1	**1**	**2**	0	20MR	0	2	20	25
MORSA/COPI 1	2.53	4.55	0.48	2.73	7.8	5.26	0.65	0.49	4.13	10.8	9.48	1	**2**	**2**	0	40MR	2	2	8	5
PACA 2/3/MUSX/LINX//STIER 12-3	5.57	3.14	0.5	5.05	6.38	5.42	0.69	0	4.88	2.58	7.17	1	**1**	**1**	TR	20MR	0	1	30	25
MASSA/NIMIR 3/3/YOGUI 1 /TARASCA 87 3//HARE 212/4/ ANOAS 3/STIER 6	3.45	3.05	0.76	7.83	7.24	6.45	0.24	0	4.28	14.72	3.97	2	**1**	**2**	40MS	20MR	1	1	3	3
Checks																				
IAPAR 23 (S-MS)	6.64	0.25	1.84	17.08	10.1	7.03			8.01	24.73	9.65	5	**5**	**3**	10MR	20MR	1	NA	15	80
BR 2 (R-R)	2.95	7.28	1.08	5.14	6.81	5.73			3.96	11.07	2.71	3	**3**	**4**	40MR	40MR	4	NA	20	20

PROGRESS IN BREEDING *FUSARIUM* HEAD BLIGHT RESISTANCE IN SOFT RED WINTER WHEAT

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ABSTRACT

A primary goal of our breeding program is to accelerate the development of adapted and commercially viable Fusarium head blight (FHB) resistant SRW wheat varieties by identifying and incorporating diverse types of resistance into elite genotypes. Breeding methods being used to accomplish this goal include topcrossing, backcrossing, doubled haploid techniques and molecular marker genotyping. In 2002, 229 segregating populations were evaluated in a mist-irrigated FHB nursery, inoculated using colonized maize seed, at Mt. Holly, VA. Seventy-seven of these populations (34%) were advanced on the basis of FHB incidence and severity, agronomic traits, and resistance to other prevalent diseases such as powdery mildew. In field tests, approximately 4500 headrows (F_3-F_8 and various backcross generations) were evaluated for agronomic traits and resistance to diseases other than FHB at Warsaw, VA. In addition, approximately 2800 F_5-F_7 headrows were evaluated for FHB resistance and agronomic traits in an inoculated, mist-irrigated nursery at Blacksburg, VA. From these headrows, 32 backcross-derived lines and 26 topcross-derived lines were selected for further testing in our scab nursery at Blacksburg and in Observation yield tests at two locations in 2003. Twelve lines from the 2001-02 Observation yield test were selected for further testing in Preliminary wheat trials. Four elite lines were selected for testing in our Advance yield trial, and two elite lines will be tested in Virginia's official variety trial. Twelve lines will be tested in the 2002-03 Uniform Winter Wheat FHB Nurseries. Two newly released varieties from the Virginia Tech Small Grains Program, 'McCormick' and 'Tribute', possess a significant level of scab resistance. Progress in transferring type II resistance into SRW wheat genotypes has been accelerated via use of the wheat by maize doubled haploid (DH) system. One DH line, VA01W-476, developed from the cross 'Roane'/W14, was found to have good scab resistance in greenhouse and field tests and also has major genes for scab resistance as determined by DNA analysis this spring. A total of 135 doubled haploid lines derived from nineteen 3-way crosses consisting of diverse scab-resistant parents were selected on the basis of field and greenhouse tests this year and will be evaluated for scab resistance in our inoculated, mist-irrigated nursery at Blacksburg and for agronomic traits at Warsaw. Type II resistance from five different sources (Futai8944, Futai8945, Shaan85, VR95B717 and W14) has been backcrossed into seven adapted SRW wheat backgrounds, and two of the recurrent parents (Roane and Ernie) possess FHB resistance other than Type II. A total of 180 BC_4F_2 and BC_5F_2 individuals were selected on the basis of scab severity in greenhouse tests and will be evaluated for scab resistance in our inoculated, mist-irrigated nursery at Blacksburg and for agronomic traits and similarity to the recurrent parent at Warsaw. Near-isogenic SRW wheat lines with Type II resistance are being developed and will facilitate pyramiding of different types of FHB resistance.

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 very well 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 resistance to FHB. However, transplanting winter wheat is a time consuming process because it involves the vernalization of seedlings in cold chambers, proceeded by hand planting. The root system is far from established in transplanted wheat, often leading to poor plant development. The laborious transplanting process also does not follow the conventional direct seeding method followed by wheat producers. This has led to the investigation of 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 winter wheat 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, 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. The cooler temperatures at anthesis may have inhibited FHB development. In 2002, 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. This planting scheme helped delay flowering by approximately two to three weeks compared to conventional timely seeding. In May 2002, the CPT trial was transplanted into the mist-irrigated field nursery. Significant correlations ($P < 0.05$) between the two types of planting techniques in 2002 were observed for FHB severity, incidence, and disease indices. Correlations between the different planting types across years were also highly significant. These results suggest that delayed direct seeding could replace transplanting. However, transplanted hills should be used if improper weather conditions prevent a successful direct seeded nursery.

STABILITY OF TYPE II RESISTANCE AND DON LEVELS ACROSS ISOLATE AND SOFT RED WINTER WHEAT GENOTYPE

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ABSTRACT

Fusarium graminearum Schwabe (teleomorph *Gibberella zeae* (Schwein.), also known as scab, is an important disease of wheat world-wide. Although host plant resistance has long been considered the most practical and effective means of control 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. The identification of new sources of resistance and their incorporation into adapted wheat varieties provides the most economical solution to this problem. Within both germplasm screening programs and breeding programs, the goal is to identify resistance that is stable over genotypes and across geographical areas. Choice of isolate may be an important factor in accomplishing this goal. We evaluated the effect of 5 diverse *Fusarium graminearum* isolates on type II resistance and DON levels in adapted winter wheat germplasm entered into the Northern Uniform Scab Nursery in 1999. Genotypes were planted in the greenhouse in a split-plot arrangement with genotypes as the main plot and isolates as the sub-plot. The experiment was replicated six times. Five plants per isolate per replication were inoculated at first anthesis with 10µL of a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Plants were then incubated in a mist chamber for 72 h and rated for type II resistance at weekly intervals post-inoculation. At maturity, inoculated heads were harvested, hand-threshed and seed were bulked for deoxynivalenol (DON) analyses. Results indicated significant differences in the aggressivity of the isolates used with the Missouri isolate being the most aggressive across all genotypes. Mean DON levels varied significantly ranging from 160 ppm to 3 ppm. Significant genotype by isolate effects were evident for DON production.

DEVELOPING FHB-RESISTANT CULTIVARS AND GERMPLASM FOR THE MID SOUTH

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INTRODUCTION

Growing resistant cultivars likely will be the primary component of any management strategy for Fusarium head blight of wheat in the Mid South. None of the currently grown cultivars have a high level of FHB resistance, but a collaborative program has identified promising resistant lines that are agronomically adapted and have resistance to other important diseases.

MATERIALS AND METHODS

A crossing program was initiated in 1991 between adapted genotypes and various sources of resistance. These populations were advanced as bulks for 5 years then lines were developed using pedigree selection. These lines have been evaluated for FHB resistance in an inoculated screening nursery along with a FHB resistant check (Ernie) to allow for FHB evaluation in a natural epidemic. These F_7 derived lines have been selected for FHB resistance, yield and other agronomic traits. Additionally, the most advanced lines from the University's wheat breeding program are being screened to determine the level of resistance (or susceptibility). For future development, many different sources of resistance have been used to develop over 450 populations (F_1- F_4) from which lines will be selected.

Ninety-three F_7, topcross F_6, or topcross F_6 germplasm lines were evaluated for FHB resistance in the greenhouse and in inoculated and misted screening nurseries at Fayetteville and Kibler. The lines also were evaluated for resitance to other diseases that are important in the Mid South and for spring freeze damage, vernalization, lodging, and agronomic phenotype.

RESULTS AND DISCUSSION

Yield plots harvested in June at Stuttgart and Marianna, AR indicated several high-yielding lines in the scab resistant nursery (Table 1). Of the 34 lines tested at two locations, 17 were not significantly different than Ernie for FHB. Five of these lines actually had lower numerical ratings than Ernie at both locations. Of those five lines, two were not significantly different in yield than the high-yielding check 'Pat.' Four lines were tested in the 2001-02 Southern Winter Wheat Scab Nursery. Field results across eight reporting locations indicated that all four Arkansas lines had ratings for FHB Index that were not significantly different than the resistant check Ernie. Percentage scabby kernels for all the Arkansas lines was also not different than Ernie. The 18 best FHB breeding lines from the 2002 FHB yield test and

8 lines from Milus' germplasm enhancement will be tested in replicated inoculated yield trials at two Arkansas locations in 2003. Four new lines were entered in the 2002-03 Southern Winter Wheat Scab Nursery. Fifty F_1 populations will be grown in the greenhouse for seed increase. The more advanced populations, which include 100 F_2, 254 F_3, and 49 F_4 populations, were planted at Stuttgart and will be grown in inoculated blocks to help natural selection shift the populations towards resistance.

Thirteen germplasm lines (Table 2) were selected based on level of FHB resistance, parentage, resistance to other diseases, and agronomic characteristics, and these would be useful parents in breeding programs. Five of the selected lines were entered in the 2003 Southern Uniform Winter Wheat FHB Nursery. For evaluation and crossing, seed of all selected lines were provided to Dr. Lucy Gilchrist of CIMMYT and Barton Fogleman of Agripro, and seed of selected lines were provided to Dr. Mohan Kohli of CIMMYT in South America. Seed of lines with Karnal bunt resistance in their parentage were sent via Dr. Art Klatt to CIMMYT for Karnal bunt screening. To determine which lines carry different genes for FHB resistance, lines are being screened by Dr. Guihua Bai for a QTL associated with FHB resistance, and six lines are included in a diallel genetic study.

Table 1. Performance of lines in inoculated scab trials at Marianna and Stuttgart, Arkansas with FHB ratings from Fayetteville and Kibler, Arkansas in 2001-02.

Entry	Yield	Test weight	Heading date	Maturity date	Plant ht	FHB Fayett.	FHB Kibler
	bu/A	lb/bu			in.	%	%
Pat (check)	79.9	56.9	4/20	5/22	39	2.0	1.8
AR 93095-4-1	75.8	56.1	4/18	5/21	38	2.5	2.8
AR 93035-4-1	75.7	56.6	4/18	5/22	35	1.5	5.5
AR 93035-4-3	74.7	57.5	4/17	5/22	36	1.3	5.5
AR 93035-4-4	71.5	55.9	4/17	5/21	35	1.3	4.3
Ernie (check)	70.5	55.3	4/15	5/20	34	4.0	4.8
AR 93035-4-2	69.1	55.6	4/17	5/22	34	2.0	6.3
AR 93188-12-1-1	68.2	55.2	4/18	5/20	34	21.3	33.8
AR 93035-7-1	67.8	55.2	4/17	5/22	35	2.3	7.5
AR 93108-8-1	66.7	52.8	4/16	5/18	36	30.0	17.5
AR 93108-1-3	65.8	54.1	4/18	5/22	34	7.5	6.3
AR 93189-3-1	65.6	55.5	4/19	5/20	33	17.5	21.3
AR 93188-1-1	65.1	53.9	4/19	5/20	35	11.3	10.0
AR 93091-4-2	65.0	56.8	4/19	5/22	39	7.5	2.5
AR 93189-4-1	63.7	54.5	4/20	5/21	35	18.8	15.0
AR 93108-9-1	63.6	52.9	4/15	5/20	35	12.5	12.5
AR 93189-7-1	62.9	55.0	4/18	5/21	34	16.3	37.5
AR 93187-6-1	62.6	54.0	4/20	5/21	34	26.3	26.3
AR 93108-3-2	62.5	56.3	4/14	5/19	36	8.8	8.0
AR 93069-5-1	62.1	58.3	4/18	5/21	37	10.0	11.7
AR 93019-2-1	62.1	57.5	4/21	5/21	40	1.6	1.5
AR 93048-8-2	61.7	51.9	4/16	5/19	35	16.3	18.8
AR 93188-7-1	60.9	53.9	4/20	5/22	34	15.0	17.5
AR 93032-6-1	60.4	56.9	4/16	5/20	37	13.8	21.3
AR 93108-1-2	60.3	53.1	4/17	5/19	36	23.8	8.8
AR 93001-3-2	59.6	56.9	4/17	5/21	36	1.4	5.0
AR 878-2-1	59.5	56.1	4/15	5/20	42	2.5	5.5
AR 93081-2-1	57.5	53.1	4/18	5/20	40	15.0	7.5
AR 93108-8-2	57.2	51.6	4/17	5/19	37	22.5	-
AR 857-1-2	57.0	54.2	4/16	5/20	37	0.1	2.0
AR 93187-4-2	56.7	54.8	4/19	5/21	34	10.0	12.5
AR 93108-4-1	56.3	51.4	4/16	5/20	34	13.8	17.5
AR 857-1-1	54.1	55.0	4/16	5/21	35	0.0	0.5
AR 880-5-1	53.2	52.9	4/18	5/21	37	2.5	7.5
AR 93035-1-1	50.1	56.0	4/18	5/20	37	3.5	5.5
AR 922-5-1	46.9	57.3	4/17	5/20	36	3.5	9.3
Mean	63.2	55.1					
CV (%)	11.9	5.4					
LSD_{05}	8.5	3.4				5.1	7.0

Table 2. Disease and agronomic ratings for F7, topcross F6, and backcross F6 germplasm lines selected during the 2002 season, compared to FHB resistant and susceptible checks.

	FHB-% Florets Infected						% Green leaves[2]			% Leaf Rust									Spring Freeze Damage	
Parentage	Fayetteville	Kibler - Early	Greenhouse	Kibler - Late	Winnsboro, LA[1]	% Scabby Seed Fayetteville	Fayetteville	Kibler - FHB Test	Kibler - LR DSN	Kibler	Greenhouse[3]	Baton Rouge	% Stripe rust	Spindle Streak[4]	Soilborne + spindle streak[4]	Lodging - Kibler[5]	Vernalization Baton Rouge[6]	Phenotype Winnsboro, LA[7]	Fayetteville[8]	Baton Rouge[9]
Mason/Catbird (G49)	0.4	0.4	26.5	47.5	1	42.5	60	43.8	22.5	0	1.3	0	0	3.0	5.0	1.8	1.5	2.5	0.1	1
Mason/Catbird (G93)	0.1	0.6	12.3	52.5	2	21.3	65	65	22.5	0	1.3	0	0	4.0	5.0	3.3	1	2.5	0.1	1
Mason/Catbird (G95)	0	0.2	11.0	42.5	1	15.0	60	65	60	0	1.0	0	25	0.0	1.5	0	1	2	0.1	1
Freedom/Catbird (G82)	0	0.5	25.8	11.3	0	6.5	55	50	50	0	0.8	0	1	3.0	4.0	4.8	0.5	3	0.0	1
Freedom/Catbird (G82)	0.1	0.7	9.3	20	1	20.0	60	50	40	0	1.4	0	3.5	4.0	5.0	4.3	1	4.5	0.3	1
Mason//Sha 3/Catbird	0.9	2.8	12.7	47.5	2	30.0	68.8	70	50	0	3.0	0	0	4.0	5.5	1.3	0	2.5	0.3	1
Mason//Freedom/Super Zlatna	1.1	1.8	22.5	56.7	2	20.0	60	63.8	22.5	18.5	2.6	25	0	4.0	1.5	4.5	1	3.5	0.0	1
Mason//Freedom/Super Zlatna	1.4	2.3	25.0	47.5	2.5	26.3	50	60	22.5	0	3.1	0	0	5.0	5.5	2	2	4	0.5	1
Mason*2//Sha3/Super Kauz	0.5	2.5	10.2	50	2.5	35.0	70	77.5	22.5	22.5	3.0	0	0	4.0	3.0	0	0.5	5	0.0	1
P2684/Er-Mai 9	0.8	1.8	18.4	45	2	55.0	60	45	22.5	4.5	0.7	81.5	7.5	1.5	4.0	1.3	2	2.5	0.5	1
Mason/Yu-Mai 7	0.7	2.0	20.8	67.5	1.5	35.0	55	26.3	15	0	2.6	15	0	1.5	5.0	3.8	1.5	4	0.5	3
Mason/3/Freedom//Clark*4/N7840	1.5	2.0	26.6	52.5	1	17.5	70	55	40	2	3.2	0	0	0.0	1.5	3.3	1	3	0.0	1
P2684/3/N7840//Parula/Veery#6	0.7	1.5	17.8	37.5	1	15.0	73.8	55	28.5	1	0.3	18.5	0	4.0	3.0	6	2	1.5	0.0	5
Checks																				
Ernie	2	3.6	10.9	59.5		25.0	49.3	4.4			5.8					4.9			0	
Patton	2	3.5	15.2	55.5		42.5	51.3	3.3			2.2					5.6			0	
Mason	4.3	10.9	64.4	75	4.5	55.0	56	30			4.1					1.4			2	1.5
P2684	22.8	26.4	62.2	89		47.5	49.3	3.8			2					2.4			0	

[1] 0-9 Scale, 0=No symptoms.
[2] Septoria tritici blotch was the principal leaf disease, but also some stripe rust, leaf rust, and spring infection of barley yellow dwarf.
[3] Inoculated with race TNRL. Infection type on flag leaves rated on 0-9 scale, 0=no symptoms.
[4] 0-9 Scale, 0=No symptoms.
[5] 0-9 Scale, 0=No Lodging.
[6] 0-2 Scale, 0=Not Vernalized.
[7] 0-9 Scale, 0=Excellent.
[8] 0-2 Scale, 0=No Damage.
[9] 0-9 Scale, 0=No Damage.

UNIFORM SOUTHERN SOFT RED WINTER WHEAT FUSARIUM HEAD BLIGHT SCREENING NURSERY

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ABSTRACT

The Third Uniform Southern Soft Red Winter Wheat Fusarium head blight (FHB) Screening Nursery comprised 28 advanced generation breeding lines and two check cultivars. Five public (Univ. of Arkansas, Univ. of Georgia, Univ. of Maryland, N.C. State Univ., and Virginia Tech) and two private (Syngenta Seeds and AgriPro) cooperators submitted entries. Ten cooperators submitted field, greenhouse, and SSR data for the annual report. Significant genotype and genotype by location variation was observed for FHB incidence, head severity, index, and percent scabby seed in combined analyses of field data and for head severity in greenhouse evaluations. No significant correlations were observed between plant height or head emergence and any of the FHB variables. Matrices containing the means of the 30 genotypes at each location for each FHB variable were subjected to GGE biplot analyses to provide insight into the underlying causes of the genotype by location interaction and to identify consistently superior genotypes across test locations. A single megaenvironment encompassing eight locations was observed for FHB incidence. Two megaenvironments (LA and Bay-AR versus OH, IL, KY, VA, NC, MD, Fayetteville-AR, and Kibler-AR) were observed for head severity. Nevertheless, there was a high degree over overlap among the most resistant genotypes in both megaenvironments. Two megaenvironments were observed for percent scabby seed (NC versus Fayetteville-AR, Bay-AR, IL, KY, and VA) and two megaenvironments were observed for greenhouse estimates of head severity (NC versus Bay-AR, MO, IL, and KY). Again, there was a high degree of overlap amongst the most resistant genotypes in both sets of megaenvironments. VA01W476, a doubled haploid line from the cross between the moderately resistant ‘Roane’ and the resistant Chinese line W14, was the most resistant genotype overall. It rated most resistant for FHB incidence, severity, index in field tests, and head severity in greenhouse evaluations overall.

DEVELOPED EVALUATION METHOD OF FUSARIUM HEAD BLIGHT (FHB) RESISTANCE IN WHEAT BY CONTINUOUS SIMULATED RAINFALL AND DIVERSITY OF FHB RESISTANCE IN DOMESTIC WHEAT

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ABSTRACT

In the Fusarium head blight (FHB) resistance evaluation test of wheat, variances and errors of FHB severities in every test are still big issues for resistance breeding. For this problem, we developed a method by continuous simulated rainfall system with injection inoculation to reduce environmental error factors of each inoculation. Sprinkler system has equipped to cover all the test plots and simulated rainfall were operated every 5 minutes for 60 seconds to keep spikes wet at all time of disease developing. Suspension of *Fusarium graminearum* spore in distilled water ($1x10^5$/ml) was injected into a single sipikelet of middle spike on the each material's flowering day and FHB disease severities were investigated after 21 days of inoculation. The correlation coefficient between field test and greenhouse test were r=0.84(n=70, P<0.01, 2001), r=0.71(n=185, P<0.01, 2002) respectively, they showed high values for 2 years. The correlation coefficient between every year's average FHB severities in the field test and the greenhouse test was r=0.71(n=30, P<0.01, 2001-2002), it also showed high value. While FHB inoculation, leaf disease by *F.graminearum* was observed, so the relationship between FHB and leaf disease was investigated. The correlation coefficient between FHB severities and leaf disease severities was significant, r=0.38(n=70, P<0.01), but it seemed to be difficult to presume FHB resistance from leaf disease severities. The highest resistance cultivar of FHB was Sumai 3 Austria line, a derivative of Sumai 3. Sumai 3 (Kyusyu) and Sumai 3 (CIMMYT) showed a little different plant height, but there observed not so much differences in FHB resistance. The relationship of FHB resistance of domestic cultivars has been investigated. 4 major cultivars of Hokkaido (Takunekomugi, Horoshirikomugi, Chihokukomugi, Hokushin) showed stable FHB severities for 2 years, so they were employed as standard cultivars. Takunekomugi showed highest resistance among of them, as the same resistance as Saikai 165 in Kyusyu. Saikai 165 was bred from a cross of Sumai 3/Asakazekomugi for the purpose of improved FHB resistant line, but the resistance was a little inferior to Sumai 3. Hokushin has a good quality for white salt noodle (Udon), and it is a leading cultivar of Hokkaido at the present time, but it was most susceptible among Hokkaido's materials. We found Kachikei 28, showed more FHB resistance than Takunekomugi, but both of their parents were susceptible to FHB. We developed a high reliability evaluation method for FHB resistance, and elucidated the relationship of FHB resistance in domestic wheat and Sumai 3.

PHENOTYPIC EFFECTS OF *QFHS.NDSU-3BS* ON FUSARIUM HEAD BLIGHT RESISTANCE IN NEAR-ISOGENIC WHEAT LINES

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OBJECTIVE

To use QTL near-isogenic lines to evaluate phenotypic effects of *Qfhs.ndsu-3BS* in multiple genetic backgrounds

INTRODUCTION

Despite high levels of FHB resistance identified in some wheat cultivars, progress in breeding for FHB resistance has been relatively slow due to complex inheritance, large environmental influences on disease development, and the resources required to conduct successful breeding nurseries. Recent QTL studies have identified chromosomal regions carrying putative genes for FHB resistance by exploiting statistical associations between molecular markers and resistance phenotypes. A major QTL, *Qfhs.ndsu-3BS*, associated with reduced pathogen spread (Schroeder and Christensen, 1963) has been identified from Chinese wheat cultivar 'Sumai 3', or its derivatives, in several studies (Waldron *et al*. 1999; Anderson *et al*. 2001; Buerstmayr *et al*. 2002; Zhou *et al*. 2002).

The consistent ability to detect *Qfhs.ndsu-3BS* and the magnitude of effect in each mapping population imply that it should be useful for marker-assisted selection (MAS). However, to justify MAS for the QTL region, increased levels of resistance due to this QTL should be observed in multiple genetic backgrounds. To test the robustness of *Qfhs.ndsu-3BS,* near-isogenic lines (NILs) contrasting for the QTL region were tested in greenhouse point-inoculation experiments and field FHB screening nurseries. NILs are particularly effective genetic stocks to study FHB resistance that is attributable to a QTL because NILs standardize the genetic background, morphology, and agronomic characters that may influence disease assessment.

MATERIALS AND METHODS

Plant materials. Co-dominant microsatellite markers gwm493, barc133, and gwm533 (Roder *et al*., 1998; Cregan and Song, 2002) were selected to develop NILs with alternate marker alleles for Qfhs.ndsu-3BS. Homozygous near-isolines were identified by genotyping the progeny of self-pollinated, heterozygous, $F_{3:4}$ lines from 17 unique cross combinations that were grown in summer breeding nurseries in 2000 and 2001. Each of the 17 selected populations had a FHB resistant parent with Sumai 3 in its pedigree and correct marker alleles that are unique to this region (Liu and Anderson, in press). In total, 33 QTL-NIL pairs were produced.

Field Screening. In the summer of 2002, all entries were tested at St. Paul and Crookston misted and inoculated nurseries. Check cultivars and NIL parents, including: 'Sumai 3' (resistant), 'Roblin' (susceptible), 'Wheaton' (susceptible), 'Alsen' (moderately resistant), 'ND 2710' (resistant), 'ND 2603' (resistant), and 'Bacup' (resistant) were included to represent a range of maturity, height, and disease resistance phenotypes. NIL pairs were randomized with check cultivars in a complete block design with 4 replications.

At St. Paul, macroconidia [1x10^5/ml] of *Fusarium graminearum* were applied at anthesis at a rate 30 ml m^{-1} row, followed by a second application 3 days later. At Crookston, infested corn-kernel inoculum was spread evenly throughout the field at a rate of 112 kg ha^{-1} at the 5 leaf stage. For both nurseries, the number of infected spikelets were counted on 20 spikes per row approximately 20 days post-inoculation. Rows were harvested by hand using a sickle and then 30 spikes per row were threshed from two replications in a manner that retained diseased kernels. The weight of seed from 30 threshed spikes was measured. Percent visually scabby kernels (VSK) was estimated based on the scale of Jones and Mirocha (1999). Data analyses were performed using SAS PROC GLM. QTL alleles were considered as fixed effects. Replications, NIL pair, and NIL pair by QTL interaction were considered random effects.

Greenhouse Screening. Approximately 15 plants per genotype were tested in two experiments by inoculation of 10µl macroconidia [1x10^5/ml] into a central spikelet at anthesis. Plants were incubated in a dew chamber (100% RH, 20°C) for 72 hours after inoculation. The number of symptomatic spikelets and total spikelet number were counted at 21 days post-inoculation. The difference in number of symptomatic spikelets between near-isolines was analyzed using t-tests.

RESULTS AND DISCUSSION

Field. Statistical analysis of individual field experiments revealed that error variances were not homogeneous; therefore, locations were not combined for ANOVA. Significantly lower disease pressure at the Crookston nursery and different inoculation methods between the two locations are the most probable causes (Table 1). The effect of *Qfhs.ndsu-3BS* was highly significant (P<0.01) for 5 of 6 trait by location combinations (Tables 2 & 3). The marginal significance (P=0.046) of *Qfhs.ndsu-3BS* in increasing 30-spike seed weight at Crookston is likely due to low disease pressure. As expected when sampling lines from diverse populations, the effect of NIL pairs was highly significant (P<0.001) for all traits in each location. The interaction between *Qfhs.ndsu-3BS* and genetic background (NIL pair) was only highly significant for disease severity at St. Paul and was marginally significant for 30-spike seed weight at Crookston. The average reduction in disease severity with *Qfhs.ndsu-3BS* present was 22% at St. Paul and 14% at Crookston. The average reduction in VSK was 19% at St. Paul and 24% at Crookston (data not shown).

Greenhouse. Sixteen of twenty-nine pairs had significantly reduced spread within the spike in isolines with *Qfhs.ndsu-3BS* across two point-inoculation experiments (Figure 1). Pairs with no statistically significant difference generally had lower disease levels, but the trend was towards more resistant genotypes with *Qfhs.ndsu-3BS*. The average reduction in

symptomatic spikelets with *Qfhs.ndsu-3BS* present was 27% in the first experiment and 36% in the second.

These results confirm that selecting for *Qfhs.ndsu-3BS* with molecular markers should enhance FHB resistance in breeding populations. The absence of consistent QTL by NIL pair interaction across 17 different cross combinations indicates that *Qfhs.ndsu-3BS* should increase FHB resistance independent of genetic background. We are producing fine mapping populations developed from the most promising NIL pairs to further define this QTL region.

Table 1. Trait means for check cultivars and *Qfhs.ndsu-3BS* NIL pairs at two nursery locations.

	St. Paul			Crookston		
Entry	Disease Severity (%)	VSK (%)	30-Spike Seed Weight (g)	Disease Severity (%)	VSK (%)	30-Spike Seed Weight (g)
Alsen	33	20	9.5	16	7	17.9
BacUp	24	20	14.2	12	9	20.6
HJ98	55	19	12.5	19	19	19.9
Ivan	34	38	9.0	9	25	18.5
ND2603	18	12	17.2	8	6	22.3
ND2710	10	14	22.4	4	3	28.1
Reeder	58	40	7.6	18	15	18.9
Parshall	39	30	9.9	17	13	20.9
Roblin	74	40	9.4	54	12	18.8
Sumai3	5	2	21.5	3	2	19.2
Verde	42	24	11.1	19	18	17.9
Wheaton	75	45	13.7	53	40	18.9
NIL pairs	28	20	14.5	15	11	23.1

Table 2. ANOVA of *Qfhs.ndsu-3BS* NILs for three FHB traits at St. Paul field nursery.

Source	Disease Severity		VSK's		Spike Seed Wt	
	df	MS	df	MS	df	MS
Replication	3	0.07***	1	172*	1	0.1
NIL-Pair	39	0.12***	39	238***	39	54.6***
Qfhs.ndsu-3BS	1	0.41***	1	677***	1	32.6**
NIL-Pair*QTL	39	0.01***	39	14	39	4.1
Error	241	0.005	81	28	81	4.4

*, **, *** Effect significant at $P < 0.05$, 0.01, and 0.001, respectively

Table 3. ANOVA of *Qfhs.ndsu-3BS* NILs for three FHB traits at Crookston field nursery.

Source	Disease Severity		VSK's		30-Spike Seed Wt	
	df	MS	df	MS	df	MS
Replication	3	0.01**	1	2	1	12.7
NIL-Pair	39	0.03***	39	132***	39	60.4***
Qfhs.ndsu-3BS	1	0.03***	1	359***	1	19.1*
NIL-Pair*QTL	39	0.003	39	19	39	7.2*
Error	239	0.003	81	18	81	4.7

*,**,*** Effect significant at $P < 0.05$, 0.01, and 0.001, respectively

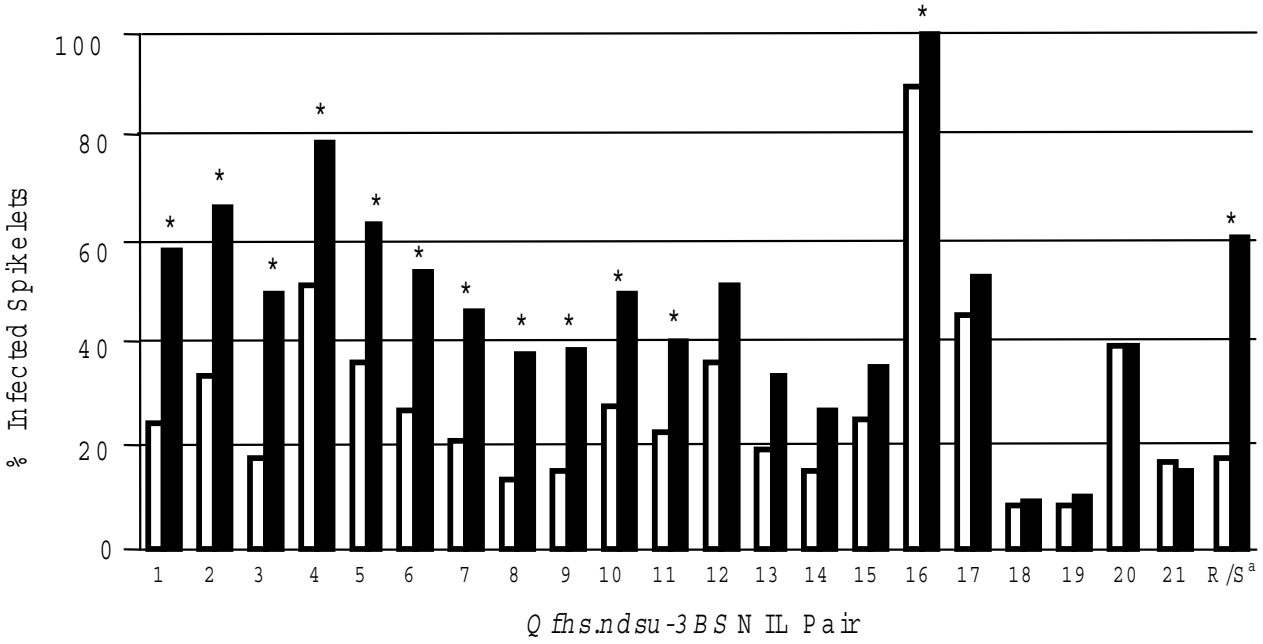

Figure 1. Results from one greenhouse point-inoculation disease screening of QTL-NIL pairs. Fifteen of the twenty-one pairs are from unique cross combinations. Open bars indicate lines with *Qfhs.ndsu-3BS* alleles; black bars indicate sib lines without *Qfhs.ndsu-3BS*. [a]Mean of resistant (R) parents with *Qfhs.ndsu-3BS* and susceptible (S) parents. *Significant at $P < 0.05$

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SSR MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused mainly by *Fusarium graminearum* Schwabe (telemorph *Gibberella zeae*), causes reduced yield and lowered grain quality. Identification of resistance sources and understanding the genetic basis of the resistance is beneficial to wheat breeding for FHB resistance. Two recombinant inbred wheat populations were developed by single-seed descent from the crosses 'Ning 894037 × Alondra' and 'Patterson × F201R', respectively. The phenotypic evaluation of the RI population Ning 894037 × Alondra displayed a continuous distribution with two peaks, suggesting a gene with large effect controlling the resistance coupled with some genes with relatively small effects. SSR marker analysis revealed three chromosomal regions associated with FHB resistance in this population, located on chromosomes 3B, 2D and 6B. The QTL on 3B accounted for 42.5% of the phenotypic variation. The three QTLs collectively explained 51.6% of the phenotypic variation. SSR marker analysis also provides evidence that the 3BS QTL in Sumai 3 was derived from Taiwan Wheat instead of the Italian line 'Funo', which was thought to be the donor of FHB resistance from previous pedigree analysis. In the RI population of Patterson × F201R, the phenotypic distribution is bell-shaped, suggesting quantitative inheritance of FHB resistance. Four chromosomal regions associated with resistance to FHB were identified in this population with SSR markers. The QTLs on chromosomes 1B and 3A have relatively large effects and accounted for 18.7% and 13.0% of the phenotypic variation, respectively. The four QTLs jointly accounted for 32.7% of phenotypic variation. The mapping results showed the genetic diversity of resistance genes in Ning 894037 and F201R, which represent the Chinese and European resistant sources, respectively. SSR markers closely linked to FHB resistance QTLs in these two parent lines may be helpful in breeding programs using marker assisted selection.

SUMMARY REPORT ON THE 2002 NORTHERN UNIFORM WINTER WHEAT SCAB NURSERY (NUWWSN)

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OBJECTIVE AND INTRODUCTION

This report is a compilation and analysis of data from the cooperative assessment of resistance to Fusarium head blight (scab) (causal agent *Fusarium graminearum* (teleomorph: *Gibberella zeae* Schwabe.)) in winter wheat germplasm adapted to the northern regions of North America. The report can be accessed in its entirety on the USWBSI web site.

METHODS

There were 46 lines and four checks in the 2002 trial (Table 1). The entries were successfully evaluated in 13 field tests and five greenhouse tests. Data was collected on heading date (HD), height (HGT), disease severity (SEV), disease incidence (INC), disease index (IND), kernel rating (KR), percent scabby seed (%SS), and DON.

Entry means were analyzed and least square estimate of means over tests were obtained. The entry x test interaction (ETI) appeared quite large for disease index, incidence, and severity from the field and greenhouse, so multivariate statistics were used to analyze the interaction and group those tests that produced similar results for each trait. Entry means were then calculated over the tests that produced similar rankings. Sets of test that produced similar rankings of entries were called megaenvironments (ME).

RESULTS

Entry was a significant source of variance for all traits. There was little ETI for heading date, height, kernel rating, % scabby seed, or DON. Thus, entry means over all tests are appropriate estimators of genetic value (Table 1). ETI seemed to be an important source of variation of disease severity from field and greenhouse trials, disease incidence, and disease index.

The ETI for incidence accounted for 30% of the treatment sum of squares. Seven of the nine tests were place into two ME. One ME consisted of IL+VA and the other consisted of IN+KY+MO+NY+OH. The correlation of entry means within an ME was generally greater than 0.50. The NE and ONT tests did not fit in any ME. The correlation between the two MEs was 0.12, suggesting that entry ranking varied between the two MEs. If we were to select the five entries with lowest incidence in each ME, only one of the selections (IL97-6755) would be the same in both MEs. Only three (IL97-6755, IL97-1828, and MO981020) of the best 10 selections would be the same in both MEs. None of five selections for highest incidence would be the same in both MEs. IL97-6755 had the lowest incidence in both MEs, but would be ranked 10th in NE and 28th in ONT. Better selection concordance would be expected between ONT and NE, or between either outlier test and either ME. None of the 10 entries selected for low incidence in either ME would be among the five worst in the other ME. The entry means for incidence in the IL+VA ME were positively correlated with heading date suggesting the earlier lines may have escaped some affect of the disease. The

opposite trend was present in the other ME (IN+KY+MO+NY+OH) and this may explain why these MEs gave different entry rankings.

The ETI for field severity accounted for 25% of the treatment sum of squares. Six of nine tests were places into two MEs (IL+KY+MO+VA and IN+OH). The remaining four tests (NE, NY, ONT, and SD) were outliers. The correlation of entry means between the two MEs was 0.43, though entry ranking was different in each ME. Only one (MO980829) of five selections for low severity would be the same in both ME. There is better concordance if selection pressure is relaxed as six of 10 selections for low severity would be the same in both MEs. None of the five entries selected for high severity were the same in both MEs. One entry (KY92C-0158-63) among the 10 entries with lowest severity in the IN+OH ME would be among the five worst in the IL+KY+MO+VA ME.

The ETI interaction for index accounted for 24% of the treatment sum of squares. The ETI pattern for index was similar to that found for severity as tests that grouped in the same ME for severity were also grouped together for index. Nine of the 13 tests were placed into two MEs: IL+KY+MO+VA and AR(2)+IN+KS+OH. The remaining tests (NE, NY, ONT and SD) appeared to be outliers. The existence of two MEs and four outlier tests show the complex ETI pattern for disease index. The correlation of entry means between the two MEs was 0.10. Assuming selection of five entries for low index in each ME, only two entries (IL97-1828 and MO980829) would be selected in both MEs. Only only four (IL97-1828, MO980829, IL97-6755, and MO981020) would be selected in both MEs if the ten entries with the lowest index were selected in both MEs. No entry would be select among the five worst entries in both MEs. None of the 10 entries selected for low index in either ME would be among the five worst in the other ME. The entry means for index for the AR(2)+IN+KS+OH ME were negatively correlated to heading date, while entry means for index from the other ME were positively correlated with heading date. Thus the interaction of heading date with index may explain why these ME provided different entry rankings.

The ETI for severity in greenhouse assays accounted for 27% of the treatment sum of squares and three of five tests were placed in a ME (AR+IL+MO). Correlation among these three tests all exceeded 0.55. The IN and KY tests were outliers, though both were more correlated to the ME ($r = 0.42$) than to each other ($r = 0.22$). Assuming selection of the best six entries in each AR+IL+MO, IN, and KY, only about 25% of the selections would be the same between any two tests. Only 20% of the entries selected for high severity would be the same between two tests.

Using entry means over all tests, heading date and height were not correlated to any disease trait (exceptions within certain ME are discussed above). There was a high correlation between the three head disease traits (incidence, severity, and index), and between the head disease traits and kernel rating and percent scabby seed. Field severity and index were correlated to greenhouse severity, and this relationship held even when severity and index were averaged within MEs.

Each entry was compared using LSD to the entries with the highest and lowest value for each of the seven disease traits. Three entries from Missouri and three from Illinois had low values for all seven traits (Tables 1 and 2). One entry from New York had low scores for six of seven traits while the two resistant checks (Ernie and Freedom) were low for five of seven traits. All nine of the resistant entries had low scores for percent scabby seed, DON, and severity in the greenhouse. Twelve entries had high scores for at least five of seven disease traits. All 12 had high scores for incidence, field severity, disease index, and kernel rating. Two entries had high scores for all seven traits, including the susceptible check (Pioneer 2545).

Table 1. Entry means for 2002 NUWWSN. Each entry was compared to the lowest (l) and highest (h) means in each column using LSD(0.05). "# low scores" is the number of disease traits for which an entry received a low score, "# high scores" is the times it received a high score.

	Trait:	HD	HGT	INC	SEV	IND	KR	%SS	DON	SEV-GH		
	# of tests:	9	7	9	10	13	4	4	2	5	# low	# high
	Units	Days	in	%	%	%	0-100	%	PPM	%	scores	scores
1	KY90C-054-6	139	37.3	55 h	34.1 h	23.2	21.4 h	22	16.6 l	54.2 h	1	4
2	KY93C-0876-66	140	35.3	64.9 h	40 h	30.1 h	30.9 h	21.5	21 h	44.8	0	5
3	KY92C-0010-17	140	37	67.2 h	38.5 h	29.5 h	31.1 h	26.7 h	26.3 h	31.2 l	1	6
4	KY92C-0158-63	142	36.3	68.3 h	31.8	27 h	21.6 h	23.6 h	19.8 l	42.8	1	4
5	VA01W447	135	35.6	61.4 h	41.8 h	31.9 h	27.3 h	27.3 h	12 l	48.8 h	1	6
6	VA01W461	137	36.6	53.8 h	28.8 l	18.8	24.9 h	14.3 l	15.8 l	40.2	3	2
7	VA01W462	135	34.6	61.8 h	38.4 h	29.4 h	27.5 h	17.5 l	13 l	34.9	2	4
8	VA01W465	139	32.6 l	66.9 h	36 h	28.4 h	20.1 h	22.5 h	28.8 h	40.6	0	6
9	VA01W469	137	35.1	62.9 h	36.8 h	30.2 h	29.2 h	24.1 h	14.3 l	55.5 h	1	6
10	P97397J1-4-1-4	135	34 l†	52.9 h	37 h	24.2	25.3 h	17.1 l	20.3	36.5	1	3
11	P97395B1-4-5-9	133 l	34.1 l	48	41.4 h	31.3 h	18 lh	22.2 h	18 l	46.7	2	4
12	P97395B1-4-2-7	134 l	34.6	46.9	33	22.3	13.3 l	14.2 l	14 l	32.7 l	4	0
13	P981128A1-23-1	137	36.3	52.9 h	37.7 h	25.3 h	22.4 h	20.5	15 l	48.9 h	1	5
14	P981238A1-1-11	137	33.6 l	44.3	28.2 l	17.2	21.8 h	19 l	18.3 l	15 l	4	1
15	OH708	140	38.1	54.9 h	41 h	28 h	16.5 l	15.2 l	15 l	48.8 h	3	4
16	OH712	141	41 h	56.4 h	38.7 h	25.3 h	23 h	22.5 h	15.3 l	56.4 h	1	6
17	OH719	142	38.9 h	53.4 h	28.8 l	19.7	21.9 h	16.9 l	14.5 l	31.5 l	4	2
18	OH720	141	39.9 h	53.2 h	41.2 h	25.6 h	24.7 h	23.5 h	11 l	44.8	1	5
19	OH685	136	37	54.6 h	45.9 h	28.2 h	26 h	23.3 h	24.8 h	63.7 h	0	7
20	IL96-6472	135	36.1	41.9 l	30.9	19	12.5 l	7.8 l	8 l	34.1	4	0
21	IL97-1828	137	36.3	29.5 l	24.6 l	13.6 l	7.7 l	11 l	11.4 l	32.8 l	7	0
22	IL97-6755	138	40.6 h	26 l	26.4 l	14.6 l	8.6 l	8.7 l	3 l	19.6 l	7	0
23	IL97-7010	136	39.1 h	38.6 l	29 l	15.7 l	14.4 l	12.7 l	16.3 l	18.6 l	7	0
24	IL98-6718	135	37.7	43.6	35.2 h	22.3	10.2 l	14 l	9.5 l	44.9	3	1
25	MILLENNIUM	142	39 h	38.6 l	30.2	15.1 l	15.6 l	25.6 h	7.5 l	43.4	4	1
26	NE98632	141	39 h	48.2	31.7	18.8	23.3 h	29.2 h	17.3 l	42.4	1	2
27	NE99543	139	38.3	40.7 l	38.1 h	21.6	23.9 h	30.7 h	14.3 l	64.3 h	2	4
28	NY89052SP-9	143 h	38.6	42.6	38.3 h	19.2	11.6 l	17.6 l	22.3 h	53.6 h	2	3
29	NY89086-7120	142	38.9 h	48.9	39.6 h	23.6	19.8 h	20.4	37.5 h	40.5	0	3
30	NY89082-7159	144 h	35.9	48.9	35.5 h	19.7	15.1 l	19.3 l	12.3 l	50.3 h	3	2
31	NY89064SP-7139	143 h	37.6	41.6 l	36.1 h	15.6 l	11.7 l	14.5 l	9.5 l	31.9 l	6	1
32	NY89088-7401	143 h	38.9 h	48.4	32.8	18	18.4 h	20.5	21.5 h	39.8	0	2
33	MDV11-52	136	32.4 l	59.1 h	41.5 h	32.9 h	30.2 h	24.8 h	28 h	46.6	0	6
34	M94*1549-1	137	34.3 l	56.7 h	35 h	26.9 h	22 h	24.6 h	14.5 l	38.6	1	5
35	M95-2994-1	140	35	46	31.3	20.2	20.9 h	23.1 h	20.3	25.5 l	1	2
36	MO980829	141	39.3 h	25.9 l	17.1 l	8.4 l	5.7 l	12.8 l	6.9 l	16.3 l	7	0
37	MO981020	137	36.7	37.2 l	23.5 l	15.9 l	8.6 l	11 l	18.3 l	19.8 l	7	0
38	MO000925	138	36.1	43.6	28.6 l	20.3	21.8 h	15.2 l	16.8 l	34	3	1
39	MO000926	136	34.4 l	40.3 l	26 l	16.9 l	13.8 l	14.3 l	17.8 l	24.7 l	7	0
40	MO000969	137	36.1	46.9	42.8 h	23.1	24.8 h	27 h	23.8 h	30.3 l	1	4
41	PATTERSON	136	37.1	50.8	40.1 h	29.6 h	18.1 lh	21.4	11.5 l	60.3 h	2	4
42	FREEDOM	140	37.6	44.6	22.4 l	15.7 l	20.4 h	17.9 l	13.3 l	16 l	5	1
43	PIONEER 2545	140	36.6	59.1 h	38.3 h	28.4 h	28.4 h	34.2 h	33.3 h	52.1 h	0	7
44	ERNIE	134 l	34.1 l	42.6	23.6 l	20	17 l	16.9 l	13.8 l	24.9 l	5	0
45	D9046-1	136	35.7	41.3 l	31.5	22.9	26.7 h	18.2 l	25.8 h	67.3 h	2	3
46	D9070-1	141	37.7	52	36.7 h	19.7	18.3 lh	13.3 l	17.5 l	33.5 l	4	2
	Average	138	37	49.3	34	22.5	19.7	19.5	17.1	38.9		
	LSD (0.05)	1.8	2.2	16.1	12.7	8.7	13.2	12.2	15	19		

† Indicates a mean that is not different from the lowest (l) or highest (h) mean in the column based on $LSD_{(0.05)}$

Table 2. Entry means for the most tolerant (top) and susceptible (bottom) entries in the 2002 NUWWSN

	Trait:	HD	HGT	INC	SEV	IND	KR	%SS	DON	SEV-GH		
	# of tests:	9	7	9	10	13	4	4	2	5	# low	# high
	Units	Days	in	%	%	%	0-100	%	PPM	%	scores	scores
21	IL97-1828	137	36.3	29.5 l	24.6 l	13.6 l	7.7 l	11 l	11.4 l	32.8 l	7	0
22	IL97-6755	138	40.6 h†	26 l	26.4 l	14.6 l	8.6 l	8.7 l	3 l	19.6 l	7	0
23	IL97-7010	136	39.1 h	38.6 l	29 l	15.7 l	14.4 l	12.7 l	16.3 l	18.6 l	7	0
36	MO980829	141	39.3 h	25.9 l	17.1 l	8.4 l	5.7 l	12.8 l	6.9 l	16.3 l	7	0
37	MO981020	137	36.7	37.2 l	23.5 l	15.9 l	8.6 l	11 l	18.3 l	19.8 l	7	0
39	MO000926	136	34.4 l	40.3 l	26 l	16.9 l	13.8 l	14.3 l	17.8 l	24.7 l	7	0
31	NY89064SP-7139	143 h	37.6	41.6 l	36.1 h	15.6 l	11.7 l	14.5 l	9.5 l	31.9 l	6	1
42	FREEDOM	140	37.6	44.6	22.4 l	15.7 l	20.4 h	17.9 l	13.3 l	16 l	5	1
44	ERNIE	134 l	34.1 l	42.6	23.6 l	20	17 l	16.9 l	13.8 l	24.9 l	5	0
1	KY90C-054-6	139	37.3	55 h	34.1 h	23.2	21.4 h	22	16.6 l	54.2 h	1	4
4	KY92C-0158-63	142	36.3	68.3 h	31.8	27 h	21.6 h	23.6 h	19.8 l	42.8	1	4
40	MO000969	137	36.1	46.9	42.8 h	23.1	24.8 h	27 h	23.8 h	30.3 l	1	4
27	NE99543	139	38.3	40.7 l	38.1 h	21.6	23.9 h	30.7 h	14.3 l	64.3 h	2	4
15	OH708	140	38.1	54.9 h	41 h	28 h	16.5 l	15.2 l	15 l	48.8 h	3	4
11	P97395B1-4-5-9	133 l	34.1 l	48	41.4 h	31.3 h	18 lh	22.2 h	18 l	46.7	2	4
41	PATTERSON	136	37.1	50.8	40.1 h	29.6 h	18.1 lh	21.4	11.5 l	60.3 h	2	4
7	VA01W462	135	34.6	61.8 h	38.4 h	29.4 h	27.5 h	17.5 l	13 l	34.9	2	4
2	KY93C-0876-66	140	35.3	64.9 h	40 h	30.1 h	30.9 h	21.5	21 h	44.8	0	5
34	M94*1549-1	137	34.3 l	56.7 h	35 h	26.9 h	22 h	24.6 h	14.5 l	38.6	1	5
18	OH720	141	39.9 h	53.2 h	41.2 h	25.6 h	24.7 h	23.5 h	11 l	44.8	1	5
13	P981128A1-23-1	137	36.3	52.9 h	37.7 h	25.3 h	22.4 h	20.5	15 l	48.9 h	1	5
3	KY92C-0010-17	140	37	67.2 h	38.5 h	29.5 h	31.1 h	26.7 h	26.3 h	31.2 l	1	6
33	MDV11-52	136	32.4 l	59.1 h	41.5 h	32.9 h	30.2 h	24.8 h	28 h	46.6	0	6
16	OH712	141	41 h	56.4 h	38.7 h	25.3 h	23 h	22.5 h	15.3 l	56.4 h	1	6
5	VA01W447	135	35.6	61.4 h	41.8 h	31.9 h	27.3 h	27.3 h	12 l	48.8 h	1	6
8	VA01W465	139	32.6 l	66.9 h	36 h	28.4 h	20.1 h	22.5 h	28.8 h	40.6	0	6
9	VA01W469	137	35.1	62.9 h	36.8 h	30.2 h	29.2 h	24.1 h	14.3 l	55.5 h	1	6
19	OH685	136	37	54.6 h	45.9 h	28.2 h	26 h	23.3 h	24.8 h	63.7 h	0	7
43	PIONEER 2545	140	36.6	59.1 h	38.3 h	28.4 h	28.4 h	34.2 h	33.3 h	52.1 h	0	7
	Average	138	37	49.3	34	22.5	19.7	19.5	17.1	38.9		
	LSD (0.05)	1.8	2.2	16.1	12.7	8.7	13.2	12.2	15	19		

† Indicates a mean that is not different from the lowest (l) or highest (h) mean in the corresponding column in Table 1 based on $LSD_{(0.05)}$

Table 3. Possible sources of resistance for the most resistant entries in Table 2.

Entry	Pedigree	Possible source of resistance
IL97-1828	P818311-16-2-1-2-3-3/IL90-4813	
IL97-6755	IL90-4813//IL85-3132-1/NING7840	Ning 7840
IL97-7010	IL90-6363//IL90-9464/NING7840	Ning 7840
MO980829	MO11769/MADISON	MO11769 which is not a descendent of Ernie, Sumai 3, or Ning 7840
MO981020	MO11769/MADISON	MO11769 which is not a descendent of Ernie, Sumai 3, or Ning 7840
MO000926	ERNIE/AP HICKORY	Ernie
NY89064SP-7139	88029(84061(6120-15/F29-76)/AUGUSTA)/HARUS	Harus and 6120-15 (Geneva) are moderately resistant

FUSARIUM HEAD BLIGHT IN HEXAPLOID WHEAT POPULATIONS DERIVED FROM LINES WITH TYPE I RESISTANCE

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ABSTRACT

Fusarium head blight (FHB) of wheat, caused mainly by *Fusarium graminearum* Schwabe, is a serious problem in North Dakota spring wheat.

An adapted, FHB resistant spring wheat, ND2710, was selected from some Sumai 3 derived lines in 1993 and been used in many crosses in the North Dakota breeding program since that time. It was a parent of the ND cultivar 'Alsen', released in 2000, and planted on over 2 million acres in 2002. The FHB resistance of ND2710 has proven durable over several years and in a wide range of environments. One flaw of the resistance pattern of ND2710 is its acceptance of individual primary infections, even though these infections do not spread to adjacent spikelets. To find a parent which will better resist primary infection we tested two populations derived from three-way crosses between adapted ND spring wheat and two lines thought to possess resistance to primary infection (= "Type 1" resistance). One parent was 'Frontana', the line in which type 1 resistance was first described; the other was 'W9207', a line derived from intercrossing of 6 of the best Chinese resistance sources. The two populations were advanced to F-5 by single seed descent and the lines were tested for FHB in an inoculated, mist-irrigated field nursery in 2001. At 3.5 weeks post-inoculation, approx. 50-60 spikes in each of two reps were individually scored for FHB on a 0-100% scale. Grain was harvested from mature spikes and proportion of visually scabby kernels and level of deoxynivalenol (DON) in the harvested grain determined. The two populations were similar in mean and distribution for all FHB measures. Overall about 5% of lines had FHB disease measures similar to or better than ND2710, but 22% and 27% of lines from these populations had FHB incidence values lower than this standard. The best lines from these populations had incidence values indicating less than half as many primary infections as ND2710.

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SCAB SCREENING USING FROZEN SPIKES

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ABSTRACT

Evaluation of Fusarium head blight (FHB) in the field environment is difficult. The amount of material in the scab nursery is large and reading the symptoms before the heads begin to mature can be problematic. To extend the days of reading symptoms a method was suggested in which spikes are harvested 21 days after flowering and frozen (Personal communication, R. Stack via D. Hershman). Spikes from two elite tests, Magnum and Mondo, along with the checks in the Kentucky variety trial were frozen and data collected from the spikes in 2002. A sample of approximately 100 spikes per row was harvested with a hand sickle at two locations, Lexington and Princeton, KY. The spikes were placed into resealable bags then placed into an ice chest. The samples were transferred into a freezer and were kept there until needed. The frozen spikes were read at various times. The samples remained green and symptoms were still visible. Disease incidence was calculated by counting the number of infected spikes per bag divided by the total number of spikes. Average head severity was assessed by evaluating 15 infected heads per bag. The preliminary data indicates that freezing infected heads is an effective tool for reading scab symptoms. The data was analyzed by comparing severity rankings of entries that were frozen in 2002 to field samples in 2001. Severity values were also compared to greenhouse severity values. Harvesting wheat at 21 days after flowering does not give peak severity of FHB on the entry row; however, the severity of the checks were similar between field rankings and frozen spike rankings in 2002 (r^2=0.88, P=0.01). The correlation from the elite test field spikes in 2001 and frozen spikes in 2002 was less encouraging (r^2=0.18 P=0.07, both locations). Further testing will occur during the upcoming year on improving the method into a efficient tool for screening and selection of resistant FHB genotypes.

FUSARIUM GRAMINEARUM AND DON IN SINGLE SEEDS FOLLOWING GREENHOUSE POINT INOCULATION

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ABSTRACT

The single floret inoculation system is commonly used to screen wheat cultivars and germplasm for FHB Type II resistance in the greenhouse by a visual rating of the spread of fungal hyphae in the spike and spikelets. Evaluation of this system in our laboratory across a wide range of germplasm has shown that the visual ratings of spikelet infection are poorly associated with the *Fusarium graminearum* (Schwabe) infection occurring in the seed, rachis and other floral components the same spikelets. The objective of this research was to use the single floret inoculation system to relate ratings of visual spikelet infection in the greenhouse to *F. graminearum* infection and deoxynivalenol levels in seeds of adjoining florets in all individual spikelets on each infected spike. The movement of fungal hyphae and DON into the various components of the spike was evaluated following point inoculation (PI) of a floret at a middle location of the spike for two susceptible (P 2555 and VA 96W-326) and three resistant (P 25R18, Roane, Coker 9474) cultivars. Although high levels of spikelet infection occurred in the susceptible cultivars in the greenhouse, the fungal movement in the spike occurred primarily in two ways; localization around the PI and movement down the spike from the PI. Thus, severity of greenhouse infection overestimated *F. graminearum* seed infection and DON presence in susceptible cultivars and underestimated fungal infection and DON in resistant cultivars. A close relationship was shown between the presence of *F. graminearum* in seed from the right floret with the presence of DON in seed from the left floret in both susceptible and resistant cultivars. Although DON was present in seed of resistant cultivars the levels were much lower than susceptible cultivars and often did not exceed 1 PPM. This investigation should allow us to evaluate the current methods for screening for Type II resistance to FHB infection in an attempt to develop more accurate methods. (This research will be presented as a poster at the annual meeting of the US Barley and Wheat Scab Initiative in Covington, KY on December 7-9, 2002.)

HOW TO MAKE INTELLIGENT CROSSES TO ACCUMULATE FUSARIUM HEAD BLIGHT RESISTANCE GENES BASED ON KNOWLEDGE OF THE UNDERLYING RESISTANCE MECHANISMS

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OBJECTIVES

To describe crossing strategies to accumulate mechanisms of resistance to FHB.

INTRODUCTION

A number of mechanisms or Types of resistance, likely coded for by specific genes, underpin wheat's final response to Fusarium head blight (FHB). Accumulating additively inherited resistance genes to enhance genetic control of FHB has been proposed (Anonymous, 2001; Singh and van Ginkel, 1997). But in the absence of specific information on these genes gene-based breeding is not yet feasible. Until the specific genes that cause these phenotypic response mechanisms are identified, disease evaluation for each mechanism has to be conducted independently.

However, in the case of FHB, intelligent crosses can be made by applying our knowledge of the various resistance mechanisms responsible for its genetic control.

Characterizing FHB Resistance

Before applying the mechanisms of resistance present in genetic stocks to a crossing scheme, they must be carefully characterized, which can be an arduous process.

A description of how the different mechanisms of resistance to FHB are determined at CIMMYT follows. We base our approach on published literature and local experience. The *Fusarium* sp. used mainly in our work is *F. graminearum*.

Specific inoculation, screening, and evaluation techniques are used for each type of resistance to avoid confounding the disease response observations of the various resistance mechanisms. Because genotype x environment interactions may affect the ranking of genotypes for any one of the resistance mechanisms, an appropriate number of replications across and within years is needed. The level of interaction appears to be linked to the level of resistance: if a genotype's resistance level is already pretty high and based on the presence of several mechanisms, it will be less affected by the environment and will express more stable resistance. Resistance based on just one mechanism is more susceptible to environmental effects.

The targeted germplasm and check cultivars are always inoculated on the same day. Later upon harvest the response of the tested lines is compared with that of the checks inoculated on the same day. If this is not done, erroneous and spurious response readings may result. A broad spectrum of checks is used, ranging from the highest level of susceptibility to the highest level of resistance, with at least two representatives at each level.

Type I (penetration by the fungus) and II (spread of mycelium throughout the spike) resistances were identified as distinct mechanisms by Schroeder and Christensen in 1963. Two other mechanisms or types were proposed by Miller and Arnison (1986), Wang and Miller (1988), and Mezterhazy (1995).

Type I resistance - To avoid interaction with height and maturity, inoculation for Type I resistance is done by spraying a fusarium spore suspension (50,000 spores per ml) on a horizontal plane onto labeled spikes at the onset of anthesis. Spikes are evaluated for resistance a fixed number of days post inoculation, depending on the prevailing conditions and the disease reaction of the resistant and susceptible checks. The interval (25 to 35 days) between inoculation and evaluation may vary from year to year. However, within the same year a fixed number of days is used. Thirty days is generally appropriate under Mexican conditions.

Type II resistance - A tuft of cotton gently soaked in inoculum (50,000 spores per ml) is inserted into a floret in the center of the spike at the onset of anthesis with a pair of tweezers; each spike is then covered with a glassine pollination bag. The lightweight, narrow bag prevents additional FHB inoculum from entering the spike through allo-infection and helps maintain a high level of humidity, which favors disease development. Spikes are evaluated for resistance 25-35 days post-inoculation.

Type III resistance - *Fusarium graminearum* produces mycotoxins, especially trichothecenes, during the infection process. Type III resistance is associated with degradation of toxins in the grain, as described by Miller and Arnison (1986). In preparation for toxin evaluation and quantification, genotypes are sprayed with an inoculum suspension when 50% of the spikes in a plot have reached anthesis. A 20-g seed sample from the inoculated spikes is collected at harvest, and resistance is evaluated by quantifying the accumulated toxin in the laboratory using the FluoroQuant Rommer method.

Resistance types IV and V - Wang and Miller (1988) described Type IV resistance as tolerance to high DON concentrations. They reported that some cultivars can tolerate high mycotoxin concentrations with no negative effects on growth. A six-year study led to the conclusion that this tolerance should be evaluated as a relative parameter of infection, and that yield response may help describe the disease reaction of the genotypes (Mezterhazy, 1995).

To identify Type IV resistance, a paired plot of each genotype is planted. One plot is treated as described for Type I, and the other is sprayed with a functional fungicide (e.g., Folicur Plus) three times during the cycle. The test weight of grain harvested from the fungicide-treated plot is compared with that of grain from the inoculated plot to calculate the percentage loss.

To evaluate for Type V resistance, grain from the two plots described above is visually scored (1=very healthy and plump; 5=diseased and shriveled) at the same time to determine relative grain filling. This parameter has not been described in the literature as a FHB resistance mechanism, but is used at CIMMYT to complement and aid in identifying Type IV resistance.

EVALUATING PARENTAL STOCKS

All germplasm being considered for crossing is first characterized for FHB resistance, as described above and as depicted in the Table 1. Commonly these materials include established sources of resistance, promising introductions contributed by colleagues, good combiners for the desired agronomic traits, and major varieties in the target region. This information is later used as the basis for making appropriate crosses.

Table 1. Parental characterization for FHB resistance mechanisms. Codings using bold/ Italics/underline/non-underline indicate the relative levels of resistance, with bold and underlined lettering representing the highest level.

		RESISTANCE MECHANISM or TYPE				
		I	II	III	IV	V
Entry	Cross	Damage (%)	Damage (%)	Toxin (ppm)	Grain losses (%)	Grain (1-5)
1	GOV/AZ//MUS/3/DODO/4/BOW	**2.51**	**2.66**	**0**	*21.16*	*2*
2	MILAN/SHA7	**0.00**	**6.07**	**0.14**	*13.29*	*2*
3	ALUCAN/DUCULA	*13.73*	*21.12*	**0.52**	*13.18*	*2*
4	CBRD/KAUZ	**3.21**	**6.43**	*2.3*	**2.36**	**1***
5	R37/GHL121//KAL/BB/3/JUP/MUS/4/2*YMI #6/5/CBRD	**1.49**	*10.53*	**0.026**	**7.68**	**1**
6	GUAM92//PSN/BOW	**4.90**	*13.16*	**0.21**	**6.62**	**1**
7	NG8675/CBRD	**0.26**	**8.20**	**0.48**	**7.67**	**1**
8	ALTAR 84/AE.SQUARROSA (224)//ESDA	**4.42**	*16.89*	**0.49**	**1.75**	**1**
9	BCN*2//CROC_1/AE.SQUARROSA (886)	*11.56*	**4.82**	**0.38**	**1.68**	**1***
10	MAYOOR//TK SN1081/AE.SQUARROSA (222)	**0.86**	**7.26**	**0.49**	**1.3**	**1***
11	SABUF/5/BCN/4/RABI//GS/CRA/3/AE.SQUARROSA (190)	**1.98**	**8.46**	**0.069**	*8.07*	*2*
12	SHA3/CBRD	**3.87**	**5.99**	**0**	**6.94**	**1**

Coding	*FHB score*
123	very good
123	good
123	moderate
123	poor

CROSSING STRATEGIES

If crossing is to be effective, the mechanisms of resistance should be complementary among parental stocks, as is evident in Table 1.

Using the information in Table 1, cross combinations can be designed to cross parents that fully complement one another in the sense that one or the other contributes high levels of resistance (scored as ‘very good’) for each of the five mechanisms. With luck and properly xecuted selection, transgressive segregants will subsequently be identified that express high levels for all five resistance types.

Following parental stock characterization and crossing, the segregating F2 generations are grown and selected. But also additional crosses can be made on the F1s. We usually opt for a top cross or, in some cases, a limited backcross, to a line with desirable agronomic type, high yield potential and yield stability, durable resistance (to other relevant diseases), good combining ability, and excellent quality. In the case of FHB, we also make doubled haploids (DH) on a limited number of simple crosses, to enhance our ability to identify homozygous transgressive progeny in replicated experiments that combine multiple resistance mechanisms.

CONCLUSION

Our approach uses data gathered on the various mechanisms of resistance on relevant parental stocks to allow more intelligent crossing. Such an approach increases, at least in theory; the chance of identifying superior progeny carrying accumulated resistance mechanisms against FHB.

EPILOGUE

An improved understanding of the FHB infection process and related resistance mechanisms reveals the potential relationship between FHB and Karnal bunt (KB) resistance. The infection processes of the two diseases are similar: in both cases, florets are infected during anthesis, and resistance is very much influenced by environmental fluctuations. Consequently, the same lines may seem resistant in some years but susceptible in others. Alleles with large effects on resistance have been noted in both diseases (Fuentes-Davila *et al*., 1995; Singh *et al*., 1995), with some lines expressing high levels of resistance following the introgression of just 2-3 desired alleles. Significant genetic variation for both diseases is available, and anecdotal evidence suggests that some genetic sources (especially among the Chinese materials) are resistant to both diseases. If the same genes confer resistance to the two diseases, this would explain why many Chinese wheats are resistant to KB, though the disease has not been reported in China. Should this be proven, then these two threats to wheat production in the USA could be addressed, at least in part, through a concerted research effort involving groups now independently engaged in research on these two diseases.

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APPARENT AND ACTUAL SEED QUALITY IN SOFT RED WINTER WHEAT INFECTED WITH *FUSARIUM GRAMINEARUM*

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ABSTRACT

Head scab caused by *Fusarium graminearum* (Schwabe) has caused significant losses in the soft red winter wheat crop in Kentucky and in small grain crops in many regions of North America. Head scab epidemics not only result in significant yield losses, but also can cause serious reductions in seed quality. To investigate associations between apparent infection based on spike symptoms, apparent damage to the kernel and the actual seed infection, we evaluated fifteen (15) lines of the 2002 Southern Scab Nursery. Seeds of each line were separated into three categories, depending on the visual aspect of the seed, so as to define "good quality" seed (without any symptom of infection), "shriveled" seed and "poor" seed (tombstones).Then, to determine the presence of *Fusarium graminearum* in these three classes of seed, seeds were plated, five (5) plates of each class, with ten seeds per plate. Plates were incubated at 20°C and at seven and fourteen days the presence of *F. graminearum* was recorded. Also a DON test was run to test the concentration of DON in these three categories of seeds. Seeds showed high levels of infection, above 90 % in seeds of poor quality, between 70-80% in shriveled seed and 40-50 % in seed of good quality. The good quality seed showed more variation, depending on the line, and conditions in the field. To evaluate the relation between this actual infection in the seed and field data, percentage of visually scabby kernels was measured for each line, and then correlated with the actual presence of *F. graminearum* in the seed. A better correspondence between apparent infection and actual seed infection could be useful in assessing varieties in the field, and predicting the real infection in the seed, depending on its quality. Correlations of seed quality and the presence of *F. graminearum* with DON will be presented.

EFFECT OF SUMAI 3 CHROMOSOMES ON TYPE II AND TYPE V SCAB RESISTANCE IN WHEAT

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ABSTRACT

Two sets of substitution lines were developed by crossing individual monosomic lines of Chinese Spring (recipient) with scab resistant cultivar Sumai 3 (donor) and then using the monosomics as the recurrent male parent for four backcrosses (without selfing after each backcross). The disomic substitution lines were separated from selfed BC_4F_2 plants. Chromosome specific SSR markers were analyzed for polymorphism between Sumai 3 and Chinese Spring. Polymorphic markers were used to identify substitution lines for specific chromosomes. Based on the specific SSR markers, chromosome substitutions occurred in thirty-six lines, and six lines segregated alleles from the two parents or were homozygous for the allele from Chinese Spring. These substitution lines were used to evaluate Type II (spread within the head) and Type V (deoxynivalenol accumulation within kernels) scab resistance. The objective was to use the substitution lines to evaluate the effect of individual chromosomes of Sumai 3 on Type II and Type V scab resistance in the greenhouse. Significant differences in Type II scab resistance and deoxynivalenol (DON) levels among different Chinese Spring (Sumai 3) substitution lines were detected. Positive chromosome substitution effects on Type II scab resistance were found on chromosomes 2B, 3B, 6B, and 7A from Sumai 3. Chromosomes 3B and 7A also reduced DON accumulation within the kernels, while chromosomes 1B, 2D, and 4D from Sumai 3 increased DON concentration. Chromosome 7A from Sumai 3 had the largest effect on resistance to scab spread and DON accumulation. Additional research is in progress on the scab resistance conferred by chromosome 7A.

Key words: Type II scab resistance, Type V scab resistance, substitution lines, SSR, *Triticum aestivum*.

ESTIMATING THE ECONOMIC IMPACT OF A CROP DISEASE: THE CASE OF FUSARIUM HEAD BLIGHT IN U.S. WHEAT AND BARLEY

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ABSTRACT

Plant diseases, particularly those affecting major agricultural crops, can have serious economic consequences, both for agricultural producers and for the regional economy. Since 1993, the spring grain producing area in the upper Midwest region of the United States has experienced a prolonged outbreak of Fusarium head blight (FHB), commonly known as scab, a fungus disease that affects wheat, barley, and other small grains. The purpose of this paper is to estimate the direct and secondary economic impacts of FHB infestations of wheat and barley during the period 1998-2000. The findings indicate that scab continues to be a major problem for U.S. wheat and barley producers. The cumulative direct economic losses from FHB in hard red spring (HRS) wheat, soft red winter (SRW) wheat, durum wheat, and barley is estimated at $870 million from 1998 through 2000. The combined direct and secondary economic losses for all the crops were estimated at $2.7 billion. Two states, North Dakota and Minnesota, account for about 55 percent of the total dollar losses.

INTRODUCTION

Plant diseases, particularly those affecting major agricultural crops, can have serious economic consequences, both for agricultural producers and for the regional economy. Fusarium head blight (FHB), commonly known as scab, is a fungus disease that affects wheat, barley, and other small grains (McMullen and Stack 1999). FHB results in yield losses, and infected grain is also subject to price discounts. FHB is recognized as a factor limiting grain production in many parts of the world. Since 1993, the spring grain producing area in the upper Midwest region of the United States has experienced a prolonged outbreak of FHB (Stack 1999). Centered on the Red River Valley of North Dakota and Minnesota, the FHB outbreak led to serious losses for farmers (McMullen et al. 1997).

The impact on the U.S. wheat and barley industries has been sufficient to stimulate a national response; a consortium of scientists and agribusiness leaders has developed a "U.S. Wheat and Barley Scab Initiative" to target research and outreach to solving this disease problem. Because the Scab Initiative must compete with other agricultural and natural resource problems to secure funding, the consortium sought estimates of the economic impact of the scab outbreak. Estimates of economic impact of scab are equally important for crop quality insurance (USDA, 2000). Traditional estimates focused on lost producer income solely due to reduced yields and abandoned acres, may lead to ineffective design of quality crop insurance instruments. In the very least, economic impact estimates of a disease must incorporate price impacts in a manner that captures the interaction between yield reduction and price increases or decreases. The purpose of this paper is to estimate the

direct and secondary economic impacts of FHB infestations of wheat and barley during the period 1998-2000.

METHODS AND DATA

Study design required choosing which crops and states should be included. The study focused on three wheat classes (hard red spring, soft red winter, and durum) and barley. For hard red spring (HRS) wheat, durum wheat, and barley, the affected states were Minnesota, North Dakota, and South Dakota. For soft red winter (SRW) wheat, the affected states were Illinois, Indiana, Kentucky, Michigan, Missouri, and Ohio.

Estimating the direct impacts (first round effects) of FHB for each type of grain entailed separately estimating (1) the effect on production and (2) the effect on prices received by producers in each year. These estimates were made for multi-county Crop Reporting Districts (CRDs) in each state. The product of the production and price effects was the estimate of the direct impact of FHB infestation.

To estimate the economic losses due to FHB in a given CRD, the value of production under 'normal' conditions was estimated (i.e., if there had been no outbreak). Normal crop value is the product of two variables: pn, the price that farmers would have received, and qn, their expected production in absence of scab. For years of scab outbreak, both variables are unobserved and must be estimated. The lost crop value is then calculated as the difference between actual and normal crop value (Johnson et al. 1998, Nganje et al. 2001). Nganje et al. (2001) provide detail methodology of estimating production loss, price impacts, and secondary economic impacts.

DATA SOURCES

Data on temperature and precipitation by region were obtained from the National Climatic Data Center (U.S. Department of Commerce). Data on planted and harvested acres, harvested yield, production, and average prices received by producers were obtained from the National Agricultural Statistics Service (U.S. Department of Agriculture). Average CBT and MGE futures prices were derived from a database of weekly quotes collected from Grain Market News (U.S. Department of Agriculture) and the Wall Street Journal. Basis was calculated as the difference between average price received in a region and the average futures price. For North Dakota, prices received were available by crop reporting district; in other states, prices are based on state averages. Prices for the 2000 marketing year were based on data available through February, 2001. Data on national wheat and barley supplies were from the Wheat Yearbook published by the Economic Research Service of the U.S. Department of Agriculture.

RESULTS

Production losses over the three-year period were estimated to total 47.8 million bushels of wheat (all classes) and 42.8 million bushels of barley. Hard red spring wheat dominated the wheat losses (27.6 out of 47.8 million bushels). Losses for both wheat and barley were most severe in 2000, followed by 1998 (data not shown).

Price effects were substantial in all three years, but generally were greatest in 1998. For hard red spring wheat, the futures price effect ranged from about 1 cent to 7 cents per bushel, while the basis effect varied among CRDs and between years from less than 10 cents to 71 cents. For other wheat classes, the futures price effect was always less than 3 cents per bushel, while the basis effect exceeded $1 per bushel in some cases. The total price effect for barley varied from 13 to 80 cents per bushel (data not shown).

The direct economic impact from FHB was greatest in 1998 ($457 million) and least in 2000 ($160 million) (Table 1). Both SRW and HRS wheat producers sustained substantial losses in 1998 ($235 million and $144 million, respectively). In both 1999 and 2000, HRS wheat growers had the largest losses. The total loss for the three-year period was estimated at $871 million, or an average of $290 million annually. Overall, the price effect accounted for 77 percent of the direct impacts of FHB (Table 1).

Table 1. *Direct Economic Effects from Fusarium head blight in the United States, by Crop and State, 1998 through 2000.*

Crop	Economic Effect	1998	1999	2000	Total, 1998-2000
			$ million		
HRS	Production Loss	25.6	19.5	36.9	82.0
	Price Effect	118.5	97.2	32.7	248.4
	Total	144.1	116.7	69.6	330.5
Durum	Production Loss	2.0	10.1	12.5	24.6
	Price Effect	18.5	18.8	8.4	45.7
	Total	20.5	28.9	20.9	70.4
SRW	Production Loss	16.0	3.2	5.5	24.8
	Price Effect	219.0	77.7	11.9	308.6
	Total	235.0	80.9	17.5	333.4
Barley	Production Loss	28.7	17.1	27.1	72.9
	Price Effect	28.8	9.8	24.8	63.4
	Total	57.5	26.9	51.9	136.4
All Crops	Total	457.2	253.5	159.9	870.6

Among the affected states, North Dakota had substantially the greatest impacts in each of the three years (Table 2). Overall, North Dakota accounted for 41 percent of all direct impacts from FHB, followed by Minnesota and Ohio. The losses sustained by North Dakota's wheat producers averaged 10.5 percent of the total cash receipts from wheat sales over the period 1998-2000. For barley growers, the losses were even more severe, averaging 25.7 percent of the value of barley production over the three-year period. In 2000, the losses associated with FHB represented 35.9 percent of total barley sales (USDA, National Agricultural Statistics Service).

Table 2. *Direct Economic Effects from Fusarium head blight in the United States, by State and Crop, 1998 through 2000.*

State	Crop	1998	1999	2000	Total, 1998-2000
			$ million		
North Dakota	HRS	80.8	53.3	48.8	182.9
	Durum	20.4	28.7	20.9	70.0
	Barley	37.0	21.7	44.2	103.0
	Total	138.2	103.8	113.9	355.8
Minnesota	HRS	38.6	38.5	11.7	88.8
	Durum	0.1	0.2	a	0.3
	Barley	19.9	5.1	7.6	32.7
	Total	58.7	43.7	19.3	121.8
Ohio	SRW	70.0	27.4	5.1	102.5
Illinois	SRW	48.6	17.5	0.3	66.4
South Dakota	HRS	24.8	24.9	9.1	58.9
	Barley	0.6	0.1	a	0.7
	Total	25.3	25.1	9.2	59.6
Missouri	SRW	38.8	8.7	4.0	51.5
Michigan	SRW	33.1	13.8	4.2	51.0
Indiana	SRW	23.8	8.0	2.6	34.3
Kentucky	SRW	20.7	5.6	1.3	27.6

a Less than 0.1

To estimate the secondary impacts of FHB infestations, the direct effects were assumed to primarily represent a reduction of producer net revenues (*i.e., the activities and expenditures associated with crop production occur with or without scab infestation*). *The direct economic effects were therefore allocated to the Households sector of the input-output model.* Over *the three-year study period, the* $*870.6 million of direct impacts resulted in an additional* $*1,809.3 million of secondary impacts, for a total economic impact of almost* $*2.7 billion* (*Table 3*). *Impacts were greatest in 1998* (*$1.4 billion*).

The distribution by state of the total economic impacts of FHB infestations was similar to that of the direct effects (*Table 4*). *North Dakota experienced the largest effects, both on average and for each year. The total economic impact for North Dakota averaged more than* $*365 million annually over the study period, almost 41 percent of the total impacts of FHB.*

Table 3. *Total* (*Direct and Secondary*) *Economic Impacts for Fusarium head blight in All Crops, by Economic Sector and Year, Northern Great Plains and Central United States, 1998 through 2000.*

Economic Sector	1998	1999	2000	Total, 1998-2000
	---------- $ million ----------			
Agriculture	43.0	23.8	15.0	81.8
Construction	41.2	22.9	14.4	78.5
Communication & Public Utilities	48.2	26.7	16.9	91.8
Retail Trade	340.5	188.8	119.1	648.3
Finance, Insurance, & Real Estate	76.9	42.6	26.9	146.3
Households	709.8	393.5	248.2	1351.5
Government	49.4	27.4	17.3	94.0
Other Sectors[1]	98.5	54.6	34.4	187.5
Total Direct Impacts	457.2	253.5	159.9	870.6
Total Secondary Impacts	950.2	526.8	332.3	1809.3
Total	1407.4	780.3	492.2	2679.9

[1]Includes sectors such as business, professional, personal, and social services, transportation, and manufa

DISCUSSION

The purpose of this study was to estimate the economic losses from FHB infestations suffered by U.S. wheat and barley producers during the period 1998 to 2000. The study was intended to provide an update to earlier work by Johnson et al. (1998), which estimated losses to wheat producers from 1993 through 1997. The present study was designed to estimate the price and yield effects of FHB, accounting for reduced yields, higher abandoned acres, and price impacts on wheat futures and basis, as well as malting and feed barley prices. The goal was to provide policy makers with estimates of the magnitude, distribution, and trend over time of FHB-related losses, as well as the secondary economic impacts resulting from these direct effects. These estimates were of special interest in the context of obtaining continuing support for the U.S. Wheat and Barley Scab Initiative, which provides funding for research to develop scab resistant varieties, as well as other research and educational efforts to solve this disease problem.

Table 4. *Total* (*Direct and Secondary*) *Economic Impacts from Fusarium head blight, All Crops, by State, in the Northern Great Plains and Central United States, 1998 through 2000.*

State	1998	1999	2000	Total	By State
	$ million				%
ND	425.4	319.4	350.5	1095.4	40.9
MN	180.6	134.6	59.5	374.8	14.0
OH	215.6	84.3	15.6	315.5	11.8
IL	149.7	53.7	0.9	204.3	7.6
SD	78.0	77.2	28.3	183.4	6.8
MO	119.5	26.9	12.3	158.7	6.8
MI	101.8	42.3	12.9	157.1	5.9
IN	73.2	24.6	8.0	105.7	3.9
KY	63.7	17.3	4.1	85.0	3.2
Total	1407.4	780.3	492.2	2679.9	---

The findings indicate that scab continues to be a major problem for U.S. wheat producers. Scab-related losses for wheat growers were estimated to average $245 million annually from 1998 through 2000, compared to $261 million annually during 1993-1997 (Johnson *et al. 1998*). *The scab effects on wheat were substantially less in 2000 than in previous years, which may reflect the introduction of FHB resistant varieties in North Dakota and Minnesota. However, it also may reflect weather conditions that were less conducive to scab development.*

Although North Dakota had substantially the greatest impacts, scab losses affect producers over a wide geographic area. Eight of the states included in the study had estimated direct impacts of at least $10 million per year over the period 1998-2000. The disease also poses major problems for producers of several classes of wheat, as well as barley.

The scab losses were substantial not only in absolute magnitude but also relative to the value of affected crops. In North Dakota, scab losses in wheat from 1998 through 2000 averaged more than 10 percent of the value of the wheat crop while barley losses averaged almost 26 percent of the total crop value over the same period.

Impacts from scab affect not only grain producers but also other sectors of the economy. Income reductions for farmers lead to reduced revenues for a variety of agricultural supply

and service businesses, and economic linkages result in subsequent effects on many sectors of local and state economies. For every dollar in direct scab losses to producers, more than two dollars in secondary economic effects are incurred.

Overall, the direct and secondary economic impacts of FHB infestations have been found to be substantial and widely distributed, both geographically and among economic sectors. Further, these losses dwarf the resources presently committed in combating the problem (funding for the U.S. Wheat and Barley Scab Initiative was $4.3 million in FY 2000). Continued support for research in this area should be relatively easy to justify.

Estimates of the economic impact of scab also have important implications in developing third party quality risk management strategies, using insurance instruments. The USDA Loss Adjustment Manual (LAM) Standards Handbook for 2001 and Succeeding Crop Years emphasize the importance of estimating the reduction in value due to scab.

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Scab Initiative