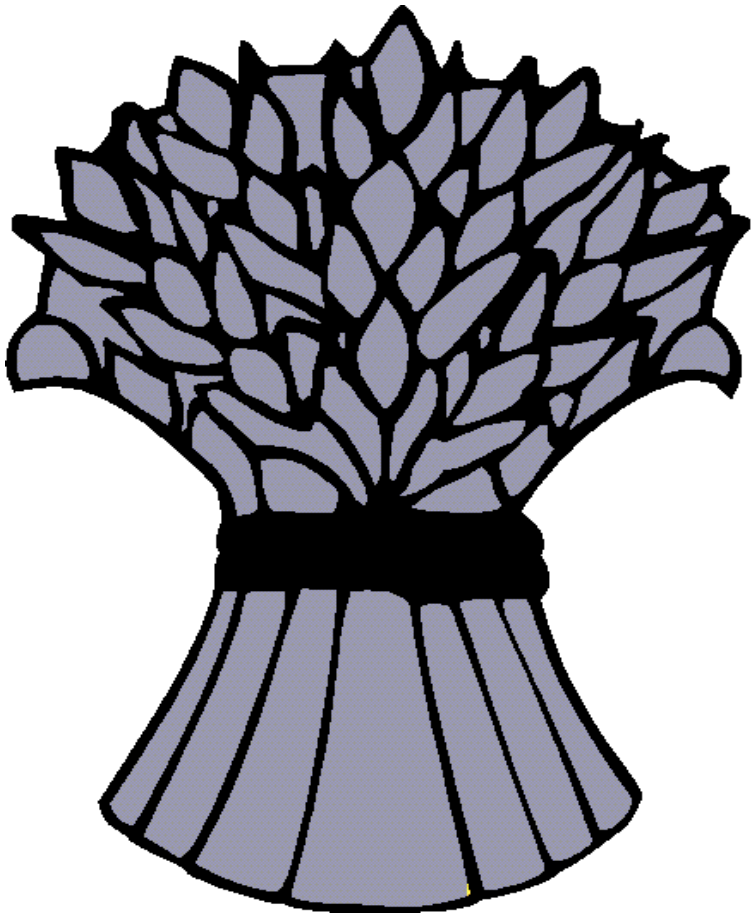
# 2001 National Fusarium Head Blight Forum Proceedings



Holiday Inn Cincinnati-Airport
Erlanger, KY
December 8-10, 2001

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Organized by:

**U.S. Wheat & Barley**
**Scab Initiative**

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# *TRI8* IN *FUSARIUM* ENCODES A TRICHOTHECENE C-3 ESTERASE

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## ABSTRACT

The elucidation of the biochemical pathway of trichothecene production by *Fusarium* species has been the focus of our laboratory for a number of years. The complex pathway begins with the sesquiterpene hydrocarbon trichodiene and consists of multiple oxygenation, cyclization, and esterification steps. As is found in a number of other organisms that produce toxic/antibiotic compounds, many of the genes involved in the pathway of trichothecene production in *Fusarium* are located within a gene cluster. Identified genes within this cluster are two genes encoding P450 oxygenases (*TRI11* and *TRI4*), a sesquiterpene cyclase (*TRI5*), two acetyltransferases (*TRI3* and *TRI7*), a pump (*TRI12*), and a gene for transcriptional regulation (*TRI6*). We have now identified the function of *TRI8*, a gene located adjacent to *TRI7*. To determine the function of *TRI8*, we disrupted the gene in both *F. graminearum* and *F. sporotrichioides*, transformed the parental strains, and analyzed transformants. Gene disruption was confirmed by PCR analysis as well as Southern hybridizations. The culture filtrate of *F. graminearum* mutants produced by genetic disruption of *TRI8* accumulated three C-3 acetylated compounds not normally seen in filtrates of the parent strain. We also conducted whole-cell and cell-free feeding experiments using acetylated trichothecenes. The wild-type parental converted the compounds into the deacetylated form whereas the disruptant mutants did not. Heterologous expression of *TRI8* and *TRI12* in yeast resulted in a strain that could remove the C-3 acetyl group from a number of trichothecenes. Based on these lines of evidence, we have identified that *TRI8* encodes an esterase that removes the C-3 acetyl group of *F. sporotrichioides* and *F. graminearum* trichothecenes.

# TRANSGENIC WHEAT OVEREXPRESSING PR-PROTEINS SHOWS A DELAY IN FUSARIUM HEAD BLIGHT INFECTION

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## OBJECTIVES

To identify and pyramid the best pathogenesis-related (PR-) protein and its combinations for enhancing resistance in wheat to Fusarium head blight.

## INTRODUCTION

Genes for pathogenesis-related (PR-) proteins are useful tools in enhancing the resistance of plants to pathogen and pest infestations. They have been successfully employed in improving resistance of plants to several fungal pathogens such as *Rhizoctonia solani* (Lin et al., 1995) and *Fusarium graminearum* (Krishnaveni et al., 2001, Chen et al., 1999). We intend to utilize combinations of these genes for enhancing resistance to wheat scab. The PR-protein group includes biochemically diverse proteins including fungal cell wall hydrolyzing enzymes (e.g. chitinase and ß-1,3-glucanase), inhibitors, peroxidases, oxalate oxidase and membrane permeabilizing proteins (e.g. thaumatin-like protein) and lipid transfer proteins. Genes/cDNAs for several of these proteins have been isolated in our laboratories from fungus-infected rice and wheat plants (Li et al., 2001). We have utilized previously a *tlp* gene to obtain transgenic wheat plants with somewhat improved resistance to scab. Our proposed research is based on the hypothesis that combinations of PR-protein genes will prove to be more effective in controlling scab than single genes. We have generated additional transgenic wheat plants with various combinations of PR-protein genes by biolistic transformation. These combinations include chitinase/glucanase and chitinase*/tlp*. Homozygous transgenic plants and their progeny with high level expression of multiple PR-proteins will be tested for resistance to the scab pathogen using standard evaluation protocols. Once the specific combinations of PR-proteins that result in maximum protection against scab infection are identified, these transgenic plants will be evaluated in a scab nursery in Kansas for scab resistance. Promising lines will be made available to breeding programs at KSU and elsewhere to generate elite wheat varieties resistant to this devastating pathogen.

## MATERIALS AND METHODS

***Wheat Transformation-*** Immature embryos (10-12 days after anthesis) of spring wheat cultivar 'Bobwhite' were co-transformed with different PR-gene (transformation cassettes in pHAC20 vector) using the particle inflow gene gun.

***Biochemical and molecular analysis of putative transgenic plants-*** The primary transformants were selected on glufosinate plates (5 mg $l^{-1}$) and the regenerated $T_0$ plants were subjected to PCR detection for *bar* gene and gene of interest. RT-PCR using gene

specific primers and western blot analyses with appropriate antisera was used for confirming the expression of the transgenes. The enzyme activity of the transgenes was assayed by incubating or overlaying the native PAGE gel with appropriate substrate. DNA blot and RNA blot analyses were carried out using the standard protocols.

***Scab bioassay-*** GZ3639, a highly virulent isolate of *Fusarium graminearum*, was used at final concentration of 5x $10^5$ spore $ml^{-1}$ to inoculate the experimental materials. The experimental materials included a resistant check (MN99112, courtesy Dr. Jim Anderson), Bobwhite non-transgenic control and the transgenic lines (32A2#3 and 26E5#6). Single floret of adult plants with spikes at anthesis were inoculated and placed under high humidity conditions in the greenhouse. The experiments were repeated thrice at different days and scored using the scoring system of Xu and Chen (1993) on a weekly basis.

## RESULTS AND DISCUSSION

Twenty-six independent transgenic $T_0$ plants were characterized and of this 10 Liberty (0.2%) resistant lines were identified for their stable expression, which are being propagated. The current status of these lines is summarized in Fig. 1. They are being tested for stable expression and inheritance of the transgenes. Some lines have been propagated to obtain $T_4$ generation plants and homozygous lines have been identified (Table 1). Preliminary bioassays with three of these lines showed a delay in the progression of scab infection (Fig. 3). Based on progeny analysis and fluorescent *in situ* hybridization (FISH) we have already identified some homozygous lines with stable expression of chitinase 383 and chitinase 383/glucanase 638 combinations. The homozygous line stably over expressing a chitinase 383/glucanase 638 combination was monitored for the expression of the transgenes periodically at weekly intervals starting from 3 weeks to 5 weeks (Fig. 2a & 2b) and the chitinase and glucanase activity in the spikes was analyzed before and after inoculation (72 hai). High expression of the transgene protein was detected about the heading stage and similarly higher activity of the enzymes was detected in the spikes before and after infection in this line. A moderate resistant reaction was observed in this line when compared with the control plants and the spread of infection was delayed for over 14 days. These lines are currently being grown for seed increase. Homozygous $T_3$ parents of 2 other lines with single (383) and combinations (289:383) of PR-protein genes were identified by progeny analyses based on Liberty painting assay.

Southern blot analysis of the 10 Liberty-resistant transgenic lines revealed that the number of copies of the transgene varied from 3 to 15 copies and they varied for each event. Fluorescent *in situ* hybridization (FISH) coupled with progeny testing (Liberty painting, and transgene PCR) was used to identify homozygous plants.

Crosses of the homozygous *tlp* transgenic wheat plants with a moderate improvement in resistance to scab and the homozygous wheat lines expressing the 383 chitinase and 383 chitinase/638glucanase gene combination have been completed. The homozygous $F_2$ LInes will be obtained and then evaluated for their resistance against *Fusarium graminearum*.

The moderately scab-resistant *tlp*-transgenic lines (Bobwhite background; Chen et al, 1999) has been crossed with Heyne. A backcross of the $F_1$ lines to Heyne, selection of Liberty-

resistant plants and production of $BC_2$ seeds was accomplished. An additional backcross, followed by selfing will be used for selecting for herbicide-resistant and herbicide-susceptible $BC_3F_3$ lines. Five lines homozygous for the transgene, and five non-transgenic lines in isogenic background will be isolated and evaluated for scab resistance. Crosses of the homozygous *tlp* transgenic wheat plants with a moderate improvement in resistance to scab and the homozygous wheat lines expressing the 383 chitinase and 383 chitinase/ 638glucanase gene combination have been completed. The homozygous $F_2$ lines will be obtained and then evaluated for their resistance against *Fusarium graminearum*.

It would thus be important to identify the specific gene combinations that are most effective against the scab pathogen. Our approach would be to pyramid the PR-protein genes and evaluate the synergistic effect (if any) of the different combinations towards scab.

## ACKNOWLEDGEMENT

We greatly appreciate the assistance of Julie Essig and Marcy Main for their tissue culture and transformation expertise, R. Adams for his preliminary screening of the transgenics and Dr. Bob Bowden and Dr. Bill Bockus in providing us the virulent strain GZ3639 of FHB and with the scab bioassay. We thank Dr. Jim Anderson for giving us the seed material MN99112, a spring wheat experimental line as an appropriate resistant check. Our sincere appreciations go out to Ms. Peng and Dr. Friebe with the FISH analyses and to Dr. Paulsen in providing us the growth chamber space.

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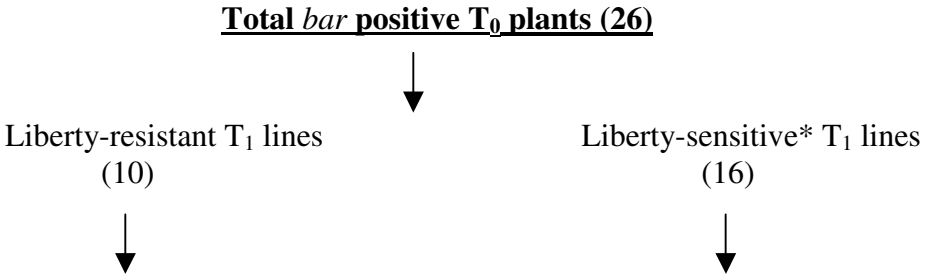



| *bar* PCR | | Transgene PCR | | RT-PCR | | Western blot | | | *bar* PCR | | Transgene PCR | | RT-PCR | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| + | - | + | - | + | - | + | - | | + | - | + | - | + | - |
| 10 | 0 | 9 | 1 | 8 | 2 | 8 | 2 | | 16 | 0 | 10 | 6 | 1 | 15 |

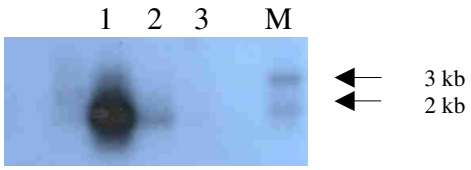
* Liberty-sensitivity is probably due to gene silencing. These lines are PCR positive for *bar*

**Figure 1.** Schematic diagram showing the total number of transgenic plants with PR-protein genes under current analysis

**Table1.** Status of the transgenic lines stably over-expressing different PR- protein transgenes

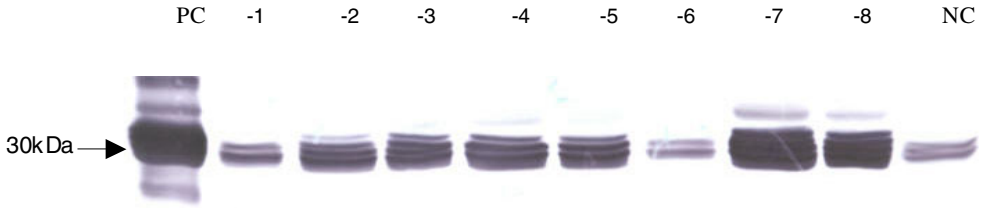
| Gene (s) | No. of lines | Type of PR-protein | Status |
|---|---|---|---|
| 383 | 2 | Wheat chitinase | Homozygous line identified in both line ($T_4$ & $T_3$) |
| 638 | 2 | Wheat glucanase | Heterozygous ($T_3$) |
| 383:638 | 2 | Wheat chitinase/Wheat glucanase | Homozygous line identified in both line ($T_4$) |
| 289:383 | 2 | Wheat glucanase/Wheat chitinase | Homozygous line identified ($T_3$) |
| tlp | 1 | Rice TLP | $T_2$ |
| tlp/chi11 | 1 | Rice TLP/rice chitinase | $T_2$ |

a) Northern blot analyses



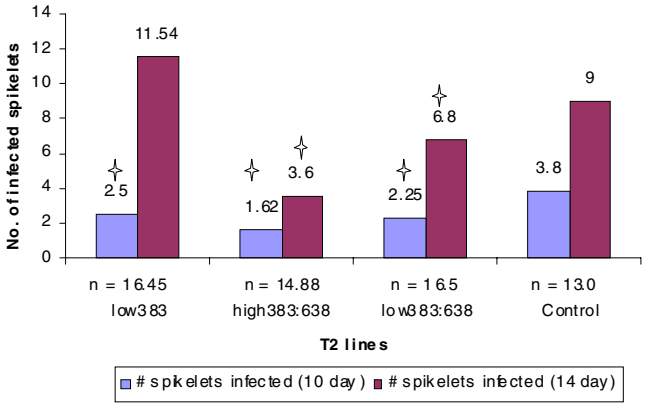

1: high expressing line; 2: low expressing line; 3: non-transgenic control and M) RNA marker. 12 µg of total RNA isolated from leaves was loaded in each lane. The expected 1.3 kb β-glucanase transcript was detected in the transgenic lines within 6 h of exposure to X-ray film.

b) Western blot analyses for glucanase expression in the homozygous line 32A2#3




PC: scab infected leaf, 32A2#3: homozygous line for 638 glucanase, NC: non-transformed Bobwhite control

**Figure 2.** β-glucanase expression in different transgenic lines




*p*=0.01; n: average number of spikelets (of 3 replicates).

Conclusion: Transgenic line expressing a combination of a wheat chitinase and a wheat glucanase at a high level showed a delay in the spread of the infection when compared to the non-transgenic line and transgenic lines with either the chitinase alone or a line with the same transgenes expressed at a lower level.

**Figure 3.** Preliminary scab bioassay data from 3 different transgenic lines

# GENETIC DIVERSITY IN SCAB-RESISTANT WHEAT CULTIVARS BASED ON MOLECULAR MARKERS

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## ABSTRACT

Wheat scab can dramatically reduce grain yield and quality. Breeding for resistance is an effective measure for disease control. Wheat cultivars with various levels of Type II resistance have been reported worldwide, however, the genetic relationships among the cultivars are not well characterized. Sixty-five wheat cultivars from eight countries varying in resistance levels were evaluated for Type II resistance and for genetic diversity based on 322 AFLP and 19 SSR marker alleles. Cluster analysis of AFLP and SSR markers linked to the major QTL on chromosome 3BS of Ning 7840 indicated that two Japanese landraces and most of the cultivars related to Ning 7840 carried the same major QTL. However, the QTL are different from those in Wangshuibai and other Chinese landraces as well as those that originated from countries other than China and Japan. Fingerprinting suggested that Taiwanxiaomai is the donor of the major QTL in Ning 7840 or Sumai 3. Combining major resistance QTL from Ning 7840 and Wangshuibai or other sources not related to Ning7840 may facilitate pyramiding different QTL. Cluster analysis based on AFLPs and SSRs provided the best estimate of genetic relationships among accessions studied. The result indicates that US cultivars are more closely related to cultivars from Europe and Argentina than cultivars from Asia; therefore, integrating scab resistance from Chinese sources may increase the genetic diversity of US wheat cultivars and combining Chinese and non-Chinese sources of scab resistance offers a way to enhance the level of scab resistance. (This poster was presented at the Annual Meeting of 2001 CSSA)

# THE EFFECTS OF HOMOEOLOGOUS GROUP 3 CHROMOSOMES ON RESISTANCE TO FUSARIUM HEAD BLIGHT IN TETRAPLOID WHEAT

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## ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* is one of the most destructive diseases of wheat in areas where the weather is warm and humid after the heading. Previous studies indicate that level of resistance to FHB varies not only among wheat cultivars but also among some of their wild relatives. No accession, however, has yet been identified to be completely immune to FHB among the Gramineae. It is know that durum wheats (*Triticum turgidum* L. conv. durum) are consistently more susceptible to FHB than common wheat (*T. aestivum* L.). The importance of D genome in conferring resistance to FHB has been emphasized. Meanwhile, recent studies using molecular markers report effective QTLs on chromosome 3BS in hexaploid population and on 3A in tetraploid recombinant inbred chromosome lines. In this study, we performed to evaluate the effects of homoeologous group 3 chromosomes of *T. turgidum* ssp. *dicoccoides* on resistance to FHB using a set of chromosome substitution lines of a durum wheat cultivar 'Langdon'. The accession of *T. turgidum* ssp. *dicoccoides* examined in this study was higher susceptible for Type II resistance (resistance to spread of FHB in the head) than Langdon. Both of the chromosome substitution lines of 3A and 3B showed same level of resistance with Langdon, but bleaching of the heads was completely prevented in the substitution lines of chromosome 3A without relationship to rachis fragility. It is concluded that the chromosome 3A of *T. turgidum* ssp. *dicoccoides* carries resistance gene(s) to head bleaching caused by FHB. (This poster was presented at the 4th International Triticeae Symposium, Córdoba, Spain, September 10-12, 2001. The abstract will be in Hereditas 2002.)

# IDENTIFICATION OF DIFFERENTIALLY EXPRESSED SEQUENCE TAGS FOR SCAB RESISTANCE IN WHEAT USING BSA AND SSH

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## ABSTRACT

Wheat scab is a destructive disease of wheat (*Triticum aestivum*). Genome-wide analysis of gene expression in response to infection by *Fusarium graminearum* may lead to discovery of novel genes for scab resistance and provide insight into further understanding of genetic mechanisms of wheat resistance to scab. To enrich differentially expressed sequence tags (ESTs) for scab resistance, cDNA subtraction libraries were generated from *Fusarium*-infected spikes of two bulked recombinant inbred lines (RILs) using the suppression subtractive hybridization (SSH) method. Two bulked RILs differing in scab resistance were formed by pooling infected spikes from five $F_{8:12}$ scab-resistant and five $F_{8:12}$ susceptible RILs, respectively, based on their Type II resistance from four greenhouse tests. The RILs derived from the cross between the resistant cultivar Ning 7840 and the susceptible cultivar Clark. The selected RILs were grown in the growth chamber and inoculated with a conidiospore suspension of *Fusarium graminearum* by single floret inoculation at early flowering stage. The inoculated spikes were enclosed in a moist plastic bag to maintain high humidity. The infected spikes were harvested for mRNA extraction at 36 and 72 hours after inoculation (HAI). To eliminate mRNA contamination from the fungus, the inoculated floret from each spike was removed before the spikes were harvested. About 550 clones were isolated from the two libraries constructed from infected spikes at 36 and 72 HAI. Eighty-five clones were randomly picked from the libraries and sequenced. Most of them (95%) were singletons. Sequence homology search using the NCBI BLAST program showed that some of them were stress and other defense-related genes or genes for PR-proteins and signal regulation. Construction of an additional SSH library at 6 HAI is underway. The clones from the libraries will be used for temporal gene expression analysis with macroarrays.

# HEREDITY AND MOLECULAR MARKERS FOR WHEAT SCAB RESISTANCE

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## RESEARCH OBJECTIVES

1) To determine the inheritance of scab resistance in identified resistance sources W14, Shaan 85 and Ernie; 2) To elucidate the genetic relationship between type II, III and IV resistance based on segregation in four $F_2$ populations for scab severity, DON content and scabby seeds; 3) To identify SSR molecular markers associated with type II, III and IV resistance in source W14 using an $F_2$ population Pioneer 2684 x W14.

## INTRODUCTION

Fusarium head blight (FHB) or scab, caused by *Fusarium graminearum* [*Gibberelle Zeae* (Schwabe) Petch], is a devastating disease of all classes of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) worldwide. Deployment of resistant varieties is an effective, economical and environmentally safe way to control FHB in wheat. However, success of this endeavor is greatly dependent on first obtaining knowledge of the amount of genetic diversity for resistance, identity of different mechanisms governing resistance, inheritance of resistance, and most importantly identifying selectable markers for incorporating and pyramiding resistance genes into wheat cultivars. DNA markers for FHB resistance QTLs have been identified in the primary resistance source Sumai 3 and its progeny Ning 7840 (Anderson, 2001; Bai et al., 1999). One major QTL was mapped on wheat chromosome 3BS, and explained 25 to 42% (Anderson et al., 2001) and 60% (Bai et al., 1999) of the phenotypic variation of disease severity in three recombinant inbred line populations. This QTL is associated with resistance to disease spread, but associations with resistance to DON production and seed colonization has not been determined. Identification of QTLs associated with resistance to disease spread (Type II), seed colonization (Type III) and DON production (Type IV) is imperative for the implementation of gene pyramiding to develop germplasm and cultivars possessing resistance levels approaching immunity and conferring near-zero losses.

## MATERIALS AND METHODS

***Plant Materials*** - Three resistance sources, W14, Shaan 85 and Ernie, identified in previous studies (Griffey et al., 1998), were crossed with susceptible soft red winter (SRW) wheat cultivar Madison and/or Pioneer 2684. W14 is an improved type II resistance source developed by recurrent selection (Jiang, 1997), which may include genes from Sumai 3 and other resistance sources. Shaan 85 is an improved type II resistance source derived from Sumai 3. Ernie is a scab resistant SRW wheat cultivar that lacks any of the known scab resistant sources in its ancestry. Four $F_2$ (Pioneer 2684 x W14, Madison x W14, Pioneer 2684 x Shaan 85, and Ernie x Pioneer 2684) and two $F_{2:3}$ (Pioneer 2684 x W14 and Madison x

W14) populations were used in genetic studies. $F_2$ population Pioneer2684 x W14 (156 DNA samples) was used in a mapping study. In order to elucidate the genetic diversity among currently deployed resistance sources, Funo, one of the parents of Sumai 3, Sumai 3, W14, Shaan85, VR95B717 (source from France), Ernie, and two susceptible parents Madison and Pioneer 2684 were used in the genotyping study.

***Disease screening -*** One to three heads per individual in $F_2$ populations, 10 to 30 individuals per family in $F_{2:3}$ populations, and 28 to 51 individuals per parent were inoculated via floret inoculation procedures. A droplet (30ul) of macro conidia ($5 \times 10^4$ spores/ml) was placed into a floret in the middle of spike at early anthesis using a SAMCO transfer pipette. Ratings of severity (percentage of infected florets) were assessed three times at 7, 14 and 21 days after inoculation. Severity rated on 21st day was used for analysis. Percentage of scabby seeds for each individual was determined based on the mean number of colonized seed per hand-threshed single spike. DON content was analyzed as ppm by a SIM Shimadzu QP5000 GC/MS system at the University of Minnesota. Severity, scabby seeds and DON content were evaluated in $F_2$ populations and only severity was evaluated in $F_{2:3}$ lines.

***Microsatellite analysis –*** A total of three hundred SSRs were synthesized and included 172 published by Roder et al., (1998); 40 by Bryan et al., (1997); and 88 kindly provided by Dr. Cregan of USDA/ARS (2001). Based on previous genetic and molecular marker work, the following linkage groups are expected to contain QTLs for FHB reaction: 1B, 2B, 3B, 3A, 5A, 6A, 6B, 6D, and 7B. Therefore, SSRs known to be located on these chromosomes were selected and used to survey DNA polymorphism among four parents (Ernie, Pioneer 2684, Madison, and W14) and the four bulked DNA samples (R and S bulks from the cross of Pion2684 x W14; R and S bulks from the cross of Madison x W14). Bulked DNA samples were obtained by mixing an equal amount of DNA from six putative homozygous resistant and six homozygous susceptible $F_2$ individuals, respectively. DNA extraction, PCR amplification and SSR assays were conducted as described by Saghai Maroof et al. 1984,1994; Bryan et al. 1997; Roder et al. 1998.

***Statistical analysis*** - Agrobase Software was used for statistical analyses. One-way ANOVA was conducted to confirm significant ($P < 0.05$) association between putative resistance-related markers and resistance to scab.

## RESULTS AND DISCUSSION

***Identification and characterization of scab resistance in four $F_2$ populations -*** Significant differences in type and level of resistance were found between parents and among individuals in $F_2$ populations (data not shown). W14 and Shaan 85 are highly resistant to disease spread, seed colonization and DON production. Ernie is moderately resistant to disease spread and highly resistant to seed colonization and DON production. Pioneer 2684 and Madison are highly susceptible to disease spread, seed colonization and DON production. Highly-resistant individuals with type II resistance were found to also possess type III and type IV resistance, having consistently lower severity, scabby seeds, and less than 10 ppm toxin accumulation. Highly susceptible individuals were found to have variable or consistently high ratings for scabby seeds and DON content. About 25 % of individuals

with type III and IV resistance were moderately resistant to moderately susceptible to disease spread. Individuals with type IV resistance were found to have type III resistance in most cases. Significant positive correlations were found between disease severity (type II resistance), toxin content (type III resistance), and scabby seeds (type IV resistance) based on analysis of segregation data from four $F_2$ populations. Correlation between scabby seeds and DON content ($r = 0.8646$) was much higher than those between severity and DON content ($r = 0.5388$), and severity and scabby seeds ($r = 0.5911$), which suggests that DON content could be predicted by percentage of scabby seeds in most cases. Therefore, assessing severity before harvest and assessing scabby seeds after harvest may be an effective and economical way to select for resistance as a whole.

***Inheritance of resistance to disease spread, seed colonization and DON accumulation in four $F_2$ populations -*** Discrete classes were not observed within the segregating populations of any crosses or for any parameter evaluated. Different frequency distributions were observed among the four populations for severity, scabby seeds and DON production. A normal distribution with transgressive segregation for both susceptibility and resistance was observed in cross Pioneer 2684 x Ernie for all parameters. This indicates that resistance in Ernie is controlled by quantitative gene. Four genes were estimated for resistance in Ernie based on a quantitative approach (Wright, 1968).

Disease severity in the $F_1$ was greater than that of the resistant parent but less than that of the mid-parent in three crosses (Pioneer 2684 x W14, Madison x W14, Pioneer 2684 x Shaan 85), and indicates that resistance is controlled by partially dominant gene effects. A right skewed distribution with two peaks coinciding to the parents was observed in crosses with W14 and Shaan 85 as the resistant source, and suggests that resistance of W14 and Shaan 85 is controlled by major genes. In W14 and Shaan 85, two major genes with complementary effects were indicated by $?^2$ analysis, and two to three genes were estimated by quantitative approach (Wright, 1968). Transgressive segregants were also observed in these three crosses with increased susceptibility based on severity and both increased susceptibility and resistance based on scabby seeds and DON content, which suggests the presence of the minor gene.

***Identification and characterization of marker-QTLs for scab resistance in common* wheat -** DNA polymorphism among four parents (Ernie, Pioneer 2684, Madison, and W14) was significant, and was observed for seventy-six percent of primers (152 out of 200); however, DNA polymorphism among parents was not always observed among bulks (R and S bulks from the cross of Pion2684 x W14; R and S bulks from the cross of Madison x W14). Therefore, all polymorphic markers were used to map scab QTLs in the population Pion2684 x W14. A total of 45 loci were mapped to five chromosomal regions in this population and a major QTL, in addition to the 3BS QTL, was identified, potentially located on 2BS. Fifteen markers identified in three QTL regions were significantly ($p < 0.05$) associated with scab resistance, and explained 23, 28, 21, and 36 % of total variation in percentage of scabby seeds, DON content, and severity in 82 $F_2$ individuals and severity in 82 corresponding $F_{2:3}$ families, respectively.

Eight DNA markers from the five chromosome regions were used to genotype six diverse resistance sources. Differences and similarities in these markers among the six lines indicate that some lines may possess different resistance genes that could be useful in pyramiding resistance from these sources and, thereby improve the level of scab resistance. W14 may possess different gene or allele than Sumai 3 on 2BS QTL region, and Ernie may possess resistance genes different from other type II resistance sources in both 2BS and 3BS QTLs regions. DNA polymorphism was found among type II resistance sources W14, Shaan 85, Sumai 3, Funo, and Ernie for marker loci that are associated with postulated resistance genes. This indicates that genetic diversity exists; however, gene interactions may adversely effect pyramiding of different resistance genes.

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# SCREENING OF WHEAT GERMPLASM FOR POLYMORPHISM OF SSR MARKERS LOCATED ON CHROMOSOME 3B

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## ABSTRACT

The development of molecular genome analysis tools has created an interest in the use of marker-assisted selection (MAS) in applied plant breeding programs. MAS may become an important aid to plant breeders in selection of superior genotypes. Instead of using phenotypic selection for a trait, once a tight linkage has been established between the trait and the marker, the marker can be used for selection. MAS may become especially useful for traits that are difficult to assay by phenotype such as scab or head blight resistance. Bai et al. (1999) identified molecular markers linked to a major QTL controlling resistance to scab present in the Chinese cultivar Ning 7840. This QTL, located on chromosome 3B, explained up to 60% of the variation in scab resistance. Knowledge of the variation for markers linked with disease resistance within wheat germplasm, however, is essential before MAS can be applied widely. In this research, we examined the variation of three SSR (Simple Sequence Repeat) markers located on chromosome 3B (Roder et al. 1998) within a sample of wheat germplasm to determine their future usefulness for selection.

# A STS MARKER FOR SCAB RESISTANCE QTL IN WHEAT DERIVED FROM PST I-AFLP

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## ABSTRACT

Large-scale field screening for scab resistance in wheat is difficult because environments significantly affect expression of the resistance genes. Marker-assisted selection (MAS) may provide a powerful alternative. Amplified fragment length polymorphism (AFLP) is an efficient marker system for mapping and tagging of quantitative trait loci (QTL). However, the complexity of AFLP procedure makes it difficult to be used as routine in breeding programs. Conversion of AFLP to sequence-tagged site (STS) may produce breeder-friendly markers for MAS. One major QTL on chromosome 3BS was mapped by using AFLPs and the population from the cross of Ning 7840/Clark. Fine mapping of the QTL identified two dominant markers individually explained up to 50% of phenotypic variation in the same population. One 35bps DNA fragment linked to the QTL in coupling phase and another 222bps fragment linked to the QTL in repulsing phase. The larger DNA fragment was cloned and sequenced. Five different DNA fragments were recovered and one with five identical copies was selected to design STS primers. A co-dominant marker was amplified and explained about 50% of phenotypic variation for scab resistance in F7 population. The STS was also validated in several other cultivars having the major QTL on 3BS. This is the first codominant STS marker for the major scab resistance QTL converted from an AFLP marker. Application of this marker in breeding programs may speed up breeding process to enhance wheat scab resistance in wheat.

# RAPID DNA EXTRACTION FROM WHEAT TISSUE FOR HIGH THROUGHPUT PCR MARKER-BASED ANALYSIS

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## ABSTRACT

High throughput marker assisted selection in plant breeding programs is limited by the ability to extract DNA from large populations of plants. The primary objective of this project was to develop a high throughput DNA extraction procedure without the need for greenhouse space or growing wheat (*Triticum aestivum* L.) plants to maturity. A sodium hydroxide rapid DNA extraction was modified for 96-well format to reduce costs. Also, compared were stored versus fresh tissue. Extracts were done on 4-day old seedling tissue from seeds germinated in 8-well tissue culture plates. Approximately 2 µg of genomic DNA can be isolated per 0.02 grams of tissue at a cost of $0.20/sample. To test the robustnest of the extraction procedure, two well characterized PCR based microsatellite markers were analyzed for size and repeatability. Data for the microsatellite markers was the same for fresh and stored tissue extracts as well as recently extracted and stored DNA. This technique will allow one person, in a single workday, to extract nearly one thousand storage stable DNA samples that are immediately ready for PCR analysis.

# PROGRESS TOWARDS SATURATION MAPPING AND BAC CONTIG DEVELOPMENT FOR *QFHS.NDSU-3AS*, A MAJOR FHB QTL IN DURUM WHEAT

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## ABSTRACT

The research conducted by our group using Langdon-*dicoccoides* chromosome 3A recombinant inbred lines (LDN-Dic 3A RICLs), has allowed the targeting of molecular markers to the region of a single chromosome (Otto et al. 2001). A major QTL, *Qfhs.ndsu-3AS*, that explains 55% of the genetic variation for FHB resistance, and a microsatellite locus, *Xgwm2*, tightly linked to the highest point of the QTL peak were identified (Otto et al. 2001). In this report we present our initial results towards development of a saturated linkage map for the region surrounding this QTL, and identification of bacterial artificial chromosome (BAC) clones from this region using a *Triticum monococcum* BAC library.

Three methods are planned for generating molecular markers in the region surrounding this QTL viz., EST (expressed sequence tag)-derived primers, synteny-derived primers and RNA fingerprinting-differential display. The first method involves design and amplification of target DNA using primers generated from publicly available wheat ESTs generated from the National Science Foundation-funded wheat EST project. As of date, 391 loci have been assigned to homeologous group 3. Our laboratory is part of this multi-institution consortium and we have been mapping wheat ESTs onto the cytogenetic deletion stocks. This valuable resource of mapped ESTs can be easily used to derive polymerase chain reaction (PCR)-based markers by designing primers from these ESTs and amplifying DNA of interest. A locus-specific marker derived from *Xgwm2* was used as a DNA-based probe on a set of 'Chinese Spring' cytogenetic deletion stocks and was assigned to the chromosomal bin location 3AS4-0.45-1.00. Primer pairs were designed from ESTs that map to this bin location, and used for polymerase chain reaction (PCR) amplification of the LDN-Dic 3A RICLs . Amplified products from four of these primer pairs have been analyzed by polyacylamide gel electrophoresis, and we report initial results for these experiments. We have also screened a *T. monococcum* BAC library with a DNA-based probe derived from the microsatellite locus *Xgwm2*, and have identified 13 BAC clones. We also report initial results from the characterization and progress towards subcloning these BAC clones.

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# UNDERSTANDING FUSARIUM HEAD BLIGHT RESISTANCE IN TETRAPLOID AND HEXAPLOID WHEAT

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## ABSTRACT

Fusarium head blight, more commonly known as scab, is a fungal disease of small-grain crops that causes yield loss and poor grain quality. The extensive damage caused by Fusarium head blight (FHB) has made it necessary to develop resistant lines of durum (AABB; 2n=4x=28) and hexaploid (AABBDD; 2n=6x=42) wheat. One species that shows promise as a source for FHB resistance in tetraploid cultivars is an accession of *Triticum turgidum* L. var. *dicoccoides* (AABB, 2n=4x=28). The Langdon-dicoccoides chromosome 3A disomic substitution line was shown to have Type II resistance to FHB. A Recombinant Inbred Chromosome Line (RICL) population of 83 individuals derived from LDN(Dic-3A) has been analyzed over multiple seasons. Phenotypic screening data, molecular marker mapping data (RFLP, AFLP, RGA, and microsatellite), and QTL analysis results have delineated the location of FHB resistance loci on the 3A chromosome. At the present time, 14 markers have been placed on the molecular map of chromosome 3A covering 133.9 cM. Quantitative trait analysis suggests 37% of the phenotypic variance, or 55% of the genetic variation for FHB resistance is explained by the locus *Xgwm2*. Effectiveness of this marker in selection of durum cultivars with improved FHB resistance is being tested.

A study was conducted in North Dakota adapted hexaploid cultivars deriving their resistance from Sumai 3 to identify markers linked to quantitative trait loci (QTL) for FHB resistance. The advantages of using ND wheat lines are three-fold. First, fewer individuals need to be genotyped for marker loci as compared with mapping populations. For in this type of analysis a priori selection has been placed on the trait of interest as opposed to mapping populations where segregation is needed. Second, the power of detecting QTL regions associated with FHB resistance is much greater than that with mapping populations. Third, the materials used in this project have been extensively screened (both field and greenhouse) over a 5-year period for their reaction to FHB, precluding the need for additional screening for resistance. Analysis of these lines for presence of markers coming from Sumai 3 indicates two significant regions (a region on chromosomes3B and 7B each). Probability of linkage between markers and introgressed resistance gene was calculated using a binomial probability formula. Probability of the 3B region being present by chance is less than $3 \times 10^{-15}$ and that on 7B is $5 \times 10^{-7}$. Markers are being utilized to select durum and hexaploid wheat lines carrying these FHB resistance loci. Populations carrying sources of FHB resistance different from those describe above are being investigated for their value in identifying different loci and pyramiding useful genes in a single genetic background.

# MAPPING GENES CONFERRING FUSARIUM HEAD BLIGHT RESISTANCE IN A MIDWEST BARLEY ACCESSION HIETPAS 5

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## INTRODUCTION

Fusarium head blight (FHB), incited primarily by *Fusarium graminearum*, adversely affected the quality of barley grown in eastern North Dakota and northwestern Minnesota the last nine years. Quality of harvested grain was reduced because of blighted kernels and the presence of deoxynivalenol (DON), a mycotoxin produced by the pathogen. A line currently used by Midwestern barley breeding programs as sources of genes for FHB resistance is C93-3230-24. This six-rowed line 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. Field tests conducted the last three summers in mist-irrigated FHB nurseries in North Dakota confirmed that C93-3230-24 has FHB resistance approaching Chevron. The genetic background of C93-3230-24 appears to be completely different than that of any of the FBH resistant accessions identified by Prom et al. (1996). Thus, this line may have alleles for FHB resistance and DON accumulation not currently identified.

## METHODS

An $F_1$-derived doubled-haploid (DH) population consisting of 300 lines was created from the cross 'Foster'/C93-3230-24. Foster is susceptible to FHB. One hundred eighteen lines were randomly chosen from the population and are being used for construction of a molecular marker map. Phenotypic data for FHB resistance, DON accumulation, heading and maturity dates, plant height, and spike angle are being collected so their respective QTL can be placed on the linkage map. Heading and maturity dates, plant height, and spike angle have been identified as traits associated with FHB resistance and DON accumulation in previous studies.

## RESULTS

Field experiments were conducted in three mist-irrigated FHB nurseries in 2000 using the 118 DH lines and parents. Two nurseries were located in North Dakota and the third was located at Zhejiang University in Hangzhou, China. Construction of a linkage map consisting of RFLP and SSR markers is in progress. Single locus analysis using available marker data identified three chromosomal regions associated with FHB resistance (Table 1). The regions are located in chromosomes 2H, 5H, and 7H. Results in this study are similar to those obtained in studies using the resistant six-rowed cultivar 'Chevron' (de la Pena et al., 1999; Ma et al., 2000). Thus, preliminary results suggest that the Midwest accession C93-3230 and the Swiss cultivar Chevron may have similar alleles for FHB resistance.

Research is continuing to complete the molecular marker linkage map. The mapping population and parents will be grown at Zhejiang University during winter 2001-2002 and at three North Dakota locations in 2002. Once the linkage map is completed, QTL analysis will be performed to obtain better estimates of the chromosomal location of QTL conferring FHB resistance and reduced DON accumulation. QTL for heading and maturity dates, plant height, and spike angle also will be mapped.

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**Table 1.** Coefficient of determination ($r^2$) values for markers significantly associated with Fusarium head blight (FHB) and other traits at three environments in 2001.

| | | Environment | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Langdon, ND | | Osnabrock, ND | | | | Hangzhou, China |
| SSR marker | Chr.† | FHB severity | Plant height | FHB severity | Plant height | Physiological maturity | Spike angle | FHB severity |
| Hvm07 | | 0.03 | | 0.05 | | | | |
| Hvlue | | | | | | | | |
| Bmag0345 | 1H | | 0.04 | | 0.04 | | | |
| Bmac0090 | 1H | | 0.04 | | | | | |
| EBmac501 | 1H | | 0.06 | | | | | |
| Bmac0134 | 2H | | | | | | | |
| Bmag0140 | 2H | 0.14 | 0.18 | 0.09 | 0.09 | 0.21 | | 0.09 |
| Ebmac521 | 2H | 0.16 | 0.23 | 0.16 | 0.08 | | | 0.17 |
| Bmag0378 | 2H | 0.13 | 0.14 | 0.09 | 0.07 | 0.26 | | 0.07 |
| Ebmac557 | 2H | 0.17 | 0.22 | 0.13 | 0.08 | 0.28 | | 0.15 |
| HVBKASI | 2H | 0.17 | 0.22 | 0.14 | 0.08 | 0.27 | | 0.15 |
| Bmac0126 | 2H | 0.17 | 0.22 | 0.13 | 0.08 | 0.28 | | 0.15 |
| Bmac0310 | 4H | 0.06 | | | | | | |
| Bmac0181 | 4H | | | | | | | |
| Hvm67 | 4H | | | | 0.05 | | | |
| Hvm06 | 5H | | | | | | | |
| Bmac0113 | 5H | | | 0.05 | | | | |
| Bmag0337 | 5H | | | | | | | |
| Bmac0163 | 5H | | | 0.05 | | | | |
| HVM65 | 6H | | | | | | | 0.03 |
| Hvm22 | 6H | | | | 0.09 | | | |
| Bmag0009 | 6H | | | | | | | |
| HVM04 | 7H | 0.04 | | 0.04 | 0.12 | 0.06 | | |
| Bmag0341 | 7H | | | | | | | |

# TRANSFORMATION OF A COMMERCIAL BARLEY CULTIVAR WITH GENES FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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## ABSTRACT

Fusarium head blight, incited primarily by *Fusarium graminearum*, has caused devastating losses to barley since the 1990's. Production of the mycotoxin deoxynivalenol (DON) by *F. graminearum* is harmful to humans and livestock. Expressing certain anti-toxin genes such as *TRI101* and *PDR*5 could improve resistance to fungal infection and reduce DON levels. *TRI101* encodes a 3-OH trichothecene acetyltransferase that converts DON to a less toxic acetylated form. *PDR5*, an ATP-binding cassette, acts as an efflux transporter, shunting DON across the plasma membrane from the interior of the cell. We have transformed the commercial malting barley cultivar Conlon with these genes to reduce DON levels in infected grain. Ten-day old calli derived from immature embryos were co-bombarded with the herbicide-resistance gene bar as the selectable marker. Putative transgenic plants were confirmed by Southern analysis. A total of seven independent events with *TRI101* and six with *PDR5* were recovered. Northern analysis indicated the expression of *PDR5*. Expression of *TRI101* was confirmed by detecting acetyltransferase activity in seeds of the transgenic plants. $T_2$ lines of three events with *TRI101* and two events with *PDR5* were field tested for disease and toxin level. Both genes appeared to reduce FHB infection and *PDR5* also may reduce DON accumulation.

# USING MOLECULAR GENETICS TO ENHANCE SCAB RESISTANCE IN WHEAT AND BARLEY

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## ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* is a major disease problem on the wheat and barley crops in the United States and around the world. Our goals are to understand the biology of this plant-pathogen interaction in order to develop biotechnology strategies for FHB resistance. Histological studies showed that *F. graminaerum* can infect wheat through multiple pathways. Examination of gene expression in wheat spikes within 48 hours after infection by *F. graminearum* showed that the classical defense response genes are upregulated at the site of infection and in a systemic fashion. Application of benzothiadiazole (BTH) to wheat spikes resulted in upregulation of the BTH-induced genes but did not result in Type I or Type II FHB resistance. Expressed sequence tags (ESTs) have been generated from cDNA libraries prepared from wheat and barley spikes infected with *F. graminearum*. Bioinformatic comparisons of these ESTs revealed four sets of genes: (1) classical biotic and abiotic stress response genes; (2) fungal genes associated with pathogenicity; (3) library specific genes; and (4) genes in common with other plant-pathogen interactions. To further characterize this plant-pathogen interaction, additional bioinformatic comparisons are ongoing and large-scale expression studies are underway.

Biotechnology approaches to developing resistance are also in progress. Our approach is to overexpress a set of antifungal protein genes in wheat and barley. We have used particle acceleration to generate transgenic wheat and barley with a set of antifungal protein genes. To date, we have generated 25, 25 and 31 wheat plants overexpressing a wheat α-1-purothionin, a barley thaumatin-like protein-1 (tlp-1), and a barley ß-1,3-glucanase, respectively. We have also developed wheat and barley plants carrying an overexpressed barley ribosome inactivating protein gene. Two FHB disease screens have been conducted on the transgenic wheat plants carrying the ß-1,3-glucanase, tlp-1 and α-1-thionin transgenes. Our results from the FHB disease screens indicate that there are lines carrying each transgene that reduce FHB severity. Transgenic plants carrying other AFP genes or AFP genes in combination will be tested in the future.

# EXPRESSION OF TWO DIFFERENT CANDIDATE ANTI-FUSARIUM PROTEIN GENES AFFORDS PARTIAL PROTECTION AGAINST THE SPREAD OF *FUSARIUM GRAMINEARUM*

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## ABSTRACT

Host plant resistance is an efficient, cost-effective and environmentally friendly way to fight plant disease and would be particularly helpful in decreasing grower losses due to Fusarium head blight. More sources of resistance, preferably tagged with molecular markers, are needed. We are using genetic transformation in a biotechnology approach to introduce novel candidate anti-*Fusarium* genes into hexaploid wheat. The genes encode proteins targeted against either the fungus itself or the mycotoxins it produces during infection. Six different candidate anti-*Fusarium* coding regions were fused to the maize *Ubi1* promoter, first intron and exon for widespread expression. Several transgenic plants have been obtained for each construct. The transgenics show a range of expression levels in semi-quantitative RT-PCR assays. On average, expression from a construct with a wheat coding region is higher than from those with fungal coding regions. Homozygous plants have been identified for many of the transgenic events. Ten of these have been tested in greenhouse trials for Type II resistance. Two transgenic lines exhibited small increases in resistance, compared to their non-transformed parent, in three independent tests. One of these lines accumulates DON acetyltransferase enzyme activity encoded by a transgene containing the *Fusarium sporotrichioides TRI101* gene. The other line contains a transgene that encodes a *Fusarium venenatum* glucanase (FvGlu). In an effort to boost the expression levels of the two most promising transgenes, we have modified sections of the FvGlu and *TRI101* coding regions to make them more wheat-like in sequence. These improved constructs are currently being introduced into wheat.

# EVALUATION OF TRANSGENIC WHEAT LINES EXPRESSING THE BACULOVIRUS OP-IAP FOR TOLERANCE TO SCAB INDUCED BY *FUSARIUM GRAMINEARUM*

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## ABSTRACT

Fusarium head blight (FHB) induced by the fungal agent *F. graminearum* is a significant problem in many wheat production areas worldwide. Chemical control strategies are currently not cost-effective and limited resistance has been observed with in the cultivated wheat germplasm. Therefore, an alternative approach is required to manage this devastating disease. A negative regulator of programmed cell death from baculovirus, Op-IAP was introduced into wheat via Agrobacterium-mediated transformation. Expression of the Op-IAP gene was regulated by the maize ubiquitin promoter coupled with its first intron. This Op-IAP cassette was subcloned to the binary vector pPZP212 which harbors a npt II cassette under the control of the CaMV 35S promoter. The resultant binary plasmid is referred to as pPTN226. The binary vector was mobilized in to Agrobacterium strain C58C1 (pPM90) and the resultant transconjugant was used for wheat transformations. Immature embryos of wheat (cv Bobwhite) were inoculated following a 4-d preculture period. The wheat transformants were selected on 10 mg/L G418 for 2 weeks followed by an additional 8-12 weeks on 25 mg/L G418 selection pressure. Primary transformants were characterized via an ELISA assay for npt II expression and subsequently by Southern blot. A total of 20 independent events were generated. A transcript corresponding to the Op-IAP transgene was detected in a subset of the wheat lines via northern blot analysis in progeny of the transgenic wheat lines. Resistance to scab was monitored in the greenhouse by inoculating immature spikes with *F. graminearum* with a conidia spore suspension (70 X $10^3$ conidia/ml) using a needle to inject the conidial suspension between the palea and lemma in the center of the spike. Included in each of the scab inoculation tests were a susceptible and resistant check, Bobwhite and ND2710, respectively. Disease severity ratings were scored 19 days post inoculation and were tabulated as percentage of infected florets per head. Over three independent inoculations the susceptible check (Bobwhite) and resistant check (ND2710) scored an average disease severity ratings of 89.4% and 14.8%, respectively. From the 20 Bobwhite transgenic lines carrying the Op-IAP transgenes we have identified four events that have consistently displayed a reduction in disease severity ratings (app. 32 to 36%). Theses events are currently at the T4 or T5 generation and homozygous lines have been identified. Field trials are being planned for this summer and the lead lines are currently being crossed to winter wheat germplasm.

# APPLICATION OF A HIGH THROUGHPUT, LOW COST, NON-DENATURING POLYACRYLAMIDE GEL SYSTEM FOR WHEAT MICROSATELLITE MAPPING

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## INTRODUCTION

Microsatellite markers, one kind of tandem repetitive DNA sequence containing a 2-5 nucleotide motif, also referred to as simple sequence repeats (SSR), have turned out to be a valuable source of highly polymorphic DNA markers. Based on differences in the length of simple sequence repeats at loci defined by locus-specific PCR primers flanking the microsatellite, microsatellite markers have begun to supercede the RFLP or RAPD to construct linkage map for many different species, including human (Dib et al. 1996), mouse (Dietrich et al. 1996), rat (Serikawa et al. 1992; Jacob et al. 1995), dog (Mellersh et al. 1997), chicken (Groenen et al. 1998), and plants such as rice (Temnykh et al 2000), maize (Chin et al. 1996; Taramino and Tingey 1996), potato (Milbourne et al. 1998), wheat (Bryan et al. 1997, Röder et al,1998) and soybean (Akkaya et al. 1995; Cregan et al. 1999). For individual scientists, one of the largest barriers to applying microsatelites to any genetic problem is the cost and technical challenges presented by traditional means of electrophoresis. We anticipate that automated and ultra-high throughput capillary electrophoretic systems will be the primary means of genotyping in the future. Meanwhile, recent innovations inspired by P. Cregan, B. Diers, and D. Wang, have led to development of a low-technology, inexpensive, and relatively high-throughput gel system. Here, we report our experience to date with this system, which is produced by C.B.S Scientific Co. (619/ 755-4959) as Model # C-DASG-400-50 (Fig 1).

## MATERIALS AND METHODS

**Primers:** Wheat microsatellite primer pairs from P. Cregan at the Beltsville Agriculture Research Center of USDA-ARS are applied for linkage mapping using the ITMI (International Triticeae Mapping Initiative) population. The allele sizes of the parents of the population, M6 and Opata are shown for six primer pairs in Table 1.

**Table 1.** Length in base pairs of allele of six primer pairs for M6 and Opata, the parents of ITMI population.

| | Barc218 | Barc124 | Barc222 | Barc137 | Barc219 | Barc196 |
|---|---|---|---|---|---|---|
| Size at M6 | 210 | 216/206 | 182 | 250 | 208 | 145 |
| Size at Opata | 212 | 219/208 | 185 | 246 | 220 | 163 |

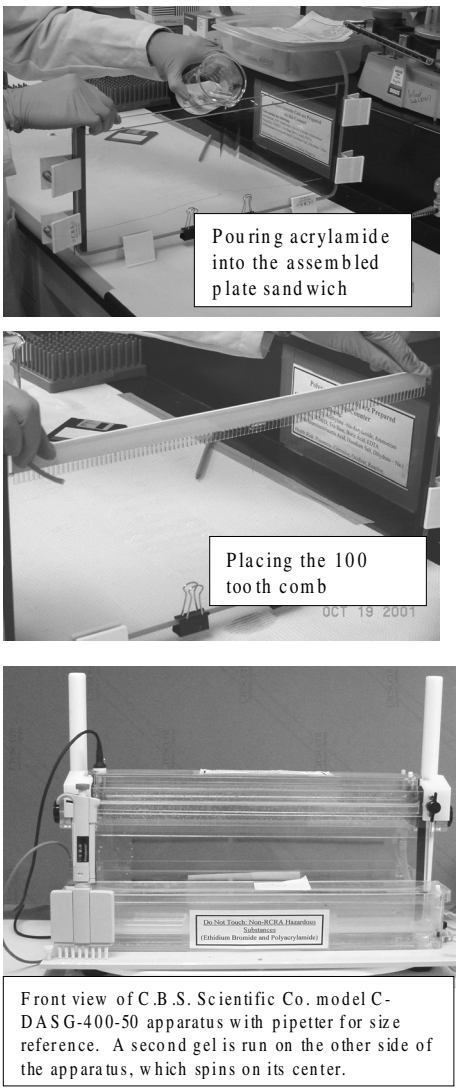


**Figure 1.** Images of the C.B.S. Scientific Co. two gel system.

**PCR reaction:** The amplification is done in a PTC-0220 DNA Engine Dyad Peltier Thermal Cycler, MJ research, using 15 ul reaction mixture, each containing 37.5 ng template DNA, 0.15 uM Primer, 1.5 mM MgCl, 0.15 mM each of dNTP and 1X PCR buffer and 2.0 U Taq polymerase. The PCR profile is followed: initial denaturation at 95 °C for 2 min; followed by 38 cycles of 94 °C denaturation for 25 sec, specified annealing temperature for 25 sec of annealing, 45 sec extension at 72 °C; after the final cycle there is a 10 min extension step at 72 °C, after which the temperature is held at 4 °C until removal from the thermal cycler.

**Plate sandwich:** The solidified non-denaturing polyacrylamide gel is created in a 'Plate Sandwich' consisting of two glass 49.0 cm wide. The back plate is UV transparent and rectangular in shape with a height of 21.5 cm. The outside edges of the front plate are the same height as the back plate. The front plate is notched so that the height between the edges is several centimeters less than that at the edges. A gel-wrap gasket is applied to the edges of the back plate. Side spacers (1.5 mm thick) are placed on both sides of the plate and then the notched front plate is set on top of the spacers (inside down) and aligned with

the back plate on each side of the plate. Two spring clamps (one on each round corner) and two medium binder clips (evenly spaced) are applied at the bottom of the plate sandwich so that the gasket forms a waterproof seal. All clamps are placed over the spacers and close to the gasket. The plate sandwich is then stood upright using the two spring clamps at the bottom for support.

Gel solution: 190 ml gel solution for 100-well gel is prepared by mixing the following solutions:

| | |
|---|---|
| 28.5ml | 40 % acrylamide solution (Final concentration in gel 6%) |
| 160.0ml | 0.5931x TBE buffer (Final concentration in gel 0.5 x) |
| 1.35ml | 10 % APS |
| 0.15ml | TEMED |

TEMED should be added right before the gel is poured.

**Gel pouring:** The gel solution is poured directly into the plate sandwich. If necessary, a thin (1 mm or thinner) plastic ruler or spacer can be used to remove any air bubbles. A 100 well comb is placed at the top of the plate sandwich between the two plates and two spring clamps (evenly spaced) are applied to hold the comb tightly against the back plate.

**Gel system setting:** After the gel has solidified (about one hour after pouring), the plate sandwich is set on a plate holder and all the clamps, binder clips and the gel-wrap gasket are removed. The plate sandwich is then placed in a vertical apparatus for eletrophoresis. The electrophoretic apparatus holds two gels, one on each side, and has a rotating base for easy access to both gels. The running buffer is (0.5x TBE) is added to both the upper and lower reservoirs. Prior to loading the gel, 50µl of Ethidium Bromide (10 mg/ml) is added to the lower reservoir, and the gel is warmed for one hour at 350 volts.

**Loading, Electrophoresis, and Visualization:** The comb is removed carefully and the samples are loaded with a multi-channel pipette with 8 or 12 tips. Loading volume can be up to 25ul. One gel can be used sequentially up to 6 times. Electrophoresis is carried out at 350 volts for 1- 2 hours. If necessary, a small fan (12 inch) may be used to cool the system. The plate sandwich is taken out and put horizontally on a UV box with the back plate (which is UV transparent) down. Note that the gel is NOT removed from the plate sandwich. Size limitations on our UV light box requires two photographs per 100-lane gel, one for the left half and the other for the right half of the gel. Digitized images of the photographs are stored on a computer.

## RESULTS

Figure 2 illustrates the resolution obtained with Barcs 196 and 222 after 1.5h electrophoresis. This result clearly demonstrates that allele size differences of 6 bp are readily distinguished with fragments in the 150 - 190bp size range. The same duration of electrophoresis (1.5h) did not, however, enable good resolution of allele sizes for Barc 218, where the ITMI parent alleles differ by only two bp (Fig. 3, top). That problem is overcome, though, with additional electrophoresis (Fig 3, bottom).

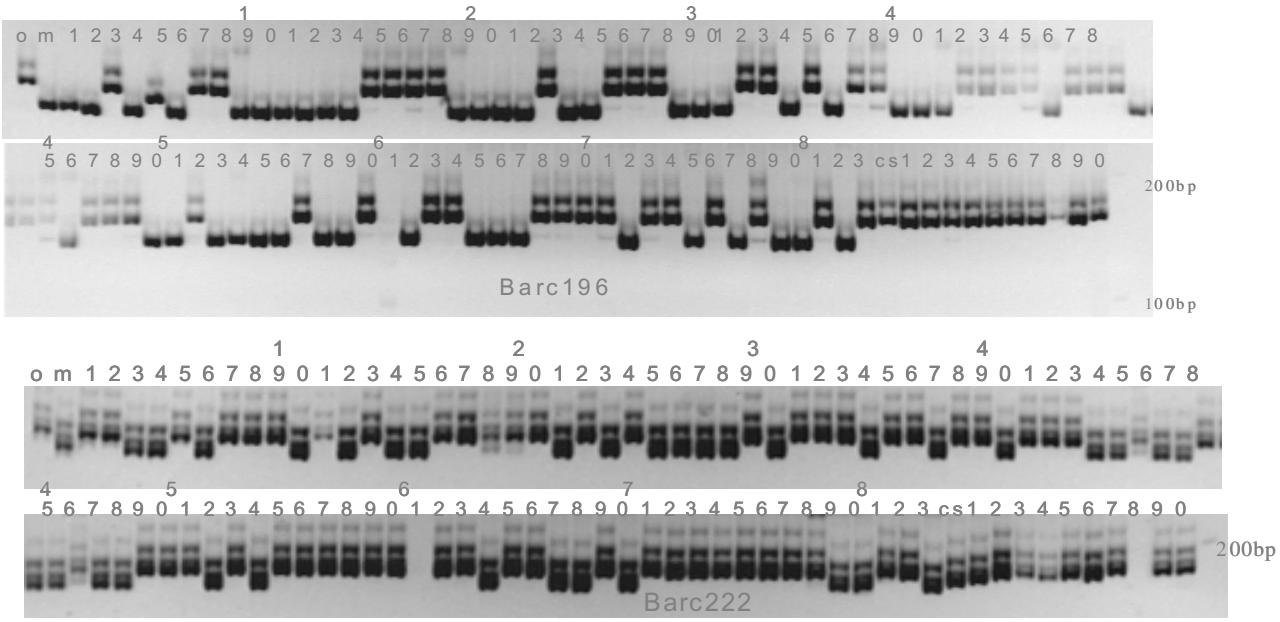


**Figure 2.** Negative images of two 100 lane gels with PCR products of Barc 196 (top), and Barc 222 (bottom) for the ITMI mapping population (o=Opata, m=M6, lanes 1-83 are progeny 1-60, and 61-84), and and 11 U.S. Wheat genotypes using the non-denaturing polyacrylamide gel system with UV exposure. Products from Barcs 196 and 222 were subjected to 1.5 hours of electrophoresis. (The allele sizes of ITMI population were 145 bp and 163 bp for Barc 196, and 174 bp and 180 bp for Barc 222)

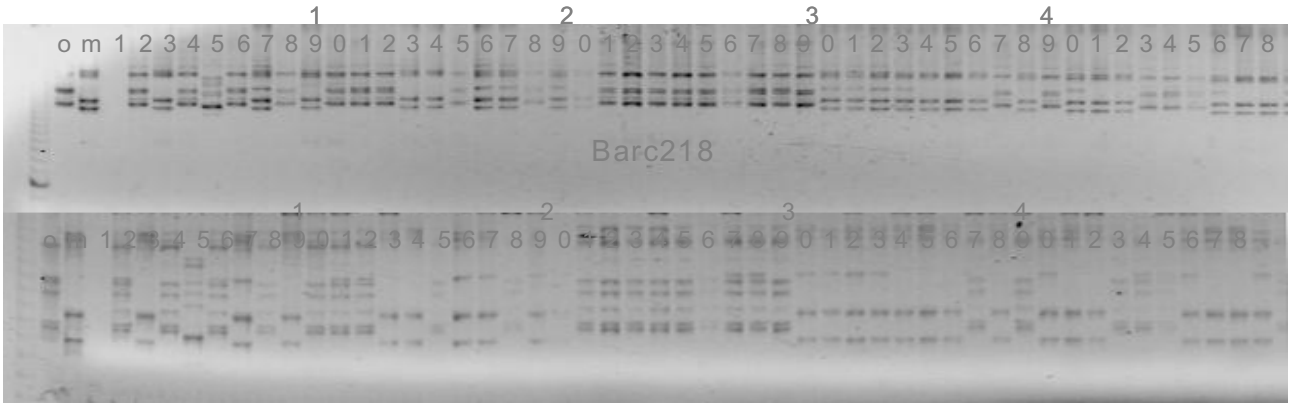


**Figure 3.** Negative mages of Barc 218 products electrophoresed for 1.5 (above) and 3.0 hours (below). The allles are 210 and 212 bp in length. Template genotypes are from from the ITMI mapping population (o=Opata, m=M6, lanes 1-48 are progeny 1-48).

Visualization with the UV light box does not involve disassembly of the plate sandwich, which permits the user to check resolution during a single run. Portability of the plate sandwich also enables re-loading of a completely new set of PCR products. We have successfully re-loaded the same plate sandwich with 6 distinct sets of PCR products, each time adding Ethidium Bromide to the lower reservoir. It is also possible to load a second set of PCR products either simultaneous to the first set (assuming no overlap in allele sizes), or after the first set is loaded but before it has moved off the gel. Figure 4 shows an image of the first 50 lanes of a single gel loaded at 1.5 hr intervals with PCR products amplified from wheat microsatellites Barc124 and Barc137. Barc124 amplifies two loci (124-1, alleles 206 bp and 208 bp; and 124-2, alleles 216 bp and 219 bp). The allele sizes of Barc137 from

Opata and M6 were 246bp and 250bp, respectively. The amplification products of Barc 124 were loaded first and Barc137 was loaded 1.5 hr later.

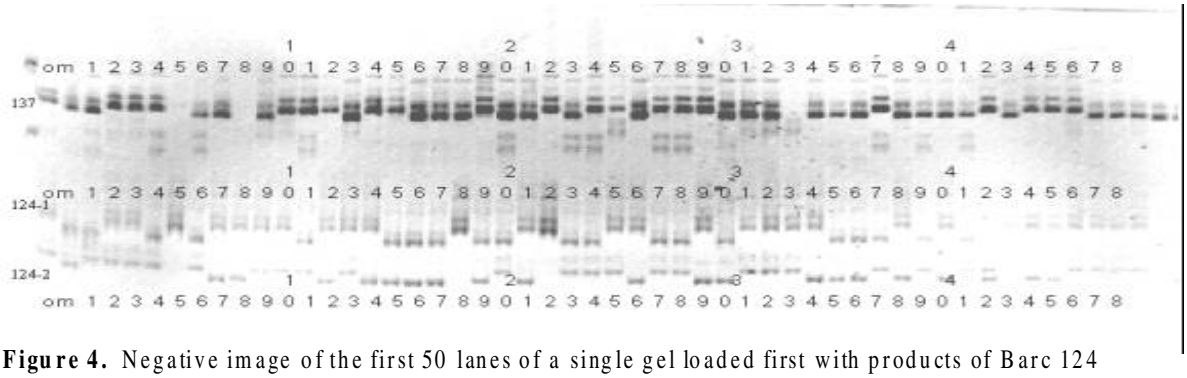


**Figure 4.** Negative image of the first 50 lanes of a single gel loaded first with products of Barc 124 followed 1.5h later with products of Barc 137. Lane layout of genotypes is identical to that for Fig.s 1 and 2.

## DISCUSSION

We have found the system described here to be extremely effective for microsatellite mapping work. At a minimum, we are able to run two sets of 100 PCR products on each gel, two times per day. Currently, we are mapping new BARC microsatellites. Since each apparatus mounts two gels, one lab worker can acquire microsatellite genotype information on eight (2 gels x 2 runs/day x 2 loadings per run) BARC primer pairs (usually 1 locus each) per day with each C.B.S. apparatus. In our experience, a skilled worker can manage two systems and therefore generate data on at least 16 microsatellite loci per day (note that PCR becomes the limiting step). Assuming only 96 lanes are used (because PCR plates have 96 wells), that amounts to at least 16*96= 1536 data points per day. Gels are never removed from the glass plate sandwich, eliminating one of the more challenging steps involved with manual polyacrylamide gels which require staining of some sort. We estimate the cost of a single gel (excluding PCR costs) to be $2.60, which compares very favorably with the costs associated with automated non-capillary sequencing gel costs. We do not currently have an automated system for data capture and rely on manual input of data acquired from the digital images.

In summary, we feel the system described here is a revolutionary development warranting serious consideration by any lab currently employing traditional polyacrylamide gels for microsatellite work. In the future automated capillary systems are likely to be the most cost effective approach to genotyping, but today, the system described here is very attractive.

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# DEVELOPMENT AND MAPPING OF MICROSATELLITE (SSR) MARKERS IN WHEAT

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## ABSTRACT

A total of 410 markers were developed from random genomic libraries, and of these, 279 were polymorphic among Chinese Spring, Opata and M6. One hundred and sixty-seven of these markers were polymorphic in the ITMI mapping population and 143 were positioned on the ITMI map, 156 were positioned using nulli-tetrasomic lines of Chinese Spring. A total of 67, 75 and 61 markers were mapped on A, B, and D genomes respectively. New microsatellites were integrated into a framework map consisting of previously published RFLP and microsatellite markers. A total of 43 of the new markers were positioned in gaps larger than 10cM.

## INTRODUCTION

Due to large genome size, high homoeology among A, B, and D genomes and high level of repetitive sequences in the genome of wheat, development of informative microsatellite markers in wheat is difficult and time consuming. Currently, approximately 350 publicly available wheat microsatellite primer sequences have been reported. This is a small number of markers relative to the huge genome size of wheat. The current ITMI map (http://wheat.pw.usda.gov/ggpages/ maps.html**)** contains a total of 1201 markers including RFLPs and SSRs. The total map length is approximately 3332 cM. There are 121 gaps of greater than 10cM. Although, there is on average one marker every 2.8cM, the likelihood that only one in three marker loci will be polymorphic in any given single cross dictates the need for much greater marker density. Clearly, development and mapping of more microsatellite markers in wheat, whose genome size is 37 times larger than that of rice, six times larger than that of corn and 15 times than that of soybean, should be important objectives of wheat geneticists.

The present paper reports the development of 269 microsatellite markers and their mapping position and also attempts to evaluate the consistency of mapping using segregation analysis versus physical mapping with cytogenetic stocks (nullitetrasomic, ditelosomic and deletion lines).

## MATERIALS AND METHODS

### Wheat library construction, screening, DNA sequencing and primer design:

For microsatellite isolation, various libraries were constructed. (1) Genomic DNA of Chinese Spring was either digested with combination of enzymes, or sheared using a nebulizer and then size selected on a 1% agarose gel. Sheared DNA was treated with mungbean exonuclease or T4 DNA polymerase to create blunt ends. DNA fragments in the 400 to 750 bp range were isolated from the gel using Geneclean II. Purified DNA fragments were ligated into the *SmaI* site of pBluescript. (2) The library was enriched based on the procedure described by (SONG *et al.* 2000; PULIDO *et al*. 1994). (3) The library was enriched for microsatellite by GIS.

Clone selection, rescreening, sequencing and primer design followed as described by Cregan et al (1994), Song et al (2001). To determine the level of polymorphism, primer sets were tested using genomic DNA of Chinese Spring; Opata 85 and M6. Opata 85 and M6 are the parents of the ITMI (International Triticae Mapping Initiative) mapping population.

### Mapping of markers using Opata 85x M6 IBL population:

The first 83 recombinant inbred lines of ITMI population were used for the segregating analysis. All published microsatellites and RFLP segregation, including 940 RFLP (NELSON *et al* 1995, VAN DEYNZE *et al* 1995) and 281 microsatellite markers (RODER *et al,*1998a), were collected, out of which 534 loci, which have an exact chromosome position, were constructed as a framework and retested for linkage (LOD = 3.0 or greater). All the new microsatellites were integrated into this framework.

### Physical mapping of markers:

Nulli-tetrasomic, and ditelosomic lines were used to assign microsatellite markers to their respective chromosome arms. Various numbers of single-break deletion lines on each chromosome were used for subarm localization of the markers (ENDO and GILL 1996).

## RESULTS

### SSR marker development and marker information

*Effect of different motifs (especially di- vs tri- and tetra-nucleotide motifs) on the level of polymorphism of markers.* Primers were designed to the sequences containing dinucleotide [CT/GA]n or [CA/GT]n, the tri-nucleotide motif [ATT/TAA], and the tetra-nucleotide [TAGA/ATCT]. The rate of polymorphic markers was 28%, 24%, 36% and 28% for [CT/GA]n, [CA/GT]n, [ATT/TAA]n and [TAGA/ATCT]n, respectively. Although the success rates of [CA/GT]n and [CT/GA]n were similar, [CT/GA]n is preferable to [CA/GT]n due to its higher frequency in the genome and higher average number of repeats in the repeat-containing sequences. Our previous work indicated that the [ATT/TAA]n motif was superior to all other trinucleotide repeats for the successful development of polymorphic microsatellite markers (Song *et al* 2001). Our current data strongly suggest that the [ATT/TAA]n motif is also superior to the two

most commonly used dinucleotide motifs and one tetra-nucleotide motif in terms of the rate with which polymorphic marker loci can be developed.

*Effect of length of repeat on the level of polymorphism of markers* As shown in Table 1, primers flanking higher numbers of repeats showed a higher probability of providing polymorphic markers.

*Functionality of primers* The percentages of primer sets that amplified the expected size product was 53%, 53%, 55%, and 56% for [CA/GT]n-, [CT/GA]n-, [ATT/TAA]n- and [TAGA/ATCT]n-containing fragments, respectively. A total of 410 markers was developed, among them, 11 were based on [CA/GT]n, 135 on [CT/GA]n, 204 on [ATT/TAA]n, 24 on [TAGA/ATCT]n and 36 contained other motifs. One hundred and sixty-seven of those amplified polymorphic products between Opata 85 and M6. The primer information for each marker can be accessible at: http://www.scabusa.org.

**Genetic mapping of SSR markers**

A total of 209 markers was mapped either using tetra-nullisomic and deletion stocks or using the 84 ITMI RILs (Table 2). Among them, ninety-eight markers were positioned on both maps, 66 and 53 were only positioned on the physical map or the ITMI map, respectively. Among the markers which had been placed on both maps, ninety were mapped on the same chromosomes by both methods, the order of 82 markers on both maps was consistent. There were 11 instances where inconsistencies wsere observed between positioning based upon the two mapping approaches. The inconsistencies were mainly confined to chromosome 5A (3 instances) and 5B (5 instances).

Forty-three of the newly developed markers were mapped in gaps greater than 10cM, nine were mapped to the ends of linkage maps. These markers extended current map length.

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**Table 1.** Effect of length of repeat on the level of marker polymorphism.

| | [CT/GA]n | | [CA/GT]n | | [ATT/TAA]n | | [TAGA/ATCT]n | |
|---|---|---|---|---|---|---|---|---|
| | Monomorphic | Polymorphic | Monomorphic | Polymorphic | Monomorphic | Polymorphic | Monomorphic | Polymorphic |
| 1-5 | | | | | 2 | | 3 | |
| 6-10 | 16 | 5 | 4 | 2 | 35 | 33 | 6 | 9 |
| 11-15 | 21 | 24 | 1 | 3 | 20 | 34 | 2 | 3 |
| 16-20 | 9 | 21 | | | 6 | 29 | | |
| 21-25 | 6 | 9 | | 1 | 9 | 14 | 1 | |
| 26-30 | 2 | 7 | | | 2 | 8 | | |
| 31-35 | 2 | 1 | | | | 3 | | |
| 40-45 | | 1 | | | | 2 | | |
| Ave. repeat length | 15.0 | 17.4 | 9.4 | 14 | 12.8 | 15.1 | 8.3 | 10 |

**Table 2.** Number of markers mapped on each chromosome

| Chromosome | Number of markers mapped on the same chromosome based on ITMI and cytological lines | Number of markers mapped based on cytological lines | Number of markers mapped based on ITMI lines | Total |
|---|---|---|---|---|
| 1A | 4 | 5 | 2 | 11 |
| 1B | 5 | 1 | 4 | 10 |
| 1D | 4 | | 5 | 9 |
| 2A | 3 | 1 | 0 | 4 |
| 2B | 6 | 5 | 3 | 14 |
| 2D | 1 | 1 | 4 | 6 |
| 3A | 5 | 5 | 2 | 12 |
| 3B | 4 | 5 | 3 | 12 |
| 3D | 2 | 2 | 1 | 5 |
| 4A | 6 | 2 | 3 | 10 |
| 4B | 2 | 3 | | 5 |
| 4D | 1 | 2 | 3 | 6 |
| 5A | 6 | 4 | 3 | 13 |
| 5B | 6 | 9 | 2 | 15 |
| 5D | 1 | 2 | 6 | 8 |
| 6A | 4 | 2 | 3 | 9 |
| 6B | 4 | 1 | 2 | 7 |
| 6D | 7 | 2 | 3 | 12 |
| 7A | 5 | 3 | | 8 |
| 7B | 6 | 4 | 2 | 12 |
| 7D | 8 | 3 | 2 | 15 |
| Total | 90 | 66 | 53 | 209 |

# PHYSICAL MAPPING OF MICROSATELLITE MARKERS ON WHEAT CHROMOSOMES

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## ABSTRACT

Microsatellite or simple sequence repeats (SSRs) consist of short DNA motifs, usually 1-6 nucleotide core elements that are repeated from two to several thousands times, have emerged as an important source of markers for molecular plant breeding. In wheat, SSRs are the marker of the choice due to their locus specificity, and co-dominant inheritance. Efforts are being made among three Institutes (ARS-USDA, Beltsville, KSU Manhattan, MSU Michigan,) through the U. S. Wheat and Barley Scab Initiative to develop a new set of microsatellite markers and map them on the ITMI mapping population and deletion stocks of wheat. In this study we analyzed 243 SSR markers on a set of deletion stocks of wheat chromosomes. Forty-four primer pairs, each amplified products of the same size from all the 21 stocks, suggesting their presence on more than one chromosome and no polymorphism among loci on homoeologous chromosomes. Two primer pairs did not amplify a PCR product and the remaining 197 primer pairs amplified a total 199-microsatellite loci, which could be assigned to all chromosome arms of seven groups (Table 1). Fifty-seven fragments were mapped to the A-genome, seventy-six to the B-genome and fifty-six to the D-genome. Of 197 primer pairs for which the corresponding loci could be assigned to a specific chromosome, 195 primer pairs each amplified only a single locus and two primer pairs amplified two loci on two different chromosomes. Thus, the primer pairs, amplifying a single locus showed high locus specificity and could be useful when chromosome specificity is desired. The two loci amplified by Barc204 and assigned to 6A and 6D chromosomes could be homoeologous loci. On other hand, the two loci amplified by primer pair Barcm70 and assigned to 2D and 4A may represent a case of interchromosomal duplication involving non-homoeologous chromosomes or a case of homoeologous segments transferred to non-homoeologous chromosomes. The markers assigned to specific chromosome bins at KSU along with their mapping in ITMI mapping population at MSU, will be useful for intra-chromosomal mapping, chromosome identification in aneuploid stocks, and targeted mapping of useful genes in wheat breeding. In addition to the microsatellite markers developed from genomic library at Beltsville, we have designed and synthesized 250 primer pairs from the sequences of wheat EST database containing SSRs. Standardizing the conditions for amplification of EST-SSRs and mapping them in wheat will be our focus in the future.

**Table 1.** Mapping of BARC and BARCM primers on wheat chromosomes

| Chromosome | Primer | |
|---|---|---|
| | Short arm | Long arm |
| 1A(10) | BARC09, BARC25, BARC28, BARC148, BARC176, BARCM48 | BARC17, BARC83, BARC158, BARCM22 |
| 1B(10) | BARC08, BARC137, BARC181 | BARC61, BARC80, BARC81, BARC174, BARC187, BARC188, BARCM15 |
| 1D(8) | BARC149, BARC152 | BARC62, BARC66, BARC99, BARC119, BARC169, BARCM42 |
| 2A(5) | BARC112, BARC124, BARC212 | BARC05, BARC15 |
| 2B(16) | BARC07, BARC13, BARC18, BARC35, BARC55, BARC91, BARC160, BARC200, BARCM24, BARCM72 | BARC16, BARC101, BARC128, BARC167, BARCM27, BARCM64 |
| 2D(5) | BARC95, BARC168, **BARCM70** | BARC159, BARC228 |
| 3A(11) | BARC12, BARC19, BARC45, BARC54, BARC67, BARC86 | BARC51, BARC57, BARC197, BARCM21, BARCM60 |
| 3B(13) | BARC68, BARC73, BARC75, BARC87, BARC102, BARC147, BARC156 | BARC77, BARC84, BARC139, BARC164, BARCM44, BARCM77 |
| 3D(4) | BARCM40 | BARC06, BARC42, BARC71 |
| 4A(9) | BARC206, BARCM52, **BARCM70** | BARC52, BARC78, BARC106, BARC153, BARC170, BARCM47 |
| 4B(5) | BARC20, BARCM01, BARCM45 | BARC60, BARC163 |
| 4D(5) | BARC98 | BARC27, BARC93, BARC225, BARCM69 |
| 5A(12) | BARC56, BARC117 | BARC01, BARC40, BARC92, BARC94, BARC100, BARC122, BARC141, BARC151, BARC165, BARC186 |
| 5B(13) | BARC04 | BARC11, BARC58, BARC59, BARC69, BARC74, BARC88, BARC89, BARC109, BARC140, BARC142, BARCM32, BARCM61 |
| 5D(9) | BARC130, BARC143, BARC205 | BARC44, BARC110, BARC133, BARC144, BARCM02, BARCM18 |
| 6A(9) | BARC03, BARC195 | BARC37, BARC107, BARC113, BARC171, **BARC204**, BARC104, BARCM55 |
| 6B(9) | BARC14, BARC48, BARC134, BARC198 | BARC24, BARC48, BARC79, BARC178, BARC180 |
| 6D(12) | BARC123, BARC173, BARC183, BARC196 | BARC21, BARC23, BARC96, BARC146, BARC175, BARC202, **BARC204**, BARCM30 |
| 7A(11) | BARC64, BARC70, BARC127, BARCM04, BARCM05, BARCM25, BARCM34 | BARC29, BARC49, BARC108, BARC121 |
| 7B(10) | BARC63, BARC72 | BARC32, BARC50, BARC65, BARC82, BARC85, BARC90, BARC182, BARCM73 |
| 7D(13) | BARC125, BARC126, BARC154, BARC214, BARCM33 | BARC26, BARC53, BARC76, BARC97, BARC111, BARC172, BARCM46, BARCM75 |

*Number given in parentheses after the chromosome indicate the total SSR markers mapped on particular chromosome and the primers mapped more than one chromosome is bolded.

# EXPRESSED SEQUENCE TAGS FROM DEVELOPMENTAL STAGES OF *GIBBERELLA ZEAE*

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## ABSTRACT

*Gibberella zeae* (anamorph: *Fusarium graminearum*) causes head scab, or head blight of wheat, barley, and oats, and foot and crown rot of corn. Recent scab outbreaks in Asia, Canada, Europe, South America and the United States highlight the increased threat this disease poses to food supplies worldwide. A better understanding of the biology of the scab organism is warranted to develop new control strategies. Our longterm goals are: (1) to understand the genetic basis for inoculum development, mycotoxin production and pathogenicity; (2) to use genomics to develop a biology-based control program for scab, using the genomics programs of wheat and corn to enhance this program. We have sequenced and analyzed a cohort of over 12,000 ESTs from 3 cDNA libraries representing different culture conditions and developmental stages. We will present the functional categorization of the genes in these libraries and a comparative analysis of gene expression.

# ASSESSING THE GENETIC DIVERSITY OF FUSARIUM HEAD BLIGHT RESISTANT SOURCES IN BARLEY

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## ABSTRACT

A number of barley varieties from around the world have been identified as potential sources of Fusarium head blight (FHB) resistance genes. All of these varieties exhibit partial resistance and, based on several mapping studies, it is known that resistance to FHB is controlled by multiple genes. To better exploit these resistant sources, we have initiated a study using simple sequence repeat (SSR) markers to determine the genetic diversity among important FHB resistant barley varieties that are being used in breeding programs and genetic mapping studies. To date, we have screened 10 resistant sources that are currently being used in mapping studies or breeding and six varieties with 59 SSR markers spanning the barley genome. SAHN cluster analysis using NTSYS-pc shows the resistant sources clustering into two groups that are 16% similar to one another. The first group consists of Atahualpa, Kitchin, Ac Oxbow, Chevron, Gobernadora, Frederickson, and Zhedar #1. Within this group Frederickson and Zhedar #1 are the most alike at 95% similarity while the others are less than 56% similarity. The second group consists of five susceptible varieties, and four resistant sources (MNBrite, CIho 4169, PFC88209, and Hor211). Hor211 is the most dissimilar line within this group with 21% similarity to the rest of the cluster. The four susceptible varieties developed in the Minnesota breeding program are greater than 80% similarity. These results show considerable differences in the relatedness of FHB resistant sources. These differences should be considered when choosing FHB-resistant sources for breeding efforts or new genetic studies. We are working to expand this SSR allele database to include more lines, both resistant and susceptible, by integrating several existing databases. In addition, we are currently incorporating 14 newly identified FHB resistant (B. Steffenson pers. comm.) accessions into the above data set.

# ESTS POSSIBLY RELATED TO VIRULENT OR AVIRULENT GENES OF *FUSARIUM GRAMINEARUM*

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## ABSTRACT

To understand molecular interaction of wheat (*Triticum aestivum*) and *F. graminearum* during the FHB development, FHB was induced on Sumai 3 (FHB-resistance) and Wheaton (FHB-susceptible) with spikelet-inoculation of isolate Fg4 or water (as control) in greenhouse. The inoculated florets were sampled in 0, 1, 16, 32 and 64 hours after inoculation. With DDRT-PCR technique, several ESTs were revealed to be expressed only in Fg4-inoculated Sumai 3, or expressed in both Sumai 3 and Wheaton but with different level of expression. We further confirmed that two of these ESTs are corresponding to the genes of *F. graminerum* with Southern and Northern analysis. These genes might be related to virulence or avirulence of the fungal. These ESTs were cloned and sequenced. (This poster was presented at 2001 ASA annual meeting.).

# QTLS MAPPING OF TYPE I AND TYPE II RESISTANCE TO FHB IN WHEAT

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## OBJECTIVES

To identify the number, the position, and the magnitude of QTLs for the resistance to initial infection (Type I) and the resistance to fungal spread within plant tissues (Type II) of Fusarium head blight in wheat.

## INTRODUCTION

Several types of the host resistance and tolerance to Fusarium head blight (FHB) have been described in wheat (Ban, 2000a, b): resistance to initial infection (Type I), resistance to fungal spread within plant tissues (Type II), resistance based on the ability to degrade the mycotoxin (Type III), tolerance to high mycotoxin concentrations (Type IV), and resistance to kernel infection (Type V). Of them, the Type I and Type II resistance are most important to screen the resistant varieties in breeding programs. The recently developed molecular techniques provide opportunities for a well understanding of the genetic mechanism of host resistance to FHB. However, previous studies for molecular mapping of resistance to FHB were mainly focused on Type II resistance since it is relatively easy to evaluate in greenhouse under controlled conditions (for a review, see Kolb et al., 2001). Only Type II resistance is not enough to prevent the FHB damages under sever epidemics. In order to increase the comprehensive resistance level to FHB, different resistance types to FHB should be pyramided in the improved varieties. In this study, we evaluated both Type I and Type II resistance for a population of double haploid lines (DHLs) and analyzed the QTLs for the two resistance types. The obtained result may give us a better understanding for the mechanism of resistance to FHB and help us to make a reasonable breeding strategy for resistance to FHB.

## MATERIALS AND METHODS

Plant materials - a segregating population of 118 DHLs developed from the $F_1$ cross of Sumai 3 (Chinese resistant culrivar) and Gamenya (highly susceptible cultivar from Australia) with the wheat x maize system (Suenaga and Nakajima, 1989).

FHB resistance evaluation - the phenotype of Type I resistance were evaluated in the field condition with a sprinkler system in 1995 and 1996 (Ban and Suenaga, 2000). After the spraying of the macroconida suspension of *F. graminearum* 'G87-36B' in the flowering stage, the severity of disease on spikes for each DHL was scored. The phenotype of Type II resistance was evaluated in green house in 2000 with two replications. The inoculum, which concentration of macroconidia was adjusted to about $5\times10^5$/ml, was injected into a central

spikelet of spikes when they were just beginning to flowering. Inoculated plants were placed in plastic chamber, which were maintained at 22-25 °C with 100% relative humidity for three days. After 21 days of inoculation, the disease severity of each line was scored based on the average value of diseased spikletes percentage over 5 spikes.

Molecular marker analysis – Ninety nine of WMS markers developed by Röder et al. (1998) that showed polymorphism between Sumai 3 and Gamenya separated on 4% Metophor agarose gel were applied to construct the linkage map with AFLP, RFLP and RAPD markers. For AFLP analysis, genomic DNA was digested with *Eco*RI and *Mse*I. A total of 30 primer combinations were used for selective amplification. The *Eco*RI side primers were labeled with florescent dyes (6-FAM, HEX, or NED), then PCR products were analyzed by an ABI 373 sequencer with Genescan 3.1 software (Perkin Elmer/Applied Biosystems). The name of each polymorphic band was assigned by the selective bases of primer combination used to amply it followed by the fragment size.

QTL analysis - Linkage map construction and QTL analysis were performed by using Map Manager QTX (http://mapmgr.roswellpark.org) software. Chromosomes assignment of the linkage maps were determined based on the SSR maps of wheat (Röder et al., 1998; Pestsova et al., 1999).

## RESULTS AND DISSCUSSION

Two hundred and thirty nine AFLP markers, together with 99 SSR, 21 RFLP, 21 RAPD markers, and one morphological marker (awnedness controlled by *B1* locus), formed 34 linkage groups covering a total genetic distance of 3739.3 cM (P=0.0001). The SSR markers assigned their chromosomes excepting chromosome 3A, 6A, and 4D.

Three genomic regions including five, one and two markers on chromosome 5AL, 5BS, and 2DS, respectively, were significantly associated with Type I resistance (Table 1). The QTLs on 5AL for Type I resistance further support our previous result that one resistance gene in Sumai 3 that was linked to *B1* locus located on 5AL (Ban and Suenaga, 2000). Two genomic regions on 3BS and 2DS assigned by three markers, respectively, were significantly associated with Type II resistance. The QTLs on 3BS were consistently detected in the populations including Sumai 3 or their derivatives in several studies by using RFLP, AFLP, and SSR markers (Kolb et al., 2001). The QTL on 2DS were associated with both Type I and Type II resistance. These results suggested that the genetic constitution for Type I and Type II resistance types is not identical. A strategy by pyramiding of resistance genes for different resistance types into an adapted background may be an effective way for FHB resistance breeding in wheat.

The QTL on chromosome 2DS showed negative effect on both Type I and Type II resistance to FHB and this negative effect was contributed by Sumai 3. Yao et al. (1997) reported that one susceptible gene in Sumai 3 located on chromosome 2D. Our study further mapped this susceptible locus originated from Sumai 3 on the chromosome 2DS. Sumai 3 is the most used resistance resource for FHB resistance in wheat breeding around the world. However, it usefulness has been hindered by its too many bad agronomic characters. The present study indicated that the Sumai 3 not only contain resistance genes but also include suscep-

tible genes for FHB. When Sumai 3 was used as a resistance resource in a breeding program, the susceptible gene should be excluded.

**Table 1.** Putative QTLs associated with Type I and Type II resistance to FHB in a doubled haploid population developed from the $F_1$ cross of Sumai 3 and Gamenya.

| Type of resistance | Putative QTLs | Marker[a] | Chr. | *P* value | LOD | V (%)[b] | Add[c] | Resistant source |
|---|---|---|---|---|---|---|---|---|
| Type I | QTL-1 | Xgwm410-5A | 5AL | < 0.001 | 2.9 | 10 | 8.22 | Sumai #3 |
| | QTL-1 | AAC/CATA221 | 5AL | < 0.001 | 3.4 | 12 | 8.78 | Sumai #3 |
| | QTL-1 | AAC/CATA416 | 5AL | < 0.001 | 4.5 | 15 | 10.01 | Sumai #3 |
| | QTL-1 | AAG/CGAC500 | 5AL | < 0.001 | 3.5 | 17 | 10.89 | Sumai #3 |
| | QTL-1 | *B1* (awn suppressor) | 5AL | < 0.001 | 5.9 | 20 | 11.24 | Sumai #3 |
| | QTL-2 | ACG/CAAC103 | 5BS | < 0.001 | 3.1 | 11 | 8.31 | Sumai #3 |
| | QTL-3 | Xgwm261 | 2DS | < 0.001 | 4.3 | 15 | -9.69 | Gamenya |
| | QTL-3 | AAG/CAGT368 | 2DS | < 0.001 | 3.8 | 14 | -9.33 | Gamenya |
| Type II | QTL-4 | Xgwm533a | 3BS | < 0.001 | 2.3 | 9 | 2.65 | Sumai #3 |
| | QTL-4 | AAG/CGAC186 | 3BS | < 0.001 | 3.1 | 12 | 8.40 | Sumai #3 |
| | QTL-4 | AGC/CACT88 | 3BS | < 0.001 | 2.5 | 10 | 7.53 | Sumai #3 |
| | QTL-3 | Xgwm296-2D | 2DS | < 0.001 | 2.5 | 10 | -7.60 | Gamenya |
| | QTL-3 | Xgwm261 | 2DS | < 0.001 | 4.2 | 17 | -9.48 | Gamenya |
| | QTL-3 | AAG/CAGT368 | 2DS | < 0.001 | 3.4 | 14 | -8.76 | Gamenya |

[a]: SSR markers are indicated by Xgwm code and *B1* is a morphorogical marker.
[b]: The amount of the total trait variance which would be explained by a QTL at this locus.
[c]: The additive regression coefficient for the association.

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# USING THE MAIZE *AC-DS* SYSTEM TO OBTAIN MARKER-FREE TRANSGENIC BARLEY PLANTS THAT STABLY EXPRESS PUTATIVE ANTIFUNGAL PROTEINS

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## ABSTRACT

Fusarium head blight (FHB) has been reported worldwide, especially in the upper Midwestern US. Infected barlely grain, contaminated by deoxynivalenol (DON), is not acceptable for malting and brewing. Introduction of recombinant antifungal proteins into barley offers the potential to suppress pathogen infection and growth. Transformation technologies were developed for the highly regenerable cultivar Golden Promise (Wan and Lemaux, 1994) and subsequently improved upon (Cho et al., 1998; Bregitzer et al., 2000). Despite these successes several hurdles exist to the use of these technologies to produce commercially acceptable germplasm. Existing methods result in the presence of selectable marker genes and plasmid sequences in the transgenic plants and introduced transgenes frequently become silenced during generation advance. To exploit potentially useful aspects of the maize *Ac/Ds* system, including the propensity of transposed sequences to reinsert into transcriptionally active chromatin, Koprek *et al.* (2001) transferred the essential parts of this system via transformation into barley. They discovered that transposase-mediated transposition of *Ds-bar* resulted in stablized expression of *bar*-mediated herbicide resistance. For example, transgene expression in $F_2$ plants with a single copy of a transposed *Ds-bar* was stable in 100% of the plants, whereas only 23% of $F_2$ plants carrying a single nontransposed copy of *Ds*-bar had stable expression of the transgene product (Koprek *et al*., 2001). We are using the *Ac-Ds* system to produce large numbers of transgenic plants containing independent insertions of genes encoding putative antifungal proteins, which will be unlinked to selectable marker genes and other plasmid sequences and which are stably expressed. The putative antifungal genes, *tlp1* (thaumatin-like protein) and *tlp4* from oat and two of the trichothecene pathway genes, *TRI101* and *TRI12*, isolated from *Fusarium sporotrichioides*, were put into a *Ds*-bordered, maize *ubiquitin*- or rice *actin* promoter-driven expression cassette. The resultant *tlp* constructs, together with pAHC20 (*ubiquitin* promoter-*bar-nos*), were introduced via bombardment into scutellar cells of immature embryos of two spring cultivars of barley, Golden Promise, a 2-rowed variety, and Drummond, an elite 6-rowed variety. The selection of putative transgenic lines is ongoing. To assist in characterizing the level of transgene expression, antibodies to the *tlp* proteins are being developed. Genes for TLP 1 and TLP 4 were inserted in vector pGEX and proteins were purified for antibody preparation. Bombardments with *TRI101* and *TRI12* genes will be initiated in the near future. In addition, *Ac*-transposase under control of maize *ubiquitin* or its own putative *Ac* promoter are being introduced into Drummond, both by direct transformation and by backcrossing from Golden Promise lines.

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# IDENTIFICATION (BASED ON MEMBRANE FATTY ACID METHYL ESTER ANALYSIS AND PARTIAL SEQUENCING OF 16S RIBOSOMAL RNA) OF BACTERIAL STRAINS USED IN THE BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT

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## ABSTRACT

Our laboratory has been working for the last several years with bacterial strains (designated as 1B-A, 1B-C, 1B-E, and 1D-3) isolated from South Dakota wheat foliage and residue which are able to antagonize *Fusarium graminearum* in laboratory plate assays and in field plot trials. Although we have known for many years that the bacterial strains are endospore formers that are able to grow aerobically, likely being members of the genus *Bacillus*, the exact identity of the strains has remained problematic. Systematics of the genus *Bacillus* have undergone great changes since modern methods for bacterial identification, such as analysis of membrane fatty acid methyl esters (FAME analysis) and analysis of small subunit 16S ribosomal RNA sequences have become available. Analysis of FAME patterns of the four strains done about six years ago suggested that bacterial strain 1D-3 was almost certainly *Bacillus amyloliquefaciens*, whereas the other three strains were not extremely similar to any bacteria in the FAME database, but were related to a degree to *Bacillus atrophaeus* (formerly *B. subtilis* variety *niger*). This year another laboratory did FAME analyses on the four strains. Results strongly indicated that strains 1B-A and 1D-3 were *Bacillus lentimorbus*, and that strains 1B-E and 1B-C were *Bacillus subtilis*. In addition, the first 500 base pairs of the 16S rRNA gene of each strain were sequenced and compared to known sequences of ribosomal RNA genes, and alignment profiles and phylogenetic trees were derived from the sequences. Usually knowing the first 500 base pairs of the 16S rRNA gene is sufficient to determine the identity of most bacterial strains. All four strains (1B-A, 1B-C, 1B-E, and 1D-3) had identical sequences in the first 500 base pairs of their 16S RNA genes, and all were most closely related to *Bacillus amyloliquefaciens* and had less but significant relatedness to *Bacillus atrophaeus*. This and other studies have found that FAME analysis will not necessarily agree with the results of 16S rRNA sequencing. Extent and completeness of the database used in each taxonomic analysis is extremely important when attempting to identify a bacterial strain, and in some cases (such as this study) further standard physiologic tests will be needed to help make a confident identification of the bacterial strains. Complete 16S RNA sequences of the strains would be very valuable in helping to determine whether the strains truly belong to known bacterial species, or are one or more new species in the genus *Bacillus*. In addition, detailed physiologic tests will be conducted to help further evaluate the relatedness of these bacterial strains to known bacterial species. Thorough understanding of the enzymatic activities of these strains will help optimize their formulation and application as biological control agents used to control FHB in the field.

# BIODIVERSITY OF MICROBIAL ANTAGONISTS TO *GIBBERELLA ZEAE* IN BRAZIL

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## OBJECTIVES

To determine the diversity of microbial antagonistic agents to *Gibberella zeae.*

## INTRODUCTION

Knowledge of biodiversity of living organisms is mainly important to determine their potential functions. Fusarium head blight (FHB), induced by *Gibberella zeae* (anamorph = *Fusarium graminearum* Schw.) is a prevalent wheat disease in Brazil, causing crop production losses varying from 10% (Luz, 1984); to 54 % (Picinini & Fernandes, 1994). Due to the difficulty in controlling the disease by chemical treatment, crop rotation and varietal resistance, biological control agents are beeing evaluated as an additional tactics for integrated management of FHB. To have an idea of potential bioprotectants, biodiversity of promining isolates need to be established.

## MATERIALS AND METHODS

Thousands of microorganisms were screened in vitro and in vivo against *G. zeae.* Biodiversity of most effective microbial strains was established by systematic determination using physiological, biochemical and morphological features as well as systems such as Biolog, GC-Fame (analysis of fat acids) and comparison of the sequences of the small subunits of the RNA 16S (for bacterial strains), comparing 500 base pairs.

## RESULTS AND DISCUSSION

The diversity of microorganisms that shows potential for managing FHB in Brazil comprises 15 different species as presented in Table 1. Searching different isolates of these microorganisms with different degree of control efficiency may be of valuable interest to investigators in other countries as well as in Brazil. This microbiota may have an important impact in inproving FHB control either alone or as an additional measure to supplement chemical, cultural and resistance control methods.

**Table 1.** Biodiversity of microorganisms for biocontrol of Fusarium head blight Wheat in Brazil[1]

| |
|---|
| *Bacillus licheniformans* |
| *Bacillus lentimorbus* |
| *Baccillus megaterium* |
| *Bacillus pumilus* |
| *Bacillus subtilis* |
| *Clavibacter michiganense insiduosum* |
| *Enterobacter cloacae* |
| *Klebsiella planticola* |
| *Kluyvera cryocrescens,* |
| *Paenibacillus macerans* |
| *Pantoea agglomerans* |
| *Pseudomonas putida* |
| *Pseudomonas fluorescens* |
| *Sporobolomyces roseus* |
| *Rhodotorula* sp. |

[1] Identifications of bacterial isolates by Microbe Inotech Laboratories, Inc. Saint Louis, Mo, USA, based on GC-FAME, Biolog, and/or 16Sr RNA sequence. Bacterial colony morphology, physiological tests, and morphological indentification of fungi by Plant Pathology Laboratory, Embrapa Trigo, Passo Fundo, RS, Brazil.

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# GREENHOUSE SCREENING OF BIOLOGICAL CONTROL AGENTS FOR SUPPRESSION OF FUSARIUM HEAD BLIGHT

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## ABSTRACT

In order to better understand the potential application of biological control agents for the suppression of Fusarium head blight (FHB), several bacterial isolates were selected that had shown evidence of biological activity against *Fusarium graminearum* in culture. These agents were selected and screened in two consecutive greenhouse trials in 2001. Oxen hard red spring wheat was planted in four replications of twenty plants each in a greenhouse ground bed for the first trial and in cone-tainers for the second trial. Treatments included untreated/uninoculated (negative control), untreated/inoculated (positive control), a chemical control standard (Folicur at 4 fl oz/A), and four biological control agents (SDSU-1BA, SDSU-1BC, TrigoCor 1448, and Trigo Cor 9790). Ten heads per treatment were exposed to the biocontrol agents and the agent was allowed to dry. The heads were then challenged with a $10^6$ CFU/ml of *Fusarium graminearum* conidia about 12 hours later. The plants were incubated in a humid chamber, with mist applied for ten minutes each hour to maintain nearly 100% relative humidity for a period of two weeks. Three weeks after inoculation, the treated heads were evaluated for FHB. In the first study, FHB damage was fairly high, about 20% incidence and 37% head severity in the untreated/inoculated check. Only Folicur significantly reduced FHB incidence, head severity, and disease index (incidence X severity) in this trial. In the second study, a lower density of challenge inoculum ($10^4$ CFU/ml of *Fusarium graminearum* conidia) was used to avoid excessively high disease severity. Incidence and severity of FHB were reduced from levels observed in the first study, about 4% incidence and 28% head severity in the untreated/inoculated check. However, no treatments significantly reduced any measurement of FHB in this trial. Additionally, a background level of FHB escaped to the negative control, making it more difficult to differentiate treatments. However, numeric differences were observed that indicate there may be cause for further study. Two biological treatments, SDSU-1BA and TrigoCor 1448 reduced disease index by 73% and 75% respectively. While this may seem unimportant due to the lack of statistical validity, the important point is that the Folicur treatment only reduced disease by 85% in the same study. A similar relationship was noted in field studies with SDSU-1BA under low disease pressure in 2000. As such, further study will be conducted on these and other potential biological control agents.

# EFFECT OF FOLIAR FUNGICIDES AND BIOCONTROL AGENTS ON FUSARIUM HEAD BLIGHT DEVELOPMENT AND CONTROL IN OHIO

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## OBJECTIVES

i) to evaluate the effect of three foliar fungicides (Folicur, AMS21619, and BAS505) and two biocontrol agents (TrigoCor 1448, and USDA/Peoria-OH182.9) against Fusarium head blight,

ii) to determine the relationships between the disease, DON and yield, and iii) to document the effect of these materials on scab development.

## INTRODUCTION

Fusarium head blight (FHB) or scab, caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*) is a major disease in many wheat and barely production regions of North America 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 Raxil-Thiram, were planted using 24 seeds/ft of row on 11 Oct., 2000 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 May 14. Plots were misted each day from one week prior to flowering to two week after flowering, using NAAN 7110 series bridge with mist sprayer head 327122 fitted with nozzles having 0.35 in. opening. Biological agents and fungicides were applied as sprays in 26.2 gal. water/A with a CO- pressurized back pack sprayer with a constant boom pressure of 40 psi and 15 in. between twinjet XR8001 nozzles mounted at a 60 degree angle forward and backward. Sprays were applied at flowering (30 May). Disease assessments were made twice a week (June 11 - June 26) for both incidence and severity in one ft. of row at 15 locations in each plot. Plots were harvested on 17 of July. 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 fungicide and biological control treatments tested (Fig. 1). Based on the coefficient of determination (R), evaluation of the residual plots, the 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 ranged from 83 to 93%). 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 per day based on disease incidence, and from 0.093 to 0.172 per day for disease severity (Table 1). Area under the disease progress curve based on disease incidence (AUDPCI) ranged from 418.0 to 804.2 and from 125.1 to 307.5 when based on disease severity (AUDPCS) (Table 1). Final disease incidence for the various treatments ranged from 55.0 to 89.6% and final disease severity ranged from 23.9 to 57.9% (Table 2).

Plots treated with Folicur at 6.0 fl oz/A, AMS21619 or BAS 505 had significantly lower rates of disease increase and AUDPCI values than the untreated control (Table 1). Only plots treated with AMS21619 and BAS 505 fungicides had both significantly lower rates of disease progress and AUDPCS values than the untreated control plots (Table 1). Additionally, only plots treated with AMS21619 and BAS 505 had significantly lower final disease incidence and severity than the untreated control (Table 2).

Plots treated with AMS21619, BAS 505 and the BAS 505 plus Folicur had significantly higher yield than the untreated plots. However, of the plots treated with these fungicides only grain from the AMS21619 and the BAS 505 treated plots had significantly lower DON levels than grain from the untreated control plots. Although the biocontrol agent USDA 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.

There were positive correlations between DON and final disease severity, AUDPCI, AUDPCS with correlation coefficient (r) of 0.79, 0.68, and 0.69 respectively. On the other hand, there were negative correlations between yield and final disease severity, AUDPCI, and AUDPCS with correlation coefficient (r) of 0.63, 0.54, and 0.55 respectively.

In conclusion, the treatments exhibited different effect on Fusarium head blight development and control. Treatments AMS21619 and BAS505 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 USDA/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 BAS505 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 for Fusarium head blight incidence (AUDPCI) and severity (AUDPCS) for fungicides and biocontrol agents tested in Ohio, in 2001.

| Treatment and rate/A | Incidence | | | Severity | | |
|---|---|---|---|---|---|---|
| | Model Fits | Rate | AUDPCI | Model Fits | Rate | AUDPCS |
| 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 |
| Folicur 3.6 EC 6.0 fl oz+ Induce (0.125%, v/v) | Gompertz | 0.158* | 592.1* | Gompertz | 0.119* | 192.6 |
| AMS21619 480SC 5.7 fl oz +Induce (0.125%,v/v) | Gompertz | 0.138* | 418.0* | Gompertz | 0.093* | 125.1* |
| BAS 505 50G 3.1 oz | Gompertz | 0.143* | 469.2* | Gompertz | 0.117* | 159.4* |
| BAS 505 50G 3.1 oz Folicur (3.6 EC 2.0 fl oz) | Gompertz | 0.166 | 647.9 | Gompertz | 0.148 | 254.7 |
| TrigoCor 1448 | Gompertz | 0.231 | 798.4 | Gompertz | 0.169 | 315.7 |
| USDA/ OH182.9 | Gompertz | 0.229 | 804.2 | Gompertz | 0.172 | 307.5 |

*Indicates means significantly different ($p \geq 0.05$) from untreated control based on LSD.

**Table 2.** Mean final disease of Fusarium head blight, yield, and DON content of grain for fungicides and biocontrol agents tested in Ohio in 2001.

| Treatment and rate/A | Mean Final Disease | | Yield (bu/A) | DON (ppm) |
|---|---|---|---|---|
| | Incidence (%) | Severity (%) | | |
| Control | 82.5 | 50.9 | 62.3 | 16.0 |
| Folicur 3.6 EC 4.0 fl oz+ Induce (0.125%, v/v) | 75.8 | 41.5 | 66.6 | 12.0 |
| Foliur 3.6 EC 6.0 fl oz+ Induce (0.125%, v/v) | 69.7 | 35.3 | 66.8 | 14.6 |
| AMS21619 480SC 5.7 fl oz +Induce (0.125%,v/v) | 55.0* | 23.9* | 74.0* | 7.2* |
| BAS 505 50G 3.1 oz | 60.1* | 28.6* | 77.1* | 8.4* |
| BAS 505 50G 3.1 oz<br>Folicur 3.6 EC 2.0 fl oz | 73.0 | 41.6 | 70.2* | 14.9 |
| TrigoCor 1448 | 89.6 | 57.9 | 56.0 | 24.0* |
| USDA/OH182.9 | 87.5 | 51.8 | 62.0 | 10.7* |

* Indicates means significantly different ($p \geq 0.05$) from untreated control.

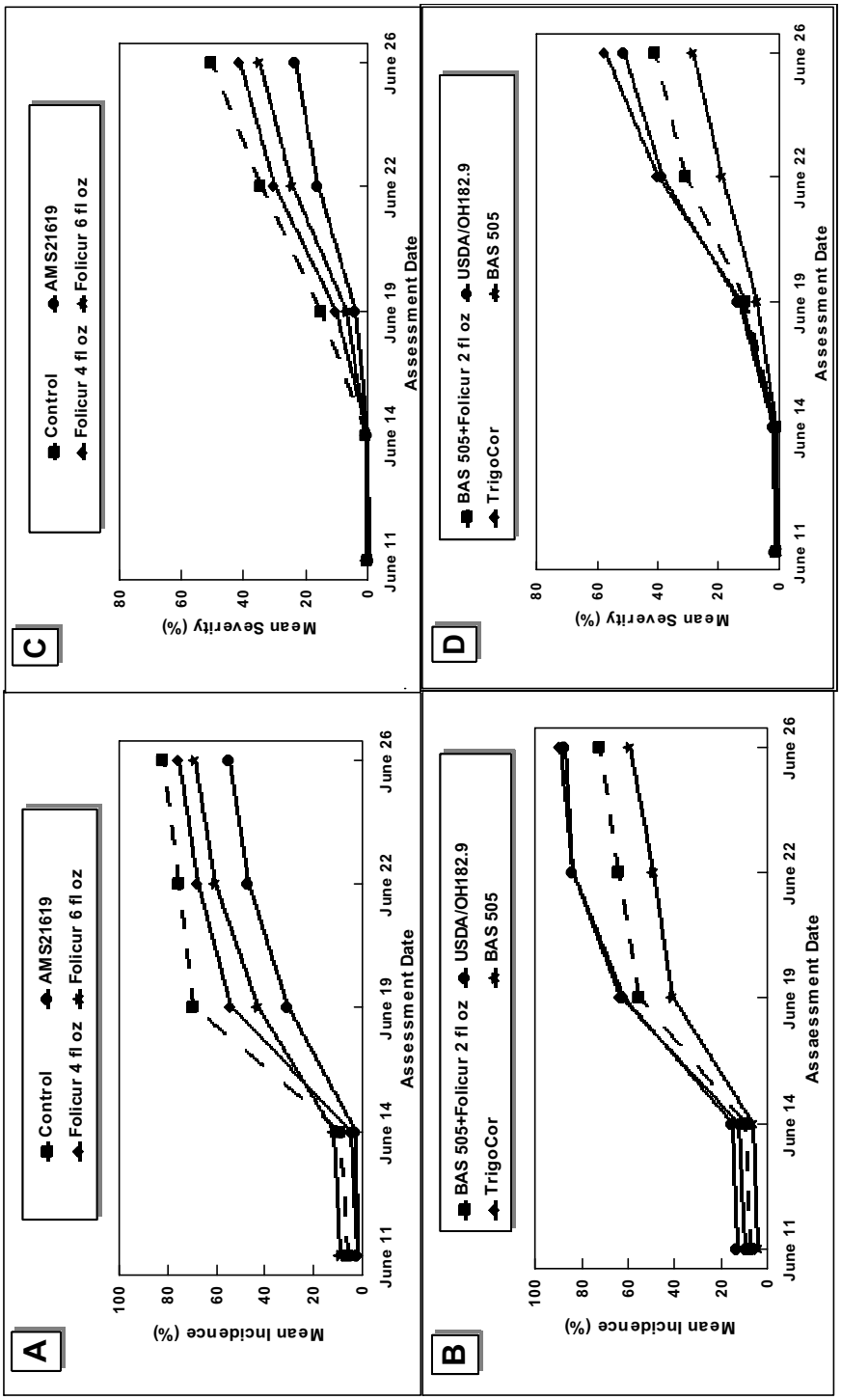


**Fig. 1** Disease progress curves for Fusarium head blight incidence (A, B) and severity (C, D) for fungicides and biocontrol agents tested in Ohio in 2001.

# UNIFORM FUNGICIDE TRIAL COLLABORATIVE STUDY 2001– MICHIGAN STATE UNIVERSITY

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## OBJECTIVE

Evaluate efficacy of fungicides on reducing incidence, severity and deoxynivalenol (DON) on Fusarium head blight (FHB) in winter wheat.

## INTRODUCTION

Fungicides were evaluated for their potential to reduce the incidence and severity of Fusarium head blight (FHB) in winter wheat, and concomitantly for a reduction in levels of deoxynivalenol (DON, vomitoxin). This project was part of an ongoing collaborative study among states participating in the FHB Initiative (Milus and McMullen, 2000; McMullen et al, 1999; and Hart, et al 1999).

## METHODS AND MATERIALS

Winter wheat varieties, Frankenmuth, Harus, and Freedom were planted in October 2000. Fertilizer and herbicides were applied as per Michigan State University recommendations. Corn inoculum infested with *Gibberella zeae* was applied on May 17th and again on June 4th. Low volume overhead irrigation was started on May 24th. Irrigation was on for 15 minutes and off for ninety minutes, 24 hours/day until June 16th. The irrigation was turned off for 24 hrs the days fungicides were applied. Fungicides were applied at Feekes growth stage 10.5 to Harus and Freedon on May 31st, and to Frankenmuth on June 7th. Fungicides were applied with a $CO_2$ backpack sprayer and flat fan nozzles directed at a sixty-degree angle above the horizontal. Each treatment was replicated four times, and replicated plot was 15 by 30 feet. Plots were rated on June 27th. Because the disease incidence was always 100 percent, the disease severity was used to calculate differences among treatments. Normally the incidence is multiplied by the severity to get a disease index value (DI). Incidence is the number of heads in a plot with symptoms, and severity is the percent of spikelets infected. Mature grain was harvested, weighed, milled and analyzed for DON by ELISA (Hart, et al, 1998).

### Treatments:

1. Untreated
2. Folicur 4 fl oz + 0.125% v/v Induce
3. AMS 21619 at 5.7 fl oz/A + 0.125% v/v Induce
4. BAS 505 0.2 lbs ai/acre + 0.125% Induce
5. BAS 505 0.1 lb ai/acre + Folicur 2 fl oz + 0.125% v/v Induce

6. Cornell biological agent
7. USDA/Peoria biological agent

A second project evaluating a prototype sprayer was held at the Michigan Bean and Beet Farm. Folicur was applied at GS 10.5 (June 8th) on the variety Harus using either a conventional boom sprayer using 25 gal of water/acre with flat fan nozzles directed at a sixty-degree angle above the horizontal; or the prototype sprayer using 5 gal of water/acre. Treatment 2 above, 4 oz of folicur + 0.125% Induce, was the only fungicide applied. Each plot was 75 x 525 feet, and the center 30 feet x 525 was harvested on July 16th. The treatment were; 1) wheat was sprayed from two sides with the prototype to ensure complete coverage of the head with fungicide; 2) wheat was sprayed on only one side with the prototype sprayer resulting in incomplete coverage; 3) Conventional flat fan sprayer with nozzles aimed downward; and 4) Untreated controls. There was only one replication per treatment. Twenty-five grain probes per treatment were collected directly from the combine at harvest. Each probe sample was analyzed was analyzed separately for DON (Hart, et al, 1998). The plots were not rated for yield or disease severity.

## RESULTS AND DISCUSSION

**Uniform Fungicide Trial.** FHB developed late in 2001, toward the end of flowering (Figure 1). Frankenmuth headed and flowered 7-10 days later than Harus or Freedom.

Heading and flowering were occurred later compared to previous years, and the flowering was longer, probably due to the cool temperature during flowering (Figure 1). FHB incidence was one hundred percent in all the plots, and severity was moderate (Table 1). Several treatments significantly reduced the severity of FHB, but did not significantly affect yield or DON. The rain and temperature data suggest that favorable infection periods probably occurred only toward the end of flowering, and may account for the limited affect of fungicide treatments on yield and DON (Figure 1; Hart, et al 1984).

**Saginaw Trial.** Treatments were not evaluated for FHB incidence, severity or yield. DON levels in the different treatments wer:

| Treatment | DON (PPM) |
|---|---|
| 1 | 0.3 |
| 2 | 0.9 |
| 3 | 0.9 |
| 4 | 0.9 |

Although these results are preliminary and not replicated, they do suggest that thorough coverage of the wheat head is essential to reduce DON, and new technologies using very low spray volumes may compete very will with conventional sprayers. The conventional sprayer used here would not have provided coverage for both sides of the wheat head.

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**Table 1.** Comparison of fungicide efficacy on FHB incidence, severity, yield, and DON in Uniform Fungicide Trials at Michigan State University.

| Treatment # | Incidence | Mean Severity | Mean Yield | DON (ppm) |
|---|---|---|---|---|
| 1 | 100 | 31.1a | 70.3a | 1.2a |
| 2 | 100 | 22.5ab | 74.8a | 1.2a |
| 3 | 100 | 19.7b | 72.4a | 0.8a |
| 4 | 100 | 21.3b | 72.7a | 1.1a |
| 5 | 100 | 20.6b | 72.3a | 1.2a |
| 6 | 100 | 29.0ab | 65.0a | 1.3a |
| 7 | 100 | 26.7ab | 70.0a | 1.2a |

**Individual Varieties**

**Frankenmuth**

| Treatment # | Incidence | Mean Severity | Mean Yield | |
|---|---|---|---|---|
| 1 | 100 | 20.3ab | 60.7a | 0.8a |
| 2 | 100 | 18.3ab | 61.6a | 0.5b |
| 3 | 100 | 18.8ab | 51.7a | 0.5b |
| 4 | 100 | 12.0a | 51.7a | 0.6a |
| 5 | 100 | 14.5a | 52.9a | 0.7a |
| 6 | 100 | 19.3ab | 48.0a | 0.9a |
| 7 | 100 | 27.5b | 50.9a | 0.7a |

**Freedom**

| Treatment # | Incidence | Mean Severity | Mean Yield | |
|---|---|---|---|---|
| 1 | 100 | 45.0a | 71.2a | 1.2a |
| 2 | 100 | 30.0b | 84.4a | 1.2a |
| 3 | 100 | 22.0b | 81.4a | 0.6b |
| 4 | 100 | 33.5ab | 84.7a | 1.0a |
| 5 | 100 | 30.0b | 78.2a | 0.9a |
| 6 | 100 | 34.8ab | 70.5a | 1.1a |
| 7 | 100 | 31.5ab | 62.1a | 1.1a |

**Harus**

| Treatment # | Incidence | Mean Severity | Mean Severity | |
|---|---|---|---|---|
| 1 | 100 | 28.0a | 79.6a | 1.7a |
| 2 | 100 | 19.3b | 83.2a | 1.8a |
| 3 | 100 | 18.3b | 88.1a | 1.3a |
| 4 | 100 | 18.3b | 87.8a | 1.5a |
| 5 | 100 | 17.3b | 87.4a | 1.9a |
| 6 | 100 | 33.0a | 77.9a | 2.0a |
| 7 | 100 | 21.0b | 79.6a | 1.7a |

**Overall Variety Comparison**

| Variety | Mean Yield | Mean Severity | DON |
|---|---|---|---|
| Frankenmuth | 53.9a | 18.6a | 0.7a |
| Freedom | 78.9b | 32.4b | 1.0b |
| Harus | 83.2b | 22.1b | 1.7c |

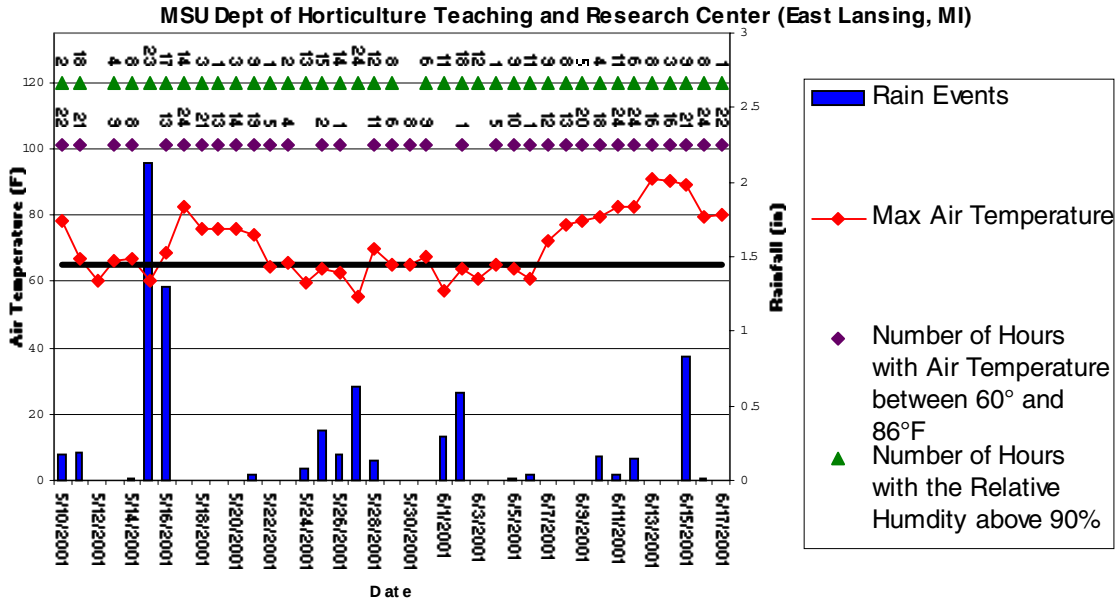


**Figure 1.** Rain fall and temperature patterns at the MSU Horticulture Farm between May 11th and June 17th, 2001. Flowering occurred between May 25th and June 15th. Temperature above 60°F coinciding with three or more days of rain and flowering may be favorable for infection of wheat by *Gibberella zeae*.

# MANAGEMENT OF FUSARIUM HEAD BLIGHT IN WHEAT USING SELECTED BIOLOGICAL CONTROL AGENTS AND FOLIAR FUNGICIDES, 2001

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## OBJECTIVES

To evaluate selected foliar fungicides and biological control agents for potential use in soft red winter wheat Fusarium head blight management programs in Kentucky. Also, to generate data as a cooperator in the 2001National Fusarium head blight Uniform Fungicide and Biocontrol Test.

## INTRODUCTION

Fusarium head blight (FHB) of wheat and barley is a significant disease concern in all wheat and barley producing regions of the United States. Statewide, epidemics in Kentucky are rare, but each year some fields are severely damaged by FHB. 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 FBH will occur under conditions that favor FHB. Preliminary studies conducted in various states indicate that foliar fungicides (Milus and McMullen, 2000) and biological control agents (BCA's) (Schisler et al, 2000) 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 program was established as a means of addressing this deficiency in data. This test involves cooperators at various test 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 2001, also evaluate unique treatments of interest locally.

## 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. Specific local treatments were also evaluated. Treatments included a variety of foliar fungicides and two BCA's. The test site was planted in a conventionally-tilled seed bed on October 18, 2000 and 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 'Clark'; maize was the previous crop grown in the test site.

Plots were inoculated on April 1, 2001 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 7pm and 9 pm. Following the onset of flowering, plots were mist-irrigated twice daily from 5-7am and 7-9pm. Fungicides were applied to plots on May 7, 2001 when the crop was in the early flowering. Treatments were applied using a $CO^2$-propelled plot 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 at the Michigan State University Don Testing Laboratory. Tests for standard germination, percent dead seed, and percent seed infected by Fusaria were conducted at Dr. TeKrony's seed technology laboratory in Lexington, KY. Percent visually scabby kernels (VSK) was determined by segregating healthy from scabby kernels for two sets of 100-seed samples for each treatment replication.

## RESULTS AND DISCUSSION

Overall conditions of the test favored moderate crop yield and significant, but not excessive, FHB pressure. At the first rating date (late milk wheat stage), all treatments except Folicur alone had significantly lower disease incidence than the non-treated check (Table 1). Disease severity and field severity, however, were similar among treatments. By the soft dough stage eight days later, the following treatments had significantly lower disease incidence ratings compared to check plots: Folicur 4.0 fl oz/A, AMS 12619 5.7 fl oz/A, BAS 505 0.2 lb a.i./A, and Tilt 4 fl oz/A plus Quadris 4.11 fl oz/A. Of these treatments, none had significantly lower severity ratings and only the treatments involving AMS 12619 and BAS 505 alone had significantly lower field severity ratings. The only significant yield difference was with the AMS 12619 treatment. In contrast, test weight values were significantly higher than the check for treatments involving Folicur alone, AMS 12619, BAS (0.1 lb. a.i.) + Folicur (2 fl oz) and BAS 500 alone. None of the treatments resulted in significantly reduced percent Fusaria as determined by culturing fungi from surface-sterilized seed (Table 2); lack of significance appeared to be the result of significant variability between treatment replications treatments. Regarding percent VSK, only AMS 12619 and BAS 505 alone resulted in values significantly below the check. AMS 12619, BAS 505 + Folicur, BAS 505 alone, and the Cornell BCA (TrigoCor 1448) each significantly reduced ppm of DON compared to the check. Standard germination of harvested seed was significantly greater than the check for treatments involving AMS 12619 and BAS 505 alone. Number of dead seed was statistically similar between all treatments.

No fungicide or BCA reduced FHB severity at either rating date. This is consistent with previous studies (McMullen et al, 1999). In our study, AMS 12619 (5.7 fl. oz) + induce (0.125% v/v) was the only treatment that resulted in a significant yield advantage when compared with the check. Foliar and other head diseases were not a factor in this test, so this yield result was apparently directly related to partial control of FBH. Several foliar fungicide treatments, including AMS 12619, suppressed FHB to a moderate extent, reduced

DON levels in grain and minimized test weight losses when compared with the check. No treatment provided excellent control of FHB. Seed quality, as indicated by standard germination and percent VSK, was maintained at higher levels in treatments involving AMS 12619 and BAS 500 alone. Other treatments, including both BCA's, had no positive effect on any of the seed quality parameters measured. Overall, the treatment involving AMS 12619 was the most consistent and effective performer across all parameters measured. In contrast, Folicur performed very poorly in this study. Specifically, there was only a slight reduction in FHB incidence (at the early but not late rating date, Table 1) and a higher test weight when compared with the check; other measurements were statistically similar to the check. This is an interesting finding considering that Folicur is usually among the most efficacious fungicides for managing FHB (E. Milus, *personal communication*; McMullin et al, 1999). The two BCA's studied where ineffective across all data sets. The one exception was a significant reduction in DON for the Cornell University BCA, TrigoCor 1448. Similarly, three treatments involving Tilt performed very poorly in the test, with the exception of a significant reduction in FHB incidence when Tilt (4.0 fl oz/A) was mixed with Quadris (4.11 fl oz/A).

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**Table 1**. Effect of various fungicides and BCA's on FHB, yield and test weight.

| | Disease Ratings* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | May 22 | | | May 30 | | | | Tst Wt |
| **Treatment and rate/A** | **Inc** | **Sev** | **Fld Sev** | **Inc** | **Sev** | **Fld Sev** | **Bu/A**** | |
| Non-treated | 19.2 | 12.9 | 2.4 | 43.3 | 43.4 | 19.0 | 61.3 | 55.6 |
| Folicur: 4.0 fl oz + Induce 0.125% v/v | 14.3 | 10.4 | 1.5 | 37.1 | 45.6 | 16.9 | 61.6 | 56.4 |
| AMS 12619 5.7 fl oz + Induce 0.125% v/v | 7.3 | 9.3 | 0.7 | 30.3 | 41.3 | 12.4 | 67.7 | 57.2 |
| BAS 505: 0.1 lb a.i.+ Folicur: 2.0 fl oz + Induce 0.125% v/v | 7.5 | 15.3 | 0.8 | 37.4 | 52.5 | 19.6 | 63.7 | 56.6 |
| BAS 505 0.2 lb a.i. + Induce 0.125% v/v | 6.1 | 24.4 | 1.3 | 24.8 | 48.5 | 10.8 | 66.1 | 57.2 |
| Cornell BCA (TrigoCor 1448) | 8.8 | 13.9 | 1.1 | 38.8 | 52.1 | 20.1 | 60.4 | 55.9 |
| USDA BCA (OH 182.9) | 11.9 | 14.3 | 1.7 | 39.5 | 55.5 | 22.0 | 63.2 | 55.7 |
| Tilt 4 fl oz + Induce 0.125% v/v | 9.1 | 11.3 | 1.1 | 42.4 | 57.8 | 24.4 | 60.9 | 55.5 |
| Tilt 4 fl oz + Quadris 3.42 fl oz + Induce 0.125% v/v | 12.8 | 9.7 | 1.3 | 43.6 | 61.5 | 26.2 | 60.4 | 55.7 |
| Tilt 4 fl oz + Quadris 4.11 fl oz + Induce 0.125% v/v | 8.5 | 18.1 | 1.5 | 31.6 | 59.9 | 16.8 | 65.1 | 56.2 |
| **LSD P=0.05** | **6.1** | **NS** | **NS** | **5.9** | **6.5** | **5.3** | **6.1** | **0.7** |

*Inc = Incidence; Sev = Severity; Fld. Sev = Field Severity. All ratings are based on 100 heads collected and rated at late milk (May 22) and soft dough (May 30) stages. ** Based on 13% moisture and 60lb/bu test weight.

**Table 2**. Effect of various fungicides and BCA's on FHB on various seed quality parameters.

| Treatment and rate/A | % Fusaria | VSK* | DON (ppm) | Std** Germ | No. Dead Seed |
|---|---|---|---|---|---|
| Non-treated | 38.0 | 25.8 | 5.7 | 75.2 | 18.0 |
| Folicur: 4.0 fl oz + Induce 0.125% v/v | 39.5 | 23.8 | 4.6 | 76.4 | 17.3 |
| AMS 12619 5.7 fl oz + Induce 0.125% v/v | 24.8 | 14.8 | 1.7 | 84.1 | 10.4 |
| BAS 505: 0.1 lb a.i.+ Folicur: 2.0 fl oz + Induce 0.125% v/v | 33.2 | 20.0 | 3.8 | 77.0 | 15.6 |
| BAS 505 0.2 lb a.i. + Induce 0.125% v/v | 26.4 | 14.4 | 3.4 | 82.5 | 12.7 |
| Cornell BCA | 42.0 | 24.8 | 3.5 | 80.3 | 14.8 |
| USDA BCA | 34.4 | 23.6 | 4.7 | 76.1 | 16.4 |
| Tilt 4 fl oz Induce 0.125% v/v | 33.2 | 26.8 | 5.7 | 77.0 | 16.3 |
| Tilt 4 fl oz + Quadris 3.42 fl oz + Induce 0.125% v/v | 41.2 | 27.2 | 6.1 | 73.5 | 18.9 |
| Tilt 4 fl oz + Quadris 4.11 fl oz + Induce 0.125% v/v | 28.8 | 21.0 | 5.5 | 74.4 | 18.7 |
| **LSD P=0.05** | **NS** | **7.9** | **1.8** | **6.2** | **NS** |

* Visually Scabby Kernels: 100 seed per plot were examined twice for scabby kernels and the average was used.
** Percent of seed germinated.

# POTENTIAL FOR BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT BY *LYSOBACTER SP*. STRAIN C3

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## ABSTRACT

Control of Fusarium head blight (FHB) remains a challenge for wheat and barley producers. Host resistance and fungicides provide only partial control of infection and are not very effective in reducing production of deoxynivalenol in seed. Biological control is being explored as another strategy for disease management. *Lysobacter sp.* strain C3 (previously reported as *Stenotrophomonas maltophilia*) is a bacterial agent that is active through chitinolysis and induced resistance. In previous field studies it exhibited efficacy against a number of turfgrass diseases and rust in common bean. C3 also was effective in greenhouse tests in controlling spot blotch (*Bipolaris sorokiniana*) and leaf rust (*Puccinia triticina*) on wheat. Application of chitin broth cultures of C3 provided the highest level of disease control; the culture fluid contained high levels of lytic enzymes, which aided in pathogen suppression, and supplied nutrients for colonization of the bacterium on the plant surface. The culture fluid also was found to elicit induced resistance in turfgrasses. Our objectives in this study were to evaluate, under greenhouse conditions, the potential for using C3 to control FHB and to determine the parameters for application of C3 in future field trials. C3 cell suspensions and whole C3 chitin broth cultures (7 days-old) were applied to 'Bobwhite' wheat heads at anthesis. Plants were then held overnight in 90-100 % relative humidity, inoculated with a sprayed suspension of *Fusarium graminearum* conidia, and then held in high humidity for another 48 hours. C3 cells suspended in distilled water exhibited little control of FHB. Treatments with C3 chitin broth cultures, on the other hand, effectively reduced the severity of FHB and were consistent between experiments. When applied to wheat heads 1 day prior to *Fusarium graminearum* inoculation, C3 treatments reduced the percent of infected spikelets to less than 10 %, whereas the controls typically exhibited greater than 50% infected spikelets. A 1:125 dilution of the whole chitin broth culture was as effective as a full strength application. FHB was controlled with a ½ strength broth culture of C3 even when the treatment was applied 7 days prior to pathogen inoculation. The results suggest there is potential for using C3 chitin broth cultures to control FHB in the field. Experiments are currently underway to test whether FHB control by C3 is due to antagonism or induced resistance. Future experiments will involve testing C3 broth cultures for efficacy in the field with spring and winter wheat cultivars.

# FURTHER STUDIES ON THE EFFECTS OF TIMING OF APPLICATION AND OF ADJUVANTS ON FUNGICIDE CONTROL OF FHB

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## ABSTRACT

Application techniques that will improve fungicidal control of Fusarium head blight in spring wheat, durum wheat and barley are needed. Early flowering (Feekes growth stage 10.51) has been defined by our research group as the optimum timing for a single fungicide application in spring wheat and durum. However, questions have arisen on whether multiple or split applications of fungicide, from early head emergence through kernel watery ripe stage, would provide better control than this single application. A greenhouse experiment was designed to test efficacy of single applications of the full label rate (4 fl oz) vs multiple applications of split rates of Folicur fungicide to durum wheat. Application timings tested were: 50% head emergence (Feekes 10.3); full head emergence prior to flowering (Feekes 10.5); early flowering (Feekes 10.51); and/or kernel watery ripe stage (Feekes 10.54). FHB disease was achieved by atomizing spores (5000/ml) of *Fusarium graminearum* onto the durum heads at Feekes 10.51. Results indicated that the greatest reduction of FHB severity was with a 2 fl oz/acre rate of Folicur applied at Feekes 10.5 followed by a second 2 fl oz application at Feekes 10.51. The second greatest reduction in FHB occurred with a split application of 1 fl oz at Feekes 10.3, followed by 2 fl oz at Feekes 10.51, and then 1 fl oz at Feekes 10.54. The smallest reduction of FHB occurred when 4 fl oz of Folicur were applied once at Feekes 10.54. In addition to studying timing of application to improve fungicide efficacy, use of various adjuvants has been examined. Field experiments in 2000 showed that some adjuvants used in conjunction with Folicur or Tilt fungicide for FHB control resulted in greater reduction in FHB than others. Further adjuvant tests in the greenhouse in 2001 showed that the addition of some experimental humectant adjuvants resulted in slightly improved control of FHB in spring wheat than did Induce or Silwet adjuvants when added to Folicur fungicide, while just the opposite results occurred when the same adjuvants were added to Tilt fungicide.

# UNIFORM BARLEY FUNGICIDE TRIALS IN NORTH DAKOTA, 2001

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## ABSTRACT

A uniform set of five fungicide treatments was evaluated on Robust barley in ND in 2001 for control of Fusarium head blight (FHB) and fungal leaf diseases. Treatments were tested in replicated plots at Fargo and Langdon. Artificial inoculum in the form of inoculated grain was dispersed in plots at both locations. Natural rainfall was augmented by mist irrigation at Langdon, but not at Fargo. All treatments were applied in 15-20 gpa at early full head emergence (Feekes 10.5) with a $CO_2$ backpack type sprayer equipped with XR8001 nozzles mounted at a 60 degree angle forward and backward toward the grain head. Treatments included Folicur (tebuconazole) fungicide, AMS 21619 (an experimental from Bayer, Corp.), BAS 505 (an experimental from BASF), a combination of BAS 505 + Folicur), and Caramba (metconazole; not registered in the US). Disease ratings were taken at soft dough stage of kernel development. Plots were harvested with small plot combines. Plots were in a RCB design with four replicates, and data were statistically analyzed across locations using ANOVA. Disease development at both locations was relatively low compared to recent years, with FHB field severity in the untreated plots averaging 6.7% at Fargo and 8.9% at Langdon. All fungicide treatments significantly reduced FHB incidence, head severity, and field severity from the untreated check, but differences among fungicide treatments were not significant. The AMS fungicide gave the greatest reduction (70.5%) in FHB field severity. DON values were less than 0.5 ppm at Fargo for all treatments, and were not yet available from Langdon at the time of this report. Leaf diseases, primarily net blotch and *Septoria passerinii*, were reduced by all fungicide treatments, but not significantly. Yield was increased by 1-6.5 bu/acre by fungicide treatments, but differences were not statistically significant. Test weights were increased by 0.6-2.7 lb/bu, but differences were not statistically significant.

# ND UNIFORM WHEAT FUNGICIDE AND BIOCONTROL TRIALS, 2001

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## OBJECTIVE

To evaluate fungicides and biological agents for control of Fusarium head blight (scab) and leaf diseases in spring wheat and durum wheat.

## INTRODUCTION

North Dakota wheat producers continue to be very interested in having effective fungicides or biological agents that will substantially reduce Fusarium head blight (FHB) severity and DON vomitoxin) levels, will reduce leaf diseases,and will increase yields. Although a recent North Dakota release of a resistant hard red spring wheat cultivar (Alsen) may reduce the need for fungicides in those spring wheat fields, acreages of susceptible spring wheat cultivars are still planted in North Dakota and durum cultivars grown are still quite susceptible to the disease. In recent years, data from the Uniform fungicide trials in North Dakota and other states have shown that, of the registered or near-registration, available fungicides, Folicur (tebuconazole) has more consistently provided better FHB control and gave greater reduction of DON than other fungicides or biologicals tested (Jones 2000, McMullen, et al. 1999, Milus and McMullen 2000). This information has been used to help obtain section 18 emergency exemptions for use of Folicur in North Dakota in recent years. Although Folicur has been consistent over several years and locations, some experimental fungicides and biologicals may provide even better control, be more cost-effective, and/or more environmentally safe. Uniform fungicide trials across North Dakota, a state with a consistent recent history of this disease, provide additional information on the performance of various products against FHB.

## MATERIALS AND METHODS

A uniform set of four fungicide treatments and a biological agent were evaluated on wheat in ND in 2001 for control of Fusarium head blight (FHB) and fungal leaf diseases. Treatments were tested across five locations and across three spring wheat and two durum wheat cultivars: Oxen hard red spring wheat at Fargo; Grandin hard red spring wheat at Langdon and at Minot; Monroe durum at Garrison; Russ hard red spring wheat at Carrington; and Munich durum at Carrington. Artificial inoculum in the form of inoculated grain was dispersed in plots at Fargo and Langdon, while infection was solely from natural sources at Minot, Garrison and Carrington. Natural rainfall was augmented by mist irrigation at Fargo and Langdon, and by 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 degree angle forward and backward, in 15-20 gpa. Treatments were applied either in the early morning hours, prior to 9 am, or in the late afternoon or early evening

hours. Treatments included Folicur (tebuconazole) fungicide, AMS 21619 (an experimental from Bayer, Inc.), BAS 505 (experimental from BASF), a combination of BAS 505 + Folicur), and a biological agent OH182.9 (developed by the USDA in Peoria, in conjunction with Ohio State University). An additional biological agent developed by Cornell University, was tested at the Fargo location, but data is not presented here.

Disease ratings were taken at soft dough stage of kernel development. Plots were harvested with small plot combines. Plots were in a RCB design with four replicates, and data were statistically analyzed across locations using ANOVA.

## RESULTS AND DISCUSSION

Disease levels varied substantially among locations; untreated durum plots at Carrington had the highest FHB field severity (42.1%), while spring wheat plots at Fargo had the lowest (5.7% untreated). Continuous rainfall and high humidities occurred at the Minot, Garrison, Carrington, and Langdon locations, beginning July 11, coinciding with flowering periods of the crops. At Fargo, the wheat crop flowered the first week of July during a hot, dry spell, and measurable rainfall in July did not begin until July 17.

All fungicide treatments significantly reduced FHB. DON levels were reduced, but not significantly, by all fungicide treatments, with the AMS product resulting in the lowest DON. All fungicide treatments also significantly reduced % flag leaf disease, predominately tan spot and Septoria/Stagnosopora leaf spots. All fungicide treatments significantly increased yield over the untreated check, and three fungicide treatments significantly improved test weight. The AMS product and the BAS 505 product look promising for further evaluation. The biological agent did not significantly improve disease control, yield or test weight. In some locations, the biological agent was applied in the early morning hours, instead of in the evening, and UV radiation may have inhibited some activity of the organism.

Results of fungicide and biocontrol tests on spring wheat and durum across ND locations, 2001

| Treatment* | % FHB incidence | % FHB head sev. | % FHB Field Sev. | DON ppm**** | % flag leaf disease*** | Yield bu/A | TWT. lbs/bu |
|---|---|---|---|---|---|---|---|
| Untreated | 64.9 | 17.9 | 13.0 | 5.1 | 58.9 | 43.2 | 55.6 |
| Folicur 4 fl oz | 42.8 | 9.6 | 4.1 | 3.4 | 32.1 | 54.2 | 57.1 |
| AMS 21619 5.7 fl oz | 42.9 | 8.4 | 3.6 | 1.4 | 20.0 | 56.1 | 58.7 |
| BAS 505 6.4 fl oz | 44.2 | 9.0 | 3.9 | 2.8 | 34.0 | 53.9 | 58.5 |
| BAS 505 3.2 fl oz + Folicur 4 fl oz | 41.4 | 8.6 | 3.5 | 2.7 | 29.9 | 55.6 | 57.9 |
| USDA biological OH182.9 | 56.3 | 15.4 | 10.1 | 5.8 | 54.1 | 44.7 | 55.8 |
| LSD P = 0.05 | 13.2 | 7.2 | 8.8 | NS | 18.3 | 8.0 | 1.7 |

*All fungicide treatments had 0.125% Induce added;

** DON (vomitoxin) levels were not available from Langdon at the time of report

*** Flag leaf disease primarily tan spot and Septoria/ Stagonospora leaf spots

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# FUNGICIDE CONTROL OF FUSARIUM HEAD BLIGHT IN WHEAT

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## OBJECTIVES

To understand betted the fungicide effect by the analysis of the influence of cultivar resistance and isolate aggressiveness and longevity of fungicide effect.

## INTRODUCTION

As the majority of cultivars is susceptible to FHB, in epidemic situations the fungicides may help to lessen the damages and toxin contamination. However, their use is often insufficient and the causes would be important to know better (Anon. 1993, Caron 1990, McMullen et al. 1997, Mauler-Machnik and Zahn 1994, Mauler–Machnik and Suty 1997). Wilcoxson (1996) agrees and lists several reasons for this from the less effective fungicides, methodical problems and application deficiencies. He represents the view that a fungicide treatment is effective when the visual grain infection (FDK) will be lower than 5 %. As agronomy gives only moderate results, especially under very favorable epidemic conditions the last hope can be the use of fungicides.

## MATERIALS AND METHODS

In the methodology we introduced the artificial inoculation of group of heads known from resistance tests (Mesterházy 1995, Mesterházy et al. 1999) that allowed testing the fungicide activity in our system on two or three cultivars differing in resistance and four isolates (two *F. graminearum* and two F*. culmorum*) with differing aggressiveness (Mesterházy and Bartók 1996, 1997). By this way 8-12 epidemic situations could be analyzed at the same time. As a mean product of these situations the results are more convincing and give a more accurate picture about the antifusarium ability of the fungicides. Each treatment contained 3 plot replicates per cultivar; in each 5 $m^2$ plot: the four isolates in three replicates was made and in each plot three control head of groups were bagged without inoculation. Besides the fungicide treatment a Fusarium check was also included without fungicide application. As control fungicide the Kolfugo Super (carbendazime 20 % a. i.) was used. The same was true for the longevity test.

Fungicide spraying was made at full flowering at rates suggested by the fungicide producers. One day thereafter the inoculation was performed. The sprayed head groups were covered for 24 hours with polyethylene bags, to secure 100 % rel. humidity for infection. Head symptoms were evaluated 10, 14, 18, 22 and 26 days after inoculation. After ripening the groups of heads were separately harvested, 10 heads of each group were randomly separated and threshed at low wind not to loose light infected scabby grains. Yield was measured; visual grain infection for scabby grains was estimated as percentages. In 1998 also the mass ratio of infected grains was measured and beside this the small grain ratio was also given.

In another test the durability of fungicide effect was studied, of the tests the 1999 and 2000 trial will be shown. Here 5, 10 and 15 days as well as 7, 14 and 21 days after spraying additional inoculations were made on separate plots. For controlling leaf disease the durability is easy to measure, we should observe only when the increase of the rusts or powdery mildew starts again to grow after treatment. For the Fusarium effect such correct data are not available in the literature only observations occur. The question is of practical importance as the necessity of a second treatment can be decided only by such data. Here the problem is whether the fungicides sprayed at flowering can combat late rainy period favoring FHB infection.

## RESULTS AND DISCUSSION

Table 1 shows the results of the 1998 trial as means across cultivars and isolates. It is important that the decrease of kernel infection, yield loss or toxin contamination correlates very closely with the decrease of FHB data. This means that a fungicide treatment is effective not only against the FHF symptoms, but similarly also to toxin contamination. It is remarkable also that the fungicides are similarly effective to both *Fusarium* spp. meaning that there is no danger to have selectivity of the fungicides to individual Fusarium species. There is a significant difference between fungicides. The best were the tebuconazole containing ones, however they differed significantly according to their tebuconazole content. The best was the combination between Folicur Top and carbendazime mixture that seems to be equivalent or better to Folicur Solo with 250 g a. i. / ha.. Amistar was very poor and it increased toxin contamination in the susceptible cultivar by 20 % related to *Fusarium* check. This effect of Amistar was not found at more resistant cultivars. In Kolfugo we found this DON increase first since ten years. The ratio of small size FDKs and total amount of infected kernels shows that 34-40 % of the total infected mass belongs to the small size group. This means that by screening only this part can be separated, the major proportion remains in the staple with its toxin contamination. This agrees well with the literature data about the 30 % effectiveness of this procedure and gives its reason.

In Table 2 we present the 1999 mean results. In the mixture the Folicur BT was replaced by Falcon 0.8 l/ha. It seems that this combination is more powerful than the Folicur BT was. The conclusions are the same we gained in 1998.

On more resistant cultivars the infection severity with the best fungicides could be decreased dawn to several percent, on susceptible genotypes, however, 20 % infection usually remained. This is in comparison with 80 % infection severity of the check is considerable, but not enough to grow a well marketable wheat. The efficacy was different among isolates, but a clear tendency was not observed at lower or higher aggressiveness. The efficacy differed also according to traits like FHB %, yield, kernel infection or deoxynivalenol content. They were lowered very parallel according to fungicide efficacy, the correlation coefficients were above 0.90 (P = 0.001) between traits indicating as much the disease severity decreased by the given fungicide, the improvement was similar also in other traits. In our tests the efficacies are significantly higher (80 % at the best entries) than published in relevant literature. It is due to the fact that we aimed a full protection of the head on its whole surface. Therefore these results show the maximal efficacy that can be achieved controlling FHB. As practical efficacy is 20-30 % lower, the application of a fungicide with 50 % efficacy

may raise problems. We could confirm data that Azoystrobin increased toxin contamination. New result that this refers on susceptible and not more resistant cultivars.

In our tests the efficacies are significantly higher (80 % or more at the best entries) than published in relevant literature. Therefore these results show the maximal efficacy that can be achieved controlling FHB. As practical efficacy is 20-30 % lower, the application of a fungicide with 50 % efficacy in our tests may raise problems. We could confirm data that Azoystrobin increased toxin contamination. It is new that this refers on susceptible and not more resistant cultivars.

The durability data show that two weeks after spraying all fungicides kept their protective effects, interestingly the Falcon 0,6 l/ha showed improvement later in 1999 (Table 3). In 2000 the two weeks data show similar results on the 14$^{th}$ day inoculation, but on the 21st day Kolfugo does not give effective protection. For the others the protective effects lasted. (Table 4).

Effective control of FHB is possible now for the cultivars that are not highly sensitive to FHB. Preventive treatment is suggested at flowering; the use of twin nozzles is important to cover correctly the heads from every side to utilize the antifungal capacity of the fungicides. The efficacy of fungicides depends besides others on cultivar resistance, isolate aggressiveness and weather conditions. The efficacy of the best fungicides exceeded 70-80 %, but differs according to the parameter (FHB %, FDK %, yield loss, DON contamination) measured. Therefore a mean efficacy is suggested to describe more correctly the fungicide effect. There was a very close correlation between decrease of toxin contamination and FHB reduction, above r=0,90 meaning that as far the FHB symptoms can be decreased, the decrease of DON content will be proportional.

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

| Fungicide and rate l/ha | Traits | | | | | | Small/ |
|---|---|---|---|---|---|---|---|
| | Grain inf.[2] | FHB % | Yield loss % | Grain inf.[1] | FDK % | DON[3] ppm | Total %[4] |
| Folicur Top 1.0+Kolf. 1.5 | 7.45 | 7.88 | 16.00 | 21.69 | 15.53 | 4.19 | 34.33 |
| Folicur Solo 1.0 | 6.74 | 8.13 | 20.87 | 18.78 | 19.73 | 3.79 | 35.90 |
| Falcon 0.8 | 9.92 | 9.85 | 20.57 | 27.51 | 28.08 | 6.24 | 36.05 |
| Falcon 1.0 | 8.60 | 11.63 | 18.43 | 24.55 | 25.86 | 5.72 | 35.05 |
| Folicur Top 1.0 | 10.73 | 12.41 | 21.26 | 27.82 | 30.81 | 5.94 | 38.56 |
| Juwel 1.0 | 9.74 | 13.44 | 30.45 | 27.06 | 28.83 | 5.86 | 36.01 |
| Duett 1.0 | 8.00 | 13.90 | 25.38 | 26.52 | 25.91 | not tested | 30.18 |
| Falcon 0.6 | 13.43 | 15.92 | 28.24 | 37.74 | 35.70 | not tested | 35.59 |
| Kolfugo Super | 14.93 | 21.72 | 38.37 | 36.58 | 41.92 | 10.42 | 40.80 |
| Amistar 1.0 | 17.48 | 24.75 | 37.60 | 42.94 | 46.39 | 10.98 | 40.70 |
| Fus.check | 23.95 | 41.55 | 50.36 | 53.98 | 58.56 | 11.79 | 44.37 |
| Mean | 11.91 | 16.47 | 27.96 | 31.38 | 32.48 | 7.21 | 37.05 |
| LSD 5 % | 1.95 | 0.71 | 2.89 | 2.90 | 3.33 | 2.07 | |

Correlations between traits

| | Grain inf.2 | FHB % | Yield loss % | Grain inf.[1] | FDK % |
|---|---|---|---|---|---|
| FHB % | 0.9678 | | | | |
| Yield loss % | 0.9247 | 0.9491 | | | |
| Grain inf.[2] | 0.9861 | 0.9514 | 0.9155 | | |
| Grain inf. %[1] | 0.9788 | 0.9522 | 0.9398 | 0.9752 | |
| DON ppm[4] | 0.9460 | 0.9080 | 0.9186 | 0.9565 | 0.9650 |

All correlations are significant at P = 0.1 %.

[1] Mass ratio of all infected grains , [2] Mass ratio of small size infected grains

[3]Correlations with DON n = 9, the others n = 11, [4]Ratio of small FDKs to total mass of FDKs

**Table 2.** Fungicides against Fusarium head blight of wheat. General means for 1999.

| Fungicide and rate l/ha | Yield loss % | Kernel inf. % | FHB % | DON ppm |
|---|---|---|---|---|
| Falc.0.8+Kolf.1 | 12.19 | 14.36 | 23.43 | 10.22 |
| Fol. Solo 1 | 13.42 | 22.37 | 27.14 | 12.85 |
| Falcon 0.8 | 14.99 | 20.47 | 28.61 | 13.29 |
| Juwel 1,0 | 23.75 | 32.69 | 38.97 | 20.84 |
| Kolfugo1.5 | 25.13 | 31.31 | 39.93 | 14.04 |
| Fus. check | 41.97 | 58.79 | 56.11 | 36.23 |
| Mean | 14.93 | 19.67 | 24.15 | 17.91 |
| LSD 5 % | 0.91 | 3.21 | 2.93 | 3.94 |

**Table 3**. Fungicide durable effect on FHB in wheat, relative grain infection data (%)to the Fusarium check, 1999.

| Inoculation: days after fungicide application | Fungicides | | | | |
|---|---|---|---|---|---|
| | Fus.check | Falcon 0.6 | Kolfugo 1.5 | Caramba 1.0 | Falcon 0.8 |
| 1 | 100.00 | 79.98 | 68.81 | 53.75 | 46.15 |
| 5 | 100.00 | 66.19 | 57.67 | 42.69 | 27.62 |
| 10 | 100.00 | 55.54 | 48.94 | 44.26 | 44.63 |
| 15 | 100.00 | 37.66 | 57.24 | 41.28 | 48.51 |
| Mean | 100.00 | 59.84 | 58.17 | 45.50 | 41.73 |

**Table 4.** Longevity of fungicide effect against FHB in wheat, Summary, 2000
Kernel infection. Related data to FHB control.

| Inoculation: days after | Fungicides | | | | | |
|---|---|---|---|---|---|---|
| fungicide application | Fus. check | Kolfugo 1.5 | Caramba 1.2 | Falcon 0.8 | Falcon 1 | Mean |
| 1 | 100.00 | 44.79 | 8.91 | 15.42 | 11.15 | 20.07 |
| 7 | 100.00 | 43.54 | 80.03 | 22.62 | 18.97 | 41.29 |
| 14 | 100.00 | 37.62 | 7.23 | 28.94 | 27.49 | 25.32 |
| 21 | 100.00 | 92.59 | 18.52 | 27.78 | 18.52 | 39.35 |
| Mean | 100.00 | 54.63 | 28.67 | 23.69 | 19.03 | 31.51 |

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# ANALYSIS OF THE 2001 UNIFORM WHEAT FUNGICIDE AND BIOCONTROL TRIALS ACROSS LOCATIONS

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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 wide spread benefits to growers and users of all market classes of wheat in the event of FHB epidemics. The overall objective of the Chemical and Biological Control Committee is to hasten the integration of fungicides and biocontrols that are effective against FHB into cost-effective and environmentally-safe wheat disease management strategies. The current objective is to identify the most efficacious treatments. Uniform trials across the range of wheat market classes and environments prone to FHB epidemics is believed to be the best means for evaluating the efficacy of treatments. This analysis will consider only variables that are directly related to FHB because other variables, such as yield, are likely to be affected by diseases other than FHB.

## METHODS

Plant pathologists in 13 states (Table 1) participated in the 2001 wheat uniform fungicide and biocontrol trials. These states represented hard red spring wheat, hard red winter wheat, soft red winter wheat, soft white winter wheat, and durum wheat production areas. The seven uniform treatments for 2001 (Table 2) included Folicur that has received several Section 18 registrations for FHB management, two experimental fungicides (BAS 505 and AMS21619), and two biological agents (TrigoCor 1448, a bacterium, and OH 182.9, a yeast). The biological agents were developed in part through USWBSI funding.

All treatments were applied at flowering stage using a CO2-powered sprayer equipped with twinjet XR8001 nozzles mounted at a 60 degree angle forward and backward. Details such as plot size, volume per acre, CO2 pressure, and number of replications varied slightly among the locations but were not considered to significantly affect the results. Inoculation and/or some form of overhead misting were used at most locations to promote head blight development, and these practices likely increased the incidence and severity of head blight. Disease variables included in this analysis were field severity (= FHB index = incidence x head severity) measured at soft dough stage and deoxynivalenol (DON) content in the grain and percentage of Fusarium-damaged kernels (FDK) measured after harvest. Cooperators analyzed results of their individual locations and provided treatment means to the authors for analysis across locations. The experimental design was a randomized complete block using the various locations as blocks. Analyses of FHB variables using all available data

were followed by analyses of the variables in high, moderate, and low categories of the variables in order to increase the probability of finding real differences among the treatments.

## RESULTS

Seventeen locations across 11 of the participating states reported some FHB data (Table 3). As expected, there were significant differences among locations for each of the FHB variables. Averaged across all of the locations with data for the FHB variables, there were significant differences among treatments for field severity, but not for FDK or DON (Table 4). Compared to the non-treated check, all treatments except TrigoCor 1448 significantly reduced field severity and the fungicides reduced field severity by about 50%. Although the difference was not statistically significant, AMS21619 reduced FDK by about 50%.

Analyzed across locations with similar levels of field severity (Table 5), all treatments significantly reduced field severity at locations with low levels of disease, and all fungicides significantly reduced field severity at locations with moderate levels of disease. The best treatments reduced field severity by about 50%. At locations with high levels of disease, none of the treatments were significantly different from the non-treated check, but AMS21619 did reduce field severity by more than 50%.

Analyzed across locations with similar levels of FDK (Table 6), there were no significant differences among treatments at high, moderate or low levels of FDK. However, the best treatments in each of the three analyses did reduce the level of FDK by about 50% or more.

Analyzed across locations with similar levels of DON (Table 7), there were no significant differences among treatments at high-moderate or low levels of DON. Compared to the non-treated check, none of the treatments reduced DON by 50% or more.

## CONCLUSIONS

All of the tested treatments had some efficacy against at least some of the FHB variables in some of the analyses. In general, fungicides were more efficacious than biological agents, and the most efficacious treatments reduced the values for field severity, FDK, and DON by about 50% compared to the non-treated check. Frequently, these differences were not statistically significant at $P = 0.05$ because of variability in the data. Perhaps it would be appropriate to use a less stringent significance level in future analyses.

Table 1. States and principal cooperators in the uniform wheat fungicide and biocontrol trials.

| | |
|---|---|
| Arkansas | Gene Milus, University of Arkansas, Fayetteville |
| Indiana | Greg Shaner, Purdue University, West Lafayette |
| Iowa | Gary Munkvold, Iowa State University, Ames |
| Kentucky | Don Hershman, University of Kentucky, Princeton |
| Maryland | Arvydas Grybauskas, University of Maryland, College Park |
| Michigan | Pat Hart, Michigan State University, East Lansing |
| Minnesota | Hala Toubia-Rahme, University of Minnesota, Crookston |
| Missouri | Laura Sweets, University of Missouri, Columbia |
| New York | Gary Bergstrom, Cornell University, Ithaca |
| North Dakota | Marcia McMullen, North Dakota State University, Fargo |
| Ohio | Pat Lipps, Ohio State University, Wooster |
| South Dakota | Marty Draper, South Dakota State University, Brookings |
| Virginia | Erik Stromberg, Virginia Technical, Blacksburg |

Table 2. Treatment, rate, and adjuvant used in the uniform trials in 2001.

| # | Treatment | Rate of product / A | Adjuvant |
|---|---|---|---|
| 1 | Nontreated | | |
| 2 | Folicur 3.6 F | 4 fl oz | 0.125% Induce |
| 3 | AMS12619 480SC | 5.7 fl oz | 0.125% Induce |
| 4 | BAS505 50DF | 6.4 oz | 0.125% Induce |
| 5 | BAS505 50DF + Folicur 3.6F | 3.2 oz + 2 fl oz | 0.125% Induce |
| 6 | TrigoCor 1448 | varied among locations | |
| 7 | OH 182.9 | varied among locations | |

Table 3. The means for field severity, Fusarium-damaged kernels (FDK), and deoxynivalenol (DON) across all seven treatments at locations that reported some level of FHB in the 2001 uniform trials.

| Location | Location | Field severity[1] | FDK[1] | DON[1] |
|---|---|---|---|---|
| (#) | (state and city or variety) | (%) | (%) | (ppm) |
| 1 | Ohio | 33.0a | 33.2b | 13.8b |
| 2 | Michigan (Freedom) | 32.4a | | 1.0c |
| 3 | Michigan (Harus) | 22.1b | | 1.7c |
| 4 | Minnesota | 19.2b | 10.5cd | 2.0c |
| 5 | Michigan (Frankenmuth) | 18.6bc | | 0.7c |
| 6 | Kentucky | 17.3bc | 21.0c | 3.9c |
| 7 | North Dakota (Carrington) | 17.2bc | | |
| 8 | North Dakota (Langdon durum) | 16.7bc | | |
| 9 | Arkansas | 12.8cd | 52.8a | 29.2a |
| 10 | Missouri | 10.1de | | 1.6c |
| 11 | Virginia | 5.1ef | | |
| 12 | North Dakota (Fargo) | 3.8f | 2.8d | 1.2c |
| 13 | North Dakota (Langdon) | 3.2f | | |
| 14 | North Dakota (Minot) | 2.7f | | |
| 15 | New York | 1.6f | 6.2d | |
| 16 | Iowa | 1.6f | | |
| 17 | Indiana | | | 0.8c |

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

Table 4. Treatment means for field severity, Fusarium-damaged kernels (FDK), and deoxynivalenol (DON) level averaged across all of the locations in Table 3.

| Treatment | Field severity[1] | FDK[1] | DON[1] |
|---|---|---|---|
| | (%) | (%) | (ppm) |
| Non-treated | 20.0a | 29.4a | 6.2a |
| TrigoCor 1448 | 18.0ab | 24.4a | 5.6a |
| OH 182.9 | 15.9bc | 23.1a | 4.4a |
| Folicur | 12.1cd | 21.1a | 5.3a |
| BAS505 + Folicur | 11.0d | 18.6a | 6.7a |
| BAS505 | 10.9d | 17.0a | 6.2a |
| AMS21619 | 9.2d | 13.9a | 4.6a |

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

Table 5. Treatment means for field severity averaged across locations with high, moderate, or low levels of field severity.

| Treatment | High severity[1,2] | Moderate severity[1,3] | Low severity[1,4] |
|---|---|---|---|
| | (%) | (%) | (%) |
| Non-treated | 43.5a | 25.2a | 5.1a |
| TrigoCor 1448 | 43.3a | 19.9ab | 2.5bc |
| OH 182.9 | 38.4a | 20.1ab | 3.5b |
| Folicur | 30.7a | 14.8bc | 2.3c |
| BAS505 + Folicur | 30.1a | 12.7c | 2.4c |
| BAS505 | 25.4a | 13.8c | 2.4c |
| AMS21619 | 17.6a | 12.0c | 2.6bc |

[1]Values within a column followed by the same letter are not significantly different by a LSD test at P=0.05. [2]Locations (by number from Table 3) in this analysis are 1 and 2.
[3]Locations (by number from Table 3) in this analysis are 3, 4, 5, 6, 7, 8, 9, and 10.
[4]Locations (by number from Table 3) in this analysis are 11, 12, 13, 14, 15, and 16.

Table 6. Treatment means for Fusarium-damaged kernels (FDK) averaged across locations with high, moderate, or low levels of Fusarium-damaged kernels.

| Treatment | High FDK[1,2] | Moderate FDK[1,3] | FDK severity[1,4] |
|---|---|---|---|
| | (%) | (%) | (%) |
| Non-treated | 60.5a | 21.0a | 6.7a |
| TrigoCor 1448 | 50.5a | 16.7a | 6.1a |
| OH 182.9 | 45.5a | 18.7a | 5.2a |
| Folicur | 44.2a | 15.7a | 3.6a |
| BAS505 + Folicur | 41.4a | 11.2a | 3.2a |
| BAS505 | 33.0a | 13.8a | 4.3a |
| AMS21619 | 25.9a | 13.4a | 2.5a |

[1]Values within a column followed by the same letter are not significantly different by a LSD test at P=0.05. [2]Locations (by number from Table 3) in this analysis are 1 and 9.
[3]Locations (by number from Table 3) in this analysis are 4 and 6.
[4]Locations (by number from Table 3) in this analysis are 12 and 15.

Table 7. Treatment means for deoxynivalenol (DON) level averaged across locations with high-moderate or low levels of deoxynivalenol.

| Treatment | High-moderate DON[1,2] | Low DON[1,3] |
|---|---|---|
| | (%) | (%) |
| Non-treated | 23.1a | 2.3a |
| TrigoCor 1448 | 21.5a | 1.9a |
| OH 182.9 | 15.2a | 2.0a |
| Folicur | 20.0a | 2.1a |
| BAS505 + Folicur | 27.7a | 1.8a |
| BAS505 | 25.1a | 1.8a |
| AMS21619 | 18.0a | 1.6a |

[1]Values within a column followed by the same letter are not significantly different by a LSD test at P=0.05. [2]Locations (by number from Table 3) in this analysis are 1 and 9.
[3]Locations (by number from Table 3) in this analysis are 2, 3, 4, 6, 10, and 12.

# EFFICACY OF FUNGICIDES AND BIOCONTROLS AGAINST FUSARIUM HEAD BLIGHT IN ARKANSAS, 2001

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## OBJECTIVE

To identify fungicides and biocontrol agents that are effective against Fusarium head blight.

## MATERIALS AND METHODS

Seeds of Hazen soft red winter wheat, treated with Gaucho (3 fl oz / cwt) and Dividend (1 fl oz / cwt), were planted at the rate of 114 lb/A on 19 October 2000 at University Farm in Fayetteville. Each plot was 7 rows (4.1 ft) by 15 ft and trimmed to 12 ft before harvest. Plots were fertilized with 40 lb N/A on 23 February and 6 March. Colonized corn kernel inoculum was spread in the field on 18, 20, and 25 April for a total of 14 kernels / sq ft. The TrigoCor1448 bacterium was grown in shake culture of nutrient broth for 10 days. The OH 182.9 yeast suspension was prepared from a frozen paste according to directions. At early flowering stage, treatments were applied in the late afternoon (to promote the establishment of the biological agents) on 27 April at 20 gal/A and 40 psi using a CO2-powered backpack sprayer equipped with three sets of twinjet XR8001 nozzles mounted at a 60 degree angle forward and backward. The design was a randomized complete block with six replications. To promote ascospore formation in the corn kernel inoculum and head infection, the plot was misted for eight 11-minute periods between midnight and 8:00 am on 23 nights between 18 April and 18 May. Fifty heads per plot were collected at soft dough stage on 25 May to determine the incidence and severity of head blight. Plots were harvested with a plot combine on 11 June. Yields were adjusted to 13% moisture and test weights were determined after passing the grain once through an air-blast seed cleaner. A 50-g sample of grain from each plot was evaluated visually for the percentage of scabby grain and then sent to Michigan State University for DON analysis.

## RESULTS AND DISCUSSION

Fusarium head blight developed later than normal, probably because ascospores were not released from the inoculum until after flowering. Septoria tritici blotch was the only other disease prevalent in the plots, but it developed late in the season and likely did not affect results. Plots treated with OH 182.9 had the lowest levels of scabby seed and DON (Table 1). Plots treated with TrigoCor 1448 also had a low level of DON. The high efficacy of the biocontrol agents relative to the fungicides may have been due to 1) treatments were applied in the late afternoon to help the biocontrol agents establish, 2) frequent mist cycles may have allowed the populations of the biocontrol agents to increase before head blight infection occurred, and 3) disease developed late after fungicide activity likely dissipated. Plots treated with BAS505 had significantly higher levels of DON than the non-treated checks.

Table 1. Results of the uniform fungicide and biocontrol trial at Fayetteville, AR, in 2001.

| Trt No. | Treatment and rate per acre | Yield (bu/A) | Test Wt. (lbs/bu) | Plot severity (%) | Head severity (%) | Incidence (%) | Scabby Seed (%) | DON (ppm) |
|---|---|---|---|---|---|---|---|---|
| 1 | Non-treated #1 | 87.1 | 54.5 | 12.7 | 16.5 | 75 | 59.2 | 29.5 |
| 2 | Folicur 3.6F 4 fl oz + 0.125% Induce | 87.2 | 55.2 | 13.7 | 18.1 | 74 | 55.0 | 27.9 |
| 3 | AMS21619 480SC 5.7 fl oz. + 0.125% Induce | 92.2 | 55.9 | 12.1 | 17.9 | 65 | 47.5 | 28.8 |
| 4 | BAS505 50DF 6.4 oz + 0.125% Induce | 88.5 | 54.3 | 12.3 | 17.5 | 69 | 59.2 | 41.8 |
| 5 | BAS505 50DF 3.2 oz + Folicur 3.6F 2 fl oz + 0.125% Induce | 84.4 | 53.9 | 13.3 | 17.5 | 76 | 65.0 | 40.5 |
| 6 | TrigoCor 1448 $1.7x10^{14}$ cfu | 87.2 | 54.8 | 14.1 | 18.6 | 75 | 49.2 | 19.0 |
| 7 | OH 182.9 $2.4x10^{14}$ cfu | 91.1 | 56.5 | 11.7 | 17.7 | 64 | 34.2 | 16.9 |
| 8 | AMS21619 480SC 3.6 fl oz. + Folicur 3.6F 4 fl oz. + 0.06% Induce | 88.2 | 56.1 | 11.8 | 17.0 | 69 | 46.7 | 22.5 |
| 9 | AMS21619 480SC 5.7 fl oz. + 1% crop oil concentrate | 88.1 | 55.3 | 12.7 | 18.2 | 69 | 55.8 | 33.5 |
| 10 | Non-treated #2 | 81.6 | 55.0 | 14.6 | 18.6 | 78 | 50.0 | 27.4 |
| | **Prob > F** | 0.32 | 0.017 | 0.71 | 0.99 | 0.002 | < .0001 | 0.0001 |
| | **LSD (P=0.05)** | NS | 1.5 | NS | NS | 7 | 11.0 | 10.1 |
| | **CV (%)** | 7.4 | 2.3 | 22.9 | 19.2 | 8.90 | 17.9 | 29.7 |

# EFFICACY OF FUNGICIDES IN CONTROLLING FUSARIUM HEAD BLIGHT ON BARLEY GENOTYPES WITH PARTIAL RESISTANCE

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## INTRODUCTION

Fusarium head blight (FHB), incited primarily by *Fusarium graminearum*, adversely affected the quality of barley grown in eastern North Dakota and northwestern Minnesota the last nine years. Quality of harvested grain was reduced because of blighted kernels and the presence of deoxynivalenol (DON), a mycotoxin produced by the pathogen. Non-detectable, or low levels of DON are needed for malting barley because DON has been found to carry through malting and brewing into finished beer (Schwarz et al., 1995). Anheuser-Busch, Inc., the largest brewer in the U.S., will not purchase malt produced from barley with DON levels greater than 0.5 ppm.

Research to test the efficacy of fungicides in reducing FHB and DON levels in barley has been conducted using cultivars susceptible to FHB. Pederson and McMullen (1999) found that the fungicides Folicur, Tilt, Benlate, Mancozeb, and Quadris significantly reduced FHB severity and DON content of barley. However, the fungicides were not successful in reducing DON content to a level that would be acceptable to maltsters and brewers. The most successful fungicide treatment reduced DON content of barley to 17.2 ppm.

In a preliminary study, Horsley et al (2000) evaluated the efficacy of Folicur in controlling FHB on barley genotypes with different levels of resistance. They concluded that Folicur did not significantly reduce FHB levels in any of the 14 genotypes included in the study.

## OBJECTIVES

The objective of this study is to further investigate the integrated use of fungicides and resistant or moderately resistant barley genotypes to reduce FHB severity and accumulation of DON to levels acceptable to the malting and brewing industry.

## MATERIALS AND METHODS

Fourteen barley genotypes with different levels of FHB resistance were grown at sites near Osnabrock, Langdon, and Fargo, North Dakota during the 2000 and 2001 growing seasons. Treatments were assigned to 35 ft2 experimental units using a randomized complete block design with a split-plot arrangement and three replicates at each location. Whole plots included; no fungicide, 4 fl oz Folicur/acre, and in the 2001 growing season only, 5.7 fl oz/acre of an experimental triazol from Bayerâ. Subplots were genotypes. Evaluated genotypes were either resistant to FHB (Chevron, Svanhals, and Kaoto Nijo 2), moderately resistant to FHB (MNBrite, F101-78, F103-61, F103-52, and F102-61), or susceptible to FHB (Foster,

Stander, Conlon, Logan, Drummond, and Legacy). Experimental units were not inoculated with *F. graminearum.*

Fungicides were applied at Feeke's growth stage 10.3 using a CO2-pressurized handheld boom sprayer operating at 40 psi, and calibrated to deliver 24 gallons of solution acre-1. Fusarium head blight severity was assessed at Feeke's growth stage 11.2 by determining the ratio of infected kernels to total kernels on 10 spikes per row. Disease severity was expressed as percent FHB severity. At maturity, each experimental unit was harvested with a small-plot combine. Grain samples were dried and cleaned prior to yield determination. Grain samples from each experimental unit were submitted to Dr. Paul Schwarz's laboratory in the Department of Cereal Science, North Dakota State University for DON analysis. To date, DON data for the 2001 Osnabrock and Fargo locations are not available.

Data from individual locations were analyzed separately using analysis of variance (ANOVA) and error mean squares from each location were tested for homogeneity of variance. Combined ANOVA's were done using data from locations in which error mean squares were homogeneous. Means were separated using an F-protected LSD (P=0.05). In the combined analyses, fungicide and genotypes were considered fixed effects and environment a random effect.

## RESULTS AND DISCUSSION

Environmental conditions at Langdon and Osnabrock were more conducive for development of FHB than conditions at Fargo. Mean FHB severity was 4.3% at Langdon, 3.7% at Osnabrock, and 0.8% at Fargo. Fusarium head blight severity was not significantly reduced by Folicur in any of the genotypes (data not presented). Deoxynivalenol content was not significantly reduced by Folicur in any of the evaluated genotypes (Table 1); however, there was a trend for slightly lower DON resulting from Folicur application. Reductions of DON to levels acceptable for the malting and brewing industry (<0.6 ppm) occurred only in genotypes with resistance or partial resistance.

Genotypes sprayed with Folicur generally had greater yield than unsprayed genotypes (Table 2). Much of the yield improvements may be due to reductions of foliar disease in genotypes sprayed with Folicur. Foliar disease severity data were collected at Langdon and Osnabrock. (Data not presented). The predominant foliar disease at each location was septoria leaf blotch, incited by Septoria spp. Significant yield increases were mainly observed for the cultivars developed and released from upper Midwest barley breeding programs (i.e.Legacy, Conlon, Drummond, Foster, Logan, MNBrite, and Stander.) This suggests that factors other that foliar diseases were limiting yield in the other genotypes.

In the barley-growing region in the upper Midwest U.S., it costs growers about $14/acre for Folicur and its application. For this cost to be recovered, a yield increase of at least 10.8 bushels/acre is needed based on a farmgate-selling price of $1.30/bushel for feed barley. Based on the yield increases observed in this study, the cost of Folicur and its application was recovered only when applied to the adapted cultivars Legacy, Conlon, Foster, Logan, MNBrite, and Stander (Table 3). If DON content could be reduced to levels required by the

malting and brewing industry (<0.6 ppm) additional net profit returns could be realized due to a $1.00/bushel premium for malting barley.

Preliminary results indicate that the efficacy of the experimental triazol was slightly better than Folicur in reducing FHB and DON (data not presented). Deoxynivalenol data were available from only one location at the time of preparing this report. The study including the experimental triazol will be continued in the next growing so that more definite conclusions can be made.

## CONCLUSIONS

Folicur application did not significantly reduce FHB severity or DON level in resistant, moderately resistant, or susceptible genotypes.

Genotypes sprayed with Folicur generally had greater yield

Yield gains due to control of foliar diseases tended to be sufficient to cover the cost of Folicur and its application on cultivars developed and released by upper Midwest barley breeding programs.

Further research is needed to determine if a fungicide with greater efficacy than Folicur for FHB control can be used with moderately resistant genotypes to reduce DON

**Table 1.** Effect of Folicur and genotype on DON content of barley.

| | Environment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2000 Fargo | | 2000 Langdon | | 2000 Osnabrock | | 2001 Langdon | |
| Genotype | Folicur | No Folicur | Folicur | No Folicur | Folicur | No Folicur | Folicur | No Folicur |
| | ppm | | | | | | | |
| Chevron | 0.1 | 0.1 | 0.5 | 1.5 | 0.7 | 0.6 | 0.2 | 0.9 |
| Svanhals | 0.0 | 0.0 | 0.5 | 0.6 | 0.4 | 0.1 | 1.2 | 0.6 |
| Kaota Nijo 2 | 0.1 | 0.1 | 1.0 | 1.9 | 1.9 | 2.4 | 0.3 | 0.2 |
| F101-78 | 0.3 | 0.2 | 0.8 | 2.0 | 0.8 | 1.2 | 0.5 | 0.4 |
| F102-61 | 0.3 | 0.4 | 0.3 | 0.7 | 0.6 | 0.7 | 1.5 | 1.5 |
| F103-52 | 0.3 | 0.5 | 0.3 | 2.2 | 1.1 | 0.8 | 0.6 | 0.9 |
| F103-61 | 0.4 | 0.3 | 1.4 | 2.2 | 0.9 | 1.7 | 0.4 | 0.4 |
| MnBrite | 0.3 | 0.4 | 1.6 | 2.9 | 1.1 | 1.4 | 0.6 | 0.7 |
| Legacy | 0.2 | 0.4 | 1.2 | 2.0 | 1.0 | 1.5 | 1.4 | 0.9 |
| Drummond | 0.6 | 0.7 | 2.1 | 3.8 | 1.3 | 1.6 | 0.6 | 0.5 |
| Foster | 0.4 | 0.5 | 1.6 | 2.4 | 2.9 | 2.6 | 0.8 | 0.6 |
| Stander | 0.7 | 0.6 | 1.8 | 2.5 | 2.3 | 2.2 | 1.4 | 1.7 |
| Logan | 0.2 | 0.1 | 1.3 | 1.9 | 1.2 | 1.6 | 0.4 | 0.2 |
| Conlon | 0.1 | 0.2 | 1.1 | 1.2 | 1.6 | 1.1 | 0.2 | 0.3 |
| LSD(0.05) | ns | | | | | | | |

**Table 2.** Effect of Folicur and genotype on yield of barley.

| | Environment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2000 Fargo | | 2000 Langdon | | 2000 Osnabrock | | 2001 Fargo | | 2001Osnabrock | |
| Genotype | Folicur | No Folicur | Folicur | No Folicur | Folicur | No Folicur | Folicur | No Folicur | Folicur | No Folicur |
| | bu/acre | | | | | | | | | |
| Chevron | 49.4 | 50.1 | 36.7 | 33.8 | 75.9 | 68.4 | 35.2 | 33.2 | 47.2 | 44.7 |
| Svanhals | 42.3 | 37.8 | 32.3 | 30.4 | 71.3 | 56.9 | 32.9 | 37.7 | 39.2 | 35.1 |
| Kaota Nijo 2 | 52.7 | 48.1 | 77.6 | 73.5 | 76.9 | 62.8 | 40.9 | 43.0 | 57.0 | 46.4 |
| F101-78 | 48.6 | 47.5 | 76.1 | 72.6 | 63.6 | 63.7 | 32.2 | 36.1 | 46.5 | 55.7 |
| F102-61 | 47.6 | 45.9 | 65.9 | 60.6 | 69.8 | 71.4 | 23.0 | 32.6 | 52.3 | 45.9 |
| F103-52 | 39.2 | 33.1 | 46.8 | 42.2 | 67.2 | 63.5 | 23.1 | 23.8 | 34.8 | 39.1 |
| F103-61 | 51.0 | 47.7 | 81.1 | 75.5 | 74.6 | 68.0 | 31.5 | 40.6 | 62.9 | 56.6 |
| MnBrite | 56.8 | 46.3 | 102.5 | 81.6 | 91.6 | 90.9 | 42.5 | 40.8 | 75.2 | 66.1 |
| Legacy | 57.7 | 57.0 | 107.4 | 91.5 | 109.3 | 94.1 | 38.7 | 36.4 | 61.7 | 55.9 |
| Drummond | 61.8 | 55.4 | 100.3 | 90.7 | 90.4 | 79.1 | 45.1 | 48.0 | 63.4 | 52.6 |
| Foster | 64.1 | 60.9 | 115.4 | 79.6 | 107.6 | 84.5 | 43.9 | 55.4 | 70.2 | 61.9 |
| Stander | 68.5 | 59.9 | 111.9 | 96.2 | 91.9 | 83.4 | 45.7 | 47.6 | 64.0 | 64.7 |
| Logan | 59.3 | 50.3 | 109.8 | 77.4 | 100.1 | 85.7 | 48.5 | 45.9 | 70.4 | 50.2 |
| Conlon | 53.4 | 45.0 | 85.6 | 68.3 | 86.4 | 75.1 | 46.7 | 49.3 | 61.4 | 54.8 |
| LSD(0.05) | 9.7 | | | | | | | | | |

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# USDA-ARS, OHIO STATE UNIVERSITY COOPERATIVE RESEARCH ON BIOLOGICALLY CONTROLLING FUSARIUM HEAD BLIGHT: PILOT-PLANT-SCALE PRODUCTION AND PROCESSING OF BIOMASS OF YEAST ANTAGONISTS

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## OBJECTIVES

To select one of two superior yeast antagonists (Cryptococcus sp. OH 181.1 and C. nodaensis OH 182.9) for use in the 2001 Uniform Wheat Fungicide and Biocontrol Trial (UWFBT) based on antagonist amenability to liquid culture production in shake flasks and 30 L fermentors. Additionally, to evaluate the suitability of two methods of freezing antagonist biomass in order to maximize viable cell counts until the time of application.

## INTRODUCTION

Research on developing strategies and microorganisms for biologically controlling Fusarium head blight (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. 2000; Khan et al., 2001). A critical step in the transition from conducting laboratory experiments on biological control agents to producing a commercially available biocontrol product is devising economically feasible procedures for large-scale, liquid culture production of biomass of the biological agent. Antagonist strains considered for commercial development must also be able survive cell preservation techniques and maintain high viable cell counts over time.

## MATERIALS AND METHODS

Growth of *Cryptococcus sp.* OH 181.1 and *C. nodaensis* OH 182.9 in liquid culture

In preliminary medium optimization experiments, a semidefined complete liquid medium (SDCL; Slininger et al., 1994) supported excellent growth of both yeast antagonists. A liter of this medium contains approximately 15 g and 1.2 g of total carbon and nitrogen, respectively. Glucose serves as a carbon source while Casamino acids provide carbon and nitrogen. In standard laboratory use, the glucose and amino acid portions of the medium are sterilized separately (A+B). A version of SDCL medium where all ingredients are autoclaved together (AB) has an enhanced commercial potential due to requiring less costly production parameters. However, the influence of heat-induced condensation products in the AB form of SDCL on microbial growth was unknown. In shake flask experiments, 125 ml flasks were

charged with 50 ml of the AB or A+B version of SDCL and inoculated with 18-24 h precultures of yeast antagonists to an optical density (ODA620) of 0.1. Cultures were incubated at 25 C and 250 rpm for 72 h in a shaker incubator. Colony forming units (CFU) per ml were determined at 48 h and 72h.

Yeast strains OH 181.1 and OH 182.9 were also produced in a B Braun D-30 fermentor charged with 20 L of either SDCL AB or SDCL A+B medium. To initiate a production run, 24 h old cells grown in the same medium as used in the production run served as a 5% seed inoculum. Reactor medium pH, temperature, dissolved O2, antifoam, agitation rate were monitored and/or maintained to insure near identical production runs. Colonized broths were sampled and plated on 1/5 strength Tryptic soy broth agar (TSBA/5) for CFU/ml after 48 h.

Processing and freezing of biomass of *Cryptococcus sp*. OH 181.1 and *C. nodaensis* OH 182.9

Cells of the yeast antagonists were produced in a 30 L fermentor as described above. After completion of biomass production at approximately 48h, cells in the broth were concentrated into a paste using a Sharples 12-V tubular bowl centrifuge. The paste was split into two parts and resuspended using either buffer or spent broth and frozen at -18 C. Samples of the frozen biomass were gradually thawed and plated on TSBA/5 every seven days for a total of 70 days to determine CFU/ml. Log10 CFU/ml data obtained over the coure of the experiment were analyzed using linear regression.

## RESULTS AND DISCUSSION

In both shake flask and fermentor experiments, CFU/ml production by OH 181.1 or OH 182.9 was not deleteriously affected by autoclaving all components of the SDCL medium together (SDCL AB)(Table 1) demonstrating the utility of a form of the medium that would be most advantageous for commercial use. Antagonist OH 182.9 actually tended to produce more CFU/ml in the AB than the A+B version of SDCL in the shake flask and fermentor experiments (Table 1). Antagonist OH 182.9 showed a trend of producing more CFU/ml than did OH 181.1 in every comparison of like medium and production vessel (Table 1). A linear relationship described CFU/ml over time for frozen OH 182.9 cells resuspended in buffer ($P<0.001$) or in spent broth ($P<0.001$)(Fig. 1). Biomass viability of OH 182.9 decayed more rapidly for cells that were resuspended in spent broth before freezing than for cells resuspended in buffer (Fig. 1). Nearly identical results were obtained for OH 181.1 (data not shown).

*C. nodaensis* OH 182.9 was chosen over *Cryptococcus sp.* OH 181.1 for use in the 2001 UWFBT due to OH 182.9 obtaining higher maximum CFU/ml and obtaining CFUmax in less time than OH 181.1. The efficacy of the frozen biomass of both antagonists in reducing FHB severity was similar in greenhouse trials (data not shown). As a result of these and other studies, cells of OH 182.9 were produced in SDCL AB medium in 20 L and 80 L quantities, harvested after 48 h, concentrated by centrifugation, resuspended in buffer, frozen, and sent frozen to participants in the 2001 UWFBT. Selected results of using antagonist *C. nodaensis* OH 182.9 in the 2001 UWFBT are presented by Milus and coauthors (2001) in this volume.

A portion of our future research on enhancing the commercial development potential of OH 182.9 will concentrate on identifying cryoprotectant compounds that further enhance the survival and shelf-life of frozen biomass of the antagonist as well as determining the feasibility of alternative biomass processing procedures such as air, fluidized bed or spray drying.

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**Table 1.** Comparison of cell production by antagonists *Cryptococcus* spp. OH 181.1 and OH 182.9 in shake flasks and 30L fermentors charged with different liquid media

| | Maximum $Log_{10}$ (CFU/ml) | |
|---|---|---|
| Antagonist/Medium[1,2] | Shake Flask[3] | Fermentor |
| OH 181.1/A+B | 8.59 | 8.63 |
| OH 181.1//AB | 8.59 | 8.63 |
| OH 182.9/A+B | 8.71 | 8.72 |
| OH 182.9/AB | 8.85 | 9.19 |

[1] OH 181.1 is a *Cryptococcus* sp. with NRRL accession number Y-30215. OH 182.9 is a strain of *C. nodaensis* with NRRL accession number Y-30216.
[2] Medium "A+B" is a semidefined complete medium (Slininger et al., 1994) where the glucose and amino acid portions of the medium are sterilized separately while in medium "AB" all media ingredients are autoclaved together.
[3] CFU/ml values are the maximum obtained and occurred between 48 and 72 hours after inoculation of liquid cultures.

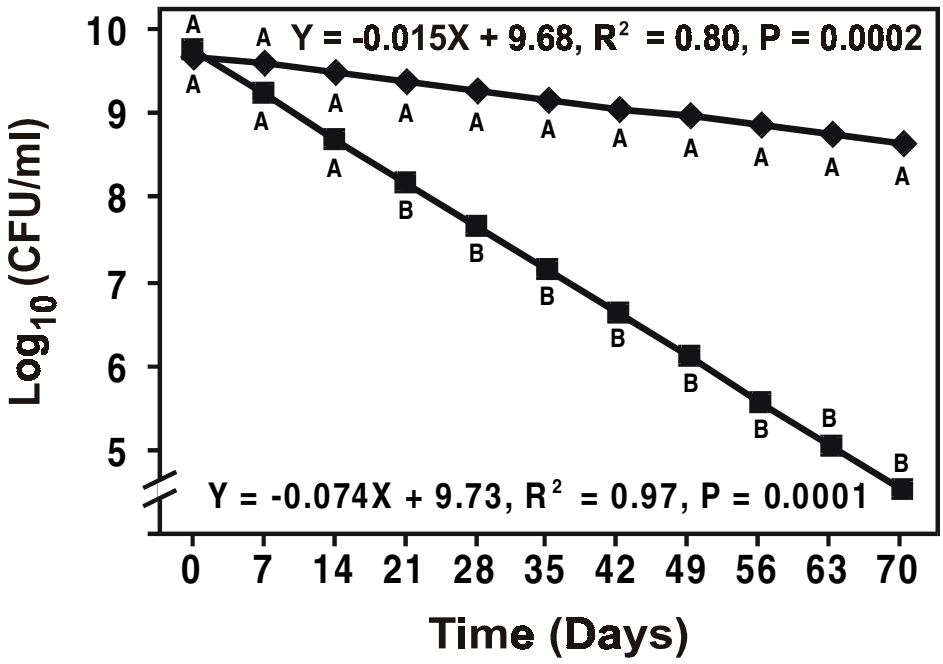


**Figure 1.** Survival of 30-liter fermentor-produced biomass of Fusarium head blight antagonist *Cryptococcus nodaensis* OH 182.9 resuspended in spent broth or weak PO4 buffer and stored at -20 C. -♦- biomass resuspended in buffer. -■- biomass resuspended in spent broth. Data points at same time that do not have identical letters are significantly different (P=0.05)

# BIOLOGICAL CONTROL OF FUSARIUM HEAD BLIGHT WITH *BACILLUS SUBTILIS* TRIGOCOR 1448: 2001 FIELD RESULTS

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## OBJECTIVES

To quantify the ability of the bioprotectant TrigoCor 1448, applied to flowering spikes, to control Fusarium head blight (FHB) and to reduce deoxynivalenol (DON) contamination of the harvested grain.

## INTRODUCTION

Efforts are being made to provide safe, affordable and efficacious biological protectants for the integrated management of FHB of wheat and barley (Schisler, et al. 2000; Luz, W.C. da 2000). The reduction of DON contamination of the harvested grain to acceptable levels remains of critical importance in the management of this disease. In previous exploratory trials the *Bacillus subtilis* isolate, TrigoCor 1448, has shown promise in field and laboratory tests (Stockwell, et al., 1997; Stockwell, et al, 2000). Repeated field trials have been done to demonstrate the efficacy of this bioprotectant when applied to the spikes during flowering in a variety of environments, under varying levels of disease pressure and over several years. Preliminary comparisons can also be made between TrigoCor 1448 and other bioprotectants based on the data generated under field conditions.

## 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 4 times and arranged in a randomized block design. In addition to 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, the resistance elicitor, Messenger (Eden Biosciences; Bothell, WA). Commercial products were applied at labeled rates. In this same trial, TrigoCor 1448 and similarly, TrigoCor 4712 were combined with tebuconazole (4 fl oz Folicur) to determine if the combination would give enhanced FHB control over either bioprotectant or fungicide alone. Bacteria were grown for 5 days in nutrient broth with yeast extract, NBYE, (2-4 X 108 cfu/ml) and applied undiluted as whole broth. 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.

**Bioprotectant Trial - McGowan Field, Ithaca, NY**

Seven treatments were included in the biocontrol trial conducted at Ithaca, NY on "Caledonia" soft white winter wheat. Treatments were replicated 5 times and arranged in a randomized block design.

Messenger (Eden Bioscience Corp., Bothell, WA) was applied on May 4 (Feekes 3.5) and again on May 23 (Feekes 9.5). The bioprotectants TrigoCor 1448 and TrigoCor 4712 were grown with constant agitation in nutrient broth for 5 days and were applied as diluted whole broth. The TrigoCor 1448-Reconstituted was prepared from frozen cells of 5 day-old cultures grown in nutrient broth that were re-suspended in sterile distilled water to the original volume of the broth. The treatments were visually rated for the incidence of Fusarium head blight and for severity. Standard data set was taken from the harvested grain.

**National 2001 Uniform Fungicide/Bioprotectant Trials**

A core set of treatments including TrigoCor 1448 were tested at 14 sites in 13 states. A culture of the bioprotectant was sent along with instructions and dry ingredients to make sufficient NBYE for field application. Undiluted broth of 3 to 5 day old cultures were applied to wheat or barley spikes during anthesis.

**RESULTS AND DISCUSSION**

**Uniform Fungicide/Bioprotectant Field Trial - Musgrave Farm, Aurora, NY**

FHB incidence was shown to be significantly different between treatments. This reflects both the elevated incidence of the Serenade treatment and a substantial decrease in incidence by Folicur (Fig. 1). There was no significant difference between treatments for all other responses including DON contamination of the harvested grain. However, some trends may be discerned for this data. TrigoCor 1448 reduced DON content by 0.6 ppm from the non-treated control (Fig. 2). When Folicur (4 fl oz) was combined with TrigoCor 1448, FHB incidence was reduced by 27% and the DON contamination was reduced by 1.6 ppm compared to non-treated wheat. Similarly, when Folicur was combined with TrigoCor 4712, FHB incidence was reduced by 16% and the DON contamination was reduced also by 1.6 ppm . In comparison, Serenade raised DON levels by 1.0 ppm while the USDA/Peoria yeast lowered DON by 1.1 ppm.

**Bioprotectant Trial - McGowan Field, Ithaca, NY**

Although FHB incidence was shown to be significantly different between treatments, this primarily reflects the elevated incidence of the Serenade and Messenger treatments rather than a substantial decrease in incidence by any treatment. Although not significantly different from the non-treated check, plants treated with TrigoCor 1448 whole broth had the lowest level of DON contamination in the harvested grain, followed closely by theTrigoCor

1448-Reconstituted (washed cells). This represents a 1.08 and 0.96 ppm reduction in DON, respectively. In a year of low scab incidence (over-all incidence of 5.7% and DON contamination of 4.4 ppm), all treatments including Folicur, generally one of the best synthetic fungicides for scab control, had little measurable effect.

**National 2001 Uniform Fungicide/Biological Trials**

At all but the Ohio site, the incidence of FHB was reduced by treatment with TrigoCor 1448 when compared to the non-treated control (Fig. 3). Reduction of DON to market acceptable levels remains the most critical challenge for integrated management of FHB. Of 10 field experiments where non-treated grain was contaminated with greater than 0.5 pmm DON, nine showed a decrease in DON or an increase of less than 0.5 ppm in response to TrigoCor 1448 application (Fig. 4). Again, only the Ohio site produced a result where TrigoCor 1448 increased significantly DON as well as FHB. By contrast, under severe epidemics at Arkansas and Kentucky, TrigoCor 1448 reduced DON by 33% and 39%, respectively. We have no explanation for the contradictory results from Ohio.

**Conclusions -** The modest success of the bioprotectant TrigoCor 1448 in reducing FHB and DON in most, but not all, locations suggests that bioprotectants may be a useful component of integrated management of FHB. The combination of the TrigoCor1448 with the fungicide Folicur gave the most promising results in New York tests and suggests one of the thrusts of future research, the combination of bioprotectants with fungicides. While the results are not spectacular, the consistent reduction in FHB incidence and DON contamination, across many test gives us encouragement to look for ways to increase the efficacy of TrigoCor 1448 and other bioprotectants. There is also a need to elucidate the conditions under which bioprotectants reduce DON levels.

**Acknowledgements -** We wish to thank all of the regional collaborators in the 2001 Uniform Fungicide Trial who included TrigoCor 1448 as a core treatment and provided us with results: Gene Milus/AR, Greg Shaner/ IN, Gary Munkvold/IA, Don Hershman/KY, Arv Grybaukas/MD, Pat Hart/MI, Hala Toubia-Rahme/MN, Laura Sweets/MO, Pat Lipps/OH, Marcia McMullen/ND, Marty Draper/SD and Erik Stromberg/VI.

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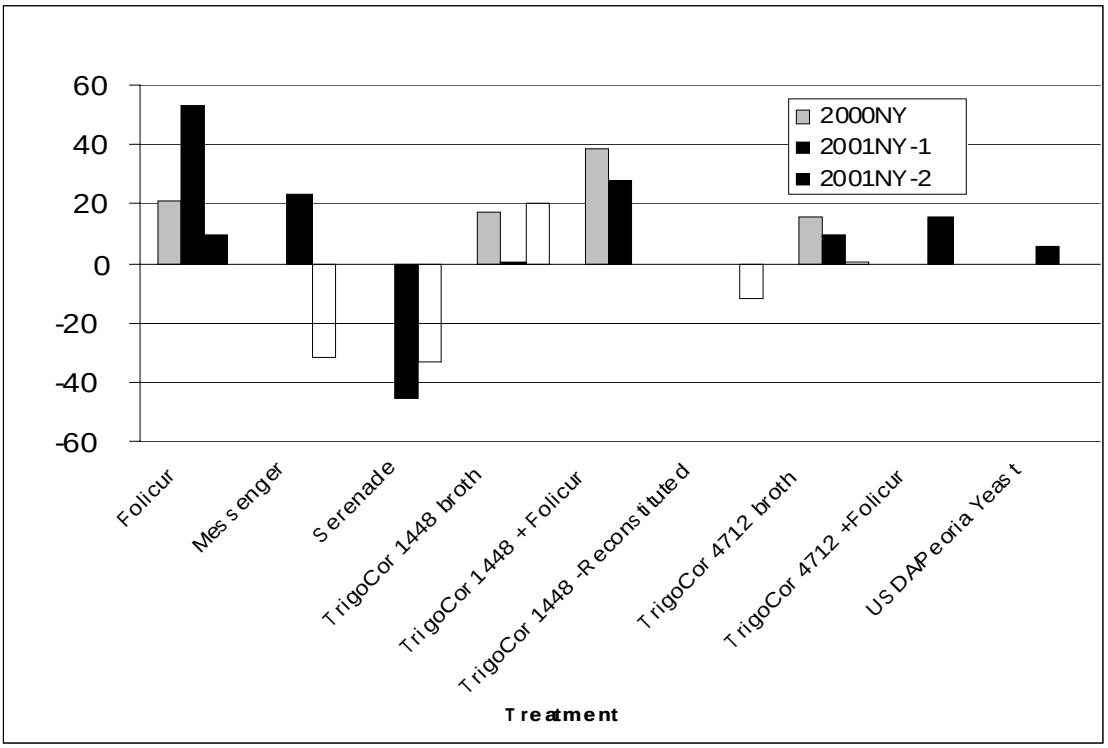


**Figure 1.** Control of Fusarium head blight in NY. Bars represent the % change in the incidence or severity of FHB for each treatment with biological or chemical control (Folicur) in trials located in NY in 2000 and 2001.

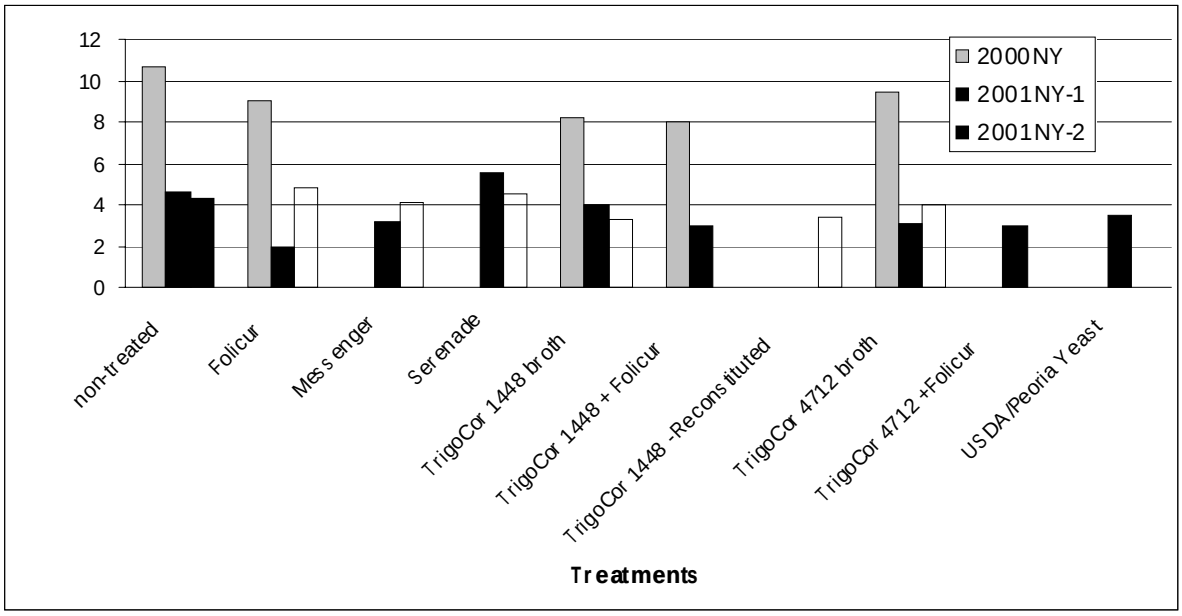


**Figure 2.** Effect of treatments to control Fusarium head blight on DON contamination of harvested grain (ppm) of 'Caledonia' winter wheat in NY in 2000 and 2001.


**Figure 3.** Control of Fusarium head blight with TrigoCor 1448. Bars represent the % change in the incidence or severity of FHB at each of the locations of the Uniform fungicide/biological trial. Trials in which disease incidence was negligible in the non-treated control have not been included in this figure.

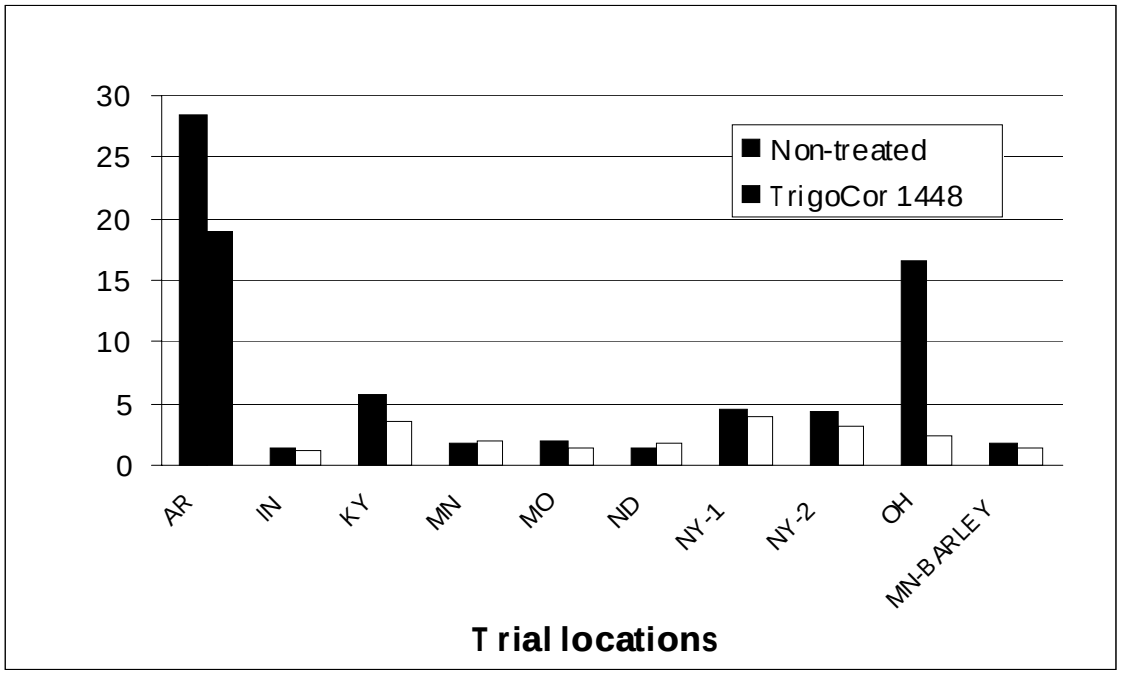


**Figure 4.** Effect of treatment of wheat spikes with TrigoCor 1448 on DON contamination of harvested grain (ppm) of wheat and barley at ten trial locations in eight states in 2001.

# EFFICACY OF FOLIAR FUNGICIDES AND BIOLOGICAL CONTROL AGENTS FOR THE CONTROL OF FUSARIUM HEAD BLIGHT IN SPRING WHEAT

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## ABSTRACT

The effect of four fungicides and two biological control agents on leaf disease severities, Fusarium head blight, grain quality parameters and the production of deoxynivalenol (DON) in the susceptible hard red spring wheat cultivar "Ivan" was investigated in a field trial in Minnesota in 2001. This study was done in collaboration with other researchers in several states that participate in the uniform fungicide trial. The objective of this cooperative study is to assess the performance of these products over a wide range of environments.

The treatments included Folicur (4 fl oz/acre), AMS 21619 (5.7 fl oz/acre), BAS 505 (0.4 lb/acre), BAS 505 + Folicur (0.2 lb + 2 fl oz/acre), Cornell biological agent (TrigoCor 1448, an antagonistic bacterium), and USDA/Peoria biological agent (*Cryptococcus nodaensis* OH 182.9, an antagonistic yeast). These treatments were applied at early flowering. The trials were planted on May 14, 2001. The plots were arranged in a randomized complete block design with four replications. Artificial inoculation of *Fusarium graminearum*, in the form of infected corn kernels were added to the plots on June 25, 2001. Treatments were applied at 40 psi in 20 gpa; using hand-boom sprayers equipped with XR8001 flat fan nozzles angled forward/backward at 30° from horizontal. Fusarium head blight incidence and severity and leaf disease severities was assessed at soft dough stage of kernel development. Plots were harvested for yield and quality measurements, and DON concentrations were determined. Fusarium damaged kernel percentages was determined on the harvested samples. Data collected were subjected to analysis of variance using SAS (Statistical Analysis System). After a significant F test (P = 0.05), treatment means were separated using a least significant difference test at P = 0.05. All treatments significantly reduced leaf diseases that were primarily Septoria and Stagonospora leaf blotches, and FHB severity compared to the untreated control. Three treatments (AMS 21619, BAS 505, and OH 182.9) reduced FHB incidence significantly. All treatments except OH 182.9 reduced significantly the percentage of scabby kernels. Two treatments (BAS 505 and BAS 505 + Folicur) resulted in significantly higher yield compared to the untreated control. Test weight and deoxynivalenol levels were not significantly affected by the treatments compared to the untreated control.

# CONTROL WHEAT SCAB WITH IMPROVED FUNGICIDE APPLICATION TECHNOLOGY - 2001

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## OBJECTIVE

Develop and field test a low-volume, air-assisted, small droplet prototype spraying system specifically applicable for spraying wheat and grasses.

## INTRODUCTION

A project evaluating an MSU prototype sprayer was held at the Michigan Bean and Beet Farm; Saginaw, MI. The MSU sprayer was a low-volume, air-assisted, small-droplet, tower sprayer that was "skid" mounted into the bed of a 4 x 4 pick-up truck. The spray plume moved horizontal to the ground and sprayed a 75 foot wide swath at 4 mph. Folicur was applied at GS 10.5 (June 8th) on the variety Harus using either a conventional boom sprayer using 25 gal of water/acre with flat fan nozzles straight down; or the MSU sprayer using 5 gal of water/acre. Four oz of Folicur + 0.125% Induce, was the only fungicide applied. Each plot was 75 x 525 feet, and the center 30 feet x 525 was harvested on July 16th. The treatments were:

1) Wheat was sprayed from two sides with the prototype to ensure complete coverage of the head with fungicide;
2) Wheat was sprayed on only one side with the prototype sprayer resulting in incom plete coverage;
3) Conventional flat fan sprayer with nozzles aimed downward;
4) Untreated controls.

There was only one replication per treatment. Twenty-five grain probes per treatment were collected directly from the combine at harvest. Each probe sample was analyzed was analyzed separately for DON (Hart, et al, 1998). The plots were not rated for yield or disease severity.

## RESULTS AND DISCUSSION

Treatments were not evaluated for FHB incidence, severity or yield. DON levels in the different treatments were:

| Treatment | DON (PPM) | Standard Deviation |
|---|---|---|
| 1 | 0.3 | 0.10 |
| 2 | 0.9 | 0.21 |
| 3 | 0.9 | 1.17 |
| 4 | 0.9 | 0.25 |

Although these results are preliminary and not replicated, they do suggest that thorough coverage of the wheat head is essential to reduce DON, and new technologies using very low spray volumes may compete very will with conventional sprayers.

The oral presentation will include a "five minute" video that illustrates the application technologies used.

| TIME (min:sec) | TOPIC |
|---|---|
| 0:00 | Original ‘field testing”of the truck-mounted sprayer in a grass field. |
| 1:50 | Operating the truck-mounted sprayer in a wheat field at the Michigan Bean and Beet Farm. |
| 2:30 | Using an alternate ‘air-assisted”spraying technology ( ®PROPTEC) in wheat. (Note: this sprayer was used for a 2001 study of fungicide application to sugar beets in a nearby field. Originally, we had intended to include it in this study.) |
| 3:05 | Self-propelled, Hagie sprayer with a ‘50 foot”wide PROPTEC boom spraying asparagus. |
| 4:00 | Spraying Christmas trees with the truck-mounted wheat sprayer. |
| 5:00 | End |

# PROGRESSION OF *FUSARIUM* SPECIES ON WHEAT LEAVES FROM SEEDLING TO ADULT STAGES IN NORTH DAKOTA

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## ABSTRACT

A more complete inventory of inoculum sources would help our understanding of epidemics of Fusarium head blight (FHB). We hypothesize that *Fusarium* species can colonize and survive on leaves in the US northern Great Plains. In 2001, two hard red spring wheat cultivars Alsen (resistant to FHB) and Oxen (susceptible) were planted in a wheat stubble or plowed area in three replicate subplots, each 1 X 2 m. Twenty healthy (asymptomatic) and diseased (with necrotic leaf spots) leaf samples of each cultivar were collected from the 4-leaf to the early milk stage. Leaf samples were collected on 6/7, 6/14, 6/21, 6/28, and 7/5. The leaves were cut into 2 cm pieces. Ninety-eight leaf pieces of each sample were plated on Komada's medium. Forty-nine of 98 leaf pieces were surface disinfected with 5% sodium hypochloride prior to plating. The leaf pieces were incubated at 22 C with an alternating cycle of 12 h light and 12 h dark for 10 days. Different colony types of Fusarium species were counted and transferred onto ½ strength PDA for species identification. Twelve *Fusarium* species were isolated: *acuminatum, avenaceum, equiseti, graminearum, moniliforme, proliferatum, poae, sporotrichioides, subglutinans, scirpii,* and *semitectum*. *Fusarium graminearum* and *Fusarium sporotrichioides* were the most prevalent species after *Fusarium equiseti* throughout the season. *Fusarium graminearum* was isolated from healthy disinfected leaves (0-6%, depending on date sampled), healthy nondisinfected leaves (2-12%), diseased disinfected leaves (2-18%), and diseased nondisinfected leaves (4-52%). *Fusarium sporotrichioides* was isolated from healthy disinfected leaves (0-14%), healthy nondisinfected leaves (0-16%), diseased disinfected leaves (0-18%), and diseased nondisinfected leaves (2-42%). *Fusarium avenaceum* was isolated from both healthy (0-6%) and diseased (0-10%) leaves but *F. poae* was observed only on diseased leaves (0-22%). Diseased leaves produced pathogenic Fusaria at a higher frequency than healthy leaf samples but a difference between the plowed and stubble areas was not detected. The results indicate that *Fusarium* species associated with FHB, such as *graminearum*, *sporotrichioides, avenaceum,* and *poae*, can survive parasitically and saprophytically on leaves throughout the season; furthermore, these leaves may contribute additional inoculum for FHB development in the northern Great Plains. Management of foliar pathogens may help in reducing FHB intensity by decreasing the amount of inoculum.

# EFFECT OF *FUSARIUM GRAMINEARUM* INFECTION DURING WHEAT SEED DEVELOPMENT ON SEED QUALITY

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## OBJECTIVES

1) Determine the time of infection of *Fusarium graminearum* during wheat seed development and its effect on seed quality.

2) Investigate effect of disease tolerance and susceptibility on severity of seed infection

## INTRODUCTION

Head scab caused by *Fusarium graminaerum* (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 can not only result in significant yield losses, but also cause serious reductions in seed quality. In contrast to seeds used for other purposes, seeds planted to regenerate the crop must be alive and possess those physiological traits that allow germination and seedling establishment (TeKrony and Egli, 1991). The pathogen may affect both the physical and the physiological aspects of seed quality, including seed size, composition, germination, and vigor. In addition, losses in food-grain quality are caused by production of the fungal mycotoxin deoxynivalenol (DON), which, in high levels, renders grain unsafe for human or animal consumption, and is detrimental to planting seed quality.

Previous studies have examined seed germination, vigor and DON concentrations of mature wheat seeds based on visual assessment of kernel disease severity. However, relatively few studies have investigated the development of *F. graminaerum* infection during seed development and maturation and its influence on planting seed quality. Likewise, little information is available regarding when peak infection occurs during seed development and maturation and how these infection levels relate to seed germination and vigor and the production of DON. Considering that the seed is the delivery system for improvements in germplasm and the source for regeneration of new cultivars, it provides a vital link between FHB research initiative and the farmer.

## MATERIALS AND METHODS

Replicated plots of four soft red winter wheat cultivars differing in Type II resistance to *F. graminearum* were established following corn in a chisel plowed and disked seedbed on Spindletop Farm in Lexington, KY in October of 2000. Cultivars included one susceptible (Pioneer 2552), two moderately resistant (Roane, Coker 9474) and one resistant (Pioneer 25R18) line. The corn seed inoculation procedure used to initiate FHB epidemic conditions was modeled after the method of Paulitz (1996) and inoculum was distributed among field plots on March 24, 2001. Mist irrigation was initiated on April 10, 2001and continued

through June, 4 to stimulate FHB epidemic conditions. Ambient air temperature and precipitation were measured at the field site. At anthesis (Feekes 10.2), approximately 1,200 spikes in each of two replications of each cultivar with anthers extruded in mid-spikelet were identified. At four days after anthesis (DAA) 75 previously marked heads were harvested, and harvesting continued at four-day intervals for a total of ten harvests in each cultivar.

Fresh weight, dry weight and seed moisture were determined at each harvest for all cultivars. Thirty heads from each cultivar were evaluated at each harvest for disease incidence and severity according to Stack and McMullen (1998). One-hundred seeds from each harvest were assigned a numerical rating as an indicator of disease severity and classified as normal, normal-discolored, brown-shriveled, chalky-shriveled, and white tombstone (levels 5 to 1 respectively) prior to plating for infection.

One hundred fresh seeds from seed of approximately 35 heads was separated from chaff, surface sterilized, plated on modified PDA medium, and evaluated for F*usarium graminearum* infection approximately seven days post-harvest. Standard germination (AOSA, 1999) accelerated aging germination, a stress vigor test, and the conductivity test for membrane integrity (Hampton and TeKrony, 1995) were conducted for seed of all harvests.

## RESULTS AND DISCUSSION

Anthesis occurred in all cultivars between May 10-14, 2001. Physiological maturity (PM, maximum seed dry weight) occurred between 30 and 32 days after anthesis (DAA). F. graminearum seed infection (freshly harvested seed) increased in all cultivars from = 20% at 10 DAA, to maximum levels (>95%) at 37-40 DAA, which were maintained until the final harvest (~50 DAA, Fig. 1A). Seed infection for Coker 9474 was slightly lower than that of P-2552, P-25R18, and Roane until 30 DAA, when all cultivars began to exhibit similar infection levels. Average seed infection over all 10 harvests was significantly higher for P-2552 and Roane than P-25R18 and 9474 (63.2 and 62.7% vs. 56 and 51% respectively). Two large precipitation events preceded the most abrupt increases in seed infection with one of these occurring after irrigation was stopped (23 DAA). Roane and P-2552 provided consistently higher visual estimates of spikelet infection than P-25R18 and 9474 at each harvest date. A significant linear relationship was shown between visual estimate of spikelet infection (severity) and *F. graminearum* seed infection for the first 5 harvests in all cultivars ($r^2$ = 0.858, Fig. 1A, inset).

Standard germination (SG) of untreated seed for the four cultivars was highly variable in early harvests (Fig. 1B) ranging from <40% (Coker 9474) at 10 DAA, to above 80% at 19 DAA. Germination declined to unacceptable commercial quality (<80%) in all cultivars by 25 DAA and continued to decline to approximately 30% at the last harvest. Although the germination of all cultivars was low, P-25R18 showed the highest germination at PM (57%), which was significantly higher than the other three cultivars for the remaining harvest dates. Standard germination was significantly lower for P-2552 (43%) across all harvests compared to P-25R18, Roane, and 9474 (mean = 51% for all 3 cultivars respectively). Standard germination of treated seeds was significantly higher than untreated (Fig. 1B, inset) for the last five harvest dates, with germination maintained above 50%. Germination of treated seed

of P-25R18 was greater than other cultivars, with SG ranging from 75% at PM, to ~65% at nearly 50 DAA. Overall means for treated seed across all cultivars was 61% compared to 48% in untreated seed. As expected, there was a significant correlation between SG (untreated) and *F. graminearum* seed infection (r2 = 0.567).

Small grain crops are most susceptible to infection during the flowering period and infection continues up to the soft dough stage of kernel development (McMullen and Stack, 1997). Type I resistance to initial infection appeared to be slightly more prevalent in Coker 9474, based on isolation of *Fusarium graminearum* from seed, and the visual rating of spikelet infection. Interestingly, this advantage was not readily obvious when measuring seed quality. Seed infection levels were very high in all cultivars, including the Type II resistant Pioneer 25R18. Concurrently, germination was very poor in all cultivars, with P-25R18 showing marginal improvement in the last five harvests compared to the others. High levels of field inoculum resulted in very high seed infection levels, which may have masked the advantages of Type II resistance ascribed to P-25R18, but provided a selective environment for evaluation of Type I resistance. Measures of seed quality (SG, seed vigor, etc.) could potentially be useful in assessing varieties earlier in development for Type I resistance and also resistance to kernel infection, as described by Mesterhazy (1995). This could serve as an additional protocol for breeder selection of promising germplasm. Retention of seed germination after PM may be of great interest to seed producers, since a crop could be harvested earlier (swathing at PM) to avoid late season disease pressure and declines in seed quality.

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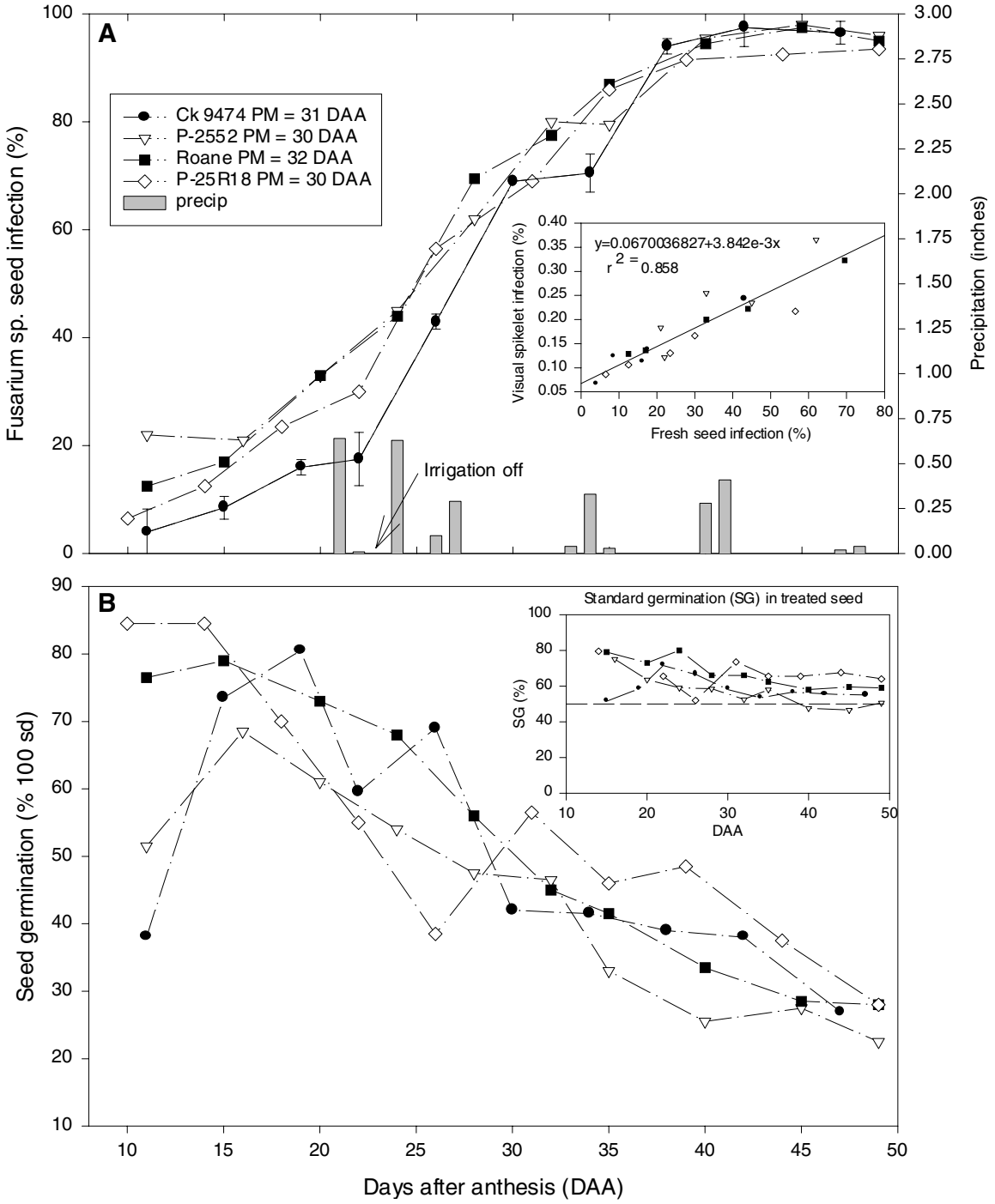


**Figure 1.** *Fusarium graminearum* seed infection during development in four varieties of wheat and effects on germination of treated and untreated seed.

# ARE *GIBBERELLA ZEAE* SEXUAL SPORES THE CRITICAL INOCULUM LEADING TO FHB EPIDEMICS?

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## ABSTRACT

*Gibberella zeae* (anamorph *Fusarium graminearum*) causes Fusarium head blight (FHB) epidemics in wheat and barley and ear rot in corn. Fungal infections decrease yield and often contaminate grains with trichothecene mycotoxins that are harmful to human and animal health. To understand and control fungal disease, the factors and conditions that lead to epidemics must be identified. The fungal life cycle in the field can include growth on plant debris in the soil during which two types of spore may be produced: sexual spores known as ascospores and asexual spores called macroconidia. Observations in the field suggest that the sexual spores may be a primary source of inoculum for FHB epidemics. In order to test the role of sexual spores, we deleted the entire mating type locus (MAT) that controls sexual reproduction. *G. zeae* MAT-deletion strains appear similar to wild-type (GZ3639) in morphology and in their ability to make macroconidia but no longer make sexual spores. In greenhouse tests, macroconidia from MAT-deletion strains caused disease and produced trichothecenes following inoculation into wheat heads. To test the importance of sexual spores in disease development, we conducted a field test in Spring 2001 in which we compared the ability of GZ3639 and a MAT-deletion strain to cause FHB on wheat. In order to focus our experiment on ascospores, we chose autoclaved corn stalk pieces as an inoculum source because GZ3639 can produce a large amount of ascospore-containing perithecia on it. We inoculated three plots (3m x 3m) of Wheaton wheat, with either sterile corn stalks, corn stalks inoculated with GZ3639 or corn stalks inoculated with a MAT-deletion strain. We scattered 100 stalks per plot between wheat rows at three weeks and at one week prior to flowering. Seed yield data and trichothecene analysis indicated that the MAT-deletion strain caused less disease and resulted in less trichothecenes than GZ3639. These results suggest an important role for sexual spores in FHB disease severity and toxin level and suggest that new control strategies that target *Fusarium* ascospore production might lead to significant reduction in the negative impact this fungus has on agricultural products.

# WHAT IS KNOWN ABOUT INFECTION PATHWAYS IN FUSARIUM HEAD BLIGHT?

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## ABSTRACT

The epidermis of the exposed outer surfaces of the florets of wheat and barley and the glumes subtending wheat spikelets consists of very thick-walled cells. These armored cells apparently are not penetrated directly from outer surfaces by *Fusarium graminearum* or other head blight Fusaria. However the glume, as well as the palea and lemma that enclose the floret, each have several rows of stomates which can be entered by hyphae of *F. graminearum.* Whether such stomatal entry leads to significant fungal invasion of florets or glumes is uncertain, although thin-walled chlorenchyma cells located beneath stomates are postulated to be sites of colonization following stomatal penetration. Another possible avenue of entry is the mouth at the apex of the floret. Exposed adaxial surfaces of the awn, palea and lemma near the floret mouth can be colonized by *F. graminearum* mycelia which then extend along adaxial surfaces basally into the mouth opening where they can colonize caught and retained anthers or the apical brush of the caryopsis (abstract of Lewandowski and Bushnell). The mycelia can also colonize interior surfaces of the palea and lemma . Another potential pathway of entry is the crevice between the palea and lemma, especially near the floret base (Kang and Buchenauer, Mycol. Res. 104:1083-1093, 2000; abstract of Lewandowski and Bushnell). Whether entering from mouth or crevice, the fungus can develop abundantly on and within interior tissues of the lemma and palea. Surface tissues of the ovary and lodicules are especially susceptible (Tu, D.S., Ph. D. Thesis, Ohio State Univ., 1950). However, the mode of penetration into these interior tissues has not been determined. Within tissues, *F. graminearum* can grow between cells instead of entering them, establishing a biotrophic relationship with host tissues. How and where the intercellular fungus penetrates cells for subsequent growth within cells is not known. Eventually, virtually all interior floret tissues can become heavily colonized. Once established within the floret, the fungus colonizes and follows vascular tissues in the floret stalk through the rachilla or rachis into other florets. Wall appositions containing callose and lignin have been implicated as factors reducing fungus spread in heads of resistant wheat (Kang and Buchenauer, Physiol. Mol. Plant Pathol. 57:255-268, 2000). Furthermore, defense response genes are known to be activated in wheat heads inoculated with F*. graminearum.* However, the molecular and physiological responses of heads to invasion by head blight Fusaria are largely uninvestigated, whether in resistant or susceptible plants. On the fungal side, *F. culmorum* produces cell wall degrading enzymes in infected floret tissues (Kang and Buchenauer, J. Phytopathology 148:263-275, 2000). Fungus-produced trichothecene toxins such as deoxynivalenol (DON) contribute to virulence of *F. graminearum*. DON, which is a potent inhibiter of protein synthesis and is postulated to inhibit activation of defense response genes, can induce complete loss of chloroplast pigments at sublethal concentrations (abstract by Seeland and Bushnell). Nevertheless, there is much to be learned about pathogenesis in Fusarium head blight at both the molecular and cellular levels.

# INFLUENCE OF ENVIRONMENT ON INOCULUM LEVEL AND FUSARIUM HEAD BLIGHT SEVERITY

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## OBJECTIVES

Develop a disease forecasting system for wheat Fusarium head blight based on the environment and inoculum level.

## INTORDUCTON

Epidemics of Fusarium head blight (FHB) have had a devastating impact on wheat production throughout North America (McMullen et al. 1997). The development of a reliable disease forecasting system would greatly increase the ability of wheat producers to make disease management and grain marketing decisions. Recent attempts to predict FHB have emphasized the importance of both inoculum and environment to disease epidemics (Francl et al. 1999, DeWolf et al. 2001). However, the interactions between environment, inoculum and disease level have not been sufficiently quantified to allow the development of a disease forecasting system. This report will summarize the information obtained during 2001 to further illustrate the findings of a cooperative effort among researchers in OH, IN, SD, ND, PA and MB to create a FHB forecasting model (De Wolf et al 1999, De Wolf et al. 2000).

## MATERIALS AND METHODS

Adapted, FHB-susceptible cultivars were grown with standard agronomic practices in replicated plots near Wooster, OH (Hopewell SRWW), Fargo, ND (Norm HRSW), and State College, PA (Hopewell). The environment at each location was monitored by an automated weather station equipped with temperature, relative humidity, precipitation, and surface wetness instrumentation. Each day, 5 wheat heads were collected from each of three replicated plots (n=15) by cutting the stem just above the first node. Heads where then transported to a laboratory for further processing. In the laboratory, the 5 heads from each rep were placed in a 250 ml flask containing 50 ml of sterile distilled water plus Tween 20 (1 drop/100ml). Flasks were shaken vigorously for 2 min to dislodge spores from the spikes, and 1 ml of the spore suspension transferred to replicate plates of Komata's media. The plates were incubated for 10 to12 days and daily inoculum level estimated from the number of *Gibberella zeae* cfu's observed. Other *Fusarium* species were also recovered from the wheat heads, but only *G. zeae* will be considered for this report. Growth stage of the wheat plots was assessed daily, and FHB incidence and severity evaluated during the early dough stage.

## RESULTS AND DISCUSSION

Disease incidence and severity were higher at the Ohio location than at the North Dakota and Pennsylvania locations during the 2001 growing season (Table 1). Inoculum level as estimated by the number of *G. zeae* colony forming units per wheat spike per day was greater at the North Dakota location relative to the other locations considered in this analysis (Figure 1). The North Dakota inoculum levels were punctuated by a single prominent peak that reached a maximum of 138 cfu's per spike per day. This peak in *G. zeae* inoculum was associated with 4 consecutive days of precipitation, and coincided with crop anthesis at this location. In comparison, the number of *G. zeae* cfu's observed at the Ohio and Pennsylvania locations ranged from 1 to 30. The highest levels at these locations did not occur until near the end of anthesis, and peaks in inoculum level were not strongly associated with precipitation events.

The average wetness duration summarized from the beginning of anthesis until the early milk stages of growth was 4 h less at the North Dakota location than at the Ohio and Pennsylvania locations (Table 1). The North Dakota location also received equal amount of precipitation as did the Pennsylvania location, and 23 mm less precipitation than the Ohio location. Average daily temperature at the Ohio location was within 2 degrees of the Pennsylvania location; however, average temperature at the North Dakota location was >9 degrees higher than the other locations.

Differences in disease level among the locations can be attributed, in part, to differences in temperature and moisture parameters, which likely influenced inoculum levels and infection periods. For example, the disease intensity observed at the North Dakota location may be explained in part by high inoculum levels, and consecutive days of favorable moisture and temperatures during crop anthesis. In contrast, the combination of low temperatures and low inoculum levels during anthesis may explain the low disease intensity observed at the Pennsylvania location. The high disease intensity observed at the Ohio location relative to the Pennsylvania location despite similarities in average wetness duration, temperature, and relative humidity were likely influenced by the frequency and magnitude of precipitation events during crop anthesis.

These results demonstrate the importance of monitoring both inoculum and environment in the development of FHB epidemics. As additional information is collected, modeling fluctuations in inoculum level and infection periods based on environment should be possible. This database development is quickened by our experimental approach that utilizes multiple locations.

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**Table 1.** Summary of weather conditions during crop anthesis and Fusarium head blight (FHB) intensity observations in research plots at three North American locations.

| Variable | Location | | |
|---|---|---|---|
| | Pennsylvania | North Dakota | Ohio |
| Avg. wetness duration (h) | 13 | 9 | 13 |
| Avg. temperature (C) | 15 | 25 | 13 |
| Avg. relative humidity (%) | 75 | 79 | 80 |
| Total rainfall (mm) | 26 | 26 | 49 |
| Plot FHB incidence (%) | 0 | 67 | 71 |
| Plot FHB severity (%) | 0 | 16 | 42 |

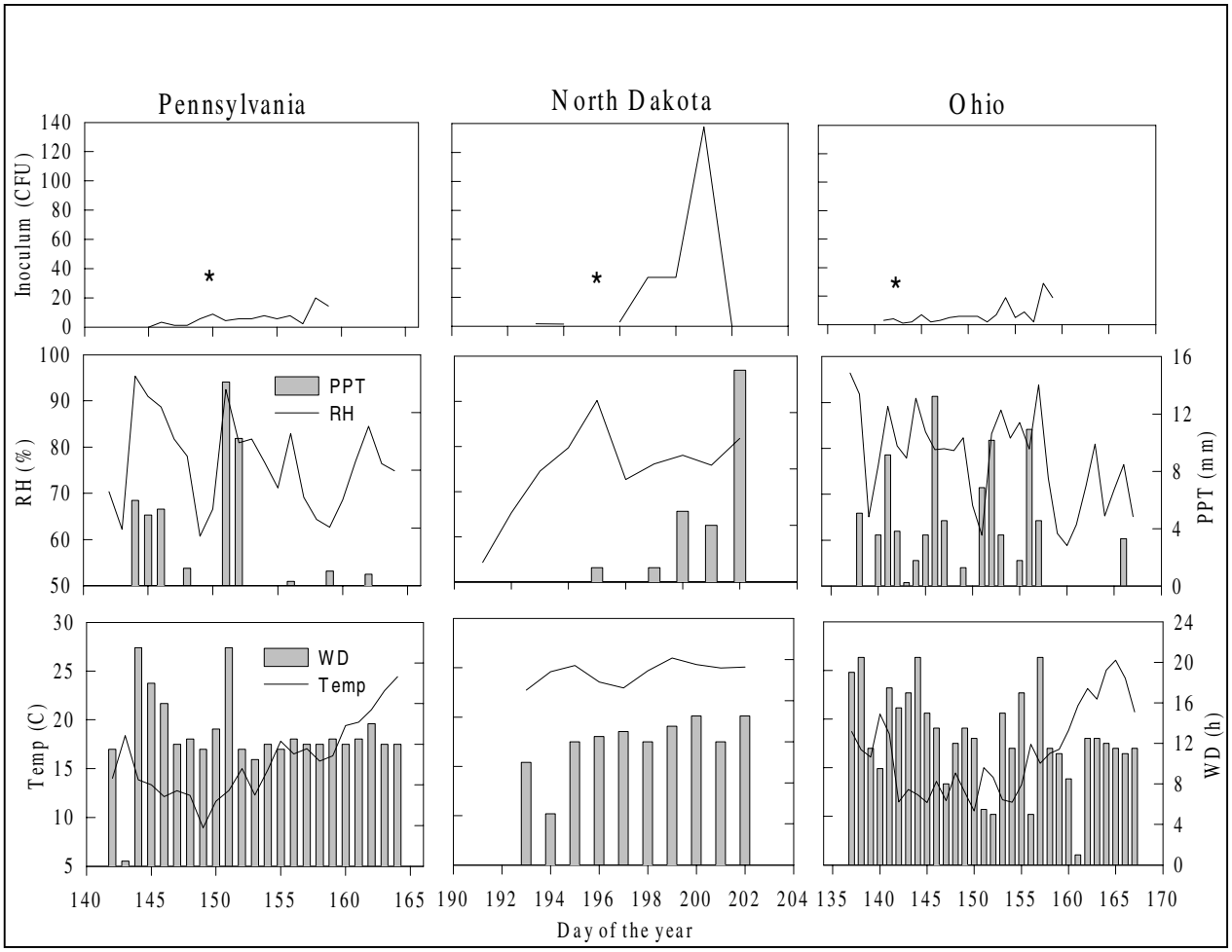


**Figure 1.** Summaries of environment and inoculum levels at research locations in Pennsylvania, North Dakota, and Ohio. Each series of plots gives the temperature (Temp), relative humidity (RH), precipitation (PPT), wetness duration (WD), and *G. zeae* inoculum level. Inoculum level was estimated from the number of colony forming units rinsed from wheat spikes collected from replicated plots of susceptible wheat varieties. The * designates the beginning of crop anthesis.

# THE NEPAL *GIBBERELLA ZEAE* PROJECT

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## ABSTRACT

Sampling of a small area (< 12 km2) of Lamjung district in the Nepal Himalaya has identified *Gibberella zeae* populations with an unusually high level of biodiversity. From smallholder farms in this area, we collected *G. zeae* strains from five kinds of representative samples: seed samples of wheat, rice, and maize, and samples of soil debris and weeds. We classified the genetic diversity of 575 strains using a species-specific, sequence characterized (SCAR), polymorphic marker, that placed all of the strains into one of five previously identified SCAR types (1). Each of the four major SCAR types, designated 1, 2, 3, and 5, was represented in each of the five kinds of samples, but the relative proportions of the SCAR types varied. On wheat, for example, SCAR type 3 comprised 62% of strains, type 5 comprised 22% of strains, and types 1, 2 and 4 were rare. In contrast, type 2 comprised half of the strains isolated from maize.

To investigate associations between genotype, virulence, and toxin production, we characterized 250 representative strains for virulence on wheat heads in the greenhouse by measuring average % spikelets blighted for ten replicate heads at 17-19 days after inoculation. We also measured production of the trichothecene toxins nivalenol (NIV) and deoxynivalenol (DON) by GC-MS analysis of seeds pooled from ten replicate heads. One-third of the strains tested produced only DON and two-thirds produced NIV. In SCAR types 1, 2, and 5, >95% of the strains in each type produced the same toxin, either DON or NIV, but in type 3 equal numbers of DON-producers and NIV-producers were present. Virulence on wheat was influenced significantly both by SCAR type and toxin type, with higher virulence on wheat associated with DON production. As previously suggested (1), types 2 and 3 may show some host specialization, since type 2 is rare on wheat and has the lowest virulence on wheat, while type 3 is relatively common on wheat and has high virulence on wheat. The Nepal population of *G. zeae* thus appears to differ from the North American populations studied to date, which have not demonstrated host specialization (2). Characterization of virulence of Nepal *G. zeae* strains on maize is being conducted by Gyanu Manandhar at the Nepal Agriculture Research Council.

Because the SCAR analysis measures genetic variability at only one locus, additional studies are in progress using amplified fragment-length polymorphisms to obtain information on fine genetic structure of the population of *G. zeae* from Nepal. These genetic studies should elucidate the level of genetic differentiation between the SCAR types and their potential for interbreeding and genetic recombination. This work should allow us to determine whether Nepal is a center of diversity for *G. zeae*, and to evaluate the potential of the Nepal *G. zeae* population for generation of novel genotypes of potential concern for wheat head blight management worldwide.

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# COMPARISON OF TWO METHODS FOR ESTIMATING SCABBY KERNELS IN FUSARIUM-INFECTED SPRING WHEAT

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## ABSTRACT

Grain from eight spring wheat lines was used to compare two methods for estimating scabby kernels. The wheat was grown in replicated field plots inoculated with six rates of Fusarium-colonized grain inoculum including a non-inoculated fungicide-protected control. Percentage of tombstones (TMB) was determined by separating grain into sound and scabby kernels and counting kernels in each category. Percentage of visually scabby kernels (VSK) was estimated by matching the sample to standards generated by mixing healthy and scabby kernels of a hard red spring wheat. Mean, minimum, and maximum values for 191 samples examined were 17.5%, 1%, and 40% for TMB and 14.3%, 0%, and 50%, for VSK, respectively. The Pearson correlation coefficient of TMB with VSK was 0.78 for the individual samples. Correlation of TMB with VSK for the means of the 48 cultivar-inoculum treatments was highly significant (r = 0.92). The results indicate that the two methods tested are comparable as visual estimates of damage to wheat grain from *Fusarium* infection.

# EFFECT OF BURNING WHEAT AND BARLEY RESIDUES ON SURVIVAL OF *FUSARIUM GRAMINEARUM* AND *COCHLIOBOLUS SATIVUS*

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## ABSTRACT

Cereal residues left on the fields due to minimum tillage increase the inoculum potential of pathogenic fungi. The effect of residue burning on the viability of Fusarium head blight (*Fusarium graminearum)* and spot blotch/common root rot (*Cochliobolus sativus*) pathogens was studied in wheat and barley residues burned one month after harvest using a flame thrower. Remaining residues were collected and stored at -10 C until isolations were made on PDA and Komada's media in late fall. Wheat node counts in straw pieces ranged from 58-137 and from 161-487 in burned plots and in control plots, respectively. Recovery of *F. graminearum* (FG) and *C. sativus* (CS) were significantly (P=0.001) reduced in burned residues (FG, 6%; CS, 10%) in comparison with the non-burned residues (FG, 26%; CS, 40%). Recovery of both pathogens was almost nil from totally charred residues. Recovery of *F. culmorum*, *F. avenaceum,* and *F. sporotrichioides*, and other fungi followed the same patterns. Our data shows that residue burning can reduce the inoculum potential of pathogens present in residues, and may assist in the management of destructive diseases such as Fusarium head blight.

# MANIPULATING ARTIFICIAL EPIDEMICS OF FUSARIUM HEAD BLIGHT IN WHEAT WITH INOCULUM CONCENTRATION

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## ABSTRACT

The poor repeatability of artificial Fusarium head blight (FHB) epidemics generated in field nurseries is likely due to methodological problems of inoculation, the variability of the fungus, and the confounding effects of environmental parameters on disease development. The ability to manipulate disease development through the application of macroconidia inoculum was examined in field experiments in St. Paul MN. Six concentrations of macroconidial inoculum (0, 6250, 12500, 25000, 50000, and 100000 macroconidia per ml) were applied to 2.4 m long two-rowed plots of the spring wheat cultivars Norm (susceptible), McVey (moderately resistant - MR), Pioneer 2375 (MR), and BacUp (resistant). The experimental design was a split-plot with four replications, with cultivar as the main plot and inoculum concentration as the sub-plot. Inoculum was applied to each plot at the appropriate concentration at anthesis and 3 and 7 days after anthesis. Plots were mist-irrigated to promote disease development for 30 minutes immediately following inoculation events and daily (8 mm per day) between inoculation and the final disease assessment. Visual assessment of twenty primary heads in each plot was used to determine disease incidence (DI) and disease severity (DS) at 14, 19, and 24 days after anthesis. Grain was harvested at maturity and used to determine 200-grain weight, visually scabby kernels (VSK) and deoxynivalenol concentration (DON). Disease levels increased with increasing inoculum concentrations irrespective of the wheat cultivar examined. The mean DI and DS (all wheat cultivars) for the six inoculum concentrations was 28%, 42%, 43%, 64%, 78%, and 93%; and 7%, 14%, 18%, 27%, 41%, and 55%, respectively. Differences among the inoculum treatments were evident in DI and DS at all three assessment and cultivar rankings were consistant with previous evaluations (DS - BacUp, 9%; McVey, 20%; Pioneer 2375, 32%; and Norm, 49%). Visual differences in disease symptoms were evident in the VSK and DON levels of harvested grain. By increasing inoculum concentrations we were able to generate increasing levels of disease, even in 2001 when a lack of rainfall between anthesis and the last inoculation date made environmental conditions highly adverse to FHB development. Information gleaned in this study will improve our ability to manipulate the level of disease in field nurseries screening germplasm for resistance to Fusarium head blight.

# MODIFICATION OF A CROP RESIDUE MOISTURE SENSOR FOR APPLICATIONS IN THE EPIDEMIOLOGY OF FUSARIUM HEAD BLIGHT

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## ABSTRACT

Corn residue remaining on the soil surface is a major source of *Gibberella zeae* inoculum in regions where small grains and corn are incorporated into crop rotations. However, the role of temperature and moisture in *G. zeae* perithecia development on corn residues have not been investigated. Sensors that can be used to continuously monitor the moisture status of crop residues were adapted for use with corn stalks. The modified sensors incorporate improvements that should improve sensor durability in a corrosive environment, and increase compatibility with other environmental monitoring instrumentation. Preliminary results indicate that the modified sensors have similar range in moisture sensitivity when compared to previously used moisture sensors. The modified sensors will be used to help evaluate the effect of temperature and moisture on perithecia development in growth chamber and wheat field environments. In the future it maybe possible to predict inoculum release events based on environmental variables including the temperature and moisture status of crop residues.

# *FUSARIUM GRAMINEARUM* INFECTION AND MOVEMENT IN FLORAL COMPONENTS OF WHEAT SPIKES

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## ABSTRACT

Many plant breeders screen wheat cultivars for Type II resistance to Fusarium head blight resulting from *Fusarium graminearum* (Schwabe) infection by visual spikelet evaluation following the single floret inoculation system. The objective of this study was to determine the relationship between the level and rate of *F. graminearum* infection in floral components of wheat spikelets and the visual ratings of spikelet infection in the greenhouse. Several spikes of VA96W-326 were inoculated in a middle spikelet at anthesis, misted at high RH for 3 days, and harvested at 5, 10, 15, and 20 day intervals post inoculation. The spikelets were labeled in relation to location above and below the point of inoculation (PI). Fresh seed from the left floret of each spikelet and dry components [glume, lemma, seed, palea] from the corresponding right floret of each spikelet and the rachis (below spikelet) were plated on a modified PCNB agar to determine *F. graminearum* infection. *F. graminearum* infection in both florets increased with maturity with the most significant change occurring between 5 and 10 days. After 5 days, *F. graminearum* infection occurred primarily at the PI for all components, except the glumes, which showed no infection. After 10 and 15 days, a large increase in infection was observed in all components in all spikelets below the PI, while infection declined sharply to zero above the PI. Rachis infection was greater than infection in all other components after 10 and 15 days. There was no significant difference in seed infection between fresh seed from the left floret and the corresponding dry seed of right floret from the same spikelet. Greenhouse ratings of individual spikelet infection showed a strong relationship with the bioassay of the corresponding seed infection at 10 and 15 day harvests, but not at 20. This study of one cultivar, VA96W-326, shows maximum infection of floral components 15 days after inoculation, which should be related to the timing of visual ratings of spikelet infection in the greenhouse.

# INFLUENCE OF CULTIVAR AND PLANTING DATE ON FUSARIUM HEAD BLIGHT DEVELOPMENT ON WINTER WHEAT IN OHIO

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## ABSTRACT

Scab or Fusarium head blight, caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*) is a devastating disease of wheat and barely in the United States and around the world. Scab outbreaks of varying intensity have been common and widespread across much of the eastern half of the United States, affecting yield and grain quality. This disease has caused losses up to $1 billion per year. In addition, reductions in grain yield, kernel size, test weight, and associated DON accumulation in the grain often result from severe disease epidemics. Host resistance and cultural practices have long been considered effective means of disease control. No single disease management strategy is likely to control this disease because of lack of availability, excessive cost, or negative impacts on soil conservation. To control Fusarium head blight, multiple disease management strategies will be required. Therefore, the objectives on this research were: i) to evaluate disease development on three wheat cultivars planted in three different dates, and ii) to determine the relationships between cultivar and/or planting date with yield and DON content.

Seeds of three wheat varieties (Hopewell, Elkhart, and Paterson) treated with Raxil-Thiram, were planted using 24 seeds/ft of row on three planting dates (18 Sep., 2 Oct., and 16 Oct, 2000) in Ravenna silt loam at the Ohio Agricultural Research and Development Center near Wooster. Prior to planting, the field was moldboard plowed, then 200 lb/A of granular fertilizer (6-24-24) was broadcast over the field and incorporated with a disc. In addition, 200 lb of ammonium nitrate was applied on 28 March. For each variety and each of the planting dates, there were three replicate plots. Each plot was 35-ft long, and consisted of 7-rows with 7 in. between rows. Corn stalks colonized by *Fusarium* sp. were placed between plots. In each plot, disease assessments were made three times a week (June 11 - June 26) for both incidence and severity in a one ft. area across the plant rows at 10 locations. Each location was marked with a tall flag that remained in the field during the period of assessment. Disease incidence was calculated as the percentage of heads with disease, and severity was calculated as the average percentage of affected florets per head. Plots were harvested on 17 of July. Yield (bu/A) was determined from harvested grain adjusted to 13.5% moisture. Harvested grain was visually assessed for the damage kernels, and grain was analyzed for DON content.

Disease development varied in the three planting dates among the three cultivars. There was more disease in the last planting date (16 Oct.). Elkhart cultivar had the maximum disease incidence and severity (80.2 and 44.3%, respectively). On the other hand, Patterson cultivar had the lowest disease incidence and severity (58.2 and 30.1%, respectively).

No significant differences were found in yield among the three cultivars through the three planting dates, but there were significant differences among cultivars in DON content through the three planting dates. In general, DON content was highest in the last planting date for the three cultivars. Elkhart had the highest level (10.4 ppm) of DON, and Patterson had the lowest level (2.2 ppm) of DON content. There were significant differences in test weight among cultivars. Elkhart and Hopewell had significantly higher test weight at the mid-planting date (59.9 and 59.7 lb/bu, respectively).

In conclusion, there were higher disease and DON level on the last planting date, but higher test weight was found at the mid-planting date. Elkhart had the highest maximum disease, the highest DON, and highest test weight. On the other hand, Patterson had the lowest maximum disease, the lowest DON level, and the lowest test weight.

# SPATIAL PATTERN OF SCAB INCIDENCE DURING FUSARIUM HEAD BLIGHT EPIDEMICS ON WINTER WHEAT IN OHIO

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## INTRODUCTION

Fusarium head blight of wheat (*Triticum aestivum L.*) caused by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae*) is a limiting factor in wheat and barely 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. Information on disease patterns is a useful ecological characteristic that helps define a population such as diseased wheat heads (Gilligan, 1988; 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 determine more efficient sampling procedures that may result in more precise estimates of mean disease intensity and properly analyzing data from different treatments. Thus, the objective of this study was to quantify the spatial pattern of Fusarium head blight incidence in wheat fields.

## MATERIALS AND METHODS

### Disease Assessments.

Epidemics of Fusarium head blight of wheat were monitored over time in two fields at the Ohio Agricultural Research Development Center (OARDC) in Wooster, and at a single time in two fields at the OARDC Northwest Branch near Hoytville, which is located in a major wheat production region of Ohio approximately 120 miles northwest of Wooster. In the Wooster fields, disease incidence was assessed twice a week in June 2001. In each field, three transects with 15 sample points per transect, spaced at 0.75-cm intervals, for a total of 45 sample points per field were established. Each sample point was marked with a flag that remained in the field throughout the assessment period. In the Northwest Branch fields, there were ten rows (transects) with ten sample points per row, spaced at 1-m intervals for a total of 100 sample points per field. 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 parameters, *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(α) 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 (Table 1). 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. 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% of the data sets and by the binomial distribution in 58% of the data sets.

The values of θ ranged from 0.00 to 0.073, with a median of 0.011. Estimated θ in over 90% of these data sets were greater than 0 (Table 1) indicating overdispersion.

The index of dispersion *D*, ranged from 0.88 to 4.50, with a median of 2.22. *D* and θ were both positively correlated with the estimated parameter *p*, with correlations of 0.52 and 0.65, respectively (Figs. 1 and 2).

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

In conclusion, it was found that heads of wheat infected with scab were aggregated to highly aggregated within the wheat fields. Moreover, the degree of aggregation was high 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 | 0.009 | 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] Analysis not preformed.

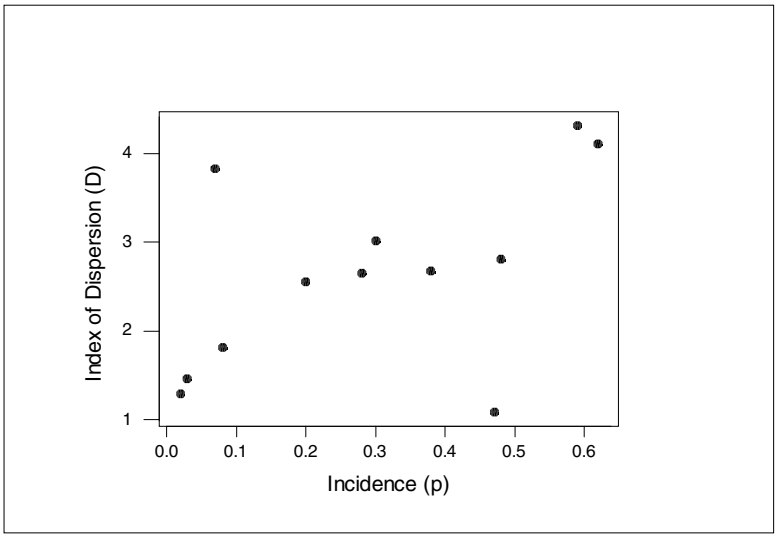


**Figure 1.** Index of dispersion (*D*) in relation to the mean incidence (P) of Fusarium head blight from four wheat fields in Ohio in 2001. Each point represents one field at one time.

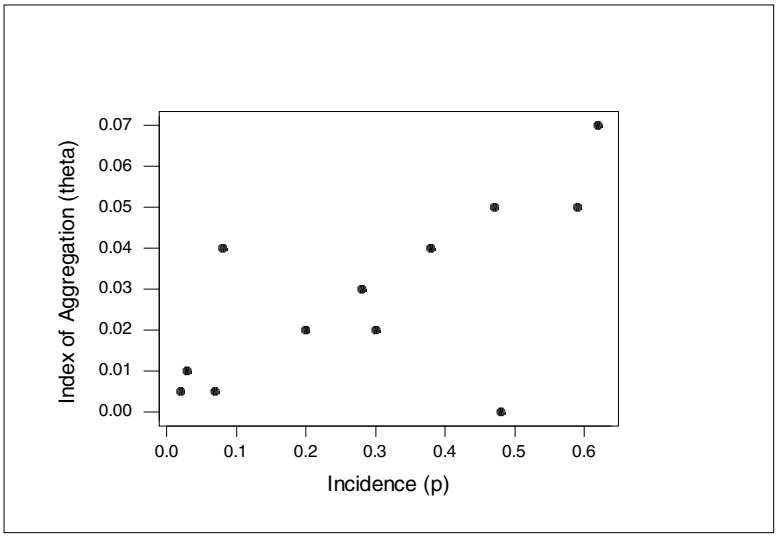


**Figure 2.** Index of aggregation (θ) in relation to the mean incidence (P) of Fusarium head blight for four wheat fields in Ohio in 2001. Each point represents one field at one time.

# PAST, PRESENT AND FUTURE OF FORECASTING SMALL GRAIN DISEASES

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Economic losses from Fusarium head blight have stimulated recent development of forecasting systems in Canada and the U.S. so it is perhaps appropriate to review what has been accomplished to date and where research may take us in the future. Diseases of high-value horticultural crops have received the bulk of forecasting system research and development effort and relatively few systems have been deployed for forecasting diseases of low-value field crops. Despite this economic disparity, small grain producers in Europe and North America have had access to several disease forecasting systems.

The first wheat disease forecaster was implemented in 1978 in The Netherlands. Called EPIPRE, for Epidemiology and Predication and Prevention, the system emphasized scouting by trained observers who forwarded information by mail to a central computer. Epidemic progress of leaf rust, stripe rust, powdery mildew, sharp eyespot, and the Septoria leaf blotch complex were forecasted together with outbreaks of aphid pests. EPIPRE used simple algorithms of population development, such as the exponential growth model, but the management recommendation incorporated many details such as cultivar, soil type, fertilizer rate, yield goal, and cost of pesticide application. The system spread to other European countries but popular usage eventually declined.

Another European model, the IPS Modell Weizen, was introduced in 1988 and is still in use. It was primarily as a method to guide control of the Septoria leaf blotch complex on wheat; leaf and stripe rusts, tan spot, powdery mildew, and eyespot were added later. Like EPIPRE, the system relies on trained scouts to diagnose diseases in the field but management decisions are based on disease incidence thresholds without reliance on computer models.

The next example is primarily an advisory system for disease management but encompasses some forecasting capability. MoreCrop (Managerial Options for Reasonable Economical Control of Rusts and Other Pathogens) was introduced to wheat growers in the U.S. Pacific Northwest in 1991. Some 30 diseases are covered for wheat and a version for barley disease management was introduced in 1999. MoreCrop bases its advice on an expert system that uses information about climatic regions, crop managerial practices, cultivar characteristics, field disease history, and departure from normal climate. The likelihood of a particular disease is suggested by the combination of factors.

North Dakota State University deployed its Small Grain Disease Forecasting System in 1999 for wheat growers and their advisors in North Dakota and Minnesota. Leaf rust, tan spot, and Stagonospora nodorum leaf blotch infection periods are predicted daily by computer models based on local weather provided by an automated weather station network. Scouting of leaf disease incidence is required for optimal disease management. Also,

airborne spores are sampled three times a week to provide information on the activity of the Fusarium head blight pathogen. Crop growth stage, extent of recent wetness from fog, rain and dew, and near-term weather forecast are suggested as guides to determine whether infection is likely. Forecasts are provided via the Internet and a toll-free number. Producers and buyers of malting barley also monitor the spore counts as a decision aid.

The Canadian provinces of Ontario and Manitoba/Saskatchewan introduced Fusarium head blight forecasts in 2000. Both systems provide contour maps of disease likelihood based on environmental data from a weather station network. Geographical information system software interpolates these point data. However, the predictive models that generate the probability of disease differ somewhat between Ontario and the Prairie Provinces. Ontario uses weather forecasts to predict the level of DON in wheat and updates this with weather station readings as flowering proceeds. The Manitoba/Saskatchewan model uses recent environmental data in a regional forecast with less sensitivity to actual flowering date.

The Ohio State University introduced a Fusarium head blight forecast in 2001. The forecast is based on a risk assessment model developed with support of the U.S. Wheat and Barley Scab Initiative. Environmental variables concerned with temperature and rainfall are summarized for the seven days prior to the onset of flowering to predict the subsequent risk of head blight. Forecasts are provided via the Internet as a county map of Ohio with risk areas highlighted.

It is difficult to foresee precisely the future of these forecasting systems but some common outcomes may be expected to recur since similar forces are at work.

Production economics and adversity to economic risk will play key roles in whether a system will be adopted. Ten years ago in North Dakota and Minnesota, approximately 100,000 hectares of wheat were sprayed with a fungicide. Today, one million hectares of spring wheat and durum are treated and barley area treated stands near where wheat was 10 years ago.

Funding for and management of system operations are often distinct from research and development. In Canada, fungicide registration is predicated on growers having available a disease advisory system so fungicide manufacturers have a compelling interest in continued operation. Grower groups and local grants fund system operations at North Dakota State University.

System reliability is an important concern because users can be rather unforgiving of failure. Reliability encompasses not only the prediction but also all parts of information delivery. To bring expectations into balance, users should be constantly educated about the accuracy rate of models, sources of variability, and other factors that influence their personal risk profile.

Conversely, the host institution benefits immensely when the system clearly makes money for the producer. Success stories told in the agricultural media enhance adoption of the system and garner political support.

Perceived value of the system will tend to increase usage. Forecasts for multiple diseases and insect pests give the user an integrated management system that will continue to hold value even if, for example, a scab-resistant cultivar is being grown.

Adoption is also influenced by factors such as cost effectiveness, ease of use, and accessibility. In-depth instructions and details of the pathogen's biology largely go unread during the decision making process. So too, scouting for disease intensity prior to treatment may be done hurriedly or left undone entirely (looking for symptoms is not an issue for scab forecasts). However, quite a few users are likely to listen to a recorded forecast from their cell phone while they are driving between chores.

Feedback is important for system viability because it allows one to catch problems before they become unwieldy. The system manager should know who is using the system, how it's being used, and if there are any obstacles to its use. Market penetration is an important benchmark of user participation. Periodic user surveys should be incorporated into the operations of a forecaster.

Research interests of scientists working in epidemiology suggest some future avenues of small grains disease forecasting. Expansion into new states is a recurrent theme: e.g., North and South Dakota plan to deploy risk assessment models in 2002. Concurrently, models of infection period are nearing completion and also should be deployed soon. New knowledge of pathogen population biology (e.g., perithecial development, spore survival, and leaf colonization) will undoubtedly contribute to future gains in forecaster accuracy. Specificity of forecasts is another broad avenue under development; e.g., environmental wetness estimates were generated at a spatial resolution scale of 4 $km^2$ with great success in a pilot study. Whereas present systems provide a uniform forecast for hundreds of square kilometers, future forecasts may aid management of diseases within a single field. It is obvious that novel methods will continue to enhance forecasting systems, given the talented scientists working on the problem and advances in information technology.

# POPULATION GENETICS OF *FUSARIUM GRAMINEARUM* FROM CHINA

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## ABSTRACT

In order to address questions of genetic diversity, gene flow and recombination among populations of the Fusarium head blight pathogen in China, a collection of strains was made from the important grain-producing region of the lower Yangtze River Valley. Diseased wheat heads were collected at 5 meter intervals along a transect in four production wheat fields in Zhejiang Province. The four fields ranged in distance from each other from 50 km to 200 km. DNA was extracted from 204 strains, transferred to solid support and probed with 6 DNA clones that hybridize to DNA fragments characteristic for particular lineages within the *F. graminearum* complex. All 204 strains from China gave a pattern of hybridization identical to that of DNA from known strains of *F. graminearum* lineage 6. The DNAs were further hybridized to 9 clones capable of detecting polymorphic loci within lineage 6 and a telomere-containing probe that further resolved genotypes. High levels of gene flow were detected among the four China populations (mean Nm = 11. 1; range 6.6 – 65) and the populations were extremely similar (Nei's unbiased genetic identity, I = 0.96 – 0.99). As a result, the strains from the four fields were considered part of a single large population. Gene diversity for the 9 single copy loci within the population was high (h = 0.35; range = 0.17 – 0.58) and the average number of alleles per locus was 3.3. Multilocus haplotypes were constructed from the allelic information of the nine polymorphic loci. Clones, having the same multilocus haplotype, originated primarily from isolates obtained from the same wheat head. Whether high genotypic diversity is caused by frequent sexual recombination is currently being explored.

# *MGV1* REGULATES FEMALE FERTILITY AND PLANT INFECTION IN *FUSARIUM GRAMINEARUM*

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## ABSTRACT

The wheat scab disease caused by *Fusarium graminearum* is prevalent worldwide and can cause severe losses during epidemics. The pathogen over-winters in debris as mycelia or perithecia and ascospores are believed to be the primary inoculum. To understand molecular mechanisms regulating the infection process of this important pathogen, we isolated a MAP kinase gene *MGV1* from *F. graminearum* strain PH-1. *MGV1* is highly homologous to the *MPS1* gene in *Magnaporthe grisea* that is involved in conidiation, plant penetration, and female fertility. The *MGV1* gene appears to be dispensable for conidiation in *F. graminearum* even though it is required for female fertility during sexual reproduction. Vegetative growth of the *mgv1* deletion mutants is normal in liquid media but is reduced when cultured on solid nutrient agar plates. Mycelia of *mgv1* deletion mutants are defective in cell wall structures and hypersensitive to cell wall degrading enzymes. In infection assays with flowering wheat heads and corn silks, *mgv1* mutants are dramatically reduced in virulence and appear to be defective in spreading *in planta*. Our data suggest that *MGV1* is involved in multiple processes in *F. graminearum* related with sexual reproduction, plant infection, nutrient sensing and cell wall integrity.

# DEVELOPMENT OF *FUSARIUM GRAMINEARUM* ON FLORET SURFACES OF FIELD-GROWN BARLEY

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## ABSTRACT

To investigate the role of mycelial colonies on floret surfaces in head blight infection, we mapped the location and number of colonies for 1-12 days after field plots were inoculated with *Fusarium graminearum*. The experiment was conducted on the St. Paul Campus of the University of Minnesota. Four plots of Robust barley (provided by K. Evans and R. Dill-Macky) were inoculated June 25, 2001 by spraying heads with aqueous suspensions of macroconidia at 50,000 or 100,000 spores/ml. At inoculation, 90% of the heads had emerged from the boot. After inoculation, plots were mist-irrigated daily from 5-9 p.m. and 4-6 a.m., totaling 8 mm of water per day. In addition, 13 mm of rain fell on the 5th day after inoculation (DAI). For the 12 day period of floret sampling, the average maximum daily temperature was 29$^{O}$ C; the average minimum was 18$^{O}$C. As viewed in the field at 13 DAI, approximately 1/3 of heads showed one or more chlorotic lesions. At 1, 2, 3, 4, 5, 6, 8, 10 and 12 DAI, florets were harvested, with bias for samples from heads showing one or more chlorotic lesions. Each day, 60 fresh (unfixed) florets were dissected and examined for presence of chlorotic or necrotic lesions; 80 additional florets were fixed and stained in lactophenol-cotton blue and then dissected and examined microscopically for presence of mycelium on floret surfaces. Mycelium and lesions were rarely seen at 1-3 DAI. At 4-6 DAI, mycelial colonies were noted on 13% of awns, 13% of lemma exteriors, 9% of lemma interiors, 57% of palea exteriors, 3% of palea interiors, and 6% of caryopses. At 4-6 DAI, chlorotic lesions were present on the lemma and palea in 8-13% of florets, and on the awn and/or caryopsis in 3-4% of florets. Thus the presence of lesions did not correlate with the presence of mycelium, especially on the frequently colonized palea exterior. There, mycelial colonies were usually located on the basal half of the palea, near the palea keel, which faces the rachis of the head. By 6-8 DAI, colonies on the palea surface often spread laterally into the crevice between the palea and lemma, sometimes extending to the interior surfaces of the palea and lemma as well as to the caryopsis. By 8-12 DAI, discrete lesions were discernible in 14% of florets on interior or exterior surfaces near the base of the lemma and palea. A second pathway to the interior of the floret was through the apical floret mouth. At 4 DAI, small discrete colonies were often present on the adaxial surface of awns, within 1-2 mm of its junction with the lemma and on adaxial tissues of the lemma and palea near the floret mouth. By 5 DAI and thereafter, colonies extended basally on the interior surfaces of the lemma and palea, sometimes colonizing any retained anthers or the brush apex of the caryopsis. By 8-12 DAI, discrete chlorotic or necrotic lesions were present on the apex of 14% of lemmas and 13% of paleas. The results indicate that under warm, mist-irrigated field conditions, colonies on the abaxial (exterior) surface of the palea (near the keel) and on adaxial (interior) surfaces of the palea and lemma facing the floret mouth serve as starting points for floret invasion.

# USE OF FUSARIUM HEAD SCAB RISK ASSESSMENT MODELS IN OHIO, 2001

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## ABSTRACT

During the 2001 wheat growing season, head scab risk assessment models were used to predict the risk of Fusarium head scab in Ohio. Logistic models were previously developed from hourly weather, crop growth and disease observations from 50 location-years representing three wheat production regions in the US. Model 1 utilized the duration of precipitation in hours and the number of hours when the air temperature was between 15°C and 30°C for 7 days prior to flowering. Cross validation prediction accuracy for this model was 78% for determining when disease was not severe (severity ≤ 10%), and its accuracy was 56% for predicting when an epidemic occurred (severity ≤ 10%). Model II utilized the: 1) number of hours when the air temperature was between 15°C and 30°C for 7 days prior to flowering; and 2) the number of hours when the relative humidity was 90% or above and the air temperature was between 15°C and 30°C for 10 days after flowering. Cross validation prediction accuracy for this model was 83% for determining when disease was severe (severity ≥10%). Hourly weather data from six weather stations in Ohio (Dayton, Columbus, South Charleston, Wooster, Hoytville and Toledo) were used to determine duration of weather events for the pre- and post-anthesis time periods. Disease risk probabilities were calculated using logistic equations determined by each model. Weather conditions in April and the first week of May were relatively dry and cool. Precipitation events became more frequent during mid to late May in many regions of the state with most locations reporting from 15 to 21 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 between 22 May and 4 June when most of the wheat was in anthesis. Calculated risk probabilities for locations ranged from 0.32 to 0.84 for Model 1 and from 0.04 to 0.78 for Model II. In no case did both models predict a high risk for head scab at any location. Based on these results the head scab risk prediction was reported to be low to moderate depending on the location 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. Field reports and disease surveys made about 3 weeks after anthesis indicated head scab was low to very low throughout most of Ohio. Most counties reported the average percentage of wheat heads with scab in fields to be below 2%. Some counties in central and northwest Ohio reported head scab levels ranging from 0 to 20% of the heads affected.

# EFFECTS OF DEW, SPRAY VOLUME AND ADJUVANT ON FUNGICIDE CONTROL OF FUSARIUM HEAD BLIGHT IN DURUM WHEAT, HRSW AND BARLEY

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## OBJECTIVES

1) Evaluate the effect time-of-day for fungicide application has on Fusarium head blight (FHB) control in respect to the presence, absence or imminent formation of dew. Applications would have morning, mid-day and evening timings.

2) Evaluate management of spray volume and/or adjuvant as a tool to compensate or enhance time-of-day effects on FHB control.

## INTRODUCTION

The volume of water present on a grain head covered by dew appears, by visually estimate, to be many times greater than the water applied during a fungicide application. The potential to manage the presence of dew to increase spike coverage or uptake of fungicide on the head offers small grain producers a technique to increase the efficacy of fungicides. Spray solution volume and adjuvant are important parts of fungicide application techniques which producers can easily adjust during the day to accommodate the environmental conditions at time of application.

Five unpublished experiments in ND have included dew as a variable and were available as personal communication. Two experiments on barley to evaluate dew effect on FHB control were conducted by J. Pederson, 1998. Head coverage by fungicide and dye solution increased from 8 to 21% when spraying dew covered heads compared to dry heads later in the day. Not enough FHB occurred for evaluation. The second experiment had head coverage by fungicide and dye of 5.8 and 1.4% for dry and rain wet heads, respectively. FHB levels were low with no difference detected. Field severity of FHB on durum was reduced from 3.4% on dew covered heads in the morning to 1.3% when spraying dry heads in the afternoon, T. Gregoire 1998. Dew effect in another trial was non-significant for FHB head severity on durum, T. Gregoire 2000. A greenhouse study found no significant differences in FHB field severity between fungicide applied to dripping wet heads and dry heads, M. McMullen 2001. FHB levels, field severity, when using 0.06% v/v Induce non-ionic surfactant were 3.5 and 1.5% for wet and dry heads, respectively, compared to levels with 0.125% v/v Induce of 1.1 and 1.3% for wet and dry heads, respectively.

## MATERIALS AND METHODS

A 3x2x3 factorial design with three replicates was used for a 'Plaza' durum experiment, which also included an untreated check when randomized. Treatments were, 1) Morning,

mid-day and evening times-of-day for fungicide application. 2) Two spray volumes, 10 and 20 gal/a. 3) Three levels of the non-ionic surfactant Induce, none, 0.06 and 0.125% v/v. Additional experiments on 'Robust' barley and 'Grandin' HRSW were conducted with only the three time of day treatments and an untreated check in a randomized complete block design with four replications. A spray volume of 20 gal/a and Induce surfactant at 0.125% v/v were used for all fungicide treatments on the Robust and Grandin. Fungicide was applied to the barley on July 10 at 6:45am, 3:30pm and 9:30pm, the HRSW on July 14 at 7:15am, 3:15pm and 7:00pm and on the durum July 18 at 8:00am, 2:30pm and 9:00pm.

Folicur (tebuconazole) at 4 fl oz/a applied at Feekes 10.51 was the fungicide treatment in all experiments. A hand held $CO_2$ pressurized boom with XR8001 flat fan nozzles oriented forward and backwards towards the grain heads at $30^{O}$ from horizontal was used to spray (6.7' x 20') plots. Dew quantity was measured by cutting about 50 heads in each of four places in each experiment. The peduncle was held just below the wet head and cut about one inch long. The heads were placed in a pan lined with tinfoil which was sealed to prevent moisture escape before weighing. The heads in a three square foot area were counted for each area sampled. The cut heads were air dried indoors until no free water was apparent and weight decrease with time dropped to a steady loss of a few hundredths of a gram over five minutes. Free water still in the head, but not readily visible, was found by slapping a head on a blotter paper and seeing the dark spots made by water droplets from between spiklets.

Sprinkler irrigation was used only for the Plaza durum study before flowering and 0.20 inch was applied on July 19 at 6:pm with rainfall occurring most days before and after fungicide application. FHB grain spawn was spread two days before fungicide application. The HRSW and barley trials were grown under natural conditions but were adjacent to irrigated/inoculated trials which increased FHB spore levels.

Foliar and FHB disease field notes were taken 21 to 25 days after fungicide application at soft dough stage. Foliar diseases were quantified as percent necrosis of individual leaves and FHB incidence and head severity were measured by counting infections on 25 individual heads from each plot. All plots were harvested by straight cutting with a Hege plot combine and drying before processing. ANOVA was used for statistical analysis.

## RESULTS AND DISCUSSION

The morning spray for Robust barley had 56.8 grams water per 100 heads and 41.7 heads per square foot, equivalent to 272 gallons water per acre on heads. There were water droplets visible in the spiklets but few water droplets on the beards. Water droplets were visible on the flag and other leaves. No visible run-off of water during the spraying operation was apparent. The morning spray for Grandin HRSW had 238.4 grams water per 100 heads with 40.5 heads per square foot, equivalent to 1111 gallons water per acre on heads. The spiklets were full and the beards covered with water droplets due to 0.61 inch rain ending at 11:00 pm the previous night. The crop canopy was covered with water droplets and run-off of water from the beards and leaves occurred during the spraying operation. The mid-day timing, 3:15 pm, had no free water visible in the head but drops showed when heads when slapped on blotter paper. Drying showed 0.9 grams water per 100 heads or 4 gallons water

per acre in the heads. The morning spray for Plaza durum had 26.3 grams water per 100 heads and 37.5 heads per square foot, equivalent to 113 gallons water per acre on heads. The beards were heavy with water droplets but no water was visible in the spiklets. Water droplets were visible on the flag and other leaves. No run-off of water during the spraying operation was apparent.

Leaf disease, FHB, yield and test weight by time of day, solution volume and adjuvant rate on durum wheat are shown in Table 1. The 3x2x3 factorial analysis had no significant interactions between the three factors. Time of day for fungicide application showed no significant difference for leaf disease, yield and test weight. FHB incidence, head severity and field severity were reduced for mid-day application compared to morning or evening. The higher adjuvant rate, 0.125% v/v of Induce with Folicur fungicide, reduced leaf disease compared to 0.06% v/v and no adjuvant. Differences due to adjuvant were non-significant for FHB, yield and test weight. No significant differences were found between spray volumes of 10 and 20 gallons per acre.

Leaf disease, FHB, yield and test weight for morning, mid-day and evening spray timings for three crops are given in Table 2. Only the 20 gal/a solution volume and 0.125% v/v adjuvant rate was used. Leaf disease and FHB were significantly reduced by fungicide for all crops but differences between timings were non-significant. Grain yields for the durum were not different while yields for the morning and evening timings of the HRSW were significantly greater than the check. Only the mid-day barley yield was significantly higher than the check. The barley trial had serious lodging which caused variability within the trial. Test weight differences due to time of day were non-significant for barley and HRSW and all fungicide treatments raised the durum test weight compared to no fungicide.

The amount of dew and its position on the head of small grains is variable from day to day. When very high amounts of dew are present, 1111 gallons per acre measured on HRSW heads, the application of spray solution causes run-off which is assumed to move fungicide to the soil. Trends from trials with dew treatments indicate the spraying of fungicide in very wet conditions may reduce efficacy. Measured effects of fungicide application in light morning dew, mid-day and evening conditions were small and variable between experiments. Time-of-day for fungicide application appears to be a minor factor for crop producers. Use of recommended adjuvant rates and high spray solution volumes when dew is present was supported by trends in this work.

**Table 1.** Fusarium head blight, Foliar Disease, Yield and Test Weight of Plaza Durum by Fungicide Timing, Solution Volume and Adjuvant Rate, Langdon, 2001.

| | | | Fusarium Head Blight | | | | |
|---|---|---|---|---|---|---|---|
| | Leaf Necrosis | | | Head | Field | | Test |
| Treatment | Flag | Flag-1 | Incidence | Severity | Severity | Yield | Weight |
| | % | % | % | % | % | bu/a | lb/bu |
| Untreated Check | 67.5 | 97.5 | 92.5 | 36.4 | 34.0 | 34.6 | 47.6 |
| **Fungicide Timing** | | | | | | | |
| Morning | 31.3 | 59.2 | 84.9 | 20.1 | 17.1 | 46.7 | 51.5 |
| Mid-Day | 30.4 | 65.0 | 77.9 | 17.9 | 14.1 | 45.0 | 51.2 |
| Evening | 30.8 | 61.7 | 87.0 | 20.7 | 18.1 | 44.1 | 51.2 |
| LSD P = 0.05 | ns | ns | 4.4 | 2.0 | 2.3 | ns | ns |
| **Solution Volume** | | | | | | | |
| 10 gal/a | 32.2 | 65.6 | 84.3 | 20.3 | 17.2 | 45.5 | 51.4 |
| 20 gal/a | 29.4 | 58.3 | 82.2 | 18.9 | 15.7 | 45.1 | 51.2 |
| LSD P = 0.05 | ns | ns | ns | ns | ns | ns | ns |
| **Adjuvant Rate v/v** | | | | | | | |
| None | 35.0 | 71.3 | 82.4 | 20.9 | 17.4 | 43.7 | 50.9 |
| Induce 0.06% | 30.4 | 60.4 | 84.0 | 19.4 | 16.4 | 46.2 | 51.7 |
| Induce 0.125% | 27.1 | 54.2 | 83.3 | 18.5 | 15.6 | 45.9 | 51.3 |
| LSD P = 0.05 | 5.4 | 9.8 | ns | ns | ns | ns | ns |

**Table 2**. Fusarium head blight, Foliar Disease, Yield and Test Weight for Robust Barley, Grandin HRSW and Plaza Durum Experiments, Langdon, 2001

| Fungicide Timing | Flag Leaf Necrosis | | | FHB Field Severity | | | Grain Yield | | | Test Weight | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Barley | HRSW | Durum | Barley | HRSW | Durum | Barley | HRSW | Durum | Barley | HRSW | Durum |
| | --------- % --------- | | | --------- % --------- | | | -------- bu/a -------- | | | ------- lb/bu -------- | | |
| Morning | 37.5 | 13.8 | 30.0 | 1.4 | 2.5 | 17.3 | 65.8 | 50.9 | 43.2 | 47.4 | 56.7 | 51.0 |
| Mid-Day | 25.0 | 15.0 | 17.5 | 1.9 | 3.6 | 14.3 | 89.9 | 45.6 | 45.5 | 47.7 | 55.8 | 51.0 |
| Evening | 42.5 | 17.5 | 27.5 | 1.5 | 5.1 | 13.9 | 76.6 | 51.3 | 45.5 | 48.1 | 57.0 | 50.9 |
| No Fungicide | 52.5 | 50.0 | 67.5 | 3.9 | 9.3 | 34.0 | 68.0 | 44.1 | 34.6 | 46.3 | 55.0 | 47.6 |
| LSD=0.05 | 27.1 | 14.9 | 13.7 | 1.7 | 3.2 | 10.2 | 11.7 | 6.0 | ns | ns | ns | 2.1 |

Fungicide was Folicur 4oz/a at 20 gal/a and 0.125% v/v Induce adjuvant for all treatments
Foliar disease predominantly Septoria species.

# INOCULUM DYNAMICS OF *FUSARIUM* SPECIES AND LEVELS OF *GIBBERELLA ZEAE* SPORE-TYPE RECOVERED FROM WHEAT SPIKE BIOASSAYS

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## ABSTRACT

Ascospores and conidia of *Gibberella zeae* are the primary causal agents of Fusarium head blight (FHB) in wheat and barley. Although *Fusarium graminearum* is the primary causal pathogen of the disease, it may be possible that other *Fusarium* species could play a role in the successful infection by *Fusarium graminearum.* In addition, some question remains concerning the importance of ascospores versus conidia as sources of inoculum. Wheat spikes clipped and vigorously shaken in sterile distilled water and Tween20 have been used in previous studies to ascertain colony-forming units (cfu) of *F. graminearum.* This wheat-spike bioassay technique was used for three years to investigate *F. graminearum* species dynamics, and for two years to compare frequencies of *F. graminearum* spore type present on wheat at the North Dakota State experiment station in Fargo, North Dakota. *Fusarium graminearum, F. sporotrichioides, F. equiseti, F. moniliforme*, and *F. sambucinum* were present in fluctuating levels during all three years. Both spore types were observed from wheat heads, with a range of 40-80% ascospores. In addition, the wheat head bioassay technique was investigated for its efficiency to recover ascospores and conidia in equal amounts when a known aliquot of either spore type was applied directly by aerial spraying and sampled at 0.5 h, 3.0 h, 7 h, and 24 h post-inoculation. Higher levels of both spore types were recovered at earlier post-inoculation times than later times, and overall levels of recovered conidia were significantly higher than those of ascopsores. To aid in interpretation of the data, known quantities of ascospores were applied directly to Komada's agar and cfu were tabulated. More cfu were observed from conidia-inoculated plates but the amounts were not significantly different than those of ascospore-inoculated plates. Observation of both conidia and ascospore in varying ratios on wheat heads suggest that both spore types may influence disease pressure; moreover, presence of other *F.* species could potentially affect the ultimate intensity of FHB caused by *F. graminearum* if there is competition among the *Fusarium* species present.

# SOYBEAN IS A HOST FOR *FUSARIUM GRAMINEARUM*

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## ABSTRACT

Routine surveys of seed quality of soybean (*Glycine max*) grown South Brazil revealed unexpected infection with *Fusarium graminearum*. Seemingly symptomless seeds were surface disinfested with 1.0 % sodium hypochlorite for 2 minutes and plated on water agar or 1/4 PDA. After 8 days, spores were examined from fungal colonies growing from the seeds and determined to be members of the *F. graminearum* species complex. Seed lots varied in the percentage of infected seed, ranging from 0 - ca.20%. To determine if the fungus was pathogenic to soybean, 8 strains of the fungus derived from soybean were added to soil at a rate of $10^3$ macroconidia/ ml or pods were inoculated each with $10^4$ macroconidia. Seedlings grown in infested soil developed small necrotic lesions in the crown and upper tap root. Pods inoculated with the fungus developed large (>1 cm), dark brown, necrotic lesions. Younger pods inoculated with the fungus blighted and dropped from the plant. Cultures of *F. graminearum* were recovered from lesions on the crown, roots and pods of inoculated plants. The lineage of the *F. graminearum* fungus infecting soybean was determined by obtaining the DNA sequence from the EF1-alpha gene from five strains and comparing it to strains of known lineage. Strains of the fungus from soybean grown in Brazil were from lineage 2 or lineage 8. Two strains of *F. graminearum* lineage 7 from the U.S. caused similar symptoms on soybean. Mycotoxin tests on naturally and artificially infected seed are being conducted.

# FUSARIUM HEAD BLIGHT: EPIDEMIC VS. NON-EPIDEMIC CONDITIONS IN SOUTH DAKOTA FOR 2001

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## INTRODUCTION AND OBJECTIVES

South Dakota State University is part of a 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 forecasting system. Primary objectives include: 1) monitoring inoculum dynamics and disease development in relation to temperature, humidity, and precipitation at locations throughout the upper mid-west; and 2) to evaluate tools and techniques for incorporation into a useful and efficient disease forecasting system.

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. FHB epidemiology monitoring plots at Brookings, SD showed distinct differences in disease incidence and severity over three planting dates in 2001. The objective of this report is to compare environment and inoculum factors that may have resulted in the differential disease 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 (11 May, 18 May, and 29 May, 2001), 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 described by Francl et al. (1999), 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 was assessed in each planting date at early to late dough stage. In each replicate, 100 spikes from primary tillers were visually rated for FHB. Diseased and total spikelets were recorded for each spike. Incidence was calculated by: infected spikes divided by total spikes per replicate. Severity was calculated for infected spikes by: diseased spikelets divided by total spikelets and reported as an average over all infected spikes per replicate.

Environmental variables (Table 1) were selected (or created using existing variables) for correlation analysis against airborne spore estimates (Burkard data), spike-borne spore estimates (spike wash data), disease incidence and disease severity over planting dates and replicates. Actual vapor pressure of the air was estimated from calculations of saturation vapor pressure and on relative humidity measurements. Variables were established to represent averages over several time periods including: flowering period, 7 days prior to flowering, 4 days post-anthesis, 7 days pre-flowering+flowering period, and flowering period+4 days post-anthesis. Within each time period, for temperature and vapor pressure, variables were established to represent averages over daytime (8:00a.m. to 8:00p.m.) hours, averages over nighttime (8:00p.m. to 8:00a.m.) hours, average daily maximums, and average daily minimums. Leaf and soil-surface wetness duration, and solar radiation were totaled, and daily averages were calculated. Precipitation was totaled and number of precipitation events larger than 3mm were also indicated as variables. Wind speed was averaged over the entire time period. For Burkard and spike washing data, correlation was examined for variables representing anthesis period and earlier. For disease incidence, correlation to anthesis period variables was examined. For disease severity, correlation was examined for variables representing flowering and after.

Table 1. Environmental variables used in correlation analysis

| Temperature in $^0$C (for each time period) | Vapor Pressure ($e_a$) in kPa (for each time period) | Additional Parameters (for each time period) |
|---|---|---|
| Avg Temp | Avg $e_a$ | Avg Daily Sol. Radiation (hrs) |
| Avg Daytime Temp | Avg Daytime $e_a$ | Daily Leaf Wet. Duration (hrs) |
| Avg Nighttime Temp | Avg Nighttime $e_a$ | Daily Soil Wet. Duration (hrs) |
| Avg Daily Max Temp | Avg Daily Max $e_a$ | Mean Wind Speed ($\mu$) |
| Avg Daily Min Temp | Avg Daily Min $e_a$ | Precipitation (mm) |
| | | No. Precipitation events > 3mm |

## RESULTS AND DISCUSSION

Major environmental parameters for each planting date are summarized in Table 2. Generally, dry conditions with moderate temperatures were experienced prior to and throughout the first and second planting date (PD1 and PD2). Leaf wetness duration (LWD) was greater during anthesis for PD2 than for PD1. Temperatures were warmer and the environment was more moist during PD3 anthesis. Inoculum levels (airborne and spike-borne) increased with time from the beginning of flowering for PD1 to end of flowering for PD3. Ascospores were present during all flowering periods, however levels were much higher for PD3. Disease levels were moderate for PD1, high for PD2 and very high for PD3 (Table 3).

Table 2. Environmental conditions over susceptible periods in each planting date.

| PD | Time period (susceptible) | Avg. air temp (°C) | Avg. $e_a$ (kPa) | Precip. (mm) / events | Mean wind spd (m/s) | LWD[a] (hrs) | SWD[b] (hrs) |
|---|---|---|---|---|---|---|---|
| 1 | DOY 182-188 | 20.4 | 1.730 | 0.3 / 0 | 2.19 | 9.8 | 17.7 |
| 2 | DOY 188-194 | 23.1 | 1.858 | 0.3 / 0 | 1.71 | 12.4 | 0 |
| 3 | DOY 200-206 | 23.8 | 2.459 | 27.4 / 4 | 2.52 | 14.33 | 24.0 |

a. Leaf wetness duration.
b. Soil wetness duration.

Table 3. Final disease ratings.

| | Plant Date 1 | | Plant Date 2 | | Plant Date 3 | |
|---|---|---|---|---|---|---|
| | Incidence % | Severity % | Incidence % | Severity % | Incidence % | Severity % |
| Rep 1 | 34 | 10.4 | 84 | 27.1 | 100 | 67.9 |
| Rep 2 | 30 | 8.1 | 89 | 18.7 | 100 | 69.9 |
| Rep 3 | 25 | 9.2 | 78 | 15.7 | 95 | 51.4 |
| PD Mean | 29.7 | 9.2 | 83.7 | 20.5 | 98.3 | 63.1 |
| Overall: | Disease Incidence = 71% | | Disease Severity = 31% | | | |

For both airborne and spike-borne inoculum, highly significant correlation was observed with anthesis-period nighttime temperature and vapor pressure variables, as well as leaf wetness duration and precipitation (r = 0.96 to 0.99). Significant correlation was noted with wind speed (r = 0.61 and 0.64 for airborne and spike-borne inoculum, respectively) and soil wetness duration (r = 0.48 and 0.51). Correlation was not as strong for the pre-flowering period variables. (Table 4.) Disease incidence showed strong positive correlation to temperature, vapor pressure and leaf wetness duration. Moderate positive correlation to precipitation was also noted. Soil wetness during anthesis showed almost no correlation with disease incidence. Disease severity showed strong positive correlation to vapor pressure, nighttime temperature, leaf wetness, and precipitation. Severity showed strong negative correlation to solar radiation and moderate to strong negative correlation to daytime temperatures (depending on evaluation period).

The results of the correlation analysis may help to identify variables that are highly predictive of the various components of a forecasting or disease development model. These results reported here indicate the potential importance of nighttime conditions (especially temperature, humidity, and dew formation) on the release of inoculum into the air. The correlation of temperature and humidity with incidence values (which can be extrapolated to infection rate) may indicate their importance for spore germination and initial infection. The correlation of disease severity (an indication of spread within an infected spike) with nighttime conditions, and the negative correlation with daytime temperatures and radiation indicate that spread within infected plants probably occurs during favorable nights, and may be hindered during unfavorable days.

Table 4. Relevant correlation values for certain environmental variables against four responses.

| | Airborne inoculum | Spikeborne inoculum | Airborne inoculum | Spikeborne inoculum | Disease incidence | Disease severity |
|---|---|---|---|---|---|---|
| | vs. anthesis period | | vs. 7 days pre flowering | | anthesis period | anthesis + 4 days |
| Night $e_a$ | 0.991 | 0.969 | 0.723 | 0.714 | 0.760 | 0.971 |
| Night temp. | 0.999 | 0.969 | 0.285 | 0.317 | 0.864 | 0.844 |
| Day temp. | 0.519 | 0.469 | 0.144 | 0.181 | 0.890 | -0.330 |
| Wind speed | 0.616 | 0.632 | -0.605 | -0.655 | 0.097 | n/a |
| Radiation | -0.300 | -0.332 | 0.826 | 0.827 | 0.256 | -0.878 |
| Precipitation | 0.962 | 0.947 | 0.003 | 0.044 | 0.660 | 0.952 |
| LWD | 0.945 | 0.906 | 0.757 | 0.805 | 0.966 | 0.823 |
| SWD | 0.483 | 0.506 | 0.061 | 0.017 | -0.061 | n/a |

Through the continued monitoring of inoculum, disease, and environment across numerous sites within several states, local and regional FHB forecasting systems and disease development models, which would provide producers with the capability of making better management decisions, are on the horizon.

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# HYPERSPECTRAL REFLECTANCE OF EIGHT SPRING WHEAT VARIETIES IN A SCAB NURSERY

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## ABSTRACT

In 2001, preliminary research was conducted on the use of hyperspectral reflectance measurements for detection of disease in spring wheat. Replicated field plots were established using eight common varieties with a range of susceptibility to Fusarium head blight (FHB). Plots were situated within the FHB field screening nursery at South Dakota State University, Brookings, SD. The nursery was artificially infested by spreading colonized grain over the soil surface to serve as an inoculum source. Mist irrigation was used to increase humidity and soil wetness in the field. Beginning at heading, measurements were taken from the plots every 7 to 10 days using a hyperspectral radiometer (CI-700, CID, Inc. Camas, WA) and infrared thermometer. Digital images of all plots were also taken. The CI-700 measured reflected radiation across a waveband from 350 nanometer (nm) to 950nm, at 1nm increments. Data was compiled for each date and analyzed by performing ANOVA at each interval wavelength. The main objective of the study was to determine the potential of such data to predict disease and/or toxin levels within infected plots through elicitation of spectral signatures indicative of disease or toxin level.

# SOIL-SURFACE WETNESS SENSOR: REPORT OF FURTHER TESTING

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## INTRODUCTION AND OBJECTIVES

Free moisture (i.e. wetness) at the soil-air interface, or within the top few centimeters (cm) of soil is a very important factor in the development of certain plant pathogens and plant diseases. This variable has been difficult to estimate or measure with current technologies such as gypsum blocks, time-domain reflectometry, neutron probe, or tensiometers which measure moisture at greater depths. *Gibberella zeae*, causal agent of Fusarium head blight (FHB) in cereal crops resides and over-winters in corn stubble and cereal residues at or near the soil surface. From this niche, under favorable environments, ascospore inoculum develops (Paulitz, 1996; Sutton, 1982, and Parry et al., 1995) and is then available for dissemination to susceptible sites on host plants. Soil-surface moisture, often associated with precipitation events or dew formation in the canopy (Rosenberg et al., 1983) is presumed to be one of the critical environmental factors affecting the development of residue-borne pathogens (Rotem, 1978). Development of epidemiological models useful in plant disease forecasting involves gathering information about critical environmental parameters. The capacity to measure soil surface wetness and wetness duration would potentially add power to current and future models. In 2000, a sensor was developed and described (Osborne and Jin, 2000) which would be used to estimate soil wetness duration through the use of data logging equipment. The primary objective of this project was to further develop an instrument which would directly measure wetness at the soil-air interface and to conduct testing to evaluate its suitability for the intended applications.

## MATERIALS AND METHODS

### *Initial testing of sensors.*

Soil-surface wetness sensors were constructed as described by Osborne and Jin (2000). Sensors were first tested on synthetic sponges. Sensing elements were held in contact with the sponge using rubber bands. The sponge and sensor were placed under a 150W lamp to speed drying. Readings were taken from the sensor using the CR10X datalogger. Several readings were taken from the dry sponge at two-minute intervals, then water was added to the capacity of the sponge. Readings were taken every two minutes until the sponge was very dry. Several repetitions of this procedure were conducted for each sensor. The data was plotted for each repetition and the results of each run were compared statistically among replicates and sensors using analysis of variance procedures.

Further testing was performed using a thin (approx. 5mm) layer of soil (screened to pass a 2mm sieve) in a plastic container. A known weight of oven-dry soil (100g) was used to facilitate moisture content calculation after addition of specific weights of tap water. The sensor was placed atop the soil layer, secured with rubber bands and the entire assembly

was placed on a digital balance. The balance was zeroed and 20g of water was added to the soil layer using a trigger-pump misting sprayer in order to evenly wet only the soil (droplets landing elsewhere were removed with absorbent paper). The system was allowed to set undisturbed for 20 minutes to allow for water absorption into soil micropores. Thereafter at five-minute intervals, the resistance across the sensor and the weight of water remaining was recorded. This procedure was repeated several times with each sensor. Data was analyzed as described for sponge trials.

Preliminary field testing of the soil-surface moisture sensors was conducted by integration into an automated weather station placed in the 2000 FHB epidemiology study field plots at South Dakota State University, at Brookings, SD. Five sensors were placed into the field shortly after planting, and operated until just before harvest, or for approximately three months. Sensors were evaluated for response to known wetting events (precipitation) and for durability of construction.

***Calibration of sensors.***

Each sensor was calibrated against both volumetric moisture content as well as tactile estimates of surface wetness using three soil types in thin layer trials. A sandy loam, silt loam, and clay loam were obtained to provide a range of soil types for calibration. For each soil type, six different gravimetric moisture contents (2, 4, 6, 8, 10, and 12% water) were established in thin soil layer pans. Soil water content levels were established by adding water to oven-dry soil, then mixing to homogenize each pan. Water was replaced as needed by weighing each pan and misting the soil until the desired weight was reestablished. Each pan was classified as 'wet' or 'dry' based on appearance and tactile estimates made by touching the soil surface with bare fingers, using slight pressure. Soils were deemed to be wet if the surface appeared darker than a dry check pan of the same soil type, and felt damp to the touch. Mottled (dark/light) surface was considered evidence of wetness if accompanied by supporting tactile estimation. By this method, sandy loam soil was determined to be wet at 4% moisture and above. Silt loam and clay loam soils were wet at 6% moisture and above. Sensors were placed onto a soil pan selected at random, allowed 30 seconds to settle, then a measurement was recorded. This was repeated until each pan had been measured 12 times by each sensor. Data were transformed using natural log transformation, then analyzed by determining mean and standard deviation for each moisture level/sensor combination. Calibration values were determined for each sensor based on the mean (transformed) for the smallest soil water content which was determined to be wet (i.e. 4% for sandy loam, 6% for clay loam and silt loam). Confidence intervals (C.I.) (90%) were calculated for the selected mean (transformed) value, and the upper limit was selected. The upper limit of the CI was then transformed back to kOhms by inverse natural log transformation, and that value was then used as the wet-dry calibration value for each sensor.

***Advanced Field Testing.***

In 2001, six sensors were again integrated into the automated weather station at the FHB epidemiology monitoring plots in Brookings, SD by placing pairs of sensors across three sites within a 100ft radius of the weather station. Additional sensors were placed into similar studies in North Dakota, Ohio, Indiana, and Pennsylvania. Data was collected every 30 minutes. The sensors were placed into study sites onto soil that had been cleared of large

debris, or large soil peds. The area was to be smooth, to allow good sensor-soil contact. The sensors were to be depressed slightly, so as to bury the wire elements partially (but not completely) into the soil. Results of the field trials were examined in spreadsheet format. Calibration values were applied to raw data for each 30-minute period resulting in a binary response (wet or dry). The consistency across sensors for indicating wetting events was noted. The duration of wetness was calculated for 24-hour periods, and totaled over the study duration and compared among sensors.

## RESULTS AND DISCUSSION

### *Initial testing and calibration of sensors.*

The sensors performed well in all trials. Visual and tactile estimates of surface moisture compared favorably to sensor measurements on the sponge and on soil. For sponge trials, resistance values across the sensing elements were plotted against time. The resultant curves were smooth, and increased more or less exponentially. Sensors were consistent over replications, however measurements differed across sensors. The variation can likely be attributed to variations in sensor construction (sensors were individually constructed). On thin soil layers in the laboratory, sensors responded in a manner similar to the sponge trials. Initial field testing showed the sensors to be quite uniform in sensing wetness events (precipitation or heave dewfall). The sensors were durable, and appeared to be in good condition after the initial field trial.

Calibration values were determined for each sensors. Values determined ranged from 30 kOhms to 120 kOhms, with variation between sensors and across soil types.

### *Advanced field testing.*

Sensors were generally very consistent for indication of wetting events. Variation of (+/-) 30 minutes was observed in some cases, however, most sensors responded to wetting events within the same 30-minute sampling period. Differences are likely attributable to sensor variation or variation in microclimate across sensor sites. Duration of wetness (Table 1) varied among sensors for specific wetting events. When considered over several events, however, the difference among sensors was insignificant (Table 2). The variation for singular events again could be attributed to microclimatic differences of sensor sites, sensor construction, or errors in calibration values. An error of (+/-) 30 minutes per event may be expected due to the sampling period used in the evaluation. The analysis shows that sensors within pairs were not significantly different over all events, and differences among pairs (or sites) were also not significant. For individual events, sensors within pairs tended to be similar while pairs tended to be different, suggesting site differences attributable to canopy or microclimate variability. Calibrations may also account for variation given that duration was estimated using the calibration values derived on specific soil types in the greenhouse. When larger calibration values were applied (potentially overestimating wetness duration) sensor uniformity increased greatly for individual wetness events. This technique may be more useful for model development as long as any potential overestimation remains consistent over locations and time.

Table 1. Soil wetness duration (hours) for six sensors following precipitation events.

| Wetness Event | Precip (mm) | SSWS 1 | SSWS 2 | SSWS 3 | SSWS 4 | SSWS 5 | SSWS 6 |
|---|---|---|---|---|---|---|---|
| 1 | <1 | 10.5 | 15.0 | 0.0 | 1.0 | 17.5 | 15.0 |
| 2 | 18 | 32.5 | 54.5 | 36.5 | 85.0 | 83.5 | 59.5 |
| 3 | 4 | 15.5 | 15.5 | 19.0 | 16.5 | 24.5 | 15.5 |
| 4 | 15 | 112.5 | 108.5 | 109.5 | 123.5 | 115.0 | 108.0 |
| 5 | <1 | 4.0 | 2.0 | 0.0 | 0.0 | 4.0 | 0.0 |
| 6 | 47 | 320.5 | 344.0 | 317.0 | 346.0 | 347.0 | 322.0 |
| 7 | 3 | 21.5 | 21.5 | 3.0 | 0.0 | 12.0 | 0.0 |
| 8 | 10 | 54.5 | 54.0 | 36.0 | 16.0 | 32.5 | 23.0 |

Table 2. Analysis of variance (ANOVA) for paired sensors.

| Source of variation | df | MS | P > F |
|---|---|---|---|
| Pairs | 2 | 270.2 | 0.9786 |
| Sensors(Pairs) | 3 | 209.1 | 0.9970 |
| Error | 42 | 12507 | |

Sensor durability was very good. Sensors remained intact and in good condition over the course of one field season. Calibrations will be checked during the winter to determine if they remain stable over time.

## CONCLUSIONS

Overall the sensors performed well. In laboratory tests, sensors responded smoothly to changes in substrate moisture content, and were consistent in replicate trials. Calibration values were determined for three soil types, and allowed for wet/dry differentiation compared to tactile estimates. Field trials were considered successful in that sensors responded in a uniform manner to precipitation or heavy dew. Care must be taken to apply proper calibration values to data in order to best estimate the field conditions. Laboratory derived values may not be representative of field conditions. Sensors were durable over the course of a field season and remained in good condition.

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# EFFECT OF *GIBBERELLA ZEAE* ASCOSPORES AND *FUSARIUM GRAMINEARUM* CONIDIA ON FUSARIUM HEAD BLIGHT SEVERITY AND DEOXYNIVALENOL PRODUCTION IN BARLEY

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## OBJECTIVE

To determine if ascospores of *Gibberella zeae* and conidia of *Fusarium graminearum* induce quantitatively similar levels of Fusarium head blight (FHB) severity and deoxynivalenol (DON) concentration in inoculated barley plants.

## INTRODUCTION

FHB, caused by *F. graminearum*, threatens the existence of the malting barley industry in the Upper Midwest region of the USA [1,2]. Researchers searching for resistance against FHB use either conidia of *F. graminearum* or ascospores of the perfect stage of *G. zeae* for inoculum. In some screening programs, ascospores are used for inoculum in field tests and conidia for growth chamber/greenhouse tests. An important factor to consider in the choice of inoculum for these tests is whether the relative infectivity of ascospores and conidia is similar. A previous study comparing the infectivity of the two spore types in wheat was conducted in the greenhouse (4). The objective of this study was to compare *G. zeae* ascospores and *F. graminearum* conidia for their ability to cause disease and produce DON in barley under both growth chamber and field conditions.

## MATERIALS AND METHODS

The six-rowed barley cultivar Stander (susceptible to FHB) and the two-rowed line CIho 5415 (moderately susceptible to FHB) were inoculated in both growth chamber and field experiments. Inocula of both spore types were derived from *F. graminearum* isolate KB176. Conidial inoculum was produced on PDA (50% potato dextrose), whereas ascospore inoculum was produced on carnation leaf agar (5). Plants at the early to mid-dough stage of development were inoculated (10,000 conidia/ml) in the growth chamber and field using methods modified from Salas et al. (1) and Prom et al. (3), respectively. Greenhouse grown barley plants were inoculated, given a 24-hr moist period (22ºC with 100% RH), and then placed in growth chambers at 25/20ºC (12 hr 5000 lux light /12 hr dark) for two weeks. Plants were misted twice a day to increase humidity and infection. In the growth chamber experiments, each treatment (ascospores and conidia) consisted of two to four replicates, and each replicate of three to five spikes. DON assays were made for each replicate.

For the field experiments, plants at the early to mid-dough stage were spray-inoculated at dusk and immediately covered with plastic bags to maintain high humidity. Bags were removed the following morning to avoid excessive heat development. Each treatment (as-

cospores, conidia, and control) consisted of four replicates, and each replicate was composed of five to ten spikes. DON assays were performed on each replicate.

## RESULTS AND DISCUSSION

Growth chamber and field data for FHB and DON were analyzed separately due to low FHB infection of field-inoculated plants. Ascospore inoculum induced slightly higher FHB levels than conidial inoculum on Stander and CIho 5415, but the difference was only statistically significant for CIho 5415 in the field test (Figs. 2a and 3a). In a similar study on wheat, Stack (4) found that ascospores and conidia induced quantitatively similar levels of FHB in single floret inoculations. No statistically significant differences were detected between the spore types for DON production, except again in the case of CIho 5415 in the field where ascospore inoculum produced a higher DON level than conidial inoculum (Figs. 2b and 3b). The general trends observed for FHB severity were not always reflected in the corresponding DON concentrations, particularly for CIho 5415 in the growth chamber test and Stander in the field test (Figs. 2 and 3). When FHB and DON data of the two barley accessions were pooled, ascospore inoculum always resulted in slightly higher levels than conidial inoculum; however, the only significant difference detected was for FHB under field conditions (Table 1). When FHB and DON data of the two spore types were pooled, cultivar Stander exhibited significantly higher DON and FHB levels than the two-rowed accession CIho 5415 in both experiments, except for FHB under field conditions (Table 2). This result was not unexpected because six-rowed accessions are generally more susceptible than two-rowed accessions. The results from the field should be interpreted with caution because natural inoculum was present in the plots as indicated by the disease levels on controls (Fig. 3).

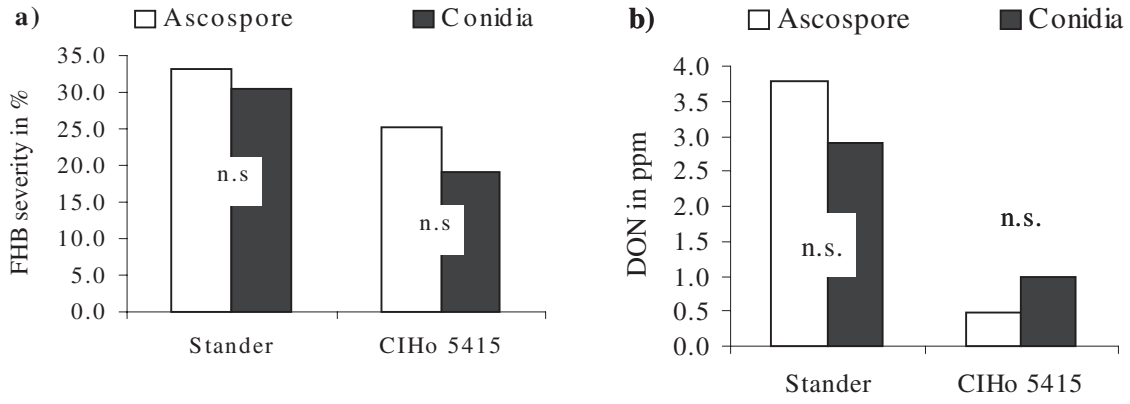


**Fig. 2.** Effect of conidia (*Fusarium graminearum*) and ascospore (*Gibberella zeae*) inocula on FHB severity (a) and DON concentration (b) in Stander (six-rowed) and CIho 5415 (two-rowed) barley under growth chamber conditions, n.s.=not significant ($P \leq 0.05$) based on LSD-test.

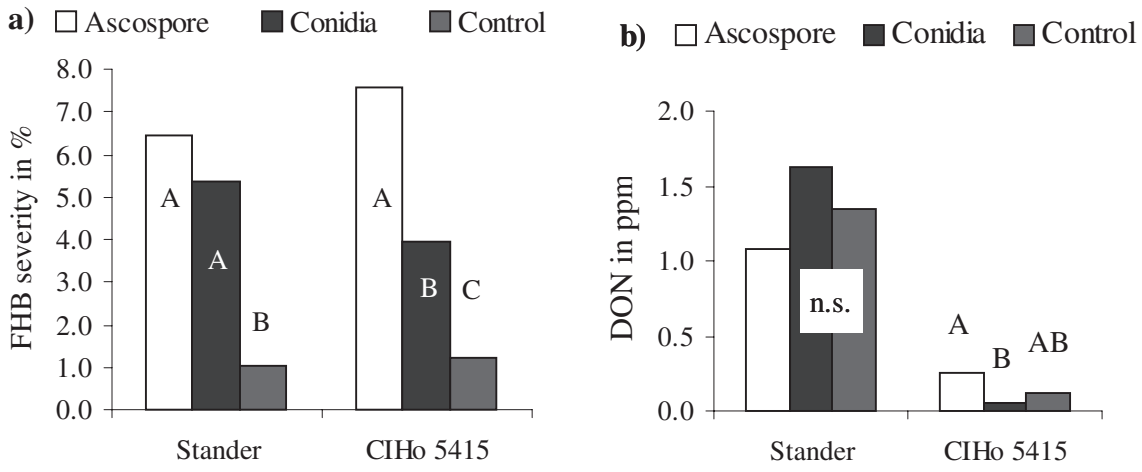


**Fig. 3.** Effect of conidia (*Fusarium graminearum*) and ascospore (*Gibberella zeae*) inocula on FHB severity (a) and DON concentration (b) in Stander (six-rowed) and CIho 5415 (two-rowed) barley under field conditions. Means followed by the same letter are not significantly different at P≤0.05 by the LSD-test, n.s.=not significant.

**Table 1.** Effect of conidia (*Fusarium graminearum*) and ascospore (*Gibberella zeae*) inocula on FHB severity and DON concentration in barley inoculated in growth chamber and field experiments, Fargo 2000.

| | Growth chamber | | | | Field | | | |
|---|---|---|---|---|---|---|---|---|
| **Spore type** | **FHB** | | **DON** | | **FHB** | | **DON** | |
| **Ascospore** | 33.8 | n.s.[1] | 2.13 | n.s. | 6.48 | A | 0.84 | n.s. |
| **Conidia** | 23.33 | n.s. | 1.95 | n.s. | 5.21 | B | 0.66 | n.s. |
| **Control** | ----- | | ------ | | 1.14 | C | 0.74 | n.s. |

Means are based on six and twelve replicates in the growth chamber and field experiments, respectively. Means followed by the same letter are not significantly different at P≤0.05 by the LSD-test. The background infection level of the control in the field was significantly lower than the inoculated treatments.

[1]n.s. = not significant

**Table 2.** Effect of barley accessions on FHB severity and DON concentration in growth chamber and field experiments, Fargo 2000.

| | Growth chamber | | | | Field | | | |
|---|---|---|---|---|---|---|---|---|
| **Spore type** | **FHB** | | **DON** | | **FHB** | | **DON** | |
| **Stander** | 33.7 | A | 3.35 | A | 4.29 | n.s. [1] | 1.35 | A |
| **CIHo 5415** | 23.11 | B | 0.73 | B | 4.26 | n.s. | 0.14 | B |

Means are based on six and twelve replicates in the growth chamber and field experiments, respectively. Means followed by the same letter are not significantly different at $P \leq 0.05$ by the LSD-test.

[1]n.s. = not significant

## CONCLUSION

The results from this study indicate that ascospores and conidia generally induce similar levels of FHB severity and DON concentration in inoculated barley under the more controlled conditions of the growth chamber. Thus, the choice of spore type for testing the resistance of barley to FHB may depend largely on the ease by which individual researchers can produce them. One other important consideration may be the genetic stability of the inoculum source. Propagation of sexual spores (ascospores) may result in greater genetic variability than with conidial inoculum.

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# EFFECTS OF DEOXYNIVALENOL ON BARLEY LEAF PIGMENTATION

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## OBJECTIVE

To determine how deoxynivalenol (DON) affects barley leaf tissues.

## INTRODUCTION

Tricothecene toxins have been implicated as virulence factors in pathogenesis of *Fusarium graminearum* in wheat head blight. Mutant fungal strains lacking ability to synthesize trichothecenes were pathogenic but gave reduced incidence and severity of disease, less bleaching of heads, and less yield reduction compared to toxin-producing strains (Desjardins et al., 1996; Eudes et al., 1997; Mirocha et al., 1997; Proctor et al., 1995). DON is known to be a potent inhibitor of protein synthesis by animal ribosomes (Feinberg and McLaughlin, 1989). In plants, DON inhibited 3H-leucine incorporation into protein in maize and wheat tissues (Casale and Hart, 1998) and also inhibited in vitro protein synthesis by ribosomes isolated from wheat leaves (Miller and Ewen, 1997). In addition, several investigators have used growth inhibition as a way to compare DON sensitivity among wheat cultivars (Cutler and Jarvis, 1985; McLean, 1996). However, information is generally lacking on the cytological and physiological effects of DON on plant cells. As a first step in characterizing the role DON plays in pathogenesis of *Fusarium graminearum* in leaf and head tissues, we treated detached barley leaf tissues with DON and examined them daily for signs of injury or other alterations. As shown here, DON had pronounced and unexpected effects on leaf pigmentation at DON concentrations that were not usually lethal.

## MATERIALS AND METHODS

Segments 1.0 cm long were cut from 7-day-old primary leaves of Robust barley plants after approximately 2/3 of the abaxial epidermis was stripped from the leaves. The stripped segments were floated (3/dish) on 1.5 ml of aqueous DON solution in glass dishes, 2.5 cm in diameter and 0.8 cm high. These dishes, in turn, were incubated in covered 9 cm Petri dishes in a plant growth chamber, usually with fluorescent light (110 µmol m-2s-1) and incandescent light (40 µmol m-2s-1) for 18 hr/day. DON was used at concentrations of 10-100 ppm at steps of 10 ppm.

## RESULTS

Regardless of DON concentration, leaf segments usually remained alive for at least 5-6 days, exhibiting little or no water soaking or necrosis. Within 3-4 days, however, the segments changed color in three different ways depending on DON concentration:

1. At 10-30 ppm, tissues turned light reddish brown while retaining a green background comparable to the green of control segments floated on water. In darkness, the brown color did not develop.
2. At 50-70 ppm, most tissues turned white, losing all chlorophyll and carotenoid pigments. In darkness, this loss of pigment did not occur.
3. At 80-100 ppm, the leaf segments usually remained dark green, in stark contrast to the white segments at lower DON concentrations. At 4-5 days after treatment, the segments remained as green as they were at the time of treatment. The green color was retained in either darkness or light. Control segments floated on water generally became chlorotic by 4-5 days, the usual senescence response of barley leaf tissues to detachment from plants.

Color responses sometimes varied both among segments floated on a given DON concentration and in different portions of a given segment. For example, at 30-40 ppm, some segments were all brown or all white, while others were mottled brown and white. At 60-80 ppm, some segments had white spots within a dark green background. Although most segments appeared to remain alive after incubation on DON at 10-100 ppm, some segments became water soaked at the specific concentration of 50 ppm. Also, in separate trials at 200 ppm, about 50% of segments became water soaked by 5-6 days.

## DISCUSSION

The results indicate that DON induced four distinct, but overlapping responses in detached barley leaf segments as follows:

1. Light dependent brown pigment formation at 10-30 ppm.
2. Light dependent loss of chlorophyll and carotenoid pigments at 50-70 ppm.
3. Light independent retention of chlorophyll at 80-100 ppm.
4. Cell death at 200 ppm and sometimes also at the specific concentration of 50 ppm.

These responses often were interrelated as, for example, the retention of chlorophyll at higher DON concentration counteracted chlorophyll loss seen at intermediate concentrations. However, the effects of DON on pigment formation, loss, or retention were not consistently related to the death of tissue. Most segments treated with DON did not die. Cells died at the very high concentration of 200 ppm, and, for unexplained reasons, at the intermediate concentration of 50 ppm.

In separate experiments (not described here), we determined that cytoplasmic streaming was not disturbed by 10-100 ppm DON in epidermal cells from barley coleoptiles. Thus, these epidermal cells, which lack chloroplasts, were relatively insensitive to DON.

The light intensity used in our experiments totaled 150 µmol m-2s-1, relatively low compared to intensities usually used to grow barley. In preliminary trials with light at 240 or 450 µmol m-2s-1, loss of chloroplast pigments occurred rapidly at all concentrations from 20-100 ppm DON. Chlorophyll was not retained at 80-100 ppm DON as it was earlier at 150 µmol m-2s-1.

We have not yet measured DON concentrations within treated tissues. Since DON inhibits protein synthesis by binding to ribosomes, we speculate that chloroplast ribosomes may be highly sensitive to DON, leading to inhibition of chlorophyll and carotene synthesis at concentrations that don't affect cytoplasmic ribosomes. In any case, the present results suggest that pigment alteration in barley heads infected by F*usarium graminearum* may be a consequence of pathogen-produced DON at concentrations which do not induce plant cell death.

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# SPATIAL PATTERNS OF FUSARIUM HEAD BLIGHT IN NEW YORK WHEAT FIELDS IN 2000 AND 2001

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## INTRODUCTION

Spatial pattern analysis is used in plant disease epidemiology for understanding the nature of inoculum sources (Madden and Hughes, 1999). We assessed the spatial patterns of the incidence of Fusarium head blight (FHB) in New York winter wheat fields in 2000 and 2001.

## MATERIALS AND METHODS

The incidence of heads showing symptoms of FHB was assessed in 60 quadrats of size 0.093 m2 (1 ft2), 20 spikes per quadrat, during the late milk to dough stages. The BBD (Beta Binomial Distribution) program (Ver. 1.3) (Madden and Hughes, 1994) was used to calculate the index of dispersion and test whether the pattern of FHB incidence was random.

## RESULTS AND DISCUSSION

In 2000, mean incidence of FHB was less than 10% in three fields, but was four to six times higher in field 4-00. Aggregation of heads symptomatic of FHB was significant in field 4-00 only (Table 1). In 2001, the incidence of FHB was less than 11% in all fields sampled. Tests of the index of dispersion indicated that the pattern of FHB was random in all six fields in 2001.

Corn residues were not observed in fields 1-00, 2-00 or 3-00. In field 4-00, a very few small remnants of corn stalks from a corn crop two years earlier were still visible on the soil. There was very little visible corn residue in fields 1-01, 2-01, 3-01, and 4-01. However, there was a fair amount of corn residue distributed on the soil surface in fields 5-01 and 6-01. The incidence of FHB was also highest in these two fields in 2001 (Table 1).

The random pattern of FHB observed in all fields (except 4-00) indicated that airborne inocula are important in contributing to FHB in New York. The observed aggregation of FHB in field 4-00, together with the observed corn residue, suggests that at least a portion of the inoculum for spike infection was derived from within-field sources. Perithecia of *Gibberella zeae* are produced on corn residue left on the soil surface for up to two years after the crop has been harvested (Khonga and Sutton, 1988). The higher incidences of FHB in fields 5-01 and 6-01 compared to the other fields sampled in 2001 indicated some role of within-field inoculum contribution to FHB. The absence of an aggregated pattern of FHB in those two fields suggests relatively low contributions of within field sources to inoculum levels.

These results present circumstantial evidence that inocula from sources external to wheat fields as well as from residues within wheat fields contribute to FHB epidemics in New York.

Further research is necessary to determine the relative contributions of external versus local sources of inoculum to FHB.

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Table 1. Incidence and spatial pattern of Fusarium Head Blight in New York winter wheat fields in 2000 and 2001.

| Field[a] | Cultivar | Previous crop[b] | | FHB incidence (%)[c] | Index of Dispersion[d] | |
|---|---|---|---|---|---|---|
| | | Year-1 | Year-2 | | *D* | *P*-value |
| 1-00 | Caledonia | oat | wheat | 5.3 | 0.80 | 0.87 |
| 2-00 | AC Ron | pea | corn | 6.0 | 1.11 | 0.27 |
| 3-00 | Caledonia | pea | corn | 4.2 | 0.86 | 0.78 |
| 4-00 | unknown | soybean | corn | 23.8 | 1.77 | <0.001 |
| 1-01 | Caledonia | pea | corn | 0.9 | 0.84 | 0.81 |
| 2-01 | Caledonia | snap bean | corn | 1.4 | 0.86 | 0.77 |
| 3-01 | Harus | cabbage | corn | 2.3 | 1.15 | 0.20 |
| 4-01 | Geneva | pea | corn | 2.7 | 1.01 | 0.45 |
| 5-01 | Caledonia | corn | snap bean | 7.8 | 0.79 | 0.88 |
| 6-01 | Caledonia | corn | soybean | 10.1 | 1.07 | 0.32 |

[a] Four fields were sampled in 2000 and six were sampled in 2001. The numbers after the dash indicate the year (-00 = 2000, -01 = 2001).
[b] Year-1 is one year previous to the current year. Year-2 is two years previous to the current year.
[c] The percentage of wheat heads showing symptoms of Fusarium Head Blight.
[d] $D > 1$ suggests aggregation of FHB incidence. *P*-values are for a test of whether *D* differs from its expected value for a random pattern of disease incidence.

# ESTIMATION OF TYPE II RESISTANCE –A DILEMMA IN NEED OF A SOLUTION

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## OBJECTIVE

The objective of this study is to develop a method of characterizing Type II resistance to Fusarium head blight of wheat that is accurate, precise, and reflects the smallest differences among genotypes.

## INTRODUCTION

At the 2000 National Fusarium Head Blight Forum, Bushnell spoke about the need to distinguish clearly among the various types of resistance in cereals to Fusarium head blight, and to develop reliable methods of measuring resistance (Bushnell, 2000). Type II resistance to Fusarium head blight in wheat is resistance to spread of symptoms from an infection. It is the type of resistance most often investigated in genetic studies. Type II resistance is commonly measured as the percentage of spikelets blighted at one or more times after inoculation of a single floret. The progression of symptoms throughout the spike is measured either by visually estimating the percentage of spikelets blighted or by counting the blighted spikelets and expressing these as a percentage of total spikelets.

For foliar diseases, severity is commonly recorded as proportion (percent) of leaf area affected. This method compensates for differences in lesion size and leaf size among treatments, and it is faster than counting lesions. The assumption that underlies this method of severity assessment is that the density of inoculum landing per unit area of leaf is, on average, the same on all leaves being assessed. A leaf that is twice the area of another leaf will receive twice as many spores of a fungus, but the resulting percent severity of disease should be the same.

Is expression of head blight severity as the proportion of blighted spikelets the best way to characterize Type II resistance? It is commonly measured by observing the progression of blight throughout the spike from a single inoculated floret (point inoculation). The assumption of equal density of inoculum, which underlies the use of percent severity as a comparative measure of leaf disease severity, is not met by point inoculation. With point inoculation, spikes with different numbers of spikelets do not receive the same relative amount of inoculum. A spike with 20 spikelets and a spike with 10 spikelets both receive the same amount of inoculum (a certain number of conidia of the fungus applied to a single floret).

We would argue that the rate of invasion of a spikelet, as reflected by the number of spikelets blighted during some interval of time, does not depend on the total number of spikelets on a spike. Consider the example above: one plant with 10 spikelets per spike and another with 20 spikelets per spike. If, after single-floret inoculation, 2 spikelets on each plant be-

come blighted, proportional severity for the first plant would be 0.2 and for the second plant would be 0.1. The first plant would be considered twice as susceptible as the second. There seems no reason to suppose, however, that the total number of spikelets governs the rate at which the fungus invades the spike. If the 2 plants each had 2 spikelets blighted after single-floret inoculation, it seems logical to conclude that these plants have an equal degree of Type II resistance. By this reasoning, severity should be expressed as the number of spikelets blighted, not the proportion of spikelets blighted.

There is a problem with expressing severity at the upper end of the spectrum from complete resistance to full susceptibility, whether as number or proportion of blighted spikelets. Consider again the example of the 2 plants. If both are completely blighted after single-floret inoculation, then the first plant will have 10 blighted spikelets and the second will have 20 blighted spikelets. When severity is expressed as the number of spikelets blighted, the first would have only half the severity as the second, and would appear to have the same degree of Type II resistance as a plant with 20 spikelets that had 10 spikelets blighted. The number of spikelets per spike places an upper limit on severity, and it is not known whether the first plant, if it had 20 spikelets per spike, would have all of them blighted, still only 10 blighted, or some number between 10 and 20. This same uncertainty, however, would apply if severity were expressed as proportion. In that case, both plants would have a severity of 100%, but it is not known whether the plant with 10 spikelets is really as susceptible as the plant with 20. If at least one spikelet (normally at the base of the spike) remains unblighted after single-floret inoculation, then the number of spikelets blighted can be used as an estimate of Type II resistance. However, once all spikelets are blighted, there is uncertainty, but expression of severity as percent spikelets blighted does not eliminate this uncertainty.

## METHODS

We used data from the 2001Uniform Winter Wheat Head Blight Nursery to compare these 2 ways of expressing severity (number of blighted spikelets versus proportion of blighted spikelets). To obtain these data, we inoculated a single floret of a well-developed spikelet near the tip of the head at the beginning of anthesis. Inoculated plants received a moist period of 48 hours after inoculation. At 10 and 20 days after inoculation, we counted the number of blighted spikelets. We also counted all the spikelets on the spike, so that counts could be converted to proportions. There were 6 replicate plants for most entries; a few had only 5.

## RESULTS

Frequency distributions for individual plant severities at day 20 were similar for count and proportional data (Fig. 1A & B), except for a peak at a severity of 100% (Fig. 1B). Plants that comprised this group had a total number of spikelets that ranged from 12 to 22, and therefore in the frequency distribution for count data, these plants were distributed over this range.

The correlation between means of entries for number of blighted spikelets and percent severity was high (R=0.97). At the low end of the range of means, the association was close, but at higher severities there were some deviations between the 2 measures of severity (Fig.

2). For example, 2 lines had similar proportional severities, but differed by 2 spikelets when severity was expressed as counts (points labeled B and C in Fig. 2). Conversely, 2 lines that had similar severities expressed as counts differed considerably when severity was expressed as percent (points labeled A and B in Fig. 2).

The correlation of entry mean ranks was close for the 2 ways of expressing severity of head blight (Fig. 3). The 2 entries that deviated most from the trend line (KS96HW115 and VA98W0593; the points enclosed in a box) had the 2nd and 3rd fewest spikelets per spike (and 14.2 and 14.7). Thus, the susceptibility of these lines was inflated when severity was expressed as the proportion of spikelets blighted.

The average number of spikelets per spike for entries in the 2001 UWWFHBN ranged from 13.5 to 23.5. We investigated the possibility that entries with larger spikes would appear to have a greater degree of Type II resistance when severity is expressed as the number of blighted spikelets. The correlation between number of blighted spikelets and total spikelets per spike was 0.21, not significant. The correlation between ranks for these 2 variables was likewise not significant (R=0.178). The correlation between percent severity and total number of spikelets per spike was low, but significant (R=-0.377, P=0.008).

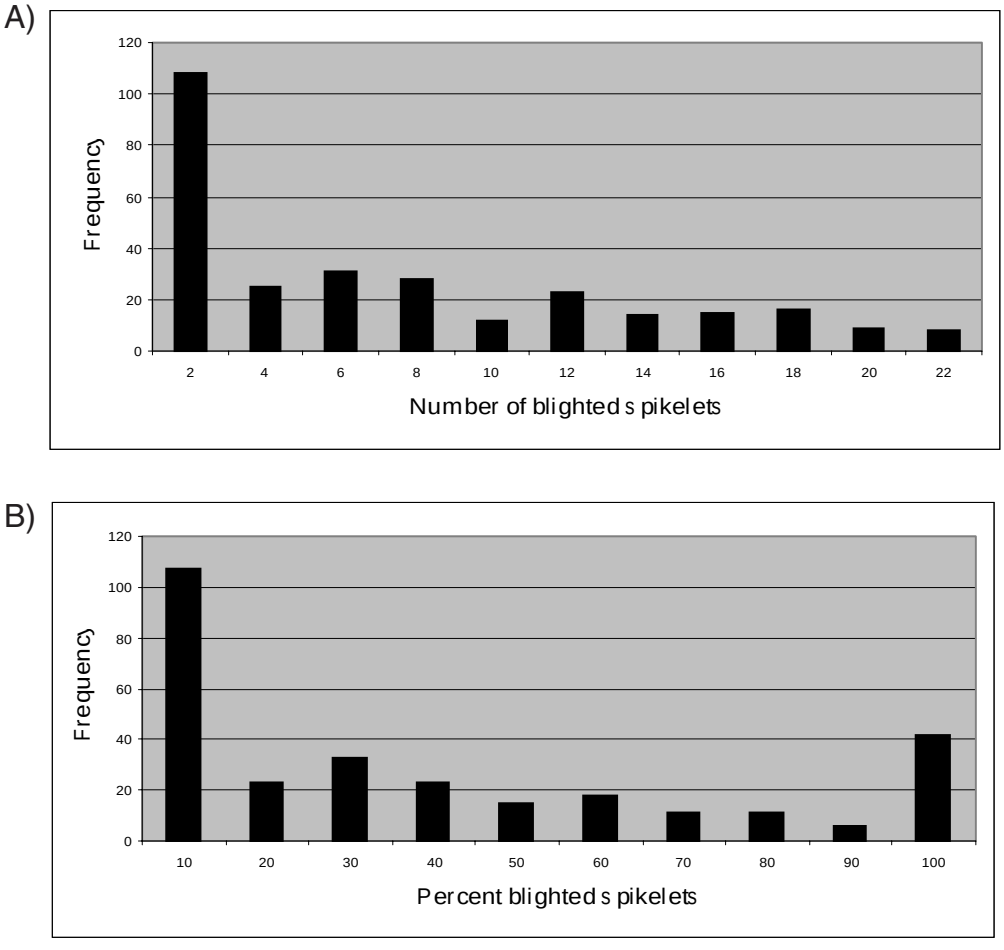


**Figure 1.** Frequency of Fusarium head blight severity values for entries in the 2001 Uniform Winter Wheat Fusarium Head Blight Nursery. Numbers on the x-axes are the upper limits of each interval. A. Frequency based on number of blighted spikelets per spike. B. Frequency based on percent of blighted spikelets per spike.

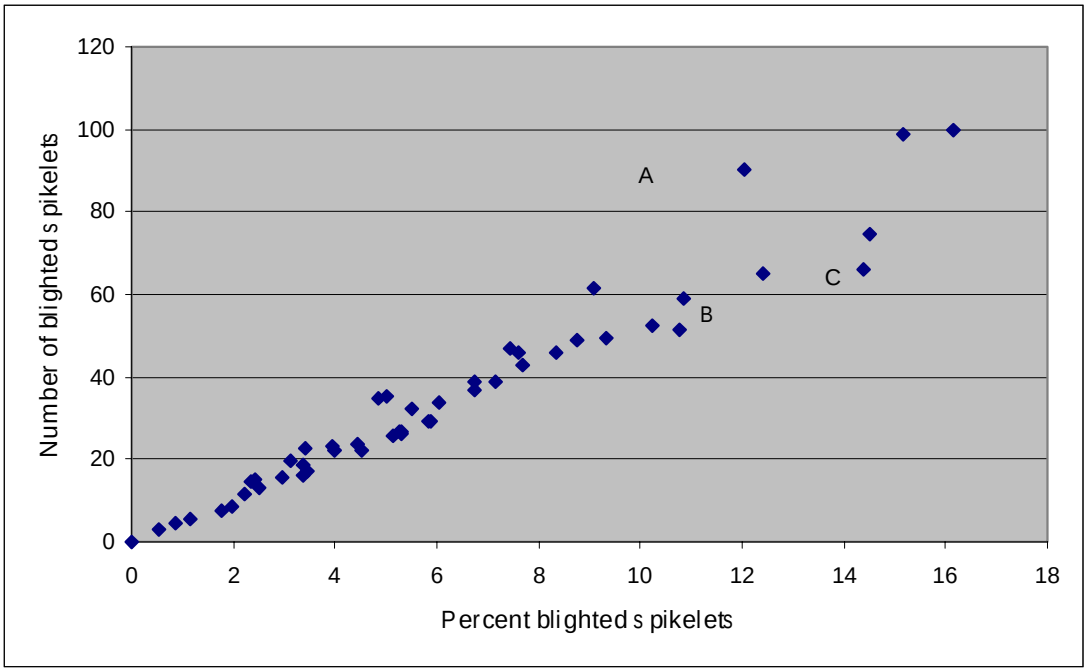


**Fig. 2.** Relation between head blight severity expressed as mean number of blighted spikelets per spike versus mean proportion of blighted spikelets for the 49 lines in the 2001 UWWFHBN.

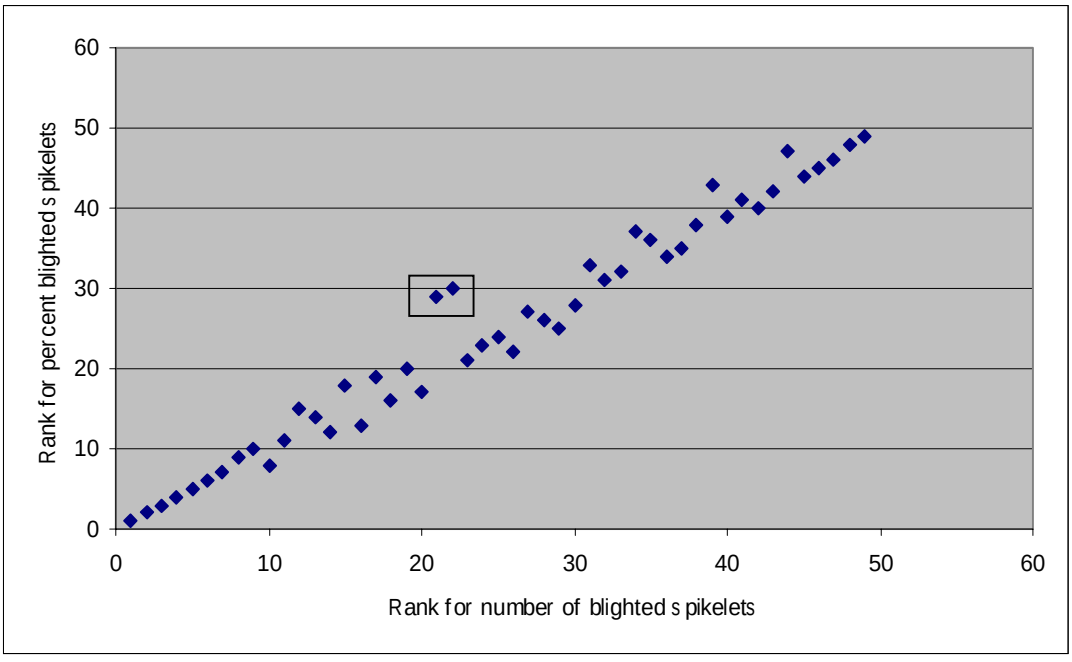


**Fig. 3.** Relation between rank of head blight severity expressed as number of spikelets blighted and rank expressed as percent of spikelets blighted for the 49 lines in the 2001 UWWFHBN.

## DISCUSSION

It seems more logical to express severity of head blight, following single-floret inoculation, as the number of spikelets blighted rather than as the proportion of spikelets blighted. Expressing severity in absolute terms reflects absolute rate of blight development rather than a rate that is adjusted for the number of spikelets on the spike. Differences in spike size are not likely to influence the degree of Type II resistance. Data from genetic experiments are only ambiguous when severity has reached a maximum: all of the spikelets blighted. This ambiguity exists for relative (percent) severity as well.

The low, but significant correlation between percent severity and total spikelets per spike is not surprising because total spikelets is the denominator for the calculation of percent severity, and therefore these two variables are not independent of each other. This association suggests that selection for Type II resistance based on percent severity would favor lines with larger spikes. While this may be good for general improvement of plant type, it could result in failure to select for some genotypes with useful resistance because they happen to occur in a plant with a small head.

The conclusions from this study need to be tested by examining a larger set of data. We are in the process of conducting analyses with data from genetic studies, involving both advanced-generation recombinant inbred lines and early generation segregating populations. It appears that use of percent severity to characterize Type II resistance will not lead to gross errors in evaluating wheat lines, but it could lead to failure to select lines with potentially useful partial resistance and could be an additional source of error in genetic studies. Because greater resistance is the goal of germplasm enhancement and breeding programs, this is the end of the spectrum of reaction that is of greater interest. It appears that severity of head blight expressed as the number of blighted spikelets rather than the percentage of blighted spikelets distinguishes differences among genotypes better toward the resistance end of the spectrum.

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# PLANT RESIDUE MANAGEMENT AND FUSARIUM HEAD BLIGHT

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## ABSTRACT

The research presented in this review is part of an ongoing investigation to establish the correlation between residue management and the survivability of *Fusarium*. Residue decomposition and *Fusarium* survival are quantified when wheat, barley and corn plant residues are placed on and below the soil surface. Cover crop and nitrogen (N) fertilizer treatments are included as variables. Parameters related to decomposition such as soil temperature and water and carbon to nitrogen ratio of the residue are monitored. If *Fusarium graminearum* survival is related to residue decomposition, then residue management strategies which enhance displacement of *Fusarium* might be developed.

Wheat, barley, and corn residues, infested with *Fusarium graminearum*, were collected at crop harvest. On September 16-17, 1999 the prepared bags were placed in a field of wheat stubble. One-half of the replicated test plots were fertilized with nitrogen. In the spring of 2000 following standard crop rotation practices, a soybean cover crop was planted on one-half the plots to establish plant canopy and soil water variables. Decomposition rates (weight loss) and *Fusarium* populations were determined at 30 day sampling intervals throughout the study period (Fall 1999-Fall 2001). Nitrogen analyses were completed on composite residue samples for each residue type. Soil samples were collected at the research site to quantify chemical and physical properties. Populations of *Fusarium graminearum* on the residue were determined by quantitative plating techniques.

In the case of all three substrates, buried residue decomposed faster than residue left on the surface. Corn residue was lost at a faster rate than either the wheat or barley residues. Nitrogen fertilizer did not enhanced the decomposition rate. *Fusarium* populations appear consistent with the level of residue present. In the Project's final report, residue decomposition rates, fusarium survivability, soil water availability, soil temperature and residue nutrient status will be correlated based on residue placement, N fertility and cover crop.

This poster was presented at the 2001 Annual Meeting of the Soil Science Society of America, Charlotte, NC, October 21-25. Support for this research has been made available from the United States Wheat and Barley Scab Initiative, Grant 59-0790-9-070.

# DEVELOPMENT OF PERITHECIA FROM *GIBBERELLA ZEAE* ON WHEAT RESIDUE

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## ABSTRACT

*Gibberella zeae* (anamorph *Fusarium graminearum*) is the major causal organism of Fusarium head blight (FHB) in the United States. This disease can affect all classes of wheat causing reduction in yield, poor seed quality and mycotoxin contamination. *G. zeae* over-winters in wheat residue as hyphae. Very little is understood about the formation of perithecia from hyphae in wheat residues. Our objectives are to characterize the early stages of perithecial development and investigate whether colonization of specific wheat tissues is important to development of perithecia. Preliminary findings on perithecium development in debris will be presented. Implications of this work towards control of FHB will be discussed.

# COMPARISON OF POPULATIONS OF *GIBBERELLA ZEAE* FROM KOREA AND NORTH AND SOUTH AMERICA

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## ABSTRACT

We isolated populations of *Gibberella zeae* (*Fusarium graminearum*) from field samples of wheat, barley, maize or sorghum from North and South America, and from South Korea. We compared the phylogenetic lineage composition from these sources using AFLP markers produced by three standard primer combinations. United States populations of *G. zeae* from wheat are composed of a single phylogenetic lineage (lineage VII) and are diverse but relatively homogeneous across the country. South Korean populations from barley were dominated by a single lineage (lineage VI). South Korean populations from maize are dominated by lineage VII, but lineage III is a relatively common component. Populations of *G. zeae* from wheat in Brazil also appear to be dominated by lineage VII, but at least one other lineage is present. We have also examined *G. zeae* populations from wheat and sorghum in Uruguay.

# EARLY DETECTION OF DEOXYNIVALENOL IN WHEAT GRAIN

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## OBJECTIVE

Evaluate the potential of pre-harvest sampling of wheat fields to provide estimates of deoxynivalenol in the harvested grain.

## INTRODUCTION

Epidemics of Fusarium head blight (FHB) on wheat and barley occur when rain and flowering coincide (Hart, et al, 1984; McMullen, et al, 1997). The resulting DON contamination of the grain is uneven from field to field and within a field**.** In 2000 FHB epidemics occurred in several Midwest states including Michigan, New York, Ohio, Indiana, and also Canada. Although the 2000 FHB epidemic in Michigan appeared to be mild, wheat processors (General Mills, Kelloggs, Jiffy Mix) indicated that 50% of the wheat they normally use from Michigan had to be imported because of high DON levels. In 2001, FHB was again widespread in Michigan, but DON levels were lower than were predicted from the incidence and severity. The poor correlation between FHB incidence and DON levels in both years suggested a need to develop in field sampling protocols to reliably estimate DON prior to harvest. Statistics can provide us with confidence levels that can be applied toward the implementation of FDA guidelines for DON. Estimates of DON based on non-statistical parameters are not acceptable where the issue of consumer food safety is concerned.

In previous studies spatial trends in deoxynivalenol concentration on truckloads of grain were not evident (Hart and Schabenberger, 1998; Hart, et al 1999). The presence of kernels with high or low concentration at a particular location in the truck yielded no information about concentrations nearby. The distribution of toxin appeared to be completely spatially random. In the study reported here, wheat fields were sampled and the grain analyzed for DON prior to harvest.

## METHODS AND MATERIALS

The research objective in 2000 was to determine if there were spatial relationships for DON concentrations between sampling sites within wheat fields prior to harvest. A relatively small, but intense sampling pattern was chosen to evaluate these relationships (Figure 1). This W pattern had four transects, and samples were collected every fifteen feet along each transect. Every thirty feet additional samples were collected on either side of the transect line. Twenty to twenty-five heads were collected at each sampling point, and the heads kept separate between sampling points. The grain was threshed from the heads using a small gasoline powered thresher.

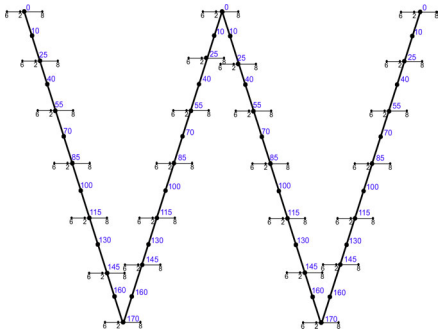

**Figure 1.** W sampling pattern used to study spatial distribution of DON in pre-harvest wheat in 2000. Each dot on the grip represents a sampling point where wheat heads were collected for DON analysis. Numbers represent distance in feet between sampling points.

In 2001 sampling in-field prior was expanded to harvest to cover entire fields. The sampling pattern was in the shape of an hourglass (Figure 2). Twenty to twenty-five heads were collected every fifty feet along each of the transect lines, and the heads kept separate between sampling points (Figure 2). The grain was threshed from the heads using a small gasoline powered thresher. For both 2000 and 2001, as the fields were harvested the grain in the trucks was sampled using commercial probes as described previously (Hart and Schabenberger, 1998), and the grain was analyzed for DON using ELISA as described previously (Hart, et al, 1998).

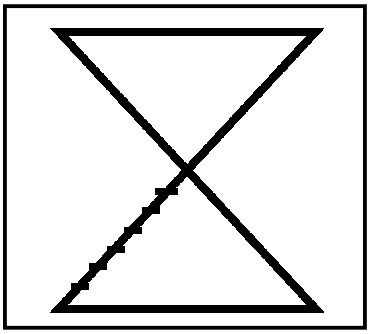

**Figure 2.** Whole field hourglass sampling pattern used in 2001. Samples were collected at 50-foot intervals along grid lines.

## RESULTS

Average DON levels in 2000 ranged from 1.4 to 8.0 ppm, and from 1.7 to 2.1 ppm in 2001 (Table 1). Randomly selecting DON data from between four and twenty of the sampling points in both years indicated that twenty samples/field could provide an estimate of average field DON levels (Table 2). Table 2 shows the 90% confidence intervals for DON levels from pre-harvested wheat. The 90% confidence interval is calculated as the range from the $5^{th}$ to the $95^{th}$ percentiles. Observations were randomly re-sampled to create samples of size 4, 6, 8, 10, 15, and 20. This process was repeated approximately one thousand times for each sample size. The percentiles of the distribution of DON sample means are then estimated from the empirical distribution of the sample means. When 20 samples per field

were randomly selected and used to calculate a field DON mean, the predicted DON mean is withn +/- 0.5 ppm of the mean calculated from all of the samples (Table 1). The 90% confidence interval decreases as the mean level of DON in the field decreases, but a larger confidence interval at high levels of DON is less important since these fields would be rejected as being outside of acceptable levels based on the FDA guidelines for food.

**Table 1**. Descriptive Statistics for Field Samples. A W sampling pattern was used in 2000. An hourglass sampling pattern was used in 2001.

| Field | Number of Observations | Mean DON ppm | Standard Deviation | Min[a] DON | Max[b] DON | Mean DON from probes |
|---|---|---|---|---|---|---|
| Field 1 2000 | 118 | 8.07 | 4.13 | 1.35 | 29.1 | 8.17 |
| Field 2 2000 | 117 | 1.48 | 1.06 | 0.15 | 5.00 | 1.47 |
| Field 3 2000 | 76 | 1.46 | 1.08 | 0.20 | 6.16 | NA |
| Field 1 2001 | 154 | 2.17 | 1.40 | 0.2 | 5.4 | 3.15 |
| Field 2 2001 | 144 | 1.93 | 1.43 | 0.2 | 5.0 | 2.6 |
| Field 3 2001 | 88 | 1.72 | 0.97 | 0.2 | 4.0 | 2.8 |

[a] Lowest level of DON from a single sample. [b] Highest level of DON from a single sample

**Table 2.** 90% Empirical Confidence Intervals for DON levels in fields sampled before harvest. See Table 1 for details.

| Sample Field | Sample size *n* = | Sample Mean | Lower Bound[a] | Upper Bound[b] | Interval Width |
|---|---|---|---|---|---|
| Field 1 2000 | 20 | 8.03 | 6.54 | 9.68 | 3.14 |
| Field 2 2000 | 20 | 1.48 | 1.13 | 1.87 | 0.74 |
| Field 3 2000 | 20 | 1.47 | 1.11 | 1.91 | 0.80 |
| Field 1 2001 | 20 | 2.17 | 1.7 | 2.7 | 1.0 |
| Field 2 2001 | 20 | 1.93 | 1.4 | 2.5 | 1.1 |
| Field 3 2001 | 20 | 1.72 | 1.4 | 2.1 | 0.7 |

[a] 5% of the DON means from randomly selected observations would be below this concentration. [b] 5% of the DON means fro the randomly selected observations would be above this concentration.

The hourglass sampling pattern (Figure 2) used in 2001 gave a better view of the spatial distribution of DON throughout a field compared to the W sampling pattern used in 2000 that represented only small-scale conditions (Figure 1). The scale of the 2000 study did not take into account the conditions across entire fields as did the 2001 study. A kriging map of DON in ppm from one of the fields sampled in 2001 is shown as Figure 3**.** In the center of the field the distribution of DON appears fairly homogeneous with a few areas showing low concentrations. Toward the edges of the field, the toxin concentrations rise sharply.

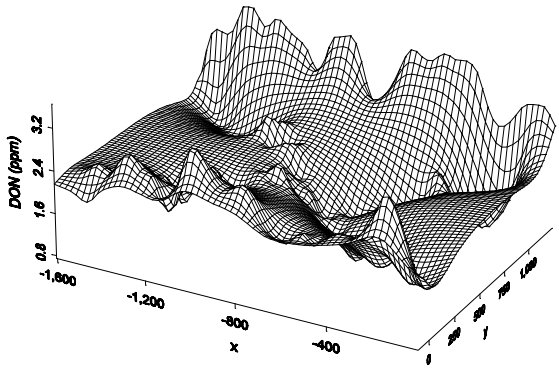


**Figure 3.** Spatial prediction of deoxynivalenol (ppm) across a Michigan field in 2001.

DON levels were similar between pre-harvest samples and probe samples in the 2000 study, but the truck probe samples tested about 1 ppm higher than the field samples in 2001 (Table 1). The results may be attributed the use of different research scale grain threshers between the two years. Small particles, it turns out, were more likely to be removed by threshing of the field samples using the new thresher in 2001, and may have contributed to the difference. Since small kernels are often associated with high deoxynivalenol concentration, this raises the important question as to "what constitutes a sampling unit?" Regardless, our studies in 2000 and 2001 suggested that infield sampling prior to harvest can be used to obtain an estimate of DON corresponding to DON levels in the harvested grain (Table 1).

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# ASSESSING THE RISK OF WHEAT CONTAMINATION BY DEOXYNIVALENOL IN BELGIUM

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## ABSTRACT

The cereals crops in Belgium are not safe from serious contaminations by mycotoxins. The years 1997 and 1998 for example were very favourable to the development of Fusarium head blight. On that account, the project main objective consists in assessing the risk of mycotoxins contamination and studying the environmental factors that contribute to increase the rate of wheat contamination and integrating them in a forecasting model. A competitive direct enzyme-linked immunosorbent assay (CD-ELISA) for the semi-quantitative analysis of deoxynivalenol (DON) has been used on 130 samples of wheat grains of the harvest 2000. In addition, a HPLC multi-residue method is in development to separate various mycotoxins. Samples, mentioned above, were contaminated mainly by *Microdochium nivale* (96%), a fungi inducing scab symptoms but not suspected to produce mycotoxins, *F. culmorum* (22%) and *F. graminearum* (15 %). A small amount of analyzed samples were contaminated by *F. poae* and *F. avenaceum*. PCR analyzes were used to verify the *Fusarium* species microscopic identification. The DON concentration determined by immunological analysis has provided values ranging from 0 to 1,2 ppm (92%: 0-0,5 ppm; 6%: 0,6-1 ppm; 2% > 1 ppm). In the other hand, two types of artificial contaminated field trials were established: 1) A multi-variety trial where different varieties of wheat were inoculated separately with *F. graminearum* and *F. culmorum*. 2) A one-variety trial where 20 *Fusarium* strains belonging to the four *Fusarium* species, above mentioned, were inoculated. For the first trial, we observed differences in both infection rates in the field and DON accumulation between varieties but not between *Fusarium* species inoculated. For the second one, differences in DON production were observed between *Fusarium* species and between some strains of the same species. Microbiological analyses and field observations show a good correlation but they were not related to the DON concentration.

# PHYSICAL TREATMENTS FOR PREVENTING THE POST-HARVEST GROWTH OF *FUSARIUM* IN MALTING BARLEY

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## ABSTRACT

We evaluated the effect of three physical treatments (hot water, UV-C radiation, and electron-beam radiation treatments) on the *Fusarium* infection rates (FIR) and germinative energy rates (GER) in Fusarium head blight infected malting barley. For hot water treatments, four different temperatures (45, 50, 55, and 60°C) for four time periods (1, 5, 10, 15 minutes) were evaluated. For UV-C radiation, three irradiation times (5, 10, and 15 minutes) were evaluated. For electron-beam radiation treatments, dry barley was irradiated at five different doses (2, 4, 6, 8, and 10 kGy). Hot water treatments caused significant reduction in the FIR and GER of barley. The decrease in FIR and GER was more pronounced with increased temperature and time of treatment. Significant reduction (compared to the untreated control) in FIR started at 1 minute for all the temperatures (45, 50, 55, and 60°C). The corresponding reductions on average were found to be 40%, 95%, 99%, and 98% at 1 minute respectively. Longer times at higher temperatures over 45°C eventually caused complete reduction (100%) of FIR. At 45°C, reductions in FIR of 97% and 96% were seen at 10 and 15 minutes respectively, with no significant reductions in GER. Significant reduction (compared to the untreated control) in GER occurred at 50°C after 5 minutes. For temperatures 55 and 60°C, significant reductions (48% and 95% respectively) in GER were seen at 1 minute. Compared to the untreated control, there were no significant reductions in the FIR with increase in UV-C irradiation times. Also, decreases in GER in the UV-C irradiated samples were not significantly different from the untreated sample. For electron-beam radiation, FIR decreased significantly with increase in the dosage used. Significant reduction in the FIR started between 2-4 kGy. Higher doses (8 kGy, and 10 kGy) achieved complete reduction (100%) of FIR. GER also decreased with increase in the electron-beam doses used. Significant decrease (7%) in GER started at a dose of 4 kGy. Higher dosage (10 kGY) caused a larger reduction (32%) in GER relative to the other dosages used. Based on the results we have obtained, further research will be done to evaluate additional treatments and combinations of treatments. Effective treatments will be evaluated further for effect on malt quality and mycotoxigenesis of surviving *Fusarium*.

# RELATIONSHIP BETWEEN FUSARIUM HEAD BLIGHT INFECTION AND THE MALTING QUALITY OF BARLEY

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## ABSTRACT

Maltsters and brewers avoid the use of *Fusarium* infected barley because of concerns over mycotoxins and gushing in the resultant beer, and the US industry has set very tight levels on DON (deoxynivalenol) as a means of regulating entry of FHB (Fusarium headbBlight) infected grain into the market. However, infection with FHB also damages barley and malt quality. These factors are likely to become more of a concern as treatments are identified that are able to eliminate mycotoxins and gushing potential (from beer), and thus encourage the use of some FHB infected grain for malting. The objective of this study was to determine the relationships between the level of FHB infection and specific barley, malt and wort quality parameters. Commercial samples of Robust barley (125) were collected in eastern North Dakota during the 1996-2000 crop years. Samples were malted (N=2) and standard quality parameters determined. Plate count (% infected kernels), DON, ergosterol, xylanase and proteinase were determined as markers of FHB infection.

# UPDATE ON DON DIAGNOSTIC SERVICES IN 2000/2001

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## OBJECTIVES

To provide Fusarium head blight (FHB) research projects with analytical services for deoxynivalenol (DON) and other mycotoxins.

## INTRODUCTION

DON analysis has been an important part of cooperative efforts to fight FHB. In 2000 and 2001, US Wheat and Barley Scab Initiative provided grants to four DON Diagnostic Centers. The centers provided free DON analysis services for all US Wheat & Barley Scan Initiative research projects. The contact information for the DON Diagnostic Centers is listed as the following:

L. Patrick Hart, Department of Plant Pathology, Michigan State University, East Lansing, MI 48824; sample type: wheat; phone: (517) 353-9428, fax: (517) 353-5598, e-mail: hart@msu.edu

Weiping Xie, Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108; sample type: wheat, barley, special samples (single kernels, small stem and leaf segments etc.); phone (612) 625-2751, fax: (612) 625-9728, e-mail: weipingx@umn.edu

Beth Tacke, Veterinary Diagnostic Laboratory, North Dakota State University, Fargo, ND 58105; sample type: wheat, barley, durum; phone: (701) 231-8309, fax: (701) 231-7514, e-mail: Beth.Tacke@ndsu.nodak.edu

Paul B. Schwarz, Department of Cereal Science, North Dakota State University, Fargo, ND 58105; sample type: barley, malt; phone: (701) 231-7732, fax: (701) 231-7723, e-mail: Paul.Schwarz@ndsu.nodak.edu

## MATERIALS AND METHODS

The analytical methods used by the centers included ELISA (P. Hart), GC/ECD (B. Tacke and P. Schwarz) and GC/MS (W. Xie). The sample preparation method used by GC/ECD and GC/MS analyses was developed by Tacke (1).

Each center conducted its own intralab quality control (QC) throughout the analysis period during the year to ensure the quality of the analysis. A collaborative quality assurance (QA) program was also carried out among the centers. Each month, from Sept. to Dec. in 2000

and Apr. to Oct. 2001, a wheat sample and a barley sample collected by the coordinator (B. Tacke) were sent to each center. Each center performed analyses in two different days and reported the replicate data to the coordinator within one week. The collected data was then sent to the centers. The QA program allowed each center to evaluate the accuracy and precision of their system.

## RESULTS AND DISCUSSION

Table 1 summarized the scale of DON analysis services conducted by the 4 centers in 2000 and 2001. Since most of samples came to the centers starting from August in each year, the data was reported as of 2000/2001 (08/01/00 to 07/30/01) and 2001/2002 (08/01/01 to 07/30/01). The total number of samples analyzed by the 4 centers was 19,672 in 2000/2001. In 2001/2002, a total of 13,136 sample have been analyzed by Nov. 10, 2001. An estimated total of 19,500 to 20,500 samples will be analyzed by the 4 centers in the 2001/2002 year.

The intralab QC information from the 4 DON diagnostic centers is shown in Table 2. The coefficients of variation (CV) varied from 7 to 16% in 2000/2001 and from 5 to 16% in 2001/2002. The average of CV of 8 QC sample pools from the 4 centers was 9.1% in 2001/2002 showing an overall improvement of precision in comparison with 11.4% of 200/2001. The Minnesota diagnostic center experienced a higher coefficient of variation for 2001/2002 (14%) than 2000/2001 (12%). The possible causes will be evaluated.

The interlab QA data of Sep. through Dec. 2000 is shown in Table 3. The average CV of the 5 tests was18% for the wheat check samples and 17% for the barley check samples. Table 4 gives the interlab QA data of Apr. through Oct. 2001. The average CV of the 7 tests was 21% for wheat and 17% for barley. The data shows that there was no major difference between the DON diagnostic centers.

Since samples sent to the DON diagnostic centers were usually in batches and the DON data was usually used to compare samples with a batch, the precision of analysis at each center in a period of a few days is important to the scab researchers. The variation between the two replicate analyses of each interlab QA sample reflects this short-term precision. Table 5 shows the average CV of two replicate QA samples from each center in 2000/2001 and 2001/2002. The data in Table 5 indicates that the reproducibility of each center within one week was quite good.

The interlab QA program will continue through December, 2001 or until most of samples for 2001/2002 year are proceed.

**Table 1.** Number of samples analyzed by DON diagnostic centers.

| Center | PI number | | State number | | Sample number | | Estimated total number of 01/02 |
|---|---|---|---|---|---|---|---|
| | 00/01 | 01/02 | 00/01 | 01/02 | 00/01 | 01/02* | |
| MI-P. Hart | 9 | 17 | 8 | 11 | 2,481 | 3,371 | |
| MN-W. Xie | 11 | 12 | 2 | 1 | 7,533 | 2,970 | 8,000 - 9,000 |
| ND-P. Schwarz | 4 | 3 | 2 | 2 | 5,222 | 4,612 | |
| ND-B. Tacke | 23 | 10 | 6 | 4 | 4,436 | 2,183 | ~4,600 |
| Total | 47 | 42 | 18 | 18 | 19,672 | 13,136 | 19,500-20,500 |

*Numbers as of Nov. 10, 2001.

**Table 2.** Intralab quality control data for Aug-Dec, 2000 and Apr-Nov, 2001.

| Center | Grain | 2000/2001 | | | 2001/2001 | | |
|---|---|---|---|---|---|---|---|
| | | Number | Mean (ppm) | CV (%) | Number | Mean (ppm) | CV (%) |
| MI-P. Hart | Wheat | 56 | 1.6 | 7 | 94 | 2.3 | 5 |
| MN-W. Xie | Wheat | 34 | 12.8 | 12 | 38 | 9 | 14 |
| ND-P. Schwarz | Barley | 120 | 6.3 | 14 | 108 | 6.3 | 11 |
| | Barley | 112 | 1.6 | 16 | 104 | 1.5 | 16 |
| | Barley | 124 | 5.3 | 15 | 104 | 5.1 | 10 |
| ND-B. Tacke | Whaet | 83 | 1.8 | 9 | 31 | 1.7 | 5 |
| | Barley | 83 | 3.1 | 9 | 31 | 2.9 | 5 |
| | Corn | 83 | 5 | 9 | 31 | 4.6 | 7 |

**Table 3.** Interlab quality assurance data of 2000/2001 (Sep. through Dec. 2000).

| Center | Grain | DON results (ppm) | | | | |
|---|---|---|---|---|---|---|
| | | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 |
| MI-P. Hart | Wheat | 10.8/10.8 | 1.5/NA | 0.6/0.6 | 7.2/7.2 | 2.4/2.5 |
| MN-W. Xie | Wheat | 11.5/11.2 | 0.6/0.7 | 0.3/0.3 | 5.9/5.3 | 2.4/2.3 |
| ND-P. Schwarz | Wheat | 11.5/14.8 | 0.8/0.9 | 0.6/0.5 | 4.0/4.5 | 2.1/2.1 |
| ND-B. Tacke | Wheat | 10.5/10.8 | 0.8/0.9 | 0.5/0.6 | 5.5/6.0 | 2.5/2.5 |
| Ave. | | 11.5 +/- 1.4 | 0.9 +/- 0.3 | 0.5 +/- 0.1 | 5.7 +/-1.1 | 2.4 +/- 0.2 |
| MI-P. Hart | Barley | 2.0/2.1 | 6.6/NA | 7.4/8.4 | 15.0/14.0 | 27.5/28.4 |
| MN-W. Xie | Barley | 2.1/1.9 | 5.6/5.5 | 7.6/7.3 | 14.5/15.3 | 28.0/27.2 |
| ND-P. Schwarz | Barley | 1.4/0.7 | 4.3/4.6 | 9.4/9.5 | 10.1/9.9 | 20.6/21.6 |
| ND-B. Tacke | Barley | 1.8/2.1 | 5.7/5.7 | 7.6/8.0 | 13.7/14.6 | 31.2/29.8 |
| Ave. | | 1.8 +/- 0.5 | 5.4 +/- 0.8 | 8.2 +/- 0.9 | 13.4 +/- 2.2 | 26.8 +/- 3.8 |

**Table 4.** Interlab quality assurance of 2001/2002 (Apr. through Oct. 2001).

| Center | Grain | DON results (ppm) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Test 1 | Test 2 | Test 3 | Test 4 | Test 5 | Test 6 | Test 7 |
| MI-P. Hart | Wheat | 1.4/1.2 | 12.0/11.2 | 6.0/6.1 | 8.8/9.0 | 3.8/4.2 | 0.4/0.4 | 7.0/9.0 |
| MN-W. Xie | Wheat | 0.8/0.9 | 4.6/8.4 | 2.4/2.5 | 6.8/7.2 | 7.0/7.4 | 0.3/0.3 | 5.9/6.2 |
| ND-P. Schwarz | Wheat | 0.8/0.8 | 7.9/8.3 | 4.0/3.7 | 6.9/6.7 | 4.9/6.0 | 0.2/NA | 7.7/7.6 |
| ND-B. Tacke | Wheat | 1.2/1.3 | 9.1/8.2 | 4.1/4.3 | 6.8/7.5 | 5.7/5.8 | 0.4/0.3 | 6.0/6.3 |
| Ave. | | 1.1+/- 0.3 | 9.1+/-1.6 | 4.1+/-1.4 | 7.5+/-0.9 | 5.6+/-1.3 | 0.3+/-0.1 | 7.0+/-1.1 |
| MI-P. Hart | Barley | 4.0/4.4 | 0.8/1.0 | 6.9/7.5 | 10.2/10.8 | 0.8/0.8 | 3.6/3.4 | 10.0/12.0 |
| MN-W. Xie | Barley | 3.0/3.4 | 0.5/0.6 | 6.1/6.1 | 8.6/9.4 | 7.0/7.4 | 3.5/3.4 | 10.3/10.6 |
| ND-P. Schwarz | Barley | 3.2/2.4 | 0.5/0.6 | 5.9/5.0 | 10.4/10.2 | 0.8/0.8 | 4.2/NA | 8.6/16.4 |
| ND-B. Tacke | Barley | 4.1/3.8 | 0.7/0.7 | 6.1/7.2 | 10.1/10.6 | 1.0/0.9 | 3.4/3.7 | 9.6/9.4 |
| Ave. | | 3.5+/-0.7 | 0.7+/-0.2 | 6.3+/-0.8 | 10.0+/-0.7 | 0.8+/-0.1 | 3.6+/-0.3 | 10.9+/-2.4 |

**Table 5.** Average CV of replicate QA samples from each center in 2000/2001 and 2001/2002.

| Center | 00/01 | | 01/02 | |
|---|---|---|---|---|
| | Ave. CV (%) | | Ave. CV (%) | |
| | Wheat | Barley | Wheat | Barley |
| MI-P. Hart | 2.9 | 4.9 | 6.2 | 7.0 |
| MN-W. Xie | 4.7 | 3.4 | 4.3 | 5.8 |
| ND-P. Schwarz | 9.5 | 11.5 | 4.4 | 16.3 |
| ND-B. Tacke | 4.5 | 6.3 | 6.9 | 5.1 |

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

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## ABSTRACT

Many Chinese wheat lines have been introduced into wheat breeding programs because of their resistance to Fusarium head blight (FHB). It is anticipated that these lines will provide diversity for resistance genes. Sumai 3, a popular resistance source from China, has been well characterized for scab resistance QTL. Fujian 5114, a Chinese wheat derived from the cross Longxi18/Ning8017, has resistance to FHB spread (Type II) as good as Sumai 3, and appears to differ from Sumai 3 in some resistance loci. The purpose of this study is to map the resistance QTL in Fujian 5114. A population of 78 $F_7$ derived recombinant inbred lines (RIL) from the cross Fujian5114/Norm was evaluated for FHB severity in mist-irrigated, inoculated field trials in the summer of 2000 and 2001. The population was also evaluated for spread within the spikelet from point inoculations in two greenhouse trials in 2001. Results from field and greenhouse trials are correlated and allow separation of lines for severity. Marker analysis will consist of simple sequence repeat (SSR) amplifications in regions containing QTL reported in Anderson et al., 2001. Preliminary analysis based on greenhouse and field FHB data shows that Fujian 5114 contains the QTL on 3BS and it explains up to 25% of the variation in this population. We hope to explain 60% or more of the variation in FHB using these or other markers.

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# GERMPLASM CONTRIBUTION OF THE CIMMYT WHEAT PROGRAM TO THE U.S. WHEAT AND BARLEY SCAB INITIATIVE

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## OBJECTIVES

Provide a genetically diverse spectrum of bread wheat and barley germplasm resistant to Fusarium head blight (FHB), for use in US breeding programs and for basic research.

## INTRODUCTION

The agreement between the CIMMYT Bread Wheat Program and the USA Scab Wheat and Barley Initiative is nearing the end of its third year. The first seed shipment consisted of 27 advanced bread wheat lines, 20 synthetic hexaploid derivatives and 10 advanced barley lines with resistance to fusarium head blight types I and II. These resistance sources were shipped to Missouri and North Dakota respectively (McKendry, 2000). In addition to the above, selected germplasm originating from Romania (7) and China (15) was confirmed to be resistant under a severe scab epidemic in Toluca (central Mexican highlands) and also sent. These preliminary results confirmed the resistance in the Romanian germplasm as noted in the USA (Ittu, 2000, personal communications). A second shipment of new and outstanding germplasm will be sent as soon as we receive the pending APHIS permit. Data on the response of these lines to FHB and other diseases are presented in below.

## MATERIAL AND METHODS

Advanced bread wheat lines were evaluated under natural conditions in Sierra de Jalisco, Guadalajara and Patzcuaro, and under of artificial inoculation in Atizapan, Toluca. Advanced barley lines were also evaluated in the last two locations. Evaluations were carried out during 1998-2000.

Stripe rust (*Puccinia striiformis*) is endemic in this location but we still conduct artificial inoculation with selected, virulent races, as determined by our rust pathologists. FHB due to *Fusarium graminearum* can also appear naturally but we also inoculate artificially to ensure good screening conditions.

The FHB inoculum is increased on mungo bean medium and its concentration adjusted to 50,000 spores/ml after growing for five days. Twenty wheat spikes per plot were inoculated at flowering, applying a liquid spore solution for type I resistance. The cotton method was used to determine type II resistance (Gilchrist et al. 1997). We counted infected spikelets per spike 25 days after inoculation for type I resistance and 35 days after inoculation for type II resistance, thus obtaining a percentage of resistant spikelets.

nvironmental conditions were optimal the past cycle for an excellent epidemic of FHB, and only rarely was additional irrigation required.

## RESULTS AND DISCUSSION

After three years of evaluation 32 outstanding advanced bread wheat lines and 12 barley lines were selected for inclusion in the second shipment. The advanced lines were selected based on their resistance over the time and the consistency of low disease responses representative group of such lines is presented in Tables 1 and 2.

The shipment will also include new lines from Argentina (107), Brazil (19), and Japan (12) that showed high levels of resistance to FHB types I and II. Brazilian germplasm also expressed good agronomic type and resistance to leaf rust. However, only 50% of the latter group carry resistance to the local stripe rust races. The Japanese germplasm was relatively poorly adapted to the Mexican conditions and was highly susceptible to stripe rust. Some of the latter lines were also susceptible to leaf or stem rust. The Argentinean collection included lines of both good and poor agronomic types, but in general expressed good resistance to stripe, leaf, and stem rust.

**Table 1.** Advanced bread wheat lines with Fusarium head blight resistance types I and II evaluated in Atizapan, Mexico, during 1998-2000.

| Crosses | Selection history | % type I | % type II | TGW loss % | Stripe rust | Grain (1 5) |
|---|---|---|---|---|---|---|
| PSN/BOW/4/MAYA/NAC/3/RPB14.6 8/PVN//PHO/5/MUNIA | CMBW91M03563T-0TOPY-13M-010Y-015M-010Y-8Y-0M-3SJ-0Y-0FGR | 6.94 | 12.75 | 5.41 | 5 MR | 2 |
| SABUF | CM95073-3Y-0M-0Y-3M-0RES-4PZ-0Y-10PZ-0Y-9PZ-0Y-0FGR | 7.47 | 8.15 | 4.58 | 0 | 1* |
| IAS64/ALDAN//URES/3/TNMU/4/TN MU | CMBW90M4487-0TOPY-14M-16AL-0AL-07Y-4M-0Y-3PZ-0Y-0FGR | 2.92 | 18.18 | 12.81 | 0 | 2 |
| SHA3/SERI//PSN/BOW | CMBW90M2470-7M-010M-010Y-015M-9Y-0M-2PZ-0Y-0FGR | 5.05 | 5.05 | 4.34 | 0 | 1 |
| PF83144/3/F3.71/TRM//VORONA | CO8687-7P-2P-0P-010Y-0M-0FGR | 10.83 | 14.97 | 19.28 | 20MR | 2 |
| RL6043/6*NAC//TNMU/3/BAU | CMSS92Y02211T-27Y-015M-010Y-010Y-10M-0Y-1SJ-0Y-0FGR | 11.14 | 7.18 | 16.87 | 30MR | 2 |
| BAU/MILAN | CM103873-2M-030Y-020Y-010M-2Y-0M-2PZ-0Y-8SJ-0Y-0FGR | 2.3 | 18.26 | 16.19 | 0 | 1 |
| SHA7/KAUZ | CM95113-8Y-0M-3FC-0FC-0FC-4FUS-0Y-6SJ-0Y-0FGR | 3.98 | 8.33 | 3.49 | 0 | 2 |
| KAUZ//PRL/VEE#6 | CM94747-27Y-0H-0SY-4M-0RES-0SY-0ECU-010Y-0M-0FGR | 8.7 | 11.2 | 19.78 | 5MR | 3 |
| ALD/PVN//YMI #6 | CM91065-2M-0Y-0M-2Y-0B-12PZ-0Y-0FGR | 4.21 | 10.38 | 3.14 | 0 | 1* |
| TINAMOU | CM81812-12Y-06PZ-3Y-1M-0Y-7AL-0Y-4AL-0AL-0M-0ECU-010Y-0M-0FGR | 4.52 | 10.77 | 2.46 | 0 | 1 |
| ALUCAN/DUCULA | CMBW89M3764-36M-0AL-2AL-2B-0Y-3SJ-0Y-0FGR | 9.12 | 17.48 | 9.52 | 60MR-MS | 2 |
| CLC89/MILAN | CMSS92Y00573S-030Y-015M-0Y-0Y-18M-0Y-0FGR | 3.23 | 13.24 | 4.99 | 0 | 1 |
| JUP73R/PVN | CMBW91M04467S-5Y-2M-2Y-10M-0Y-0FGR | 8.9 | 18.79 | 6.55 | 0 | 2 |
| LIRA//AU/UP301/3/2*KAUZ | CMBW91Y02983M-030TOPM-19Y-010M-010Y-015M-7Y-0M-3SJ-0Y-0FGR | 3.87 | 8.05 | 8.3 | 0 | 1 |
| XIANG82.2661/2*KAUZ | CMBW91Y02917M-030TOPM-24Y-010M-010Y-015M-2Y-0M-6SJ-0Y-0FGR | 3.01 | 12.43 | 12.45 | 0 | 1 |
| TUI/MILAN | CMSS92Y00540S-030Y-015M-0Y-0Y-2M-0Y-1PZ-0Y-0FGR | 8.75 | 19.41 | 11.72 | 0 | 2 |
| WUH1/VEE#5//CBRD | CMSS92M01863S-015M-0Y-050M-0Y-13M-0Y-1SJ-0Y-0FGR | 1.1 | 8.13 | 1.55 |  | 1* |
| OCEP14/BAU | CMBW90M1656-48M-1AL-0AL-07Y-3M-0Y-1PZ-0Y-0FGR | 5.18 | 16.34 | 3.36 | 0 | 1* |
| BAU/DUCULA//BAU | CMBW91M03579T-0TOPY-23M-010Y-015M-010Y-7Y-0M-2PZ-0Y-0FGR | 13.13 | 15.96 | 27.59 | 60MR-MS | 3 |
| WUH1/VEE#5//CBRD | CMSS92M01863S-015M-0Y-050M-0Y-18M-0Y-0FGR | 6.87 | 8.05 | 4.01 | 0 | 1 |
| GUAM92//PSN/BOW | CMSS92M01860S-015M-0Y-050M-0Y-11M-0Y-0FGR | 4.9 | 13.16 | 6.62 | 0 | 1 |
| R37/GHL121//KL/BB/3/JUP/MUS/4/2 *YMI#6/5/CBRD | CMBW91Y01575S-4Y-010M-010Y-015M-9Y-0M-0FGR | 1.49 | 10.53 | 7.68 | 0 | 1 |
| DESC/3/ALD/PVN//YMI #6 | CMBW90M2417-9M-010M-010Y-015M-8Y-0M-0FGR | 6.29 | 12.93 | 6.53 | 0 | 1* |
| CBRD/KAUZ | CMBW90M2494-8M-010M-010Y-015M-10Y-0M-0FGR | 3.21 | 6.43 | 2.36 | 0 | 1* |
| WUH1/VEE#5//MUNIA | CMSS92M01862S-015 M-0Y-050M-0Y-15M-0Y-0FGR | 3.19 | 5.08 | 6.61 | 0 | 1 |
| WUH1/VEE#5//CBRD | CMSS92M01863S-015M-0Y-050M-0Y-13M-0Y-0FGR | 0 | 12.15 | 9.08 | 0 | 1 |
| BCN//DOY1/Ae SQUAROSA | Check S-S | 24.07 | 32.93 |  | 0 | 5 |
| FLYCATCHER (MS) | Check S-MS | 29.8 | 21.04 |  | 0 | 4 |
| SUMAI #3 (MR) | Check R-MR | 1.84 | 9.2 |  | 10MS | 2-Jan |

**Table 2.** Advanced barley lines with resistance to Fusarium head blight types I and II evaluated in Atizapan, Mexico, during 1998-2000.

| Crosses | Selection history | % type I | % type II | % TGW | Grain (1-5) |
|---|---|---|---|---|---|
| AZAF/KYOTO NAKATE//ALELI | CBSS96WM00287T-D-3M-4Y-2M-0Y | 6.3 | 29.03 | 7.08 | 2 |
| GOB/HUMAI10//ALELI/3/AZAF | CBSS95M00623T-C-1M-3Y-17M-1Y-2M-0Y | 7.91 | 27.23 | 5.68 | 1 |
| GOB/HUMAI10//ALELI/3/AZAF | CBSS95M00623T-C-1M-3Y-18M-1Y-1M-0Y | 5.32 | 6.13 | 3.36 | 1* |
| AZAF/KYOTO NAKATE//ALELI | CBSS96WM00287T-D-3M-3Y-2M-0Y | 9.07 | 32.32 | 6.25 | 2 |
| GOB/HUMAI10//ALELI/3/AZAF | CBSS95M00623T-C-3M-3Y-3M-3Y-1M-0Y | 4.83 | 34.93 | 1.8 | 2 |
| GOB/HUMAI10//ALELI/3/AZAF | CBSS95M00623T-C-1M-3Y-20M-1Y-1M-0Y | 9.69 | 16.1 | 2.15 | 1* |
| AZAF/KYOTO NAKATE//ALELI | CBSS96WM00287T-D-2M-1Y-1M-0Y | 8.91 | 34.02 | 9.01 | 1 |
| FRANKLIN-BAR//GOB/HUMAI10/3/AZAF | CBSS96M00672T-C-2M-1Y-1M-0Y | 4.91 | 36.9 | 4.88 | 1 |
| NE175-B/GOB96DH//AZAF | CBSS96M00675T-H-2M-1Y-1M-0Y | 7.74 | 8.83 | 3.55 | 2 |
| ALELI/KANTO NIJO 2//MSEL | CBSS96WM00438T-F-7M-1Y-1M- | 7.2 | 10.36 | 6.27 | 2 |
| ATACO/ACHIRA//HIGO/3/KANTO NIJO 2/4/SHYRI | CBSS96WM00270T-D-1M-3Y-1M-0Y | 6.68 | 5.54 | 2.44 | 3 |
| GOB96DH/3/ND10277/SHYRI//ND11231/SHYRI/4/AZAF | CBSS96M00681T-Y-2M-1Y-2M-0Y | 2.74 | 6.87 | 3.8 | 2 |
| ATAHUALPA | CHECK (MR-MR) | 10.2 | 4.26 | | 2 |
| AZAFRAN | CHECK (MR-R) | 8.52 | 6.44 | | 2 |

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# IDENTIFICATION OF QTL ASSOCIATED WITH RESISTANCE TO FHB IN NING 7840 AND FREEDOM

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## ABSTRACT

Our objectives were 1) to map QTL for FHB resistance from Ning 7840 and Freedom and 2) to determine molecular basis for transgressive segregation. Two populations of $F_{2:4}$ families were developed from the crosses Ning 7840/OH542 and Ning 7840/Freedom. OH542 is a highly susceptible line while Freedom and Ning 7840 are resistant. FHB severity was rated in two years of greenhouse tests using hypodermic syringe inoculation technique and one year of field tests using corn kernel inoculum and mist irrigation. Families were genotypes with SSR markers and QTL analysis was performed using single point marker analysis.

A QTL for FHB severity was identified at 3BS in both populations with resistance conferred by the Ning 7840 allele in both cases. The Ning allele at 3BS appeared to be recessive to the susceptibility allele from either Freedom or OH542. This was observed in both greenhouse and field trials. The 3BS QTL accounted for 13 to 15% of the phenotypic variation. A QTL at 3AL was also detected in both populations, with resistance being conferred by Ning 7840. Resistance at this locus appeared to be additive and it accounted for 7 to 12% of the variation.

A QTL was identified on 2AS locus in the Ning 7840/Freedom population. This QTL explained 20% of the phenotypic variation for severity (field). Resistance was conferred by the Freedom allele and appeared to be additive. This QTL was also detected using greenhouse data. The $R^2$ value for this QTL was greater than what we found for the 3BS QTL in either population. This suggests that the 2AS region from Freedom is of equal or greater importance than the 3BS region from Ning 7840 in explaining resistance. This conclusion needs to be confirmed.

The importance of the 2AS QTL allele from Freedom is supported by genetic analysis of resistant transgessive segregants for severity from the Ning 7840/Freedom population. Nearly 80% of these resistant (field or greenhouse) lines were homozygous for Freedom alleles at 2AS. Only 50% of the resistant lines based on field data were homozygous for Ning 7840 alleles at, while 80% of such lines based on greenhouse data were homozygous for Ning7840 alleles at 3BS.

A major QTL for resistance from Ning 7840 was identified at 3BS. The QTL was not population specific. A major QTL for resistance from Freedom was identified at 2AS. The magnitude of the QTL at 2AS from Freedom appeared to have equal or greater effect on resistance than 3BS from Ning 7840. A combination of the Ning 7840 allele from 3BS and the Freedom allele at 2AS seemed important in obtaining resistant transgressive segregants.

(A PDF file of this poster will be available from the Ohio State University wheat breeding program web site.)

# MOLECULAR AND PEDIGREE ANALYSIS OF SOURCES OF RESISTANCE TO FHB IN WHEAT

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## ABSTRACT

There are multiple sources of FHB resistance, with 30+ sources being used in various prebreeding and variety development breeding programs. Many breeders plan to pyramid different FHB resistance genes to maximize resistance. This will require that we know the allelic nature of resistance in the many sources. It is difficult to ascertain this information when there are so many sources and phenotyping is cumbersome. Our objective was to determine if markers can be used to separate sources of resistance that are likely to have unique FHB resistance genes. Twenty-three lines were selected based on pedigree relationship and resistance to FHB, including Ning 7840, Sumai 3, Freedom, Mentana, Frontana, and Bezostaja. Each was genotyped with SSR markers from regions known to be associated with FHB resistance as well as other regions. Principal component analysis was performed to produce biplots of the data for each region of the genome.

Markers from the 3BS region clearly separated Ning 7840 and Sumai 3 from all other lines. This separation agrees with mapping reports that Sumai 3, Freedom, and Frontana all appear to have different FHB genes at the 3BS region. The unique nature of these sources of resistance is supported by pedigree analysis. Markers on chromosome 2AS, which has been associated with resistance from Freedom, also separate the resistant sources. Mapping studies also show that Freedom and Ning 7840 have different FHB genes in this region. The 2AS analysis suggests that Freedom and Bezostaja may possess similar FHB genes at 2AS, a notion supported by pedigree analysis. Thus, the marker analyses of 3BS and 2AS conform to what we know from other studies on allelism of FHB resistance genes. Other analysis suggests that Bezostaja may possess different alleles at 5A than other sources of resistance and that Ning 7840 and Sumai 3 are quite distinct from other sources of resistance at 7A. Only markers at 7B differentiated Ning 7840 and Sumai 3, suggesting that they could have different FHB gene at 7B. This may also be inferred from the literature where 7B has been found to be significantly associated with resistance to FHB from Ning 7840, but not Sumai 3.

The analysis of marker diversity for at key chromosome segments grouped sources of resistance. In some cases the differences between groups corresponded to known genetic differences for FHB resistance between some members of different groups. This indicates that SSR genotyping may be useful as a prescreening tool for new sources of FHB resistance. This presecreeing could be used to reduce the number of new lines that would be tested for uniqueness relative to our current sources of resistance.

(A PDF file of this poster will be available from the Ohio State University wheat breeding program web site.)

# DEVELOPMENT OF SYNTHETIC HEXAPLOIDS WITH FUSARIUM HEAD BLIGHT RESISTANCE FROM *TRITICUM TURGIDUM* L. VAR. *DICOCCOIDES*

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## ABSTRACT

Fusarium head blight (FHB) continues to significantly impact grain production of barley (*Hordeum vulgare* L.) and wheat (*Triticum aestivum* L.) throughout the world. Incorporating host plant resistance from secondary gene pool species such as *Triticum turgidum* L. var. *dicoccoides* (2n=4x=28, AABB) may result in the transfer of unique sources of FHB Type II resistance to cultivars and consequently provide genetic diversity for resistance. Molecular mapping of FHB Type II resistance in a recombinant inbred chromosome line (RICL) population derived from a Langdon-*dicoccoides* chromosome 3A disomic substitution line [LDN(Dic-3A)], identified a quantitative trait locus (QTL) on *T. dicoccoides* chromosome 3A. The QTL explained approximately 55% of the genotypic variance for resistance. Incorporation and expression of the (Dic-3A) QTL in synthetic hexaploids and utilization of the tightly linked microsatellite marker *Xgwm2* for future marker-assisted selection may expedite the integration of this new source of resistance into hexaploid cultivars. Crosses were made between seven individuals of the LDN(Dic-3A) RICL and eight accessions of *Triticum tauschii* (2n=2x=14, DD). Hybrids (n=3x=21, ABD) were subsequently embryo rescued and colchicine treated for chromosome doubling. Accessions of *T. tauschii* were selected for hybridization based on previous studies demonstrating their resistance to other wheat pathogens. Currently, we have 45 synthetic hexaploids (2n=6x=42, AABBDD) for screening of Type II resistance in 2002 spring greenhouse and field experiments.

# PROGRESS IN BREEDING FOR SCAB RESISTANCE IN ROMANIA ON WHEAT

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## OBJECTIVES

In this report are reviewed the main results obtained in Romania on the breeding of resistance to scab (FHB) in winter bread wheat. The main topics of researches performed in two centers located in the South (Fundulea) and the North (Turda) of the country, have been:

- development of reliable screening methods under artificial field inoculation;
- identification of new FHB resistant sources;
- recombination of resistance genes from various sources;
- combining FHB resistance with yield capacity, quality and adaptation by stepwise hybridization, recurrent selection or the DH approach.

As results of these breeding approaches, a reliable screening protocol for resistance to FHB in winter bread wheat and improved resistant lines, not related to the sources previously described in spring wheats of Asian and Brazilian origin, were obtained. Some of the most advanced lines, particularly *Fundulea 201 R* and *Turda 195* have shown in several environments high levels of resistance Type I, Type II and reduced DON content as well. Following successive years of selection at Fundulea, promising derivatives of crosses between the scab resistant parent F 201 R and donors for good bread making quality (Dropia and Delabrad), combining reasonable levels for the both traits, were identified. Selection of new sources of resistance to scab in bread winter wheat derived from complex crosses is in progress.

## INTRODUCTION

Fusarium head blight (FHB, scab) has emerged in the past decades as a very destructive wheat disease with a worldwide distribution. In addition to the significant reduction of grain yield and quality, accumulation of toxic compounds (DON, NIV etc) in infected grains and in end use products, is detrimental for health of humans and animals upon consumption. Breeding wheat cultivars that combine high levels of resistance to FHB with other agronomic traits, remains the most reliable and friendly to environment strategy for scab control. Current resistance to scab reduces to just a few sources, mostly spring types of Asian and Brazilian origin, containing each only a few genes (1,2, 10,11). In Romania natural occurrence of *Fusarium* scab (FHB) in wheat is not very common. However the damaging potential of this disease is considerable when humidity is high at anthesis. In the past decades breeding researches for resistance to FHB are conducted under artificial field inoculation in two centers located in the South (Fundulea) and the North (Turda) of the country (4). As results of breeding approaches to develop new winter bread wheat cultivars with improved resistance to FHB, advanced lines, not related to the sources previously described in spring

wheats of Asian and Brazilian origin, were identified (7,8). The objective of this report is to review the main results obtained in Romania according to the following topics: screening for resistance; identification of new FHB resistant sources and recombination of resistance genes from various sources; analysis of resistance; combining FHB resistance with other important agronomic traits.

## RESULTS

*Screening for resistance to FHB.* Our breeding strategy for resistance to FHB is based on combining this character with other desirable agronomic traits: complex resistance to pathogens, yield capacity, quality and adaptation. In this respect, we start the field evaluation for resistance to FHB in F5 at Fundulea and Turda. Following single head artificial inoculation, two assessment criteria are frequently utilized to characterize the FHB resistance Type II (resistance to fungal invasion within plant tissues): the area under disease progress curve (AUDPC, % of bleached spikelets at 10 and 20 days postinoculation) and Relative Weight of Inoculated Spikes (RWIS, % of control). RWIS, % of control provides also reliable information on tolerance to FHB being constantly found to be correlated with other yield components. Selected entries with higher levels of resistance to FHB are artificially inoculated, in parallel, in advanced yield trials and local FHB nursery, as single head rows. After trials performed for two years in each center, the best entries from each location are interchanged, in order to be evaluated also in the other center (Fig. 1).

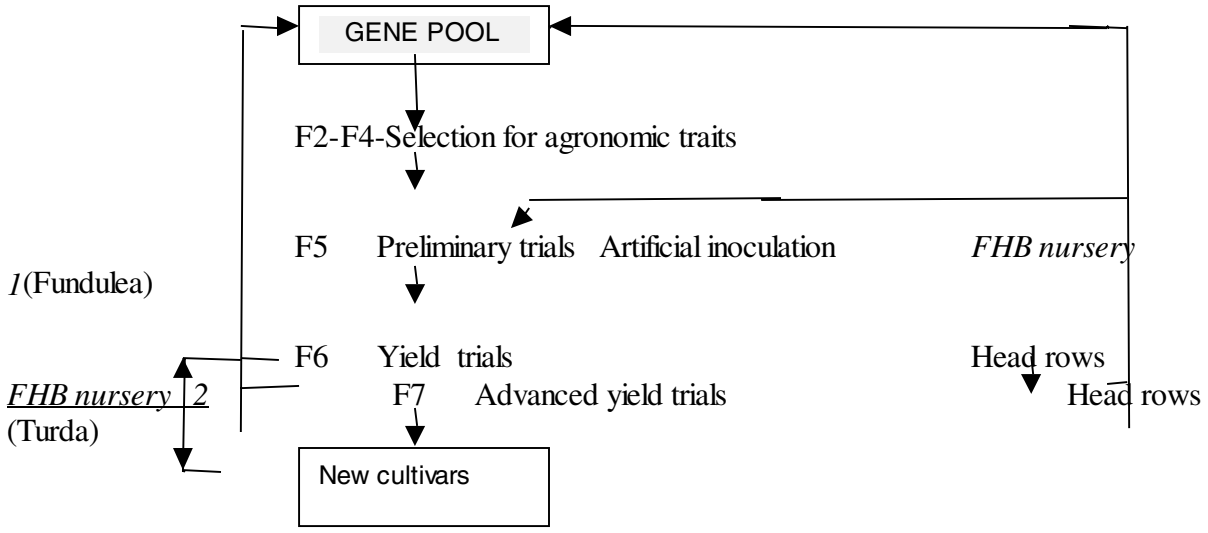


**Fig. 1** Screening protocol for resistance to FHB in wheat

***Evaluation of aggressiveness in local Fusarium isolates.***
The current evaluation of aggressiveness among local *Fusarium* isolates became quite necessary in order to determine the pathogenic potential and to select the most suited isolates for assessment of resistance under artificial inoculation. Evaluation of aggressiveness in a collection of 57 Romanian *Fusarium* isolates revealed a large diversity of this trait in two bread wheat cultivars with different responses to FHB, *Fundulea 29* (moderately resistant) and *Fundulea 4* (very susceptible). Experiments were performed in seedling stage by a rapid screening method (3). ANOVA of data regarding the length of coleoptyle (% of control) showed significant differences between wheat cultivars and Fusarium isolates. The

main sources of variance were the genotypes of host (F=319:68**) and pathogen (F=54:03**), respectively. However, we found some differential host plant/pathogens combinations among the *Fusarium* isolates from our collection. These results suggested that preliminary selection of *Fusarium* isolates, according to their pathogenic potential to wheat be recommended in order to reduce the risks of non-accurate estimation of resistance. In the same experiment evidence for the existence of association between resistant host genotype/aggressive isolate was observed. The isolates originated from the moderately resistant cultivar *Fundulea 29* reduced more drastically the length of the coleoptyle in seedlings of susceptible *Fundulea 4*, as compared to its own isolates. This could be the effect of selection pressure into the pathogen population, as result of cultivation host genotypes with higher resistance (5).

***Identification of new FHB resistance sources and recombination of resistance genes from various sources.***

Efforts to select new sources of resistance to FHB in winter wheat were initially directed toward the breeding gene pool. We identified new sources of resistance in old Romanian (Montana, Turda 195) and foreign more or less adapted wheat cultivars (Libelulla, NS 732, Amigo). New cultivars with improved resistance to FHB obtained at Turda (Turda 95) have in their pedigree old Romanian wheat breeding lines, not particularly bred for resistance to FHB. As a result of a program to recombine resistance to FHB from various sources, several winter bread wheat lines with better levels of resistance were obtained (9). *Fundulea 201 R*, (F 201R) is a selection of complex cross between several sources of resistance *(F156I5-2112/F2076 W12-11)*. It cumulates resistance genes derived from cultivars NS 732 and Amigo, having no relation to any of previously described sources of resistance. It has shown high levels of resistance, in several environments from Romania and other European countries, Mexico etc, when various methods of inoculation were utilized. HPLC analysis of DON content, performed in Poland, showed low amounts of DON (0.70µg $g^{-1}$), in kernels of F201 R from heads inoculated with two Romanian *Fusarium graminearum* isolates (6). Repeated multiple location investigations on resistance to FHB including other advanced lines are in progress.

**Analysis of resistance to FHB.**

*Recombinant inbred lines (RIL's) approach*. Recombinant inbred lines derived from the cross F 1054 W (*susceptible*)/Sincron (*moderately resistant*) were investigated for three years, in order to study the genetic control of resistance to FHB and to identify possible associations of resistance with several marker loci. Frequency distributions for AUDPC (area under disease progress curve) and RWIS (relative weight of inoculated spikes) indicated different patterns among years and a quantitative inheritance of resistance. When lines were grouped according to alternative alleles at marker loci for height-reduction (*RhtB1 & Rht 8*), gliadin content (*Gli B1* & *Gli D1*) and waxy appearance of leaves (*W2*), significant association between FHB resistance and some gliadin loci (*Gli D1 b & Gli R1)* was documented. These results emphasized the involvement of chromosome *1B* in the control of FHB resistance and provided additional evidence for the possible role of *1D* (7).

***Double haploid lines (DH) approach.***

Based on DH analysis, the inheritance of resistance to FHB in winter bread wheat line *Fundulea 201 R* was investigated. Three years of investigation of response to FHB in 108

DH lines from crosses between this line and both, medium resistant (*F 249 T*) and susceptible (*F 135 U*) parents, suggested a quantitative inheritance. Transgressive segregation was observed in both crosses with *F 201 R*, mainly when the response to FHB was assessed as relative weight of inoculated spikes (RWIS, % from control). These data demonstrated the reliability of DH approach in breeding wheat for resistance to FHB. On the other hand the possibility to improve concomitantly the economical level of resistance to FHB and to maintain the desirable agronomic type was demonstrated (8).

***Combining FHB resistance with bread making quality.***

Line *F 201 R* is characterized not only by a good level of resistance to FHB, but also to other pathogens as rusts, powdery mildew and Septoria leaf blotch, but has a low bread making quality. Following successive years of selection at Fundulea, promising derivatives of crosses between the FHB resistant parent F 201 R and donors for good bread making quality (*Dropia and Delabrad*), combining reasonable levels for the both traits were obtained.

The distribution of 15 lines derived from crosses of *F 201R* with two high quality parents Dropia and Delabrad, according to AUDPC and sedimentation index suggests that, although a higher resistance is usually associated with low quality, several lines combining good levels of both resistance and quality were obtained.

Next step of breeding is expected to recombine higher values for both traits, FHB resistance and bread making quality respectively. All evidence reported to date suggests that further progress in FHB resistance is possible through the cumulation of resistance genes from different sources.

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# PROBLEMS ENCOUNTERED IN TRANSFERRING SCAB RESISTANCE FROM WILD RELATIVES INTO DURUM WHEAT

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## THE PROBLEM

Scab or Fusarium head blight (FHB), caused primarily by the fungus *Fusarium graminearum* Schwabe, is a serious disease of both hexaploid and tetraploid wheats. The fungus infects wheat heads from flowering through grain fill, causing enormous losses to growers in the northern plains area of the United States (McMullen et al., 1997). It is estimated that the losses may approach a billion dollars in the U.S. alone in some years. The combined direct and secondary economic losses suffered by wheat and barley producers in scab-affected regions of the U.S. during the 1998 to 2000 period were estimated at 2.7 billion dollars (Nganje et al., 2001); North Dakota and Minnesota account for about 55 percent of the total dollar losses. Reduction in kernel density and the presence of tombstone kernels (Figure 1) make wheat unmarketable. Moreover, the accumulation of the mycotoxin deoxynivalenol (DON) in the grain renders it unfit for human or even animal consumption. The need for breeding FHB resistance into wheat cultivars cannot, therefore, be overemphasized. Some of the problems encountered in transferring FHB resistance from wild species into durum wheat are outlined in this article.

**Figure 1.** Healthy and scab-infected seeds of durum wheat. [Photograph by Jim Miller, USDA-ARS]

## SOURCES OF SCAB RESISTANCE

Most modern wheat cultivars lack resistance to FHB. Durum cultivars, in particular, have no resistance to this devastating disease. However, some of the wild relatives of wheat are rich sources of genes for resistance to diseases including FHB (Jauhar and Peterson, 1996, 1998, 2001; Friebe et al., 1996). We found that two wild relatives of wheat – the tetraploid wheatgrass (*Thinopyrum junceiforme* (Löve & Löve) Löve, $2n = 4x = 28$; $J_1J_1J_2J_2$ genomes) and diploid wheatgrass (*Lophopyrum elongatum* (Host) Á. Löve, $2n = 2x = 14$; EE) – are excellent sources of resistance to FHB. Thus, *L. elongatum* shows a mean infection of

3.8%, compared to infections of about 60% to 90% in susceptible durum cultivars. Several other wheatgrasses, e.g., *Thinopyrum bessarabicum* (Savul. & Rayss) Á. Löve ($2n = 2x = 14$; JJ), may also prove excellent donors of FHB resistance to wheat. Chinese scientists have attempted to transfer scab resistance from *Roegneria komoji* Koch. ($2n = 6x = 42$; SSHHYY) into bread wheat (Liu et al., 2000). From the tertiary gene pool of wheat, Canadian workers have found one accession of *Elymus humidus* to be immune to FHB (Fedak, 2000).

It is not known whether the resistance in wild species is due to genetic factors or mechanical reasons. In diploid *Th. bessarabicum*, for example, the resistance may be due to the powdery coating on its leaves and stems. This powdery substance seems to be toxic to the fungal pathogen and even to aphids. But then the production of this metabolic by-product must be under genetic control. Other wild species like *Th. junceiforme* and *L. elongatum* must have genetically controlled resistance to FHB. An appropriate donor of FHB resistance must be selected. The problem with polyploid donors is the high number of undesirable chromosomes they bring into the wheat genome. It is preferable, therefore, to use diploid species as potential donors of FHB resistance. We are and plan on using the diploid wheat grasses *L. elongatum* and *Th. bessarabicum* for resistance breeding into durum wheat.

## LACK OF CHROMOSOME PAIRING

Synthesis of hybrids between wheat and alien donors is an essential first step for alien gene transfer. Using embryo rescue techniques, wheat can be crossed with most wild grasses in the tribe Triticeae. Pairing between wheat chromosomes and alien chromosomes is a prerequisite for gene transfer into wheat. However, such chromosome pairing is generally low or even absent primarily because the pairing regulating gene, *Ph1*, located in the long arm of wheat chromosome 5B, suppresses pairing among homoeologous (less related) or unrelated chromosomes (Sears, 1976; Jauhar and Joppa, 1996). This poses a serious problem from the standpoint of alien gene transfer into wheat.

This problem may be overcome in durum wheat by using a Langdon 5D(5B) substitution line that lacks chromosome 5B and hence the *Ph1* gene. Hybrids between the 5D(5B) substitution and most alien species show high chromosome pairing, accelerating the chances of alien gene transfer into wheat. Another method of at least partially solving this problem is to use appropriate genotypes of wild species that suppress the activity of *Ph1*, resulting in promotion of chromosome pairing in wheat - alien species hybrids (see Jauhar, 1992; Jauhar and Almouslem, 1998; Jauhar and Peterson, 2001). In very difficult cases when chromosomes fail to pair in synthetic hybrids, gamma radiation may be employed to induce translocations between wheat and alien chromosomes.

## STABLE INTEGRATION OF ALIEN CHROMATIN

Occurrence of pairing between wheat chromosomes and alien chromosomes leads to genomic reconstruction in wheat, resulting from integration of alien chromatin into the wheat genome (see Jauhar and Peterson, 2000, 2001). Such integrations may confer FHB resistance but may not be stable and hence lost in subsequent generations, resulting in loss of FHB resistance. This is a serious problem. It is advisable to synthesize a relatively large

number of hybrids with alien integrations, thereby increasing the chances of finding a stable integration. In some cases, a monosomic addition (full chromosome complement plus one alien chromosome, i.e., $2n = 28 + 1$) has been found to have resistance to scab. However, monosomic additions are not stable because the unpaired alien chromosome is lost. To obviate this problem, monosomic addition lines may be selfed to produce stable disomic additions. The production of a disomic addition would, however, depend upon the transmission of the monosome through the male and female gametes.

## ASSOCIATION OF UNDESIRABLE TRAITS WITH FHB RESISTANCE

In some (perhaps most) cases, alien chromatin conferring FHB resistance may, at the same time, bring undesirable traits to the recipient parent. The severity of this problem may depend on the size of the alien chromosome fragment integrated. The smaller the size of alien chromatin, the lower are the chances of bringing undesirable characters into wheat. Smaller integrations would also be generally stable.

## PROBLEM OF SCREENING FOR TYPE-2 FHB RESISTANCE IN WILD GRASSES

Screening for Type-2 FHB resistance is done by introducing 10 ¼l of inoculum (100,000 spores per ml) of three different isolates (biotypes) of *F. graminearum* into the florets at the time of anthesis. Inoculated plants are then grown under warm, humid conditions favoring *Fusarium* growth and spread (see Jauhar and Peterson, 2001) and individual spikes are scored for percent infection once after two weeks and then after three weeks (Stack and McMullen, 1994).

The grass florets are generally small and tightly compact, making it difficult to introduce the desired amount of inoculum, thereby posing a problem for optimal screening. Increasing the concentration of spores per ml may help solve this problem. Spraying the florets with the inoculum may offer another alternative.

## CONCLUSION

In transferring FHB resistance from wild gasses into wheat we confront several problems, which are not insurmountable. Wide hybridization does offer an excellent tool for breeding scab resistance into wheat cultivars. Transgenic approaches to combating scab are also being pursued in several laboratories including ours (Dahleen et al., 2001). Standardization of transgenic technology for durum wheat in our laboratory (Bommineni et al., 1997) has paved the way for direct introduction of antifungal genes into otherwise desirable durum cultivars. All available approaches should be adopted to combat Fusarium head blight in cereals.

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# CHARACTERIZATION OF WHEAT GERMPLASM FOR SSR MARKER ALLELES NEAR THE FUSARIUM HEAD BLIGHT RESISTANCE QTL ON CHROMOSOME 3BS

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## ABSTRACT

Previously, we reported SSR markers associated with Sumai 3-derived FHB resistance. The objective of this research was to characterize wheat germplasm using SSR markers in the region of a major QTL on chromosome 3BS. Seventy-four FHB resistant and susceptible lines from throughout the world were genotyped with four SSR markers. Cluster analysis of a genetic similarity matrix was performed using the unweighted pair-group mean algorithm (UPGMA). The SSR markers GWM389, GWM533, GWM493 and BARC102, detected 14, 12, 9 and 12 alleles, respectively. Sumai 3 type alleles are unique. Only 19 out of 54 FHB resistant lines have Sumai 3 type alleles for at least one SSR marker, and none of the FHB susceptible lines have the Sumai 3 type alleles. Six FHB resistant lines have the same genotype as Sumai 3 for all four SSR markers. All of these lines contain Sumai 3 or derivative in their pedigree, and, therefore most likely contain this major QTL. Five FHB resistant lines have the Sumai 3 type alleles at three of the four marker loci. The five lines likely have the same QTL as Sumai 3 in this 3BS region. All the other 42 FHB resistant lines and all 20 susceptible lines tested were distinct from Sumai 3 in this region. These resistant lines may carry novel FHB resistance genes, and further genetic study is worthwhile.

# MAPPING OF FUSARIUM HEAD BLIGHT RESISTANCE QTL IN THE WHEAT LINE WUHAN3

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## ABSTRACT

The best method of controlling Fusarium head blight (FHB) is through resistance and therefore, it is necessary to identify sources of resistance. The objectives of this study were to determine the associations between disease (symptomatic spikelets) and molecular markers and to confirm QTLs already reported in other populations. A population of 110 F7 derived RIL from the cross Wuhan 3 (resistant) /Norm (susceptible) was evaluated for reaction to the fungus *Fusarium graminearum*, in inoculated mist-irrigated field conditions, (2 locations, 2 years) and under greenhouse point-inoculation, (2 experiments, 5 replications). The number of symptomatic spikelets was recorded 21 days post inoculation in both the field and greenhouse experiments. Wuhan 3/ Norm displays a normal distribution, transgressive segregants and significant variation among RILS 7for FHB severity. The FHB severity evaluations are well correlated and the correlations are statistically significant. Preliminary mapping of this population has been done with DNA SSR markers located on chromosomes 3BS, 2A, and 5A. The interval analysis of the 3BS markers shows a putative QTL that is associated ($P<0.01$) with FHB resistance. This confirms the findings of Anderson et al (2001). The marker has an average R2 value of 0.27 and a range of 0.11 to 0.41 over the five experiments.

# RESISTANCE TO FUSARIUM HEAD BLIGHT IN ACCESSIONS FROM THE BALKANS: A PROGRESS REPORT

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## OBJECTIVES

To evaluate, under greenhouse and field conditions, accessions from the Balkans contained in the National Small Grains Collection at Aberdeen, ID for resistance to *Fusarium graminearum.*

## INTRODUCTION

Host resistance has long been considered the most economical and effective means of control for *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.), also known as scab (Schroeder and Christensen, 1963; Martin and Johnston, 1982), but breeding has been hindered by a lack of effective resistance genes and by the complexity of the resistance in identified sources (Mesterházy, 1997). No source of complete resistance is known, and current sources provide only partial resistance, often in genetic backgrounds with inferior agronomic type. The identification of different sources of resistance and their incorporation into adapted wheat varieties is critical to the continued improvement of Fusarium head blight resistance in winter wheat. Research funded by the National Wheat and Barley Scab Initiative has led to the systematic evaluation of resistance to scab in winter wheat accessions from targeted geographical regions of the world where resistance has been identified or where environmental conditions are conducive to scab development including those from Eastern Europe. Approximately 2,000 winter wheat accessions from the Balkans were identified in the USDA National Small Grains collection for evaluation.

## MATERIALS AND METHODS

In 1999 and 2000, approximately 2000 accessions from the Balkans representing winter wheat landraces, breeding lines, cultivars and cultivated genotypes from Yugoslavia, Croatia, Macedonia, Bosnia Herzegovina, Serbia, and Montenegro were acquired from the USDA-ARS Small Grains Collection at Aberdeen, Idaho. Lines were screened in both field and greenhouse screening programs at Missouri. A sub-sample of lines were jointly screened in the greenhouse at North Carolina State University in order to expedite verification of resistant accessions.

***Disease Resistance Screening - Greenhouse****:* Vernalized seedlings (4 per accession) were planted in the greenhouse. At first anthesis, plants were inoculated with 10µL of a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was placed in a single central floret using an Oxford 8100™ repeat dispensing syringe. For all inoculations, a single isolate was used which had been previously

determined to be the most aggressive Missouri isolate on our most 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 disease spread in the spike were made at 21 d after inoculation. Plants were identified for further evaluation that had low spread in the head (mean spread $\leq$ 2 spikelets), and good kernel quality relative to an uninoculated head. Resistant check cultivars were Ernie, Sumai 3, Ning 7840, and MO 980525. The susceptible check was MO 94-317.

***Disease Resistance Screening – Field:*** Accessions were planted as head rows in the field at the Agronomy Research Center near Columbia, MO. Plants were sprayed at 75% heading with a macroconidial suspension concentrated to 50,000 macroconidia/mL. Head rows were maintained under overhead mist irrigation through heading and evaluated for scab incidence 7-10 d post inoculation and severity18 - 21 d after inoculation. A field scab index was determined as the product of incidence and severity. Checks were again Ernie, MO 980525 and MO 94-317.

## RESULTS AND DISCUSSION

Table 1 provides information on the accession, improvement status, and resistance data for accessions identified as having good field and greenhouse resistance in both the Missouri and North Carolina evaluation programs. Data are presented for accessions that had either reduced spread in the spike following greenhouse inoculations, a low scab index in the field (determined as the product of incidence and severity) or both. Resistance in approximately 90 additional accessions will be verified in 2002. Resistance identified in a further group of accessions screened and verified only at Missouri are given in Table 2. The majority of resistant accessions (>90%) from the Balkans are landraces. Many are tall and late and significant pre-breeding will be required to transfer their otherwise excellent levels of resistance into adapted backgrounds.

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**Table 1.** Winter wheat accessions, originating from Balkans, screened at the University of Missouri and at North Carolina State University in and 2001.

| | | | Missouri Data | | | | North Carolina Data |
|---|---|---|---|---|---|---|---|
| | | Improvement | 2001 Field Data | | | Greenhouse | Greenhouse |
| MO ID | Accession | Status | Incidence | Severity[1] | Index | Type II[1] | Type II[1] |
| 1947 | Cltr 11214 | Breeding | 80 | 0.29 | 23.28 | 0.09 | 0.09 |
| 2012 | PI 278623 | Cultivated | 70 | 0.27 | 18.72 | 0.14 | 0.07 |
| 2036 | PI 345009 | Landrace | 100 | 0.29 | 28.68 | 0.32 | 0.04 |
| 2037 | PI 345010 | Landrace | 100 | 0.27 | 26.70 | 0.21 | 0.22 |
| 2048 | PI 345023 | Landrace | 100 | 0.15 | 15.18 | 0.15 | 0.22 |
| 2070 | PI 345047 | Landrace | 100 | 0.14 | 14.00 | 0.07 | 0.08 |
| 2084 | PI 345063 | Landrace | 80 | 0.13 | 10.79 | 0.11 | 0.08 |
| 2105 | PI 345086 | Landrace | 100 | 0.28 | 27.85 | 0.09 | 0.23 |
| 2122 | PI 345110 | Landrace | 90 | 0.18 | 16.28 | 0.24 | 0.06 |
| 2170 | PI 345168 | Landrace | 50 | 0.32 | 16.10 | 0.10 | 0.07 |
| 2229 | PI 345235 | Landrace | 100 | 0.21 | 21.10 | 0.20 | 0.09 |
| 2230 | PI 345236 | Landrace | 100 | 0.26 | 26.23 | 0.15 | 0.08 |
| 2255 | PI 345274 | Landrace | 90 | 0.26 | 23.48 | 0.16 | 0.23 |
| 2294 | PI 345334 | Landrace | 100 | 0.11 | 11.34 | 0.17 | 0.07 |
| 2295 | PI 345335 | Landrace | 80 | 0.15 | 12.31 | 0.27 | 0.08 |
| 2304 | PI 345346 | Landrace | 80 | 0.24 | 18.96 | 0.06 | 0.14 |
| 2314 | PI 345356 | Landrace | 40 | 0.14 | 5.69 | 0.08 | 0.06 |
| 2329 | PI 345377 | Landrace | 90 | 0.15 | 13.25 | 0.18 | 0.21 |
| 2416 | PI 345490 | Landrace | 100 | 0.16 | 15.53 | 0.18 | 0.14 |
| 2429 | PI 345504 | Landrace | 100 | 0.28 | 27.51 | 0.28 | 0.19 |
| 2539 | PI 350118 | Landrace | 90 | 0.16 | 14.26 | 0.13 | 0.11 |
| Ernie | Resistant check (cultivar) | | 82.5 | 0.22 | 18.2 | 0.14 | |
| Sumai 3 | Resistant check | | not grown in the field | | | 0.06 | |
| Ning 7840 | Resistant check | | not grown in the field | | | 0.16 | |
| MO 94-317 | Susceptible check (cultivar) | | 100.0 | 0.47 | 47.0 | 0.93 | |

[1] Mean ratio of infected spiketlets to total spikelets on inoculated heads.

Table 2. Winter wheat accessions, originating from Balkans, screened and verified at the University of Missouri in 2000 and 2001.

| MO ID | Accession | Improvement status | 2001 Field Data[1] Incidence | Severity[3] | Scab Index | 2000 Field Data[2] Scab Index | Greenhouse Type II[3] |
|---|---|---|---|---|---|---|---|
| 939-2 | CI 11225 | Landrace | 100.0 | 0.21 | 21.1 | 20.0 | 0.30 |
| 940-1 | CI 11228 | Landrace | 90.0 | 0.26 | 23.3 | 30.0 | 0.16 |
| 1083-1 | PI 316425 | Breeding | 100.0 | 0.42 | 42.0 | 9.5 | 0.19 |
| 1143-1 | PI 349928 | Landrace | 100.0 | 0.45 | 45.0 | 22.5 | 0.08 |
| 1158-2 | PI 349964 | Landrace | 80.0 | 0.36 | 28.6 | 18.0 | 0.05 |
| 1173-1 | PI 350002 | Landrace | 100.0 | 0.29 | 29.3 | 25.5 | 0.11 |
| 1182-1 | PI 350018 | Landrace | 90.0 | 0.33 | 29.6 | 27.0 | 0.19 |
| 1184-1 | PI 350020 | Landrace | 90.0 | 0.35 | 31.8 | 38.0 | 0.16 |
| 1186-1 | PI 350022 | Landrace | 100.0 | 0.34 | 33.5 | 38.0 | 0.17 |
| 1223-3 | PI 350134 | Landrace | 100.0 | 0.25 | 24.5 | 28.5 | 0.34 |
| 1225-1 | PI 350146 | Landrace | 100.0 | 0.19 | 19.2 | 24.0 | 0.20 |
| 1240-2 | PI 350258 | Landrace | 100.0 | 0.37 | 37.3 | 36.0 | 0.11 |
| 1274-2 | PI 362459 | Landrace | 70.0 | 0.31 | 21.8 | 14.0 | 0.14 |
| 1280-2 | PI 362469 | Landrace | 100.0 | 0.29 | 28.8 | 95.0 | 0.15 |
| 1301-1 | PI 362503 | Landrace | 70.0 | 0.12 | 8.5 | 22.5 | 0.07 |
| 1306-1 | PI 362512 | Landrace | 100.0 | 0.16 | 16.3 | 35.0 | 0.08 |
| 1312-3 | PI 362519 | Landrace | 80.0 | 0.21 | 17.0 | 18.0 | 0.23 |
| 1423-4 | PI 362676 | Landrace | 100.0 | 0.15 | 15.5 | 27.0 | 0.16 |
| 1487-2 | PI 374515 | Landrace | 100.0 | 0.27 | 26.8 | 17.0 | 0.07 |
| 1507-1 | PI 374545 | Landrace | 60.0 | 0.09 | 5.6 | 18.0 | 0.21 |
| 1529-1 | PI 374577 | Landrace | 90.0 | 0.46 | 42.0 | 38.0 | 0.12 |
| 1579-2 | PI 374676 | Landrace | 90.0 | 0.41 | 37.0 | 25.5 | 0.09 |
| 1580-1 | PI 374677 | Landrace | 100.0 | 0.42 | 42.0 | 25.5 | 0.10 |
| 1588-2 | PI 374689 | Landrace | 100.0 | 0.24 | 24.0 | 27.0 | 0.30 |
| 1600-1 | PI 378265 | Landrace | 90.0 | 0.33 | 29.6 | 18.0 | 0.13 |
| 1607-1 | PI 378278 | Landrace | 100.0 | 0.22 | 21.8 | 25.5 | 0.16 |
| 1644-2 | PI 378330 | Landrace | 100.0 | 0.43 | 43.0 | 45.0 | 0.07 |
| 1645-1 | PI 378331 | Landrace | 100.0 | 0.32 | 32.0 | 38.0 | 0.07 |
| 1652-3 | PI 378342 | Landrace | 90.0 | 0.40 | 36.4 | 25.5 | 0.10 |
| 1673-1 | PI 378398 | Landrace | 100.0 | 0.32 | 31.7 | 34.0 | 0.14 |
| 1682-1 | PI 378415 | Landrace | 100.0 | 0.20 | 20.2 | 12.0 | 0.12 |
| 1734-2 | PI 378528 | Landrace | 80.0 | 0.18 | 14.1 | 18.0 | 0.22 |
| 1750-1 | PI 405862 | Landrace | 80.0 | 0.16 | 13.0 | 36.0 | 0.28 |
| 1752-1 | PI 405864 | Landrace | 50.0 | 0.13 | 6.5 | 10.0 | 0.14 |
| 1838-1 | PI 420587 | Landrace | 80.0 | 0.30 | 23.7 | 24.0 | 0.20 |
| 1842-2 | PI 420591 | Landrace | 60.0 | 0.20 | 11.9 | 27.0 | 0.12 |
| 1845-2 | PI 420594 | Landrace | 90.0 | 0.19 | 17.4 | 8.0 | 0.18 |
| 1846-2 | PI 420595 | Landrace | 90.0 | 0.17 | 15.4 | 20.0 | 0.16 |
| 1891-3 | PI 434658 | Breeding | 90.0 | 0.22 | 19.7 | 27.0 | 0.03 |
| Ernie | Resistent check | Cultivar | 82.5 | 0.22 | 18.2 | 11.5 | 14.0 |
| MO 94-31´ | Susceptible check | Cultivar | 100.0 | 0.47 | 47.0 | 60.0 | 92.0 |
| 980525 | Resistent check | Cultivar | 63.3 | 0.08 | 5.2 | - | 10.0 |

[1] Data are for progeny from individual plant selected for good type II greenhouse score.

[2] Data are for the accession prior to selection for an individual plant within the accession exhibiting a good type II greenhouse score.

[3] Mean ratio of infected spiketlets to total spikelets on inoculated heads.

# TYPES I AND II RESISTANCE TO FUSARIUM HEAD BLIGHT IN ASIAN AND ITALIAN GERMPLASM

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## ABSTRACT

*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 germplasm research area. The objective of this research was to confirm type I and II resistance in 45 accessions from Asian and Italy that we had previously identified as having potentially useful levels of type I and/or type II resistance. 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 using an Oxford 8100™ repeat dispensing syringe. For all inoculations, a single isolate was used which had been previously determined to be aggressive on our most 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. For evaluation of type I resistance, heads were inoculated with a macroconidial suspension of *Fusarium graminearum* concentrated to 50,000 macroconidia/mL. Inoculum was sprayed directly on the head 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. The type I 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 (incidence x severity) for the head. The type I rating (10 d) was taken as a measure of incidence. The 21-d rating (total number of infected spikelets/total spikelets in the inoculated head) provided an estimate of severity on the inoculated head. Data presented indicated independence of type I and II reactions in this range of plant material. Correlations with field based evaluation of incidence and severity will be presented.

# FUSARIUM HEAD BLIGHT RESISTANCE IN FALL-SOWN TRITICALE

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## OBJECTIVES

1) To evaluate Type II resistance in advanced generation breeding lines and cultivars of triticale adapted to the Southeastern United States and,
2) to evaluate the impact of inoculation with different spore concentrations on estimates of Type II resistance.

## INTRODUCTION

Fall-sown triticale (X *Triticosecale* Wittmack) is grown on a limited acreage in North Carolina and is utilized as a winter cover crop, a forage and a grain component in hog and poultry diets. Much of the wheat crop in eastern North Carolina is utilized as an animal feed and, in the event of repeated FHB epidemics, triticale may offer an alternative for the small grain producer.

N.C. State has a small cultivar development effort in triticale and we released our first cultivar, Arcia, in 2000. The methodologies we utilize to develop breeding populations include cultivated x cultivated triticale crosses and the development of primary triticales [(wheat x rye) x triticale] combinations. An evaluation of advanced generation lines and cultivars is appropriate. In addition to providing information on the inherent resistance of these triticales, novel sources of resistance may be identified in triticale germplasm which can be incorporated into wheat breeding populations.

The head morphology of triticale differs from that of soft red winter wheat. The larger and more lax heads of triticale may provide a different environment for disease establishment in infected florets. An evaluation of inoculation techniques, particularly number of macroconidia per inoculation, was considered timely.

## MATERIALS AND METHODS

Three triticale cultivars, two wheat check cultivars and 28 advanced generation triticale breeding lines were evaluated in greenhouse tests during spring 2001 (Table 1). Twenty-four triticale breeding lines ('NC') were selected from conventional triticale x triticale crosses and four breeding lines ('NCPT') were selected from primary triticale populations.

A randomized complete block design with two replications was utilized. An experimental unit was a single plant. Four plants were evaluated per entry. Ten ml of a spore suspension containing 50,000 spores per ml were injected into a floret in the center of the head at the time of the first anther extrusion. The inoculation suspension consisted of three aggressive North Carolina isolates of *Fusarium graminearum* identified by Walker et al. (2001). Following inoculation, the plants were placed in a mist chamber for 3 days. Twenty-one days post-

inoculation, heads were rated for the number of diseases spikelets/total number of spikelets/ head and expressed on a percentage basis. After harvest, inoculated heads were threshed and kernels were divided into five categories: 1 = sound, 2 = slight shriveled, 3 = moderately shriveled, 4 = very shriveled, and 5 = tombstone. A kernel quality score was calculated as the weighed average of the number of kernels in each category.

**Table 1**. Type II resistance to Fusarium head blight and kernel quality of 31 fall-sown triticale cultivars and breeding lines plus two wheat cultivars evaluated in greenhouse tests in spring 2001.

| Cultivar/ designation | Infected spikelets | Total spikelets | Type II resistance | Rank | Kernel quality | Rank |
|---|---|---|---|---|---|---|
| NC 99-424 | 1.2 | 25.3 | 4.6 | 1 | 4.0 | 5 |
| NCPT 97-1017 | 2.0 | 21.8 | 9.2 | 2 | 4.1 | 6 |
| NC 97-1311 | 2.4 | 25.8 | 9.3 | 3 | 4.7 | 21 |
| NC 99-609 | 2.5 | 25.0 | 10.0 | 4 | 4.9 | 28 |
| NC 99-728 | 2.5 | 24.8 | 10.1 | 5 | 4.7 | 24 |
| NCPT 97-1005 | 2.5 | 24.3 | 10.4 | 6 | 4.1 | 7 |
| NC 99-937 | 2.0 | 18.0 | 11.7 | 7 | 4.2 | 8 |
| NC 99-786 | 3.2 | 25.8 | 12.1 | 8 | 4.3 | 12 |
| NC 99-643 | 2.8 | 23.0 | 12.5 | 9 | 4.8 | 26 |
| ARCIA | 3.0 | 23.0 | 13.0 | 10 | 4.2 | 9 |
| NC 97-1305 | 3.9 | 28.3 | 14.2 | 11 | 4.4 | 14 |
| NC 99-530 | 3.6 | 25.0 | 14.2 | 12 | 4.5 | 16 |
| NC 99-797 | 3.2 | 21.5 | 14.3 | 13 | 4.6 | 19 |
| NC 99-618 | 3.3 | 22.5 | 14.4 | 14 | 4.9 | 29 |
| TRICAL498 | 4.0 | 26.3 | 15.1 | 15 | 4.6 | 20 |
| NC 99-647 | 3.8 | 25.0 | 15.2 | 16 | 5.0 | 31 |
| NCPT 97-1008 | 3.9 | 25.0 | 15.7 | 17 | 4.5 | 17 |
| NC 99-810 | 2.5 | 15.8 | 15.9 | 18 | 5.0 | 32 |
| NC 99 348 | 4.3 | 25.3 | 16.5 | 19 | 3.9 | 4 |
| NC 99-11 | 3.9 | 23.0 | 16.7 | 20 | 4.2 | 10 |
| NC 99-745 | 4.3 | 25.0 | 17.1 | 21 | 4.3 | 13 |
| NC 97-1425 | 4.5 | 26.0 | 17.3 | 22 | 4.2 | 11 |
| FLPFT 215 | 3.7 | 20.8 | 17.5 | 23 | 2.4 | 1 |
| NC 99-628 | 4.6 | 23.0 | 20.0 | 24 | 4.8 | 27 |
| NC 99-647 | 4.3 | 21.5 | 20.0 | 25 | 5.0 | 33 |
| NC 98-1737 | 4.7 | 23.0 | 20.2 | 26 | 3.9 | 3 |
| NC 99-859 | 4.2 | 20.3 | 20.7 | 27 | 4.5 | 18 |
| NC 99-554 | 5.2 | 23.3 | 21.8 | 28 | 5.0 | 30 |
| ERNIE (check) | 2.3 | 10.0 | 22.2 | 29 | 3.4 | 2 |
| NC 99-815 | 5.9 | 22.0 | 27.0 | 30 | 4.8 | 25 |
| NC 99-857 | 5.7 | 19.8 | 28.0 | 31 | 4.4 | 15 |
| NCPT 98-103 | 6.7 | 21.8 | 31.2 | 32 | 4.7 | 22 |
| C9663 (check) | 6.7 | 13.3 | 50.4 | 33 | 4.7 | 23 |
| Mean | 3.7 | 22.5 | 17.2 | | 4.4 | |
| LSD | -- | -- | 17.0 | | 0.5 | |

A subset of three triticale cultivars and seven advanced generation lines was chosen for the spore concentration study. The experimental protocol was similar to that described above, except a split block design was utilized. Whole plots were three spore concentrations: 50,000 spores/ml, 100,000 spores/ml and 150,000 spores/ml. Subplots were the 10 cultivars and breeding lines.

## RESULTS AND DISCUSSION

The two wheat check cultivars performed as expected; Ernie, the resistant check, had significantly better Type II resistance and kernel quality than the susceptible check C9663 (Table 1). The greater overall resistance of the triticale germplasm to these isolates is reflected in the relative rankings of Ernie (29$^{th}$) and C9663 (33$^{rd}$) for Type II resistance. All of the triticale entries were significantly superior to C9663.

No triticale had significantly superior kernel quality to Ernie, the resistant wheat check, but six had superior kernel quality to C9663, the susceptible check. A difficulty we observed with rating kernel quality for triticale versus wheat was the inherently greater shriveling of triticale seed that is observed, even in the absence of FHB infection. This resulted in a nonsignificant rank correlation between Type II resistance and kernel quality ratings.

No significant differences were observed for mean Type II resistance or kernel quality at the three inoculation concentrations (Table 2). Neither genotype nor genotype x spore concentration sources of variance were significant for Type II resistance estimates. Genotype variance was significant for kernel quality, but genotype x spore concentration was not significant.

**Table 2**. Mean Type II and kernel quality scores for 10 triticale cultivars and breeding lines evaluated at three spore concentrations.

| Spore concentration | Mean Type II (%) | Mean kernel quality (1-5) |
|---|---|---|
| 50,000 spores/ml | 13.6 | 4.4 |
| 100,000 spores/ml | 19.2 | 4.4 |
| 150,000 spores/ml | 14.9 | 4.5 |
| LSD | ns | ns |

The results of this preliminary greenhouse evaluation of southeastern triticale cultivars and breeding lines suggested that triticale germplasm is inherently more resistant to FHB isolates in the region than is wheat germplasm. In addition, triticale germplasm may serve as a source of resistance alleles that could be introgressed into wheat breeding populations. Finally, it appears that current wheat protocols regarding spore concentration in greenhouse evaluations of Type II resistance are adequate for similar evaluations of triticale.

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# NEW SOURCES OF RESISTANCE TO FUSARIUM HEAD BLIGHT OF WHEAT

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## OBJECTIVE

The goal of this research is to identify wheat accessions whose resistance to Fusarium head blight differs from that in Sumai 3 and Ning 7840.

## INTRODUCTION

Scab has become a serious disease of wheat and barley in many areas of the US. Resistant cultivars will be an important component of an integrated disease management strategy. Many wheat-breeding programs are utilizing resistance from Sumai 3 or the closely related cultivar Ning 7840. While this resistance appears to be the best available, and reasonably effective, it does not totally prevent disease development. Total reliance on this one source of resistance is also a concern. If any of the *Fusarium* species capable of causing scab were to adapt to this resistance, millions of acres of wheat could become vulnerable to the disease. This project is designed to find other sources of resistance to scab, with two objectives: to provide genetic diversity for resistance and to enhance the degree of resistance conferred by the Sumai 3 source of resistance.

## MATERIALS AND METHODS

We selected several lines from accessions in the USDA wheat germ plasm collection for resistance to *F. graminearum*. We also evaluated accessions from other sources. The original accessions were heterogeneous for reaction, but by repeated cycles of inoculation and selection, we developed lines with a high degree and consistent expression of Type II resistance (Table 1).

**Table 1.** Wheat accessions from which lines with a high degree of Type II resistance to *Fusarium graminearum* were selected.

| Accession | Accession |
|---|---|
| Chokwang | CIMMYT 211 |
| Futai 8944 | Funo |
| Mentana | Paula VZ 434 |
| Oscar V | Y 5418 |

We crossed these resistant selections to susceptible cultivars, to Sumai 3 or Ning 7840, and to each other. During the spring of 2001, we evaluated progeny from several test cross and

backcross populations for Type II resistance. We also evaluated Typemon ($10^4$ conidia/ml) was used as inoculum. The inoculated spike was covered with a small, clear polyethylene bag for 48 hours to provide moisture for infection. Blighted spikelets were counted 10 and 20 days after inoculation.

## RESULTS

The initial goal of the crossing program with these new sources of resistance was to combine them in order to seek transgressive segregates for greater resistance than shown by either parent. Because of the unreliability of single-plant selection, even following controlled inoculation in the greenhouse, identification of transgressive segregates must await confirmation in progeny tests, which are now underway. Nonetheless, several crosses included some progeny that were highly resistant. These showed no symptoms, or discoloration of only part of the inoculated floret. Based on this preliminary observation, genes for resistance in Mentana and Paula VZ 434 may differ from those in Sumai 3. CIMMYT 211, Futai 8944, and Y5418 probably share at least one resistance gene with Sumai 3.

Genes for resistance do not appear to be completely dominant. For example, when the F1 of Futai 8944 x Norm was backcrossed to Futai 8944, progeny ranged from highly resistant to moderately susceptible. Likewise, when the F1 of Futai 8944 x Paula VZ 434 was backcrossed to Futai 8944, progeny ranged from highly resistant to moderately susceptible. When the F1 of Futai 8944 x Sumai 3 (or Ning 7840) was backcrossed to Futai 3, all progeny were resistant or moderately resistant, further suggesting that Futai 8944 has one or more genes in common with Sumai 3.

Distributions of the backcross of the Chokwang x Clark F1 to Clark and of the test cross to Norm were trimodal (Figs. 1 and 2)

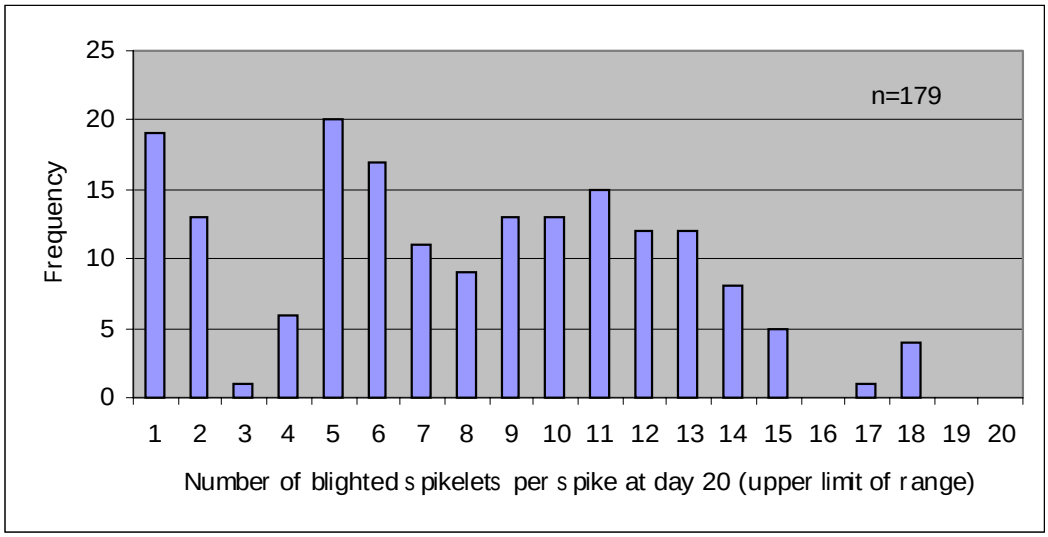


**Fig. 1.** Distribution of Fusarium head blight severity among F1 progeny of Clark//Chokwang/Clark

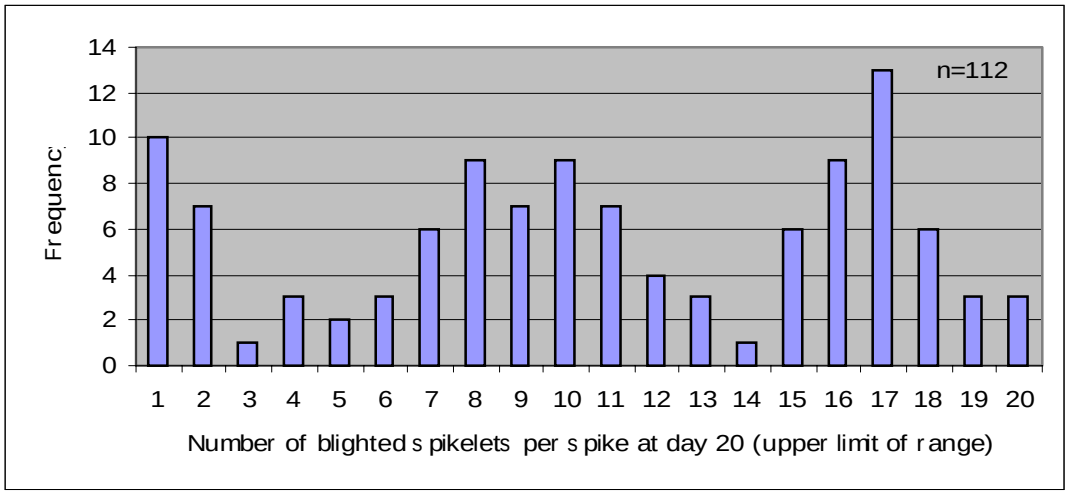


**Fig. 2.** Distribution of Fusarium head blight severity among F1 progeny of Clark/Chokwang// Norm

None of the progeny of the backcross to Chokwang were fully susceptible, but 62% fell into an intermediate category (Fig. 3).

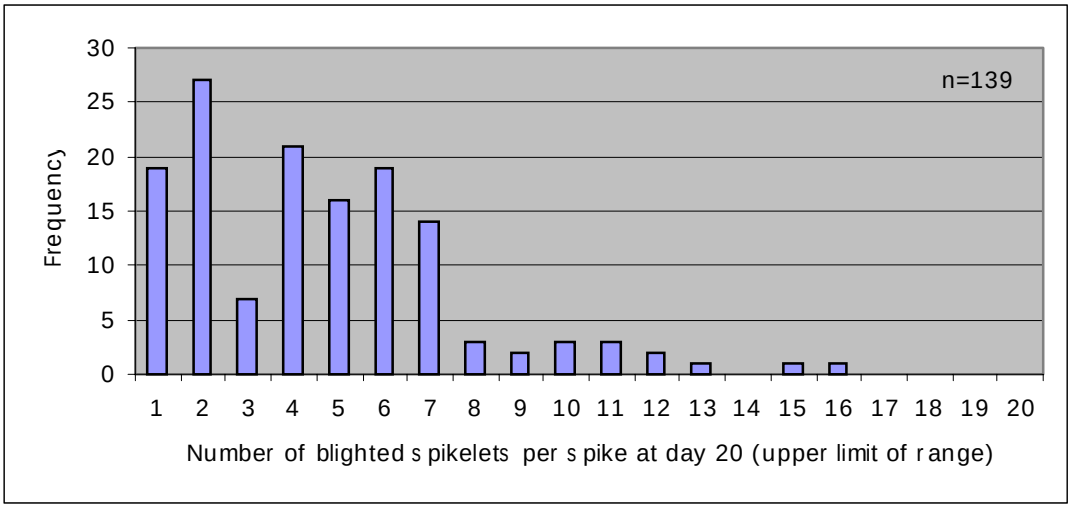


**Fig. 3.** Distribution of Fusarium head blight severity among F1 progeny of Chokwang// Chokwang/Clark

## DISCUSSION

Preliminary results suggest that at least some of the accessions selected for resistance to *Fusarium graminearum* have genes not found in Sumai 3 or Ning 7840. The Type II resistance of these latter cultivars is generally quite good in greenhouse tests, leaving only a limited range for detection of transgressive segregation for enhanced resistance. Progeny tests may allow us to confirm whether backcross F1s with essentially complete resistance will be stable for this trait.

Based on evaluation of a small recombinant inbred population derived from Chokwang/Clark, we speculated that at least 2 genes in Chokwang conferred resistance to *F. graminearum*, but that the interactions among genes were not simply additive (Buechley and Shaner, 1999). In the study reported here, highly resistant progeny were most frequent in the backcross to Chokwang, and least frequent in the test cross to Norm. The fact that all 3 crosses gave rise to some highly resistant progeny further supports the notion that these genes do not act in a simple additive manner.

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# COMBINING ABILITY OF FHB RESISTANCE FROM DIFFERENT SPRING WHEAT SOURCES

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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. In response to this problem, both public and private spring wheat breeding programs have directed considerable resources to adding FHB resistance to their lines. As sources of FHB resistance, many programs have used the Chinese lines such as Sumai 3 or its derivatives. FHB resistance in wheat is a quantitative trait of complex inheritance. None of the known FHB resistance source lines possess sufficently strong resistance to prevent economic damage under conditions of high disease pressure. This study was an attempt to determine if the testing of F1's would be a suitable method to decide which crosses have potential for major improvement in resistance to FHB. In this study we used a half-diallele set of five parents: all pairings were included but not the respective reciprocal crosses. Three parents were derived from Sumai 3 through different lineages and two were of other origin. Using spikelet inoculation in the greenhouse, we tested the ten F-1 hybrids among these 5 lines. Sufficient hybrid seed was made so that there could be replicated row plantings of F1 plants. Values for FHB severity for each parent and F1 represent the mean of 45-50 individual head scores Analysis of Variance for the FHB severity scores were partitioned by Griffing's model to show general and specific combining ability effects. Some pairings of lines showed major additivity of resistance while others, supposedly of different origin, did not. All but one parent showed significant general combining ability for FHB severity. The two crosses that showed the greatest differences between F1 severity score and parent mean score were also those with the largest effect for specific combining ability. The ability of partially resistant lines to show useful combining ability for FHB response is encouraging. It also suggests that that various partially resistant lines are likely to have different genetic makeup for that resistance – a factor which may aid the stability of resistance to FHB. The lack of combining ability between the two least susceptible lines was disappointing, and may suggest that major improvement in FHB resistance may be more difficult to achieve. Proper choice of parents will be needed to obtain major improvement in FHB resistance.

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# EVALUATION OF *HORDEUM* ACCESSIONS FOR RESISTANCE TO FUSARIUM HEAD BLIGHT

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## OBJECTIVE

To identify diverse sources of resistance to Fusarium head blight (FHB) in Hordeum.

## INTRODUCTION

FHB has devastated the barley (*Hordeum vulgare*) crop in the Upper Midwest region over the past nine years (1993-2001) (Salas et al. 1999; Steffenson 2002). Deployment of resistant cultivars is the most effective and environmentally sound means of managing the disease. Thus, the objective of this study was to identify diverse sources of resistance to FHB in the primary (*H. vulgare* and *H. vulgare* subsp. *spontaneum*) and secondary (*H. bulbosum*) gene pools of cultivated barley.

## MATERIALS AND METHODS

The primary and secondary *Hordeum* gene pools are potential sources of useful resistance genes for cultivated barley (Pickering 2000). Over the past three years, we evaluated about 8,100 six-rowed spring and 600 six-rowed winter accessions of *H. vulgare* for resistance to FHB in the United States (North Dakota and Minnesota) and China (Zhejiang Province), respectively. Additionally, 510 accessions of wild barley (*H. vulgare* subsp. *spontaneum*) and 28 *H. vulgare ´ H. bulbosum* (bulbous barley grass) introgression lines were screened in China in 2000-2001. FHB nurseries were inoculated using the "grain spawn" method, except in St. Paul where the "foliar spray" method was used (Steffenson 2002). For the grain spawn inoculation, equal amounts of two to six regional *F. graminearum* isolates were applied uniformly to plots. The first inoculation was made when the flag leaves of the earliest maturing plants were expanding. One to four additional inoculations were made during the course of the season to ensure that sufficient inoculum was available for infection of later maturing accessions. To promote FHB infection, overhead irrigation was applied to plants in the morning and evening. For the foliar spray method, plants were uniformly inoculated twice with a back-pack sprayer at the late milk stage of development. Overhead irrigation was applied to promote infection. Disease severity was assessed on each accession at the mid-dough stage. Accessions exhibiting low levels of FHB infection were also assayed for deoxynivalenol (DON) concentration.

## RESULTS AND DISCUSSION

**Six-rowed spring barley evaluations.** Over 8,100 six-rowed spring barleys were initially screened for FHB resistance at two locations in North Dakota in 1999 and 2000 (~4050 per

year). Only 27 accessions exhibited FHB severities less than 30% in one of the evaluations in 1999 or 2000 (Scholz and Steffenson 2000). These accessions were then evaluated in the greenhouse under more controlled conditions and also in additional field environments. Data on the infection levels of the five most resistant accessions identified in each year are given in Tables 1 and 2, along with the resistant (Chevron and CIho 4196) and susceptible (Foster or Stander) controls. FHB severity and DON concentration varied considerably in the selected accessions across different evaluation tests (Table 2); however, a few accessions (CIho 6613 and CIho 11526) consistently exhibited resistance levels that were similar to Chevron. Several of the most resistant six-rowed accessions identified to date originate from Switzerland. This includes PI 370919, PI 371317, the resistant control Chevron (CIho 1111) and a Chevron selection (CIho 11526)(Table 1).

**Table 1.** Accvessions of six-rowed spring barley exhibiting resistance to FHB in initial field screeing in Langdon and Osnabrock, North Dakota in 1999 and 2000.

| Accession Number | % FHB Severity | | | Origin | |
|---|---|---|---|---|---|
| | Langdon | Osnabrock | Name | Country | Region |
| **Screening 1999** | | | | | |
| CIho 4095 | 40 | 20 | --[2] | Georgia | |
| CIho 4530 | 40 | 20 | 7603 | China | Jilin |
| CIho 6613 | 30 | 10 | Seed Stocks 1148-1 | USA | WI |
| CIho 9114 | 40 | 20 | 184 | Yugoslavia | Serbia |
| CIho 11526 | 10 | 10 | Chevron Selection | USA | MD |
| | | | | | |
| CIho 1111[1] | 17 | 14 | Chevron | Switzerland | |
| PI 592758[1] | --[2] | 54 | Foster | USA | ND |
| **Screening 2000** | | | | | |
| CIho 9699 | 20 | 30 | 9241 | Ethiopia | Gonder |
| PI 328642 | 20 | 20 | HOR 1390 | Romania | |
| PI 370919 | 30 | 30 | 569C | Switzerland | Valais |
| PI 371317 | 30 | 40 | 194D | Switzerland | Uri |
| PI 565567 | 40 | 30 | Chun Gong Mai | China | Shandong |
| | | | | | |
| CIho 1111[1] | 28 | 18 | Chevron | Switzerland | |
| PI 592758[1] | 60 | 47 | Foster | USA | ND |

[1]Chevron (CIho 1111) and Foster (PI 592758) are resistant and susceptible six-rowed controls, respectively.

[2]Not available or not tested.

**Table 2.** FHB severity (%) and DON concentration (ppm) of selected six-rowed barley accessions evaluated in greenhouse and filed tests, 2000-2001.

| | Greenhouse 2000 | | Field 2000 | | Field 2001 | |
|---|---|---|---|---|---|---|
| | Fargo | | Fargo | | St. Paul | |
| Accession | FHB | DON | FHB | DON | FHB | DON |
| CIho 9114 | 1.4 | 0.1 | 4.2 | 13.7 | 4.5 | 11.6 |
| CIho 11526 | 2.8 | 0.0 | 3.9 | 17.6 | 6.0 | --[2] |
| CIho 6613 | 4.7 | 0.9 | 1.7 | 14.7 | 2.9 | 9.2 |
| CIho 4530 | 6.4 | 1.1 | 14.9 | 60.4 | 2.8 | 16.0 |
| CIho 4095 | 11.8 | 2.2 | 13.1 | 55.1 | 2.9 | 12.2 |
| | | | | | | |
| Chevron[1] | 4.7 | 0.6 | 3.7 | 16.4 | 3.4 | 3.6 |
| Stander[1] | 39.7 | 7.0 | 14.5 | 81.6 | 5.3 | 35.6 |
| CIho 4196[1] | 2.3 | 1.4 | 6.4 | 7.0 | 4.4 | 12.8 |
| | | | | | | |
| CIho 9699 | 10.1 | -- | -- | -- | 9.8 | 17.2 |
| PI 328642 | 7.1 | -- | -- | -- | 9.9 | 10.0 |
| PI 370919 | 8.0 | -- | -- | -- | 5.4 | 3.7 |
| PI 371317 | 16.4 | -- | -- | -- | 2.3 | 7.4 |
| PI 565567 | 14.7 | -- | -- | -- | 0.7 | 6.0 |
| | | | | | | |
| Chevron[1] | 7.4 | -- | -- | -- | 3.4 | 3.6 |
| Stander[1] | 73.6 | -- | -- | -- | 5.3 | 35.6 |
| CIho 4196[1] | 8.1 | -- | -- | -- | 4.4 | 12.8 |

[1]Chevron (CIho 1111) and Stander (PI 564743) are resistant and susceptible six-rowed controls, respectively. CIho 4196 is the resistant two-rowed control.

[2]Not tested.

**Six-rowed winter barley evaluations.** Six hundred winter barley accessions from diverse regions of the world were evaluated for FHB resistance in China in 2001. Less than 1% (56) exhibited FHB severities less than 30% under heavy disease pressure. Many of these accessions headed very early and may have escaped severe infection. Only three accessions (CIho 39516, CIho 2339, and CIho 14296) in this group of 56 had an intermediate heading time and a DON concentration less than 3 ppm (susceptible control Stander=5.4ppm).

***Hordeum vulgare* subsp. *spontaneum* evaluations.** One hundred and ten wild barley accessions from Israel and Jordan were tested in China in 2000. Twenty-three accessions from this group exhibited less than 10% FHB incidence and severity under light disease pressure. In 2001, four hundred additional *H. vulgare* subsp. *spontaneum* accessions from across the Fertile Crescent were evaluated, and forty exhibited FHB severities less than 30% under heavy disease pressure. Two accessions from Israel (PI 391056 and PI 466519) had DON concentrations that were less than 3 ppm compared to 16 ppm in the susceptible cultivar Stander. *Hordeum vulgare* subsp. *spontaneum* exhibited a high degree of genetic diversity for FHB reaction as disease severities ranged from <10% to over 80%. This wild

species may be a useful source of alternative FHB resistance alleles in barley breeding programs.

***H. vulgare´ H. bulbosum* evaluations.** Data were obtained on 26 of the 28 introgression lines planted in China in 2000. Only two lines (38P18/22/3 and 219W3) exhibited less than 10% FHB incidence and severity under light disease pressure. It is not know whether the lower disease severity in these lines was due to the *H. bulbosum* introgression because the *H. vulgare* parent also exhibited relatively low disease levels.

An emphasis was placed on screening six-rowed barley germplasm for resistance to FHB because this type is preferred by the malting and brewing industries in the Upper Midwest. Our results indicate that resistance in six-rowed *H. vulgare* germplasm (both spring and winter types) is very rare. To achieve the highest level of FHB resistance possible, it is best to pyramid resistance genes from different sources including two-rowed types, six-rowed types, and wild barley.

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# CHARACTERIZATION OF FUSARIUM HEAD BLIGHT RESISTANT GERMPLASM WITH SSR MARKERS LINKED TO FHB RESISTANCE IN SUMAI 3

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## INTRODUCTION

Fusarium head blight (FHB) resistant materials have been identified from the USDA spring wheat collection (Zhang et al. 2000a). Analysis of geographical origin and pedigree indicated that diversity exists in this group of resistant materials (Zhang et al. 2000b). DNA markers linked with FHB resistance in Sumai 3 related materials have been identified (Waldron et al. 1999, Bai et al. 1999, Anderson et al. 2001). The most significant genomic region associated with FHB resistance in Sumai 3 has been located on chromosome 3BS. SSR markers Gwm533, Gwm493 and Gwm389 were reported to be closely linked with FHB resistance from Sumai 3 in this region (Anderson et al. 2000, 2001, Zhou et al. 2000). We used these SSR markers to screen putative new sources of FHB resistance identified from the USDA spring wheat collection in an attempt to characterize the relatedness of these new resistant sources with resistance from Sumai 3.

## MATERIALS AND METHODS

One hundred and thirty-nine accessions of spring wheat from the USDA spring wheat collection, selected for resistance to FHB based on field and greenhouse inoculations, were used in this study. Sumai 3 and Wheaton were used as the positive and negative check, respectively. DNA was extracted from 0.3g young leaf tissue. SSR primers of Gwm533, Gwm493 and Gwm389 were synthesized by Life Technologies Inc. according to the sequence information published by Roder et al. (1998). The PCR products were visualized by silver staining.

## RESULT AND DISCUSSION

The materials were highly polymorphic for these three markers. The average allele at a given locus is 1.1. The DNA fragments amplified by primers Gwm389, Gwm493, and Gwm533 were 176bp, 135bp, and 145bp, respectively. Nine percent of the materials (13 accessions) were positive for all three markers (Table 1). Three of these lines are landraces from Europe, and the rest are from South America. Five of the lines from Uruguay, PI 225375, PI 225376, PI 225384, PI 225444, and PI 225448 shared same pedigree, Sinvalocho/Petiblanco. One of the parents, Petiblanco was moderately resistant in the field, and very resistant to point-inoculation in the greenhouse (Zhang, *unpublished*). Two of the lines in this group, Excelsior and 69Z108.42, have exhibited resistant levels equivalent to, or better than, Sumai 3 in the field.

Twelve lines (8.6%) were positive for the two tightly linked markers, Gwm493 and Gwm533 (Table 2), including several of the most resistant lines (i.e. Tokai 66, Nobeoka Bozu, and Abura) identified from our germplasm project (Zhang et al. 1999, 2000a). Three of the lines from Uruguay had the same pedigree, Sinvalocho/Petiblanco as those in Table 1. Ban (2000) reported that Nobeoka Bozu has one FHB resistance gene identical to Sumai 3, and our result may corroborate that finding. Seven lines (5%) were positive for Gwm389 and Gwm493 (Table 3). Fifty-eight lines (41%) had one of the three markers, and 50 lines (35%) did not have any. Part of the FHB resistant lines that were negative for any of these markers is listed in Table 4. This group of materials is of particular interest because the FHB resistance is likely not related to the major component of resistance from Sumai 3. Results from this study further support our hypothesis that genetic diversity for FHB resistance exists in the USDA spring wheat collection.

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**Table 1.** Accession, origin, and pedigree of lines with three SSR markers, Gwm389, Gwm493, and Gwm533.

| Accession ID | Origin | Pedigree |
|---|---|---|
| Citr 5103 | 274 | Argentina Landrace |
| PI 163424 | Argentina | |
| PI 104131 | EXCELSIOR | Argentina Arminda/Virtue |
| PI 214394 | COLOTANA 1085/50 | Brazil Colonista/Frontana |
| PI 225375 | Uruguay | Sinvalocho/Petiblanco |
| PI 225376 | Uruguay | Sinvalocho/Petiblanco |
| PI 225384 | Uruguay | Sinvalocho/Petiblanco |
| PI 225444 | Uruguay | Sinvalocho/Petiblanco |
| PI 225448 | Uruguay | Sinvalocho/Petiblanco |
| PI 264927 | 220 | Greece Landrace |
| PI 337151 | MAGNIF 100 | Argentina |
| PI 349494 | 1112 BS | Switzerland Landrace |
| PI 35076 | 869Z108.42 | Austria Landrace |

**Table 2**. Accession ,origin, pedigree of lines with Gwm493 and Gwm533.

| Accession ID | Origin | Pedigree |
|---|---|---|
| PI 182561 | SIN CHUNAGA | Japan |
| PI 225396 | Uruguay | Sinvalocho/Petiblanco |
| PI 225519 | Uruguay | Sinvalocho/Petiblanco |
| PI 225525 | Uruguay | Sinvalocho/Petiblanco |
| PI 344465 | A. A. L. | Argentina complex |
| PI 351998 | TA 3332 | Finland |
| PI 382140 | ABURA | Brazil |
| PI 382153 | NOBEOKA BOZU | Japan |
| PI 382161 | TOKAI 66 | Brazil |
| PI 584926 | PANTANEIRO | Brazil Sonora63*2/Lagoa Vermelha |
| PI 83729 | MAGYAROVAR 81 | Hungary |
| PI 92387 | BELOZIORNAYA NO. 604 | Russian |

**Table 3.** Accession, origin, and pedigree of lines with Gwm389 and Gwm493

| Accession ID | Origin | Pedigree |
|---|---|---|
| PI 214396 | COLOTANA 2107/50 | Brazil Colonista/Frontana |
| PI 344454 | BUCK AUSTRAL | Argentina complex |
| PI 344467 | ONCATIVO INTA | Argentina complex |
| PI 35086969Z108.164 | | Austria landrace |
| PI 351743 | CLUJ 49-926 | Romania |
| PI 351748 | JASI 10T | Romania |
| PI 81791 | PI 81791 | Japan |

**Table 4.** Accession, origin, pedigree of lines negative for Gwm389, Gwm493, or Gwm533.

| Accession ID | Origin | Pedigree |
|---|---|---|
| Citr 11215 | Belgrade 4 | Yugoslavia |
| Citr 12002 | RenacimientoUruguaycomplex | |
| Citr 13136 | Rio Negro | BrazilSupresa/Centenario |
| PI 163429 | Argentina | |
| PI 168727 | BahienseArgentina | K./E. |
| PI 184512 | H 51 | ArgentinaA./Favorito//U. |
| PI 185383 | 3084 | Argentina |
| PI 185843 | SurpresaBrazi | Polyssu/A. |
| PI 192634 | TrintecincoBrazil | A./A. |
| PI 192660 | Prodigio Italiano | Italy |
| PI 197128 | Shinchunaga | Japan |
| PI 197664 | ArgentinaThatcher | //L./R6 |
| PI 213682 | Buck 62/52 | Argentina |
| PI 225457 | Uruguay38 | M.A./Petirrojo |
| PI 225504 | Uruguay | Sinvalocho/Petiblanco |
| PI 225516 | Uruguay | Sinvalocho/Petiblanco |
| PI 285933 | Chudoskaja | Poland |
| PI 349534 | 533b | Switzerland |
| PI 351476 | Vaulion | Switzerland |
| PI 351649 | 263.25-2 | SwitzerlandHuron/Newthatch |
| PI 351993 | Z.88.54 | Switzerland |
| PI 352000 | Z.89.37 | Switzerland |
| PI 382154 | Nyu Bai | Japan |
| PI 434987 | Estanzuela Y. | Uruguaycomplex |
| PI 519798 | PF79782 | Brazilcomplex |

# A PROCEDURE OF PRODUCING *FUSARIUM GRAMINEARUM* CONIDIA IN LARGE QUANTITIES

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## INTRODUCTION

*Fusarium graminearum* is the major pathogen causing Fusarium head blight (FHB) on wheat. We apply conidial inoculum of *F. graminearum* in the field screening nurseries to ensure successful disease development. However, field spray inoculation requires large quantities of conidia. We use solid medium to produce conidia because it is easier to make and culture is less likely to be contaminated than liquid medium. On the solid medium, it was observed that the amount of mycelium growth is negatively associated with the ability to develop conidia and streaking the surface of the culture can suppress the mycelium growth and promote conidial production (Zhang, *unpublished*). This report summarizes our research efforts in developing a procedure of producing large quantities of inoculum.

## MATERIALS AND METHODS

Four isolates of *F. graminearum*, Fg1, Fg4, Fg62, and Fg63, part of the composite consisting of 10 isolates for field inoculation, were used in this study. They were cultured on three types of medium, 1/2 PDA (19.5g potato dextrose, 10g agar, 1L $dH_20$), 1/2 PDA with 0.5ml/L (or 0.05% in volume) of lactic acid, and 1/2 PDA with 2.0ml/L (or 0.2%) of lactic acid. Each petri plate contained 20ml of medium. Each isolate was cultured on three plates of each medium. Isolates were transferred and spread evenly onto agar plates with a sterile glass hokey stick. Plates were incubated at 23C with 12hr light. Conidia were harvested every three days until day 15 by adding 5ml of sterile distilled water into a plate, and sweeping the agar surface with a sterile glass hockey stick. The conidial suspension was collected with a micropipette. The number of spores from each plate was determined based on haemacytometer sampling. The number of spores/plate on each harvest day was the average of the three plates. After each harvest, the plates were re-incubated. The experiment was conducted three times at one-week interval. Data were subjected to ANOVA after proper data transformation.

## RESULTS AND DISCUSSION

The number of conidia harvested over time was presented in Figures 1 to 4. Log-transformation was conducted for conidia/plate on each isolate before data were subjected to analysis. Analysis of variance did not detect differences among the three trials, thus, the number of conidia/plate on each medium was averaged over the three trials. The effect of isolates was significant ($P<0.0001$). Among the four isolates of *F. graminearum*, Fg1 and Fg4 were most productive. After first three days of incubation on 1/2 PDA, for instance, $1.1 \times 10^8$ conidia/plate were produced by Fg1 (Fig. 1) and Fg4 (Fig. 2) while half of that were produced by isolates Fg62 (Fig. 3) and Fg63 (Fig. 4). The number of conidia harvested from different days varied greatly, as expected. The second harvest after another three-day incubation period could still

yield substantial number of conidia. The number of spores produced after the second harvest, however, was minimal.

Although medium with a high concentration of lactic acid (0.2%) suppressed conidial production slightly at the beginning, this medium sustained a higher spore production in the subsequent harvests. For example, the second harvest of isolate Fg4 on the acid medium yielded 70% spores of the first harvest (Fig. 2), while only 28% was obtained from the medium with no addition of lactic acid. Similar trends were also observed on other isolates (Fig. 3 and Fig. 4). Analysis of variance indicated that the number of conidia harvested from different media was significantly different for a given isolate except for the first harvest.

## SUMMARY

Results from this research suggested that secondary harvests of *F. graminearum* conidia on solid media could be productive, and addition of lactic acid (0.2%) could sustain conidial production better than regular medium. Based on our experience, this is a time/supply saving protocol for large quantity inoculum production.

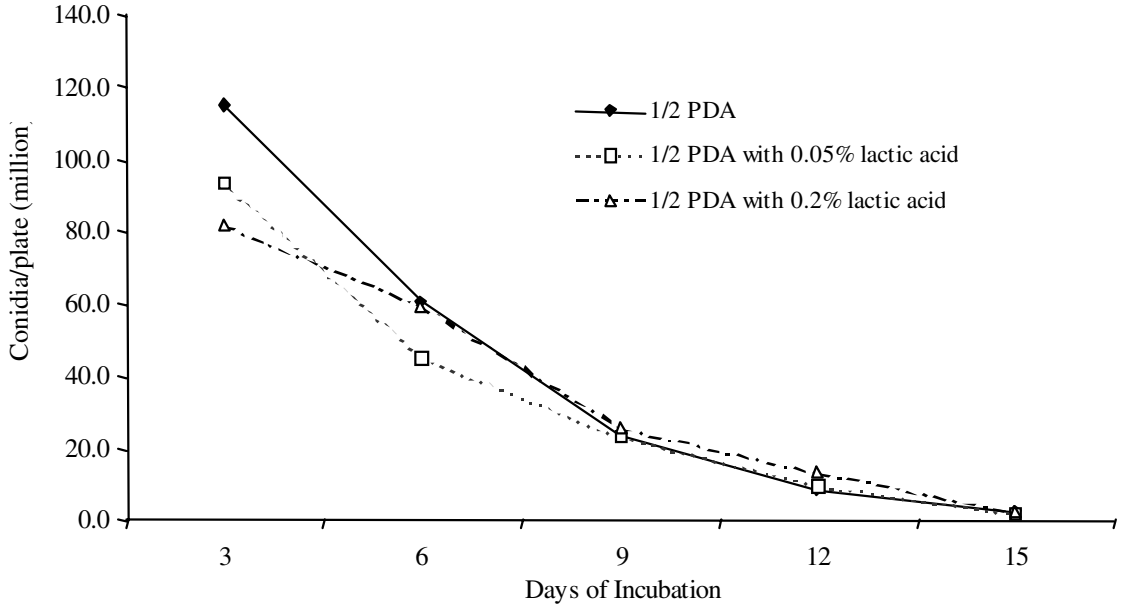


**Fig. 1.** Conidial production of isolate Fg1.

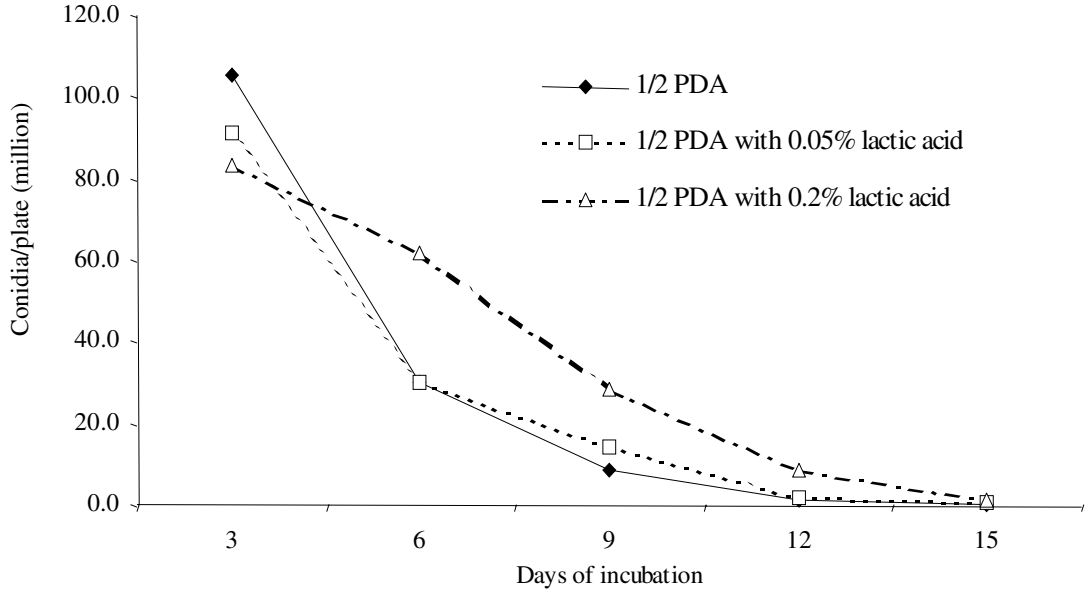


**Fig. 2.** Conidial production of isolate Fg4.

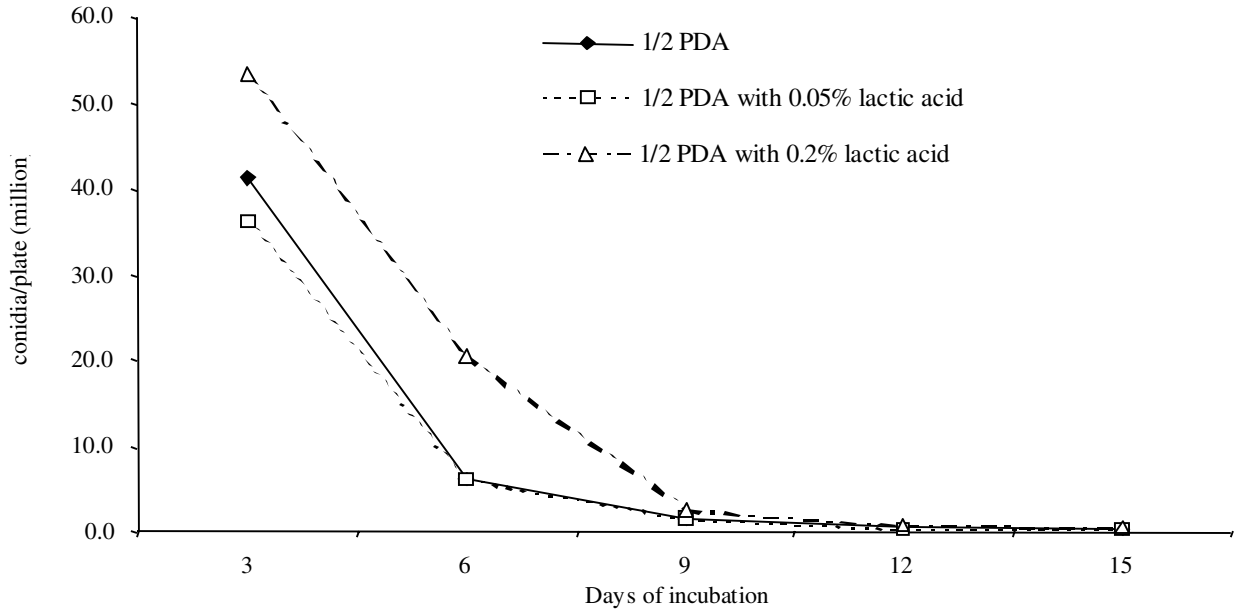


**Fig. 3** . Conidial production of isolate Fg62.

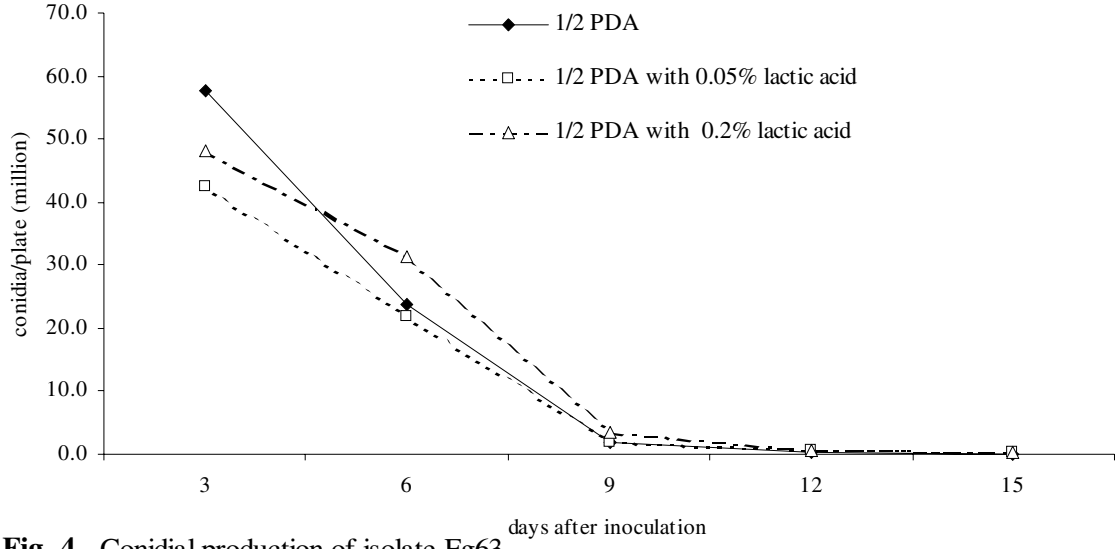


**Fig. 4**. Conidial production of isolate Fg63.

# EVALUATION OF USDA SPRING WHEAT GERMPLASM FOR FUSARIUM HEAD BLIGHT RESISTANCE.

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## INTRODUCTION

The use of host resistance will be one of the major components in managing Fusarium head blight (FHB) of wheat. Since 1998, we have been systematically evaluating the USDA spring wheat collection in an attempt to characterize the variations for FHB resistance in this germplasm pool, initially focusing on germplasm originated from regions where FHB has been problematic. A number of accessions were found to be moderately resistant to resistant (Zhang et al. 2000a). Analysis of geographical distribution and pedigree of these selections indicated that considerable diversity for FHB resistance exists in the USDA spring wheat collection. Since 2000, our search for new sources of resistance has been extended to germplasm from most of the European countries while we continue our evaluation of the eastern Asian and South American materials. This report summarizes the progress on the USDA germplasm screening, and the screening methodology we used in 2001.

## GERMPLASM SCREENING METHODS

In 2001, our FHB screening project followed a germplasm screening system we have developed (Zhang et al. 2000a) with a few modifications as described bellow.

**Preliminary Screening Nursery (PSN):** Spring wheat germplasm from the USDA collection (Aberdeen, ID) were first evaluated in the PSN nursery. 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 management, inoculation, and data collection were as described previously (Zhang et al. 1999, 2000a) except second spray inoculation was applied four days after the first spray. Selections were made based on FHB index and seed infection and increased in off-season nursery. After row/plant selection, the entire PSN was harvested in bulk, and mass selection was made based on kernel weight. The composite will be evaluated and selected under FHB pressure for several cycles (years) before individual lines will be derived.

**Elite Germplasm Nursery (EGN):** Resistant selections from the previous year's PSN is advanced to EGN. The entries of EGN were planted into row plots (one meter in length) with three replicates and arranged into split-plot design, with maturity as the main plot and genotype as the subplot. In 2001, there were four maturity groups based on days between planting and flowering: early (£55), intermediate early (55-60), intermediate late (61-65), and late (³66). Nursery management, inoculation and data collection were the same as in PSN. Data on Fusarium damaged kernels (FDK), DON, and seed weight/row were collected after

harvest. Selections evaluated for two years in EGN were entered into an advanced EGN nursery (with doubled plot size) for a final evaluation.

**Greenhouse confirmation and selection:** Selections from PSN except the composite are evaluated in the greenhouse with point and spray inoculations (Zhang et al. 1999). A floret at the middle of a spike is injected with a *Fusarium graminearum* conidial suspension (ca. 70,000 conidia/ml) when the plant is at full heading to beginning of anthesis. Spray inoculation (ca. 50,000 conidia/ml) was done at the beginning to half anthesis stage. Inoculated plants are incubated in a mist chamber in the greenhouse for 72h. Approximately 15 to 30 spikes of each entry were tested by each of the two methods.

**Uniform Regional Scab Nursery (URSN):** Each year, five most FHB resistant lines in second year EGN nursery are selected and contributed to the URSN for spring wheat. These entries are selected based on origin, pedigree, and agronomic traits, which may imply uniqueness of the resistance in addition to selections based on FHB resistance. Testing these resistant sources across the region allows a vigorous evaluation of the elite germplasm through replicated trials over multiple locations. This approach also provides the accessibility to individual programs for utilizing these resistant sources if they desire to do so.

## RESULTS AND DISCRUSSION

In 2001, a total of 1262 spring wheat accessions were evaluated in PSN and the origin of the germplasm is listed in Table 1. One hundred forty-one accessions were selected for further testing in the greenhouse and as entries of the 2002 EGN.

One hundred thirty-one entries of the 2001 EGN were from the 2000 PSN selections. A small portion (11 lines) were eliminated. Interestingly, only one of the discarded lines entry was in the early nursery maturity group. Apparently  Thus,  maturity had a significant effect on FHB field infection.  Although a combination of resistance to field floret tissue infection, seed infection, point-inoculation, and toxin was not common, lines possess a few of those components were readily found in this group of materials. A few of these linesLines with high level of resistance in the field (low FHB index and low FDK) and resistant to point inoculation in the greenhouse are listed in Table 2.

Table 3 lists lines evaluated for two or more years in the EGN. Selections were grouped into three categories: 1) lines with low FHB indices (≤40%) AND low FDK (≤40%); 2) lines with low FDK (≤40%) but high FHB indices (>40%); and 3) lines with low FHB indices (≤40%) but high FDK (>40%). We have observed that most lines from group 1 exhibited stable FHB reaction over years, whereas materials in group 2 were variable. Materials in group 3, namely Sin Chunaga, Norin 61 and several other lines originated from Japan, consistently showed a lower disease index, but high seed infection (in the form of bleached seed, not tombstone kernels). The level of geographical diversity was unexpectedly high. Diverse origins of these selections may imply that potential genetic diversity for FHB resistance exists in the spring wheat gene pool (Zhang et al. 2000c). A number of selections were landraces, likely possess different resistant genes. Studies are in progress to elucidate the

genetics of resistance and allelic relations among these new resistant sources (Zhang et al. 2000a).

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**Table 1** . Country of origin and number of accessions tested in the 2001 PSN

| Country[a] of origin | Number of accessions | Country of origin | Number of accessions |
|---|---|---|---|
| Europe | 705 | South America | 557 |
| Austria | 51 | Argentina | 50 |
| Finland | 44 | Bolivia | 53 |
| France | 30 | Brazil | 44 |
| Greece | 54 | Chile | 100 |
| Portugal | 150 | Colombia | 50 |
| Sweden | 50 | Ecador | 66 |
| Switzerland | 57 | Uruguay | 27 |
| Yugoslavia | 38 | | |

[a]Countries with less than 20 entries were not listed.

**Table 2**. Accessions in the 2001 EGN exhibiting low infection in the field and greenhouse evaluations.

| Accession or ID | Origin | FHB index (%) | FDK (%) | DON (ppm) | FHB (%) with point inoculation |
|---|---|---|---|---|---|
| PI 225396 | Uruguay | 19.0 | 10.0 | 13.3 | 17.0 |
| PI 225516 | Uruguay | 18.8 | 6.0 | 5.9 | 32.6 |
| PI 225467 | Uruguay | 15.4 | 8.3 | 5.8 | 17.2 |
| PI 225382 | Uruguay | 16.4 | 13.0 | 8.4 | 18.6 |
| ND 2710--CK | ND | 14.2 | 15.0 | 11.1 | 15.0 |
| Wheaton--CK | MN | 85.0 | 95.0 | 36.7 | 95.0 |

**Table 3**. Fusarium head blight resistant accessions of spring wheat selected from the USDA collection that have been evaluated for the second year in the EGN.

| Accession | ID | Country of origin | FHB[c] index (%) | FDK[d] (%) | DON[e] (ppm) | Maturity[f] group |
|---|---|---|---|---|---|---|
| PI 478282 | Sonalika --CK | India | 85.7 | 79.9 | 37.0 | Early |
| PI 469271 | Wheaton--CK | MN, USA | 87.7 | 95.7 | 22.5 | Early |
| | ND 2710--CK | ND. USA | 11.5 | 19.4 | 5.0 | Early |
| PI 596533 | BacUp--CK | MN, USA | 21.0 | 30.4 | 7.7 | Early |
| PI 382161[a] | Tokai 66 | Brazil | 8.5 | 14.0 | 1.9 | Interm. |
| PI 349478[b] | 193C | Switzerland | 8.9 | 14.0 | 5.0 | Early |
| PI 382154 | Nyu Bai | Japan | 10.6 | 15.0 | 2.2 | Interm. |
| PI 350768[b] | 69Z108.42 | Austria | 12.8 | 19.8 | 4.6 | Early |
| PI 382153 | Nobeoka Bozu | Japan | 14.0 | 14.9 | 4.2 | Interm. |
| PI 382140[b] | Abura | Brazil | 16.4 | 35.7 | 3.7 | Early |
| PI 182568 | Norin 34 | Japan | 18.7 | 38.3 | 6.8 | Interm. |
| PI 382167[a] | 16-52-9 | Brazil | 19.7 | 20.7 | 4.9 | Late |
| CItr 5103 | 274 | Argentina | 20.1 | 21.2 | 11.2 | Late |
| PI 434987 | Estanzuela Young | Uruguay | 21.5 | 42.0 | 10.1 | Early |
| CItr 12002 | Renacimiento | Uruguay | 23.0 | 39.2 | 9.3 | Interm. |
| PI 519790 | 274-1-118 | Uruguay | 24.1 | 36.7 | 8.5 | Early |
| PI 345731 | Tezanos Pintos Precoz | Argentina | 24.9 | 20.0 | 9.2 | Early |
| PI 462151 | Shu Chou Wheat No. 3 | China | 24.9 | 19.4 | 5.8 | Interm. |
| PI 192660 | Prodigio Italiano | Italy | 27.3 | 20.8 | 10.8 | Late |
| PI 163429 | | Argentina | 28.4 | 27.5 | 10.0 | Early |
| PI 185380[b] | Prodigio Italiano | Italy | 28.6 | 21.8 | 8.2 | Late |
| PI 351256 | Japon 2 | Japan | 28.7 | 34.2 | 8.0 | Early |
| PI 81791a | Sapporo Haru K. | Japan | 30.2 | 19.8 | 15.3 | Late |
| CItr 12021 | Centenario | Uruguay | 32.0 | 35.8 | 8.4 | Early |
| PI 285933 | Chudoskaja | Poland | 32.6 | 24.2 | 16.8 | Interm. |
| PI 382144 | Encruzilhada | Brazil | 33.5 | 39.2 | 8.0 | Late |
| PI 351221 | Newthatch Sel. | Switzerland | 34.6 | 20.0 | 9.9 | Late |
| PI 104131[b] | Excelsior | Argentina | 37.4 | 17.8 | 5.8 | Interm. |
| PI 168727 | Bahiense | Argentina | 37.8 | 22.5 | 12.9 | Early |
| PI 192634 | Trintecinco | Brazil | 38.4 | 34.2 | 9.5 | Early |
| CItr 13136 | Rio Negro | Brazil | 39.0 | 36.0 | 12.6 | Early |
| CItr 2492 | Manchurian | China | 40.0 | 22.5 | 9.4 | Early |
| PI 264927 | 220 | Greece | 40.0 | 18.3 | 4.6 | Interm. |
| CItr 17427 | 16-52-2 | Brazil | 41.6 | 30.0 | 19.4 | Early |
| PI 362437 | III/14-B | Yugoslavia | 43.9 | 27.9 | 8.1 | Late |
| PI 83729 | Magyarovar 81 | Hungary | 44.1 | 34.5 | 9.0 | Late |
| PI 185843 | Surpresa | Brazil | 44.4 | 33.3 | 10.8 | Interm. |
| PI 264998 | 628 | Greece | 45.8 | 28.3 | 8.2 | Interm. |
| PI 294975 | Artemowska | Bulgaria | 45.8 | 20.0 | 10.4 | Late |
| PI 163428 | | Argentina | 47.3 | 37.3 | 7.1 | Late |
| PI 344467 | Oncativo Inta | Argentina | 49.2 | 31.9 | 5.2 | Early |
| PI 256958 | Academia 48 | Romania | 50.7 | 23.3 | 11.8 | Interm. |
| PI 264940 | 111a | Greece | 51.5 | 33.8 | 9.3 | Early |
| PI 351743 | CLUJ 49-926 | Romania | 52.0 | 28.0 | 9.1 | Late |
| PI 519798 | PF 79782 | Brazil | 53.8 | 25.8 | 14.3 | Interm. |
| PI 351748 | JASI 10T | Romania | 56.3 | 36.7 | 10.2 | Late |
| PI 184512 | H 51 | Argentina | 57.2 | 26.2 | 14.8 | Early |

**Table 3.** (Continued)

| Accession | ID | Country of origin | FHB[c] index (%) | FDK[d] (%) | DON[e] (ppm) | Maturity[f] group |
|---|---|---|---|---|---|---|
| PI 351993 | Z.88.54 | Switzerland | 57.8 | 27.5 | 6.8 | Interm. |
| PI 344465 | Laureano Alv. Laah | Argentina | 59.3 | 33.3 | 7.8 | Interm. |
| PI 362043 | Arnaut De Toamina | Romania | 60.5 | 28.3 | 10.7 | Late |
| PI 349534 | 533B | Switzerland | 60.8 | 23.3 | 11.8 | Interm. |
| PI 168716 | Klein Condor | Argentina | 61.8 | 33.0 | 14.0 | Interm. |
| CItr 11215 | Belgrade 4 | Yugoslavia | 61.9 | 32.0 | 9.9 | Late |
| PI 584934 | Whestphalen | Brazil | 63.5 | 37.1 | 11.8 | Interm. |
| PI 192219 | Hatvani | Hungary | 64.0 | 30.0 | 8.7 | Late |
| PI 351187 | Taillens Velu Sel. | Switzerland | 64.5 | 30.3 | 15.0 | Late |
| PI 351476 | Vaulion | Switzerland | 65.6 | 32.5 | 13.4 | Late |
| PI 113949 | Stepnjachka | Ukraine | 66.9 | 31.2 | 8.6 | Late |
| PI 352000 | Z.89.37 | Switzerland | 67.2 | 35.0 | 8.1 | Interm. |
| PI 192229 | Gran Commune Ung. | Romania | 67.8 | 35.8 | 22.7 | Late |
| PI 349447 | 1882A | Switzerland | 69.1 | 33.3 | 17.5 | Late |
| PI 344454 | Buck Austral | Argentina | 70.8 | 29.4 | 14.2 | Late |
| PI 113948 | Kooperatorka | Ukraine | 76.3 | 34.8 | 7.1 | Late |
| PI 182561 | Sin Chunaga | Japan | 21.4 | 81.7 | 14.4 | Interm. |
| PI 360869 | Fujimi Komugi | Japan | 22.2 | 51.7 | -- | Late |
| PI 182586 | Norin 43 | Japan | 25.3 | 53.0 | 11.4 | Early |
| PI 411132 | Gogatsu-Komugi | Japan | 25.9 | 72.1 | 10.6 | Early |
| PI 197128 | Shinchunaga | Japan | 26.6 | 78.3 | 11.4 | Interm. |
| PI 182583 | Chuko | Japan | 29.9 | 76.9 | 7.1 | Interm. |
| PI 351816 | Froment Du Japon | Japan | 31.1 | 58.5 | 8.4 | Early |
| PI 182591 | Norin 61 | Japan | 34.6 | 53.8 | 7.1 | Interm. |

[a] & [b] Lines were entered into the URSN for Spring Wheat in 2000, and 2001, respectively.
[c] & [d] FHB index and FDK data were averages between 2000 and 2001.
[e] DON data were based on 2001 field nursery only.
[f] Maturity groups were based on days between planting and flowering: ≤60 (early), ≥64 (late), and 61-63 (intermediate).

# VARIETY DEVELOPMENT AND UNIFORM NURSERIES: PROGRESS IN FHB RESISTANCE IN HARD SPRING WHEAT

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## ABSTRACT

Evaluation of varieties and breeding materials for FHB resistance has been a high priority in the Upper Midwest spring wheat breeding programs since 1993. A Uniform Regional Scab Nursery for Spring Wheat Parents was initiated in 1995 and is coordinated by USDA-ARS. In 2001, more than 40 lines were contributed to the nursery by breeding programs with Agriculture and AgriFood Canada, AgriPro, the North Dakota State University, South Dakota State University, University of Minnesota, and Western Plant Breeders. A combination of field screening in inoculated, misted nurseries and greenhouse evaluations are used to characterize resistance levels. Varieties with enhanced levels of FHB resistance have emerged during the past several years, including 'BacUp', 1996 and 'McVey', 1999 (University of Minnesota); 'Parshall', 1999 and 'Alsen', 2000 (North Dakota State University); 'Gunner', 1996 and 'Hanna', 2001 (AgriPro); 'Ingot, 1998, Ember, 1999, and Walworth, 2001 (South Dakota State University); and 'Keystone', 2001 (Western Plant Breeders). The resistance source in BacUp is the Japanese variety Nuy Bay. Sumai 3 or derivatives are in the pedigrees of Alsen, Keystone, and McVey. Programs in the region are using additional resistance sources including other Chinese lines, European winter wheats, and spring types screened by Dr. Yue Jin at South Dakota State University.

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

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## ABSTRACT

Wheat germplasm that is resistant to Fusarium head blight (FHB, scab) will be the basis for cultivar development in high rainfall and irrigated acreage, which is at high risk to FHB infection, in the Central Great Plains. Wheat cultivars released by the University of Nebraska's wheat breeding program are widely grown in South Dakota and Kansas and are grown on 80% of Nebraska's acreage. Approximately one third of these acres, between 600 and 700, 000 acres are considered to be at risk to FHB infection.

The primary objective was to identify and develop elite winter wheat varieties that are tolerant to Fusarium head blight (FHB, scab). This will be accomplished using conventional breeding methods. Sources of FHB tolerant germplasm include transgenes from our biotechnology efforts, spring and soft wheat germplasm, and exotic materials. These sources will be incorporated into hard winter wheat germplasm (white and red) by crossing (initial crosses have been made and additional crosses will be made annually), they shall be screened for beneficial agronomic traits. The germplasm is being advanced to elite line status through modified bulk breeding or backcrossing methods. An effective greenhouse screen, using the injection method was implemented. It was used mainly for better parent identification. A field screening nursery, inoculated with *F. graminearum* infected corn and receiving mist irrigation with appropriate controls was used to screen 1000 lines.

The second objective was to determine the level of FHB and need for FHB resistant varieties under diverse environmental conditions. A survey of Nebraska, allowed the level of FHB resistance in common varieties, grown under irrigation and on dryland to be determined. In addition, we surveyed throughout Nebraska for FHB, as part of a monitoring system for foliar diseases such as leaf rust, caused by *Puccinia triticina* in wheat.

The third objective was to screen elite hard winter wheat lines in the Regional Germplasm Observation Nursery (RGON). This nursery was screened in the field. In 2001, only 3 lines in the RGON showed a high level (<10%) of tolerance. In the three Nebraska nurseries approximately 1% of the lines had promise for FHB tolerance in these preliminary tests, more lines (20%) were FHB tolerant, if one allowed a more lenient (<20%) FHB tolerance. However with the range of flowering dates among these materials, additional testing will be needed to confirm our putative tolerances.

# RANKINGS OF WHEAT CULTIVARS AFTER USING DIFFERENT TIMES AND METHODS TO RATE 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 the development of resistant cultivars. Host reaction to FHB can be quantified at different times and by several different methods. This research sought to determine how different rating times and methods correlated with each other. Twenty different winter wheat cultivars were screened in field nurseries over a 2-year period with 16 cultivars common to both years. Experimental design was a randomized complete block with four replications. Corn grains colonized by *Fusarium graminearum* were applied to the soil surface (108 g/m2) about 4 wk prior to heading. During heading and flowering, plots were sprinkler irrigated (3 min/hr) from 9:00 p.m. until 6:00 a.m. FHB index (percentage diseased spikelets) was determined either four (2000) or six (2001) times. Other rating methods included FHB index averaged across all rating times, the slope of the linear part of the disease progress curve, FHB index at 28 (2000) or 21 (2001) days after heading, and the percentage scabby kernels in combine-harvested samples. Cultivars were ranked (1=best, 20=worst) for eight (2000) or 10 (2001) different rating times or methods and correlations of rankings among all times and methods calculated. Variation occurred among different rating times and methods in the rank of a particular cultivar. For example, Hondo was the most resistant cultivar during the two years; however, it ranked as only the ninth best cultivar for percentage scabby kernels during 2000. Similarly, Heyne consistently ranked in the top six cultivars during both years; however, it ranked 11 for FHB index 21 days after heading during 2001. The lowest range of ranks was three for Heyne during 2000 (ranking 1-3 for all parameters) and 2137 for 2001 (ranking 16-18). The highest range of ranks was 16 for Larned during 2000 (ranking 4-19). Early ratings for this cultivar ranked low while the 28-day rating was high, resulting in the large range of ranks. The average range of ranks for all cultivars combined was 7.6 for 2000 and 7.3 for 2001. However, even with occasional significant departure in rank, cultivars tended to rank similarly across rating times, methods, and years. A notable exception across years was Big Dawg, which had a rank average of 12.0 in 2000 but only 2.4 in 2001. Correlations among rating times and methods were all significant ($P<0.10$, with 71 out of 73 having $P<0.05$) although some correlation coefficients for the slope parameter were low. When all coefficients for individual rating times and methods were averaged, the highest average was for average FHB index (0.8857). This was expected because most comparisons involved the same rating method (FHB index) but determined at different times. The lowest average across all correlations was for the slope parameter (0.6792). While there is adequate consistency among rating times and methods, the choice of a time or method can sometimes significantly affect the conclusions concerning the reaction of a particular cultivar to FHB.

# GENETIC ANALYSIS OF FUSARIUM HEAD BLIGHT RESISTANCE IN WHEAT LINE HUAPEI 57-2

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## INTRODUCTION

Fusarium head blight (FHB), also known as scab, is presently one of the most important fungal diseases affecting wheat (*Triticum aestivum L.*) in the Midwest and Upper Great Plains of the USA, as well as in many others parts of the world. FHB has the potential to greatly reduce grain yield and quality (Bai and Shaner, 1994), resulting in important economic losses (McMullen et al., 1997). Since evaluation of resistance to FHB requires laborious inoculation and evaluation procedures, DNA markers could be an efficient means to select desired genotypes. As new sources of resistance are identified, it becomes important to develop breeder-friendly markers to effectively introgress and pyramid resistance genes into adapted wheat cultivars.

## OBJECTIVE

Identify and map DNA markers controlling FHB resistance in a wheat population developed from the cross Huapei 57-2 (resistant) / Patterson (susceptible)

## MATERIALS AND METHODS

### Plant materials

A population of 163 F5:6 recombinant inbred lines (RIL) was developed by single seed descent from the cross Huapei 57-2 / Patterson. Patterson has been characterized as susceptible to FHB.

### FHB inoculation and evaluation

RILs and parents were artificially vernalized and transplanted to the field on 10 March as single hill plots of 8-10 plants per line. At flowering, a single floret located toward the top of spikes was inoculated with a conidial suspension of *F. graminearum*. Then, a plastic bag was placed over the spike for 3 days. Total number of spikelets and the number of diseased spikelets were counted 25 days after inoculation. Fusarium head blight severity was calculated as the percentage of infected spikelets.

### DNA marker analysis

Primers for the SSRs published by Röder et al. (1998) were screened for polymorphism among the parents. Using polymorphic markers, bulked segregant analysis (Michelmore et al., 1991) using 8 putative resistant and 8 putative susceptible RILs was initiated

## RESULTS AND DISCUSSION

### FHB evaluation

There was significant variation among RILs for FHB severity (data not shown). Broad-sense heritability and heritability on an entry mean basis were 0.43 and 0.87.

The distribution of FHB severity was heavily skewed towards resistance (Fig. 1). The weather particularly cold (maximum temperature < 25°c) for almost two weeks during the experiment might explain this pattern. The environmental conditions were not favorable to the establishment and development of the fungus. Thus, Patterson which is known to be susceptible had a mean FHB severity of only 40%. Therefore, we suspect that certain RILs that appeared resistant in this test are in fact susceptible.

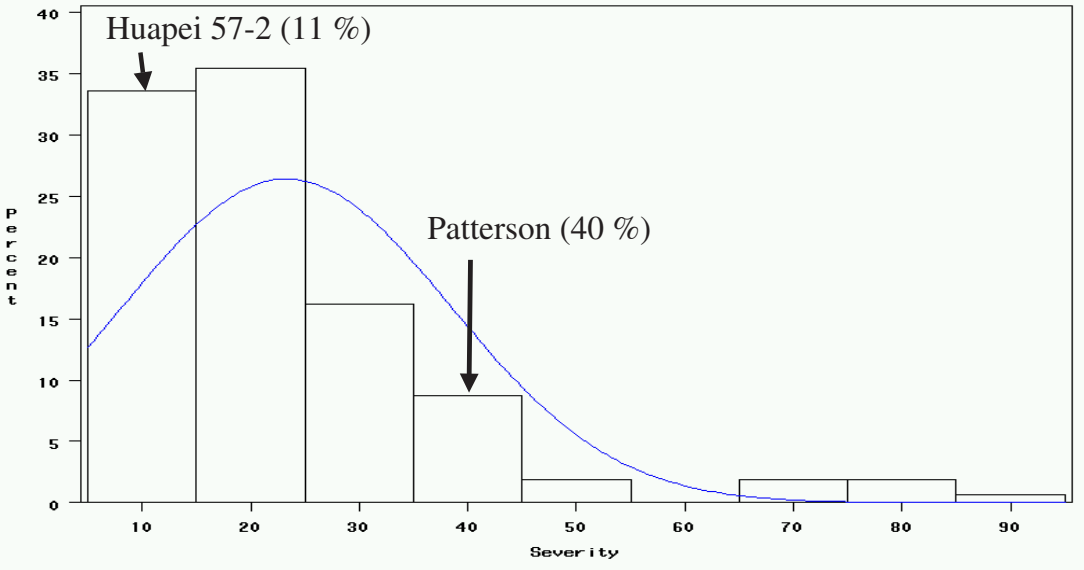


**Fig. 1: Histogram of mean Fusarium head blight severity for the parents and RILs from the cross Huapei 57-2/Patterson (May-July 2001)**

### DNA marker analysis

Based on the phenotypic data collected during the field testing, 8 resistant and 8 susceptible RILs were selected to initiate bulked segregant analysis. A major QTL conferring resistance appears to be located on chromosome 3BS close to SSR markers Xgwm 533 and Xgwm 493 which have been previously identified (Anderson et al., 2001). A single line (# 4) does not have at both marker loci the expected allele (Fig. 2). It could be due to double crossing over or to misclassification of that line.

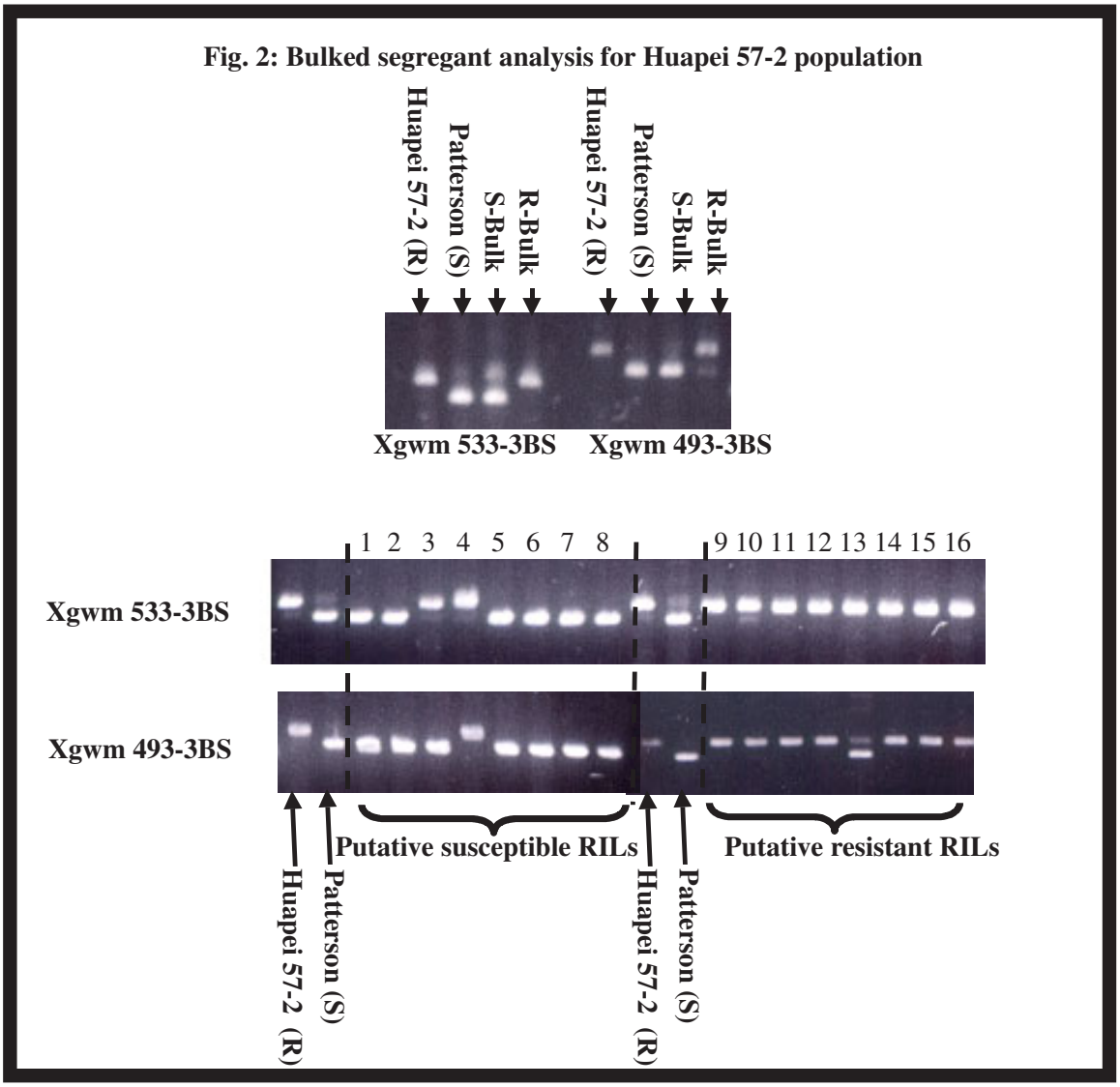


**Fig. 2: Bulked segregant analysis for Huapei 57-2 population**

## CONCLUSION

Preliminary results suggest that Huapei 57-2 does not have novel resistance genes. A major resistance locus is located in the 3BS region already identified by Waldron et al. (1999). The population is currently tested again this fall under greenhouse conditions. All RILs will be screened using the markers identified by bulked segregant analysis to confirm marker-QTL linkages and estimate the distance between markers and putative resistance loci.

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# DEVELOPMENT OF DURUM WHEAT RESISTANT TO FUSARIUM HEAD BLIGHT

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## ABSTRACT

Fusarium head blight (FHB) caused by the fungus *Fusarium graminearum* Schwabe (telomorph *Gibberella zea* (Schwein.) Petch. has been seriously attacking durum wheat (*Triticum turgidum* L. var. *durum*) in North Dakota and the surrounding states. There is continuous decline in harvested durum acreage and production in ND because of FHB. The harvested acreage and durum production in the year 2001 were 22% less than the year 2000. The decline in harvested acreage and durum production in ND is disastrous to the farm economy and has a direct impact on the national pasta industry and the international export market since ND, on average, produces 75% of the durum in the United States. Fungicides may reduce the disease but the most environmentally safe and economical way to control the disease is with genetic resistance. Our objective is to develop FHB resistant durum wheat cultivars/germplasm with good agronomic and quality traits. We have identified a Langdon *Triticum dicoccoides*-3A (LDN (Dic-3A)) substitution line, a line from a FHB recurrent selection program established in 1995 and a doubled haploid line, all having moderate levels of resistance to FHB. Six segregating populations were developed from crossing the three lines to the durum cultivars Maier and Ben. All six populations were evaluated for type II resistance in the 2000 FHB screening nursery at Prosper, ND and 30% of lines were selected. In the Fall 2001 greenhouse only the $F_3$ selected lines from the Ben/LDN(Dic-3A) were evaluated because of space limitation. Eighteen lines had disease severity lower than 18%. These lines will be evaluated for FHB and agronomic traits. We also have identified 20 durum lines that have FHB type II resistance from crosses of durum with the hexaploid wheat Sumai 3. We are in the process of evaluating these lines for agronomic and quality traits for possible release. We have developed 25 populations from crossing the best three FHB resistant lines with new North Dakota released durum cultivars. Only two populations were evaluated as $F_3$ lines for FHB in the Fall 2000 and Spring 2001 greenhouses because of space limitations. Approximately 30% of lines from each population were selected for further evaluations. One population was sent to the Academy of Agricultural Science, Plant Protection Institute in Shanghai, China (AASPPI) for evaluations in the 2000-2001 season. Fifty lines were selected for further evaluations in the greenhouse. The remaining populations are being advanced in greenhouses and the winter nursery in New Zealand to be evaluated either at AASPPI or greenhouses in North Dakota. We will be using the two molecular markers *Xgwm2* and *Xgwm533* for screening some of the populations.

# VARIATION IN WHEAT GENOTYPE REACTION TO *FUSARIUM GRAMINEARUM* DUE TO INOCULATION TECHNIQUE

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## ABSTRACT

There have been several different inoculation protocols proposed to distinguish among types of resistance in wheat to *Fusarium graminearum*, but the nature of resistance mechanisms and procedures to test for the different types have not been determined. This study evaluated different inoculation techniques for assessing reaction of wheat genotypes to *F. graminearum*. Six wheat cultivars with resistant and intermediate reactions, as determined in field tests in the 1999 Northern Uniform Winter Wheat FHB Screening Nursery, were selected for evaluation in the greenhouse based on their mean disease severity and incidence ratings. A known susceptible cultivar (2545) was included as a control. Four inoculation procedures were tested; 1) injecting a macroconidial suspension (6 x $10^4$ conidia /ml) with a hypodermic syringe into a single central floret on the spike, 2) atomizing a macroconidial suspension (6 x $10^4$ conidia/ml) onto the entire spike surface to the point of run-off, 3) applying 1 µl drops of ascospore suspensions (6 x $10^6$ spores/ml) on the junction of the glume, lemma, and palea of all emasculated florets on one side of the spike, 4) applying a 1 Føl drop of ascospore suspensions (6 x $10^6$ spores/ml) on each group of extruded anthers of all spiklets on one side of the spike. The control inoculation was a central floret hypodermic injection of de-ionized water. In the greenhouse experiments conducted to evaluate inoculation techniques, disease development varied among genotypes tested and the disease level depended on the inoculation technique used. Across the genotypes tested, statistically (P=0.05) higher levels of disease (severity 14 days after inoculation (DAI), AUDPC and rate of disease progress) were recorded when conidial suspensions were atomized onto the heads at anthesis than when other inoculation techniques were used. The central floret injection technique and placing a drop of inoculum on the outside of the glume initiated statistically similar levels of disease. Analysis of variance for the interaction between inoculation technique and genotype indicated that all genotypes responded similarly (severity 14 DAI and AUDPC) when inoculated by the single floret injection technique and when a drop of inoculum was placed on the exterior of the glume. Both OH609 and IL94-1909 had significantly lower severity 14 DAI and AUDPC values than 2545 when conidia were atomized onto the head at 50% anthesis. When drops of ascospores were placed on extruded anthers and assessed for disease severity at 14 DAI, Goldfield and IL94-1909 had significantly less disease than 2545, but according to AUDPC values only Goldfield was identified as having a statistically lower level of disease than 2545. Results of these tests indicate that selection of genotypes with resistance to FHB is dependent on the inoculation technique used.

# INFLUENCE OF MIST-IRRIGATION VOLUME OVER TWO SEASONS ON THE SEVERITY OF FUSARIUM HEAD BLIGHT AND SEED CHARACTERISTICS IN SELECTED CHECK CULTIVARS AND LINES OF WHEAT AND BARLEY

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## ABSTRACT

A field experiment was conducted in 2000 and 2001 at the St. Paul campus of the University of Minnesota to investigate the influence of mist-irrigation treatments that might improve our ability to screen for resistance to Fusarium head blight (FHB) in wheat and barley. Mist-irrigation was applied to the barley cultivars and lines Stander (susceptible, S), Robust (S), MNBrite (resistant, R), MNS93 (R) and the wheat cultivars Norm (S), McVey (moderately resistant, MR), P 2375 (MR), and BacUp (R). The experimental design consisted of four separate randomized complete split-blocks with four replications. One randomized complete split-block was non-misted as a control. The mist-irrigation treatments were: non-misted, 2, 4, and 8 mm of water per day. Split-block treatments were inoculated versus non-inoculated. Plots were inoculated with macroconidia in an aqueous suspension adjusted to 100,000 spores per ml using a $CO_2$ powered backpack sprayer. Variables measured in barley plots over the different mist-irrigation treatments included FHB incidence, severity, discolored kernels, and concentration of deoxynivalenol (DON) in harvested grain. Differentiation among the barley cultivars for incidence of infection was clearer under no mist whereas differentiation among the barley cultivars for the other three variables was more consistent at the 8 mm per day volume. The variables measured in wheat plots over the same mist-irrigation treatments included FHB incidence, severity, visually scabby kernels (VSK), and concentration of DON in harvested grain. Differentiation among the wheat cultivars over two years for the four variables was more consistent than among the barley cultivars and was most consistent under no mist or at the 2 mm per day volume. We feel the lower disease levels reflect conditions in years where natural epidemics generate FHB severity below a mean of 20%. These preliminary data also suggest that breeders could obtain useful information regarding promising breeding lines by screening for resistance to FHB utilizing inoculated plots that would be non mist irrigated.

# SELECTIVE BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN SOFT RED WINTER WHEAT

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## ABSTRACT

Highly effective type II scab resistance has not been identified in soft red winter (SRW) wheat. Most type II resistance sources currently used are progeny of Sumai 3, and are spring habit, low yielding and susceptible to other endemic diseases. A major objective is to transfer type II resistance from these sources into SRW wheat backgrounds to develop scab resistant germplasm and varieties with high yield potential and resistance to other prevalent diseases including powdery mildew, leaf rust and glume blotch. Strategies being used to accelerate development of scab resistant wheat genotypes include: 1) Incorporation and pyramiding of type II and other types of resistance into adapted SRW wheat backgrounds via selection of progeny from top-cross, backcross and doubled haploid populations; 2) Screening and selection for type II and other types of resistance in inoculated mist-irrigated greenhouse and field tests and; 3) Simultaneous evaluation of progeny for resistance to other diseases and agronomic traits. Thirty-six scab resistant sources (21 Chinese, 2 French, 1 Japanese, 2 Canadian and 10 SRW wheat lines) have been used as parents in the breeding program and over 500 populations have been developed. In 2001, 234 populations were evaluated in a mist-irrigated scab nursery, inoculated using colonized maize seed, at Warsaw, VA. On the basis of scab incidence and severity, 68 of the 234 (29%) populations were advanced. In headrow tests, 2960 $F_5$ lines, derived from 53 $F_4$ populations previously screened for scab resistance, were evaluated for agronomic traits and resistance to diseases other than FHB at Warsaw, VA. From these headrows, 47 top-cross derived lines and 3 DH lines were selected for further testing in our scab nursery at Blacksburg, VA and in Observation Yield Tests at two locations. Twenty-three advanced $F_6$ lines and 13 doubled haploid (DH) lines were evaluated simultaneously for scab resistance in a mist-irrigated nursery, inoculated by spraying a spore suspension, at Blacksburg, VA and for other agronomic traits in a non-inoculated observation yield test at Warsaw, VA. Nine of the $F_6$ lines and two of the DH lines were advanced for testing in Preliminary Wheat Trials. Three elite lines were evaluated in Preliminary Yield Trials; one of these lines was selected for further testing in our Advanced Yield Trial and another will be evaluated in Virginia's Official Variety Trial. Seven lines were tested in the Uniform Winter Wheat FHB Nurseries. Advanced lines developed in our program possess scab resistance derived from one or more type II resistance sources or type II resistance combined with other types of resistance. Some of these scab resistant lines also have high yield potential and resistance to other prevalent diseases. Progress in transferring type II resistance into SRW wheat genotypes has been accelerated via use of the wheat by maize double haploid system. To date, 109 doubled haploid lines and 113 haploid plants have been derived from 3-way crosses consisting of diverse scab resistant parents. Type II resistance derived from five different sources is being backcrossed into seven SRW wheat backgrounds, of which two are adapted sources with other types of resistance. Approximately 495 backcrosses were made

between resistant progeny derived from three $BC_3F_1$ and 16 $BC_2F_1$ populations and their recurrent parents. Near-isogenic lines with type II resistance incorporated into adapted SRW wheat backgrounds will be developed and facilitate pyramiding of different types of resistance.

# COMPARISON OF FHB DEVELOPMENT ON HARD RED WINTER WHEAT USING DIFFERENT PLANTING SCHEMES

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## ABSTRACT

Fusarium head blight (FHB) is a destructive fungal disease of wheat causing yield loss and poor grain quality. Recent changes in winter wheat production in South Dakota may lead to an increase in scab. Producers have adopted a reduced tillage cropping system and have increased production of winter wheat in traditional corn-soybean rotations. The winter wheat breeding program at South Dakota State University has screened transplanted hill nurseries for scab resistance 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 vernalization in chambers, proceeded by planting the germinated seedlings by hand. The root system is far from established in transplanted wheat, often leading to poor plant development. The 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 May, 2001. Significant correlations among the two types of planting techniques were observed for FHB severity, FHB disease indices, and percentage of tombstone kernels. These results might provide a basis for using direct seeding as an alternative to transplanting. Because this is inconclusive, however, further studies are needed. Transplanted seedling performance in the CPT will also be compared to dormant seeded CPT lines in 2002. These lines as well as other elite and preliminary lines will also be evaluated under greenhouse conditions in the future.

# EVALUATING PHENOTYPIC AND MARKER-ASSISTED SELECTION IN THE F2 GENERATION FOR CHEVRON-DERIVED FHB RESISTANCE IN BARLEY

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## ABSTRACT

Early generation selection for resistance to Fusarium head blight (FHB) in barley could significantly increase the efficiency of breeding efforts to develop FHB resistant varieties. In previous studies, we have identified and validated two FHB QTL derived from the resistant source Chevron. In the summer of 2000, we initiated an experiment to determine the effect of selection in the F2 generation by marker-assisted selection (MAS) and by visual disease assessment. We grew the F2 generation of two populations (~800 individuals per population) and imposed selection based on two criteria: 1) low disease severity in a misted and inoculated FHB nursery; and 2) simple sequence repeat (SSR) marker genotype. In addition, a random sample was taken from each population. In population C119, we selected SSR genotypes as either homozygous Chevron (resistant allele) or homozygous Lacey (susceptible allele) for a marker linked to a FHB QTL on chromosome two discovered in the Chevron x M69 mapping population. In population C113, we selected SSR genotypes as either homozygous Chevron or Lacey for a marker linked to a FHB QTL on chromosome six. For each population, twenty F4:5 lines for each of the four selection classes were evaluated in a disease nursery (3 reps line) for FHB severity and heading date (HD). In addition for population C113, we measured grain protein (GP) in a separate field trial. Mean FHB severity was the same in the phenotypic and random selection groups as were the means for the other traits measured for both populations. MAS for the Chevron allele on chromosome two in the C119 population resulted in a 43% reduction in FHB severity compared to the random control. We also observed a correlated response of 4% increase (~ 2 days) in HD. This was expected since the FHB QTL mapped to chromosome two was coincident with a QTL for HD. MAS for the Chevron allele on chromosome six in the C113 population resulted in a 11% reduction in FHB severity. We also observed a correlated response of 5% increase (~ 0.7% GP) in percent GP. This was also expected since the chromosome six FHB QTL was coincident with a QTL for GP. The future success of exploiting these genes using MAS will require either breaking the linkages between FHB and HD and GP using more tightly linked markers or using other genes to reduce HD and GP to compensate for the correlated response to selection for FHB resistance.

# DIALLEL ANALYSIS OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN SOFT RED WINTER WHEAT

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## OBJECTIVES

Objectives for this study included to gain a better understanding of Fusarium head blight (FHB) resistance in Kentucky adapted soft red winter wheat (SRWW) lines, to identify promising combinations of parents for the selection of improved breeding lines, and to study the effect of greenhouse versus field screening techniques.

## INTRODUCTION

FHB is a destructive disease of wheat and barley around the world. The disease caused by the fungus, *Fusarium graminearum*, reduces test weight and yield, and produces deoxynivalenol (DON), a harmful toxin in harvested grain. The release of genetically resistant varieties is considered to be the most effective control against FHB. Resistance to FHB is quantitative in nature and found in several spring wheat cultivars including the Chinese Sumai #3. However, resistance to FHB in adapted winter wheats is not as well defined.

## MATERIALS AND METHODS

***Plant Materials*** - A diallel crossing scheme was constructed using nine soft red winter wheat lines from the 1999 Kentucky Wheat Variety Trial. Crosses were made in the greenhouse in 2000 using the approach method of pollination. The $F_1$ progeny, reciprocals, and parents were screened for resistance in the greenhouse. Reciprocals were combined and only $F_1$ progeny and their parents were screened in the field environment. Analysis of data was completed via SAS Version 6.12.

***Greenhouse Injection Screening*** - Seed was vernalized on August 17, 2000 and seedlings were transplanted in the greenhouse on October 12, 2000. Ten heads per cross or parent were injected at anthesis following the procedure as reported in Van Sanford et al. (1999).

***Field Injection Screening*** - The same $F_1$ progeny and their parents were planted in small 5 seed hill plots on October 15, 2000 in Lexington, KY with three replications per cross or parent. The experiment was grown under an automated mist irrigation system and 10 heads per hill plot were injected at anthesis. Injected heads were covered with glassine bags to guard against a natural infection. These field injection procedures are modeled after techniques used at CIMMYT. Symptoms were read 21 days after injection by counting the number of infected spikelets and total spikelets.

***Deoxynivalenol Analysis*** - Ten injected heads were harvested from one replication of the field experiment. These heads were hand threshed in bulk. A five gram sample of grain from each $F_1$ and parent was then analyzed for DON using the EZ-Quant Vomitoxin Test Kit from the Diagnostix Company. Each sample was ground in a coffee grinder for 15 seconds. The coffee grinder was vacuumed between samples to protect against any cross contamination. Twenty-five ml of distilled water was then added to each ground sample and the remainder of the test was completed following the protocols contained within the EZ-Quant Vomitoxin Test Kit. Two aliquots from each DON extraction were pulled to provide replication. Only seed from the field experiment was subjected to the DON analysis.

## RESULTS AND DISCUSSION

***Greenhouse Screening*** - Table 1 shows the parent and cross severity means of the 10 injected heads from the greenhouse screening. General combining ability (GCA) effects were calculated from Griffing's Model 1 Method 4 (Griffing, 1956). Negative GCA effects for a certain parent indicate that parent's $F_1$ progeny were more resistant, and a positive GCA effect indicates they were more susceptible. Roane and NK CK 9663 showed positive GCA effects for severity while Freedom showed a negative GCA effect.

Table 2: ANOVA for FHB severity in a 9x9 diallel cross of SRWW screened in the greenhouse at Lexington, KY 2000.

| Source | df | SS | MS |
|---|---|---|---|
| Crosses | 35 | 58749.9 | 1678.6** |
| GCA | 8 | 41278.3 | 5159.8** |
| SCA | 27 | 17471.6 | 647.1** |
| Error | 553 | 197005 | 349.9 |

**P<0.01

Mean 18.47% CV 101.29

Highly significant differences among crosses were observed (Table 2). General combining ability and specific combining ability (SCA) effects were both significant (Table 2). The majority of variation among the crosses was due to the general combining ability effects and thus most of the variation is attributed to additive effects. The overall greenhouse severity mean was 18.47% with a very large coefficient of variation (CV) (Table 2). Variation among individual heads within a cross can only be derived from environmental effects due to the fact that the genotypes being tested were $F_1$ progeny.

***Field Screening*** - In the field, Kaskaskia and its progeny were not planted due to insufficient $F_1$ seed. The parent and cross severity means and DON means in the field environment are presented in Table 3. Again GCA effects were calculated for both traits using Griffing's Model 1 Method 4 (Griffing, 1956). In the field, Roane's GCA effect for severity was negative while in the greenhouse the GCA effect was positive. This is unexplained. DON GCA effects were not significant and no parents produced GCA effects that exceeded the

estimate of the standard error (Tables 3 and 5). It should be noted that the material tested for DON consisted only of injected heads and thus DON levels were very high (C=31.31 ppm). The DON data presented in Table 3 can therefore be regarded as a potential upper limit for these parents and crosses.

The correlation coefficient between DON and severity from the field screening was r = 0.3 (P<0.5). This low correlation does not support the selection of FHB resistant lines based on DON data alone. Bai et al (2001) reported a higher correlation coefficient of 0.65 between proportion of scabbed spikelets and DON in a greenhouse screening where only injected heads were analyzed for DON.

The ANOVA table for the field experiment also shows highly significant differences among crosses for the severity data (Table 4). The GCA effects and SCA effects were also highly significant. However in the field, the amount of variation attributed to SCA effects increased from that shown in the greenhouse. This leads to the hypothesis that along with additive effects, dominance effects may also control some of the variation expressed in the field.

There were significant differences among the crosses for DON (P<0.10) (Table 5). The GCA effects were non significant (P<0.10).

Table 4: ANOVA for FHB severity in a 8x8 diallel cross of SRWW screened in the field at Lexington, KY 2001.

| Source | df | SS | MS |
|---|---|---|---|
| Crosses | 27 | 93533.4 | 3464.2** |
| GCA | 7 | 28388.3 | 4055.5** |
| SCA | 20 | 65145.1 | 3257.3** |
| Error | 791 | 280102 | 354.1 |

**P<0.01

Mean 67.05% CV 28.07

Table 5: ANOVA for DON levels in a 8x8 diallel cross of SRWW screened in the field at Lexington, KY 2001.

| Source | df | SS | MS |
|---|---|---|---|
| Crosses | 27 | 1873.4 | 69.4* |
| GCA | 7 | 188.3 | 26.9 |
| SCA | 20 | 1685.2 | 84.3** |
| Error | 27 | 1060.9 | 39.3 |

*P<0.10 **P<0.01

Mean 31.31 ppm CV 20.02

***Greenhouse Screening Versus Field Screening*** - Disease intensity was highest in the field. The overall severity mean for the field experiment was very high at 67.05% when compared to the greenhouse severity mean of 18.74%. The field environment was obviously more favorable for infection. The CV in the field environment (28.07%) was much lower than that of the greenhouse (101.29%) for the severity data. With this drastic reduction in the CV it can be recommended that the field environment provided the better screening environment.

The correlation coefficient between the two environments for the severity data was very low at r = -0.087 and was not statistically significant. The greenhouse and field environments were not related and thus resistance that is noted in the greenhouse may not hold up in the field. This is an important point for breeders to remember when selection is practiced based on greenhouse data alone.

**Table 1:** Mean FHB Severity (%) for parents (diagonal), $F_1$ crosses (above-diagonal) and reciprocals (below diagonal) and GCA effects in a 9x9 diallel cross of SRWW screened in the greenhouse at Lexington, KY 2000.

| | 25R26 | CK 9663 | CK 9474 | Roane | KY 89C 804 | KY 86C 127 | Freedom | Kaskaskia | Patton | GCA Effects |
|---|---|---|---|---|---|---|---|---|---|---|
| 25R26 | 9.6 | 17.8 | 15.7 | 21.0 | 5.4 | 9.9 | 5.3 | 6.0 | 10.54 | -4.13 |
| CK 9663 | # | 40.7 | 16.6 | 58.0 | 29.0 | 21.0 | 9.6 | 9.7 | 26.45 | 7.42* |
| CK 9474 | # | 28.2 | 19.8 | 22.6 | 6.8 | 29.5 | 14.2 | 14.4 | 9.52 | -2.07 |
| Roane | 24.6 | 49.4 | 29.8 | 8.5 | 32.2 | 40.2 | 19.9 | # | 12.78 | 11.70* |
| KY 89C 804 | 18.9 | 31.7 | 18.7 | 42.8 | 43.9 | 25.5 | 17.6 | # | 14.47 | 2.87 |
| KY 86C 127 | 18.0 | 23.7 | 15.7 | 25.8 | 18.6 | 20.3 | 12.9 | # | 14.13 | 1.91 |
| Freedom | 9.7 | 17.4 | 6.2 | 5.3 | 19.5 | 11.9 | 5.8 | 12.6 | 5.76 | -7.14* |
| Kaskaskia | 16.1 | 10.7 | 5.2 | 22.1 | 5.0 | 13.1 | 4.2 | 14.2 | 13.26 | -6.55 |
| Patton | 8.0 | 19.3 | 8.0 | 18.7 | 28.8 | 16.5 | 5.5 | 16.3 | 10.05 | -4.00 |

# $F_1$ was not made. *Exceeds standard error

**Table 3:** Mean FHB Severity (%) (above diagonal) and deoxynivalenol production (ppm) (below diagonal) for parents (diagonal) and $F_1$ crosses and GCA effects in a 8x8 diallel cross of SRWW screened in the field at Lexington, KY 2001.

| | 25R26 | CK 9663 | CK 9474 | Roane | KY 89C 804 | KY 86C 127 | Freedom | Patton | FHB Severity GCA Effects | DON GCA Effects |
|---|---|---|---|---|---|---|---|---|---|---|
| 25R26 | 66.2<br>31.6 | 66.2 | 56.5 | 46.1 | 66.1 | 75.9 | 54.2 | 68.7 | -5.93 | -0.13 |
| CK 9663 | 38.5 | 71.6<br>30.7 | 71.2 | 70.3 | 78.8 | 69.9 | 81.4 | 67.4 | 5.99 | -0.14 |
| CK 9474 | 30.4 | 29.9 | 73.8<br>32.7 | 54.4 | 86.7 | 66.5 | 81.6 | 69.9 | 2.93 | 0.59 |
| Roane | 22.3 | 28.4 | 24.3 | 56.8<br>21.2 | 66.9 | 50.3 | 66.3 | 65.6 | -8.24* | -2.82 |
| KY 89C 804 | 20.4 | 36.5 | 38.3 | 42.1 | 78.1<br>34.4 | 47.1 | 67.6 | 59.4 | 0.56 | 0.72 |
| KY 86C 127 | 45.0 | 30.4 | 28.8 | 22.9 | 32.1 | 81.7<br>22.5 | 77.2 | 84.8 | 0.39 | 1.58 |
| Freedom | 30.3 | 25.6 | 34.9 | 30.7 | 25.0 | 31.5 | 54.6<br>23.1 | 60.1 | 3.17 | -1.37 |
| Patton | 31.6 | 29.1 | 36.1 | 31.6 | 29.2 | 38.0 | 33.0 | 75.6<br>37.3 | 1.13 | 1.57 |

*Exceeds standard error

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# BREEDING FOR FUSARIUM HEAD BLIGHT RESISTANCE IN SPRING WHEAT (*TRITICUM AESTIVUM* L.)

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## ABSTRACT

Recently, the importance of Fusarium head blight (FHB) has increased worldwide and major epidemics have occurred in southern Manitoba in most years during the last decade. Annual losses to Manitoba wheat producers have been estimated at $50 million and losses to the grain industry are estimated at $100 million. Developing varieties resistant to FHB is an important breeding objective because few registered spring wheat varieties have a useful level of tolerance to the disease and none is resistant to the pathogen. At the Cereal Research Centre (CRC), a collaborative effort between wheat breeders and pathologists has lead to the development of effective screening nurseries and improved germplasm. Initially crosses and backcrosses were made directly with exotic germplasm such as Sumai 3 or Ning8331. More recently, convergent crossing strategies have been used in population development. Recent crosses have used improved FHB resistant germplasm from the CRC and US spring wheat breeding programs. F2 and F4 generation breeding material is screened in a FHB screening nursery where an artificial epidemic is generated using Fusarium-infected corn inoculum and the application of 6 mm of water 3 times per week using a Renke irrigation system. Later generation breeding material is screened under closely controlled conditions of inoculation. Using a macroconidial spore suspension, rows are spray inoculated at 50% heading and four days later to infect later tillers. FHB ratings are carried out 21 days after inoculation using a FHB index. The index accounts for both incidence (% of heads infected) and severity (% of spikelets infected) of the FHB infection. Breeding progress has been slow due in part to weak agronomic potential and low functional quality of FHB resistant germplasm such as Sumai 3. Breeding lines with improved FHB resistance produced by CRC include: FHB#21, FHB#37, BW278, BW310, BW311, BW312 and HY644.

# GREENHOUSE EVALUATION FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN WHEAT

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## OBJECTIVES

To establish a refined greenhouse screening system for scab resistance;

To identify resistant germplasm and breeding lines through assessment of the resistance on a large scale.

## INTRODUCTION

Scab or Fusarium head blight caused by *Fusarium graminearum* (*Gibberella zeae*) is a serious disease in wheat all over the world. Development and use of resistant cultivars is the most effective and economic approach to control this disease. Identification and utilization of the resistant germplasm sources is one of the critical steps to reach this goal. Schroeder and Christensen (1963) first defined two types of scab resistance in wheat: resistance to initial infection and resistance to the spread of pathogen within the tissue or spike. The two types have subsequently been designated as Types I and II, respectively, and have been widely accepted. Based on these definitions, additional types of resistance have also been proposed (Wang and Miller, 1988; Mesterhazy, 1995). So far, however, very few sources with real Type I resistance have been found and almost all the germplasms with other additional types of resistance have proved to possess type II resistance at the same time. Therefore, we focused the scab screening on assessment of Type II resistance in the greenhouse.

## MATERIALS AND METHODS

### *Host Planting*

One hundred and eighty genotypes (200 entries) were included in the scab screening in greenhouse during the crop season 2000/2001 by means of single-floret inoculation. Thirty-one spring materials were introduced from China and the remaining 169 cultivars or breeding lines were taken from those being tested by the MSU Wheat Breeding Program. 'Ning 7840' and 'Norm' were used as controls. A completely randomized design with three or four replications was adopted. For spring types, no vernalization treatment was given. Eight pots (11x11 cm) per replication and three seeds per pot were planted for each entry. For winter types, seeds were sown into petri dishes or plastic pots. There were two replications for pot planting and one replication for petri dish planting. Vernalization treatment was conducted at 2-4 $^{o}$C in the dark for eight or ten weeks. After vernalization, seedlings were transferred to and kept in a room for two days where temperature was maintained at 13-15 $^{o}$C to avoid de-

vernalization. All the materials were transplanted into 11x11 cm pots and placed in the greenhouse. Eight pots per replication and three seedlings per pot were transplanted for each entry. In order to compare and compute the relative resistance, the checks Norm and Ning 7840 were planted once a week until all the assessed materials had jointed (Feekes stage 6). At the beginning, the temperature in the greenhouse was adjusted to 15-18 °C until jointing stage. Then the temperature was kept at 22-26 °C. From seedling stage to flowering stage, the plants were watered daily and fertilized once every other week. About 12 hours of artificial light was provided every day by a combination of halogen and fluorescent lights.

***Inoculation and Disease Scoring***

During heading and flowering stages, single-floret inoculation was conducted. For each entry, about 12-15 spikes (sampled at random from different plants) were inoculated by pipette injecting 12-15 ul of *F. graminearum* conidiospore suspension (50,000 spores/ml) into a basal floret in the central part of the spike. Then the pots with inoculated plants were placed for three days in a mist-irrigation system programmed to deliver 10-second mist periods at intervals of 10 minutes. Temperature in the mist system was 22-26 °C. After mist-irrigation, the pots were transferred to another greenhouse compartment with conditions similar to those in the greenhouse where plants were placed after jointing. Then the inoculated heads were watered three to four times a day until the disease observation in order to maintain high humidity. For each time of inoculation, the controls Norm and Ning 7840 were always included. The number of scabby spikelets on the inoculated heads was recorded at three weeks after inoculation as described by Jiang (1998) and Jiang et al (1995):

0.5: only the inoculated floret showed the symptom;
1.0: only the inoculated spikelet showed the symptom;
1.8: inoculated spikelet and main rachis showed the symptom;
2.0 or more: number of the total scabby spikelets on the inoculated spike.

For the winter materials, the number of total spikelets was counted and percentage of diseased spikelets was calculated.

***Statistical Analysis***

Firstly the average of scabby spikelets was calculated for each replication and then ANOVA was computed based on the replication means. For the percentage of scabby spikelets, arc sine transformation was undertaken in order to approach the homogeneity. Broad-sense heritability was estimated based on the results of ANOVA.

## RESULTS AND DISCUSSION

ANOVA showed that there were significant differences in scab resistance (Type II) among entries for each trial. For all the entries, the range for the number of scabby spikelets was 0.6-17.6. For the winter types (U.S. materials), the range for the percentage of scabby spikelets was 14.3%-100%. In order to classify the resistance and group the materials into different resistance levels, the scab resistance was divided into the following 6 levels according to the mean of number or percentage of scabby spikelets and the standard deviation in the controls Norm and Ning 7840.

| HR | R | MR | MS | S | HS |
|---|---|---|---|---|---|
| <1.3 | 1.3~2.6 | 2.6~4.0 | 4.0~7.4 | 7.4~13.0 | >13.0 |
| <7.5% | 7.5~15% | 15~25% | 25~45% | 45~70% | >70% |

Most of the newly introduced Chinese germplasm resources proved to have higher resistance (Table 1). 'W14' and its sister lines 'CJ 9306' and 'CJ 9311' were further verified to possess higher Type II resistance than the well-known 'Sumai 3', which was consistent with previous results (Jiang, 1997; Jiang et al, 2000). It could be reasoned that more resistance genes have been likely integrated in these lines because they were developed from a recurrent selection population with diverse resistance sources and complex genetic background (Jiang and Wu, 1996). Additionally, other Chinese materials listed in Table 1 also possess improved resistance and desired agronomic traits such as lower plant height, higher yielding potential, better lodging resistance and ripening performance. However, their spring habit should be considered when they are used as parents.

Although most of U.S. cultivars and lines were susceptible to scab, there were some that exhibited some resistance. Among all the U.S. materials, the most resistant materials were Pioneer Brand '25R18', MO 980525, MO 981020 and TW 97613. In addition, MO 980525, 'Patton', 'Roane', IL 95-4162, VA96W-250, 'Ernie', NY87048W-7388 and TW 97613 showed a stable reaction to scab in different trials or years (Jiang et al, 2000). The U.S. materials with moderately susceptible or better reaction to scab were listed in Table 2. Although they do not have high levels of resistance, these materials can be used in U.S. breeding programs because of their adaptability.

The coefficient of correlation between the number and percentage of scabby spikelets for all the 169 entries of winter types was 0.9344. It is seen that both measures could be used in scab screening. Comparatively speaking, however, the number of scabby spikelets seems to be simpler and the percentage of scabby spikelets reflects more accurate economic loss. The estimates of broad-sense heritability ($h_B^2$) depended on the source of materials. In the experiment with China-introduced materials and Norm, $h_B^2$ was 83.4%. Among the entries from different trials of MSU Wheat Breeding Program, the $h_B^2$ was lowest in the experiment with MSU advanced lines (31.3% for the number of scabby spikelets and 21.5% for the percent of scabby spikelets), and the highest estimate was obtained in the Uniform Scab Nursery (57.4% for the number of scabby spikelets and 40.3% for the percent of scabby spikelets). The estimates of heritability for number of scabby spikelets were always greater than those of percentage of scabby spikelets in different experiments. For all the 169 entries of winter types, $h_B^2$ was estimated as 50.6% for the number of scabby spikelets and 38.7% for the percentage of scabby spikelets, respectively. This indicates that the percentage of scabby spikelets within a spike is more affected by the environment than the absolute number of scabby spikelets in a spike.

Cultivars Norm and Ning 7840 were included as replicated checks in 12 different experiments between November 2000 and June 2001. We calculated the means for those cultivars for each of the 12 experiments. The overall mean (n=12), range, and coefficient of variation for the average number of scabby spikelets were 2.0, 0.8~3.3 and 33.8% for Ning 7840, and 10.7, 7.4~14.0 and 21.0% for Norm, respectively. This indicates a relatively high level of repeatability and therefore power to resolve differences between genotypes 'S' and

'R' Type II FHB reactions. Moreover, similar results were produced with other cultivars or lines, such as D6234, D8006, 'Caledonia', Pioneer Brand '25W60' and Roane for 8-11 times of evaluation. Therefore, stable and reliable results of scab assessment could be obtained by single-floret inoculation in the greenhouse. Greenhouse based evaluation is practical for screening of scab resistance on a large scale in breeding program. Inclusion of resistant and susceptible checks and stable environmental conditions are necessary and critical.

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**Table 1.** Scab resistance of wheat germplasm introduced from China

| Entry | Origin | No. Scabby spikelets | | Resistance | 1999/2000 | |
|---|---|---|---|---|---|---|
| | | Average | Range | level | No of SS | R. Level |
| W14 | Nanjing Agricultural University | 0.7 | 0.5~0.8 | HR | 0.8 | HR |
| TFSL037 | NAU | 1.2 | 0.8~1.5 | HR | 2.7 | R |
| CJ 9807 | NAU | 1.4 | 1.1~1.8 | R | | |
| CJ 9602 | NAU | 1.7 | 1.6~1.8 | R | | |
| CJ 9403 | NAU | 1.3 | 0.7~2.8 | R | | |
| CJ 8809 | NAU | 1.2 | 0.7~1.6 | HR | 3.7 | MR |
| CJ 9306 | NAU | 0.6 | 0.5~0.8 | HR | 0.8 | HR |
| CJ 9311 | NAU | 0.7 | 0.5~1.1 | HR | 0.8 | HR |
| CJ 9815 | NAU | 1.5 | 0.6~3.7 | R | 3.1 | MR |
| CJ 9811 | NAU | 1.8 | 1.0~3.1 | R | | |
| CJ 9819 | NAU | 2.7 | 1.2~4.7 | MR | | |
| CJ 9804 | NAU | 3.2 | 1.2~7.3 | MR | 4.6 | MS |
| Nantai 7 | NAU and Nanping Agr Institute | 3.7 | 2.8~4.4 | MS | | |
| SH 19089 | Shanghai Academy of Agr Sci | 1.1 | 0.8~1.4 | HR | | |
| Lunhui 116 | SAAS | 3.2 | * | MR | | |
| Lunhui 201 | SAAS | 9.3 | 7.3~1.2 | S | | |
| Emai 9 | Hubei Academy of Agr Sci | 1.8 | 1.1~2.6 | R | | |
| E 61506 | HAAS | 2.7 | 1.9~3.9 | MR | | |
| Yang 158 | Lixiahe Agricultural Institute | 1.4 | 1.3~1.5 | R | | |
| 93-111 | NAU and Lixiahe Agr Institute | 1.4 | 0.9~1.8 | R | | |
| Ningzi 28 | Jiangsu Academy of Agr Sci | 1.1 | 1.0~1.2 | HR | | |
| Wan 8704 | Anhui Academy od Agr Sci | 1.3 | 0.6~2.1 | R | | |
| T 531 | South China Agr University | 2.0 | 1.3~3.0 | R | | |
| Sumai 3 | Suzhou Agricultural Institute | 0.9 | 0.5~1.5 | HR | | |
| Ning 7840 | Jiangsu Academy of Agr Sci | 1.6 | 0.8~2.3 | R | 2.0 | R |
| Shaan 85-2 | Northwest Agr University | 1.2 | 1.0~1.4 | HR | | |
| WZHHS | Land race, Zhejiang Province | 1.6 | 1.5~2.0 | R | | |
| FSXM | Land race, Fujian Province | 1.0 | * | HR | | |
| NK | Japan | 1.8 | 1.1~2.4 | R | | |
| Veery | CIMMYT, Mexico | 11.2 | 9.8~14.0 | S | | |
| Norm | U.S.A. | 10.4 | 8.6~13.0 | S | 10.4 | S |

* Only one replication.

**Table 2.** Reaction of some American wheat varieties and lines to Scab

| Entry | No. scabby spikelets | | Percentage of | Resistance | 1999/2000 | |
|---|---|---|---|---|---|---|
| | Average | Range | scabby spikelets | level | No of SS | R. Level |
| E0029 | 5.1 | 5~5.2 | 33.1 | MS | | |
| E0039 | 6.3 | 4.1~9.4 | 37.6 | MS | | |
| Patton | 5.8 | 4.5~7.4 | 32.2 | MS | 5.8 | MS |
| TW97613 | 3.8 | 1.9~5.4 | 21.3 | MR | | |
| Freedom | 6.0 | 3.1~12.4 | 36.3 | MS | 9.5 | S |
| Roane | 7.1 | 3.3~10.8 | 42.2 | MS | 3.7 | MR |
| Valor | 6.3 | 5.0~9.5 | 36.1 | MS | | |
| VA96W-250 | 7.2 | 5.0~9.9 | 36.4 | MS | 5.7 | MS |
| MO980525 | 3.5 | 2.0~5.9 | 19.8 | MR | 2.8 | R |
| IL95-4162 | 5.7 | 3.6~7.1 | 41.5 | MS | 5.0 | MS |
| M97-1171 | 6.9 | 3.4~9.1 | 41.6 | MS | | |
| Ernie | 5.0 | 5.0~5.0 | 40.1 | MS | 1.8 | R |
| MO981020 | 3.1 | 2.3~4.3 | 19.7 | MR | | |
| MO980429 | 6.4 | 5.2~8.3 | 52.5 | MS/S | | |
| IL96-6472 | 6.0 | 3.7~10.4 | 47.6 | MS/S | | |
| 25R18 | 2.0 | 1.8~2.2 | 14.3 | R | | |
| NY87048W-7388 | 5.8 | 3.2~10.2 | 38.7 | MS | 3.7 | MR |
| 9793A1-5 | 6.5 | 3.7~9.4 | 42.1 | MS | | |
| 97397B1-4-5 | 4.8 | 3.7~5.9 | 32.0 | MS | | |
| 97463A1-17-1 | 5.1 | 3.9~6.3 | 32.5 | MS | | |

# BREEDING FOR RESISTANCE TO FUSARIUM HEAD BLIGHT IN SOFT RED WINTER WHEAT

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## OBJECTIVES

1) To identify resistance to Fusarium head blight in field and greenhouse screens.
2) To evaluate the role of morphological traits in Type I resistance.

## INTRODUCTION

Fusarium head blight (FHB) has caused significant losses in Kentucky's wheat crop in most years since 1991. The prevalent rotation in which growers are planting wheat after corn into minimally or no-tilled soil ensures abundant inoculum in most years. Therefore, breeding for FHB resistance is an essential component of the wheat breeding project at the University of Kentucky.

## MATERIALS AND METHODS

Two inoculated field nurseries were established in 2001. At Lexington, entries in the 2001 Uniform North and South Scab Screening Nurseries, the state variety trial, and a number of advanced breeding lines were planted in a randomized complete block design with four replications on 17 October 2000. Each plot consisted of two rows planted on 7-inch centers with approximately 20 inches of space on either side of the rows. This method was adopted from the CIMMYT scab screening protocols. At Princeton, KY, our second nursery, consisting of a single replication of the state variety trial and several breeding line trials was planted on 25 October 2000. The previous crop at both locations was corn (Zea mays L.) and the seedbed had been chisel plowed and disked. Entries in the greenhouse were planted on 17 November 2000 in a completely randomized design with a variable number of replications.

**Field Inoculation** - *F. graminearum* colonized field corn was spread in wheat plots prior to heading (GS 7) on April 10. To keep the grain inoculum hydrated, plots were directly mist irrigated for approximately 15 minutes and then 3 times daily for a week. Abundant perithecia were first observed on the corn on April 26. Once the wheat started to head, the irrigation system was set on the disease development schedule. Beginning on April 30, plots were mist irrigated for 5 minutes with 15-minute intervals between the hours of 6 to 10 AM and 10 minutes with 20-minute intervals between the hours of 8 and 10 PM. Heading and anthesis notes were taken daily. Those plots at 50% anthesis were sprayed with a macroconidial suspension (110,000 sp/ml). Macroconidial inoculum was prepared from previously frozen stocks used during the current spring greenhouse screening cycle.

Disease evaluations were initiated on May 31, when scab symptoms were detected on several of the susceptible cultivars, approximately 3 weeks post anthesis of the earliest maturing lines. Those lines that flowered first were read first. Disease incidence was calculated by counting the number of infected heads per plot divided by the total number of heads per plot. This was accomplished by using a fixed rectangular measuring tool made from PVC pipe. Once the sample area was defined, the total number of heads was counted within the box. Counts were taken from ten random plots to get an average number of heads per plot. Likewise, the measuring tool was used to define the sampling area for counting the number of diseased heads per plot. Average head severity was assessed by evaluating 25 infected heads per plot. This was determined by counting the number of infected spikelets divided by the total number of spikelets per head.

**Greenhouse Inoculation** - As reported in Van Sanford et al. (1999).

**Greenhouse Evaluation of Type I resistance** - Fifty individuals in four $F_2$ populations were characterized for flowering type (open vs. closed) in the field, 2000. Seeds from each head ($F_{2:3}$) were planted in the greenhouse on 12 Nov. 2000. A macroconidial suspension of *F. graminearum* (400,000 sp/ml) was sprayed on the flowering wheat head at 50 % anthesis. Spray volume was 100ml. Heads were misted with water prior to spraying. The spore suspension was sprayed once on both sides of heads. Plants were kept in the humidity chamber for three consecutive nights. Disease severity was recorded as number of infected spikelets per head at 8 and 21 days after spraying. The percentage of infection is the ratio of number of infected spikelets over total number of spikelets per head.

## RESULTS AND DISCUSSION

**Field Nurseries** - Our goal in 2001 was to create a severe FHB epidemic in at least one of our inoculated nurseries. In the nursery at Lexington, we were successful in reaching this goal. Infection levels in 2001 were much greater than in previous years due to optimal timing of inoculum (Table 1). The nursery at Princeton was a partial success, but infection levels were reduced due to a later than optimal application of the scabby corn in the field. One promising outcome is the strong performance of a Kentucky breeding line, which is under increase for possible release. KY 90C-054-6 showed low severity of infection in two inoculated field nurseries and in the greenhouse in response to Type II injection screening.

**Greenhouse Evaluation of Type I resistance -** In conclusion of this study, there was one population which showed significantly different type I resistance according to flower type and awn type. The close flower type had significantly less infection than open flower type (14 vs. 22 %), the awned type has significantly less infection than the awnless type (13 vs 21 %). Lines from this population are being developed and will be evaluated further in the field and greenhouse.

Additional data and scab screening protocols can be found at: http://www.uky.edu/Ag/Wheat/wheat_breeding/scabpage.html

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**Table 1.** FHB symptoms in entries from the 2001 Kentucky variety trial at Lexington, Princeton, and in the Greenhouse.

| | LEXINGTON | | | PRINCETON | | | GH |
|---|---|---|---|---|---|---|---|
| Entry | % Disease Incidence | Mean Severity | FHB Index | % Disease Incidence | Mean Severity | FHB Index | Mean Severity |
| CLARK | 47.86 | 42.96 | 20.56 | 30.36 | 11.48 | 3.48 | 92.0 |
| PATTERSON | 34.19 | 30.79 | 10.53 | 5.36 | 7.77 | 0.42 | 55.0 |
| MADISON | 90.60 | 51.58 | 46.73 | 17.86 | 10.80 | 1.93 | 53.5 |
| ROANE | 95.73 | 21.78 | 20.84 | 60.71 | 32.42 | 19.69 | 28.1 |
| KAS INDEPENDENCE | 74.36 | 19.46 | 14.47 | 44.64 | 15.54 | 6.94 | 30.2 |
| KAS REVERE | 44.44 | 14.68 | 6.53 | 8.93 | 6.96 | 0.62 | 12.7 |
| Hopewell | 38.46 | 24.65 | 9.48 | 48.21 | 35.42 | 17.08 | 7.4 |
| Exsegen Esther | 97.44 | 43.62 | 42.50 | 26.79 | 9.10 | 2.44 | 43.5 |
| Exsegen Rebekah | 88.03 | 32.76 | 28.84 | 42.86 | 15.92 | 6.82 | 61.1 |
| Exsegen Sarah | 28.21 | 16.80 | 4.74 | 28.57 | 15.35 | 4.39 | 4.6 |
| SS 522 | 100.00 | 70.25 | 70.25 | 25.00 | 16.62 | 4.16 | 32.9 |
| SS 566 | 27.35 | 32.16 | 8.80 | 26.79 | 21.12 | 5.66 | 100.0 |
| SS 555 | 69.23 | 47.77 | 33.07 | 17.86 | 10.64 | 1.90 | 81.1 |
| SS 558 | 90.60 | 28.54 | 25.85 | 58.93 | 12.44 | 7.33 | 67.5 |
| SS535 - Raxil | 97.44 | 34.28 | 33.40 | 25.00 | 7.59 | 1.90 | 81.5 |
| SS535- Gaucho | 92.31 | 39.01 | 36.01 | 33.93 | 10.28 | 3.49 | 90.8 |
| Stine 422 | 86.32 | 29.91 | 25.82 | 16.07 | 12.44 | 2.00 | 25.5 |
| Stine 454 | 33.33 | 30.32 | 10.11 | 42.86 | 25.05 | 10.74 | 54.3 |
| AGRIPRO FOSTER | 33.33 | 20.79 | 6.93 | 21.43 | 20.39 | 4.37 | 30.8 |
| AGRIPRO PATTON | 89.74 | 16.58 | 14.88 | 21.43 | 12.49 | 2.68 | 27.7 |
| AGRIPRO GIBSON | 95.73 | 31.35 | 30.01 | 75.00 | 18.44 | 13.83 | 25.7 |
| M95-2883 | 88.89 | 39.56 | 35.16 | 51.79 | 21.84 | 11.31 | 26.6 |
| NK COKER 9663 | 28.21 | 22.23 | 6.27 | 50.00 | 27.62 | 13.81 | 74.2 |
| NK COKER 9474 | 93.16 | 26.27 | 24.47 | 28.57 | 17.57 | 5.02 | 9.2 |
| NK BL930390 | 100.00 | 41.80 | 41.80 | 37.50 | 15.96 | 5.98 | 72.9 |
| NK BL940582 | 90.60 | 37.09 | 33.60 | 44.64 | 31.12 | 13.89 | 72.4 |
| NK BL940812 | 100.00 | 51.64 | 51.64 | 16.07 | 15.34 | 2.47 | 100.0 |
| Croplan Genetics SR218 | 92.31 | 29.01 | 26.78 | 64.29 | 20.80 | 13.37 | 60.9 |
| Croplan Genetics SR204 | 88.03 | 20.73 | 18.25 | 71.43 | 20.36 | 14.54 | 55.6 |
| BECK 101 | 86.32 | 32.70 | 28.23 | 126.79 | 71.83 | 91.07 | 72.9 |
| BECK 104 ( EX 6820) | 56.41 | 25.06 | 14.13 | 76.79 | 28.91 | 22.20 | 84.7 |
| USG 3209 | 88.89 | 41.54 | 36.92 | 80.36 | 30.52 | 24.53 | 54.7 |
| VA96W-270 | 97.44 | 52.84 | 51.49 | 62.50 | 50.13 | 31.33 | 83.8 |
| SISSON | 100.00 | 81.33 | 81.33 | 42.86 | 36.22 | 15.52 | 81.4 |
| 25R18 | 36.75 | 12.10 | 4.45 | . | . | . | 6.6 |
| 2568 | 100.00 | 51.83 | 51.83 | . | . | . | 43.0 |
| 25R37 | 90.60 | 29.89 | 27.08 | 44.64 | 17.50 | 7.81 | 13.4 |
| 25R44 | 99.15 | 35.57 | 35.27 | 26.79 | 13.62 | 3.65 | 25.2 |
| 25R49 | 90.60 | 60.30 | 54.63 | 57.14 | 19.61 | 11.20 | 100.0 |
| XW692 | 96.58 | 30.91 | 29.85 | 80.36 | 22.26 | 17.89 | 46.2 |
| 25W60 | 98.29 | 46.17 | 45.38 | 50.00 | 17.86 | 8.93 | 40.4 |
| 25W33 | 88.89 | 38.18 | 33.94 | 60.71 | 18.45 | 11.20 | 29.3 |
| Cropland Genetics SR211 | 100.00 | 45.37 | 45.37 | 41.07 | 19.91 | 8.18 | 61.3 |
| KY 90C-054-6 | 58.97 | 16.76 | 9.88 | 10.71 | 7.61 | 0.82 | 5.4 |
| Ernie | 94.02 | 21.69 | 20.39 | 32.14 | 16.33 | 5.25 | 18.2 |
| Ernie | 97.44 | 30.33 | 29.55 | 39.29 | 13.10 | 5.15 | . |
| Ernie | 81.20 | 17.41 | 14.14 | 32.14 | 10.28 | 3.30 | . |
| Ernie | 97.44 | 32.79 | 31.95 | 41.07 | 15.13 | 6.21 | . |
| 2555 | 97.44 | 70.41 | 68.60 | 78.57 | 30.60 | 24.04 | . |
| 2555 | 98.29 | 60.68 | 59.65 | 73.21 | 36.51 | 26.73 | . |
| 2555 | 93.16 | 44.26 | 41.24 | 100.00 | 22.22 | 22.22 | . |
| 2555 | 97.44 | 58.62 | 57.12 | 92.86 | 31.84 | 29.57 | . |
| | | | | | | | |
| **Mean** | 80.41 | 36.25 | 30.98 | 45.86 | 20.59 | 11.58 | 49.9 |
| **LSD $_{(0.05)}$** | 19.49 | 31.39 | 34.01 | 32.05 | 15.74 | 8.29 | 38.8 |
| **C.V.** | 6.95 | 25.75 | 32.24 | 20.63 | 21.86 | 21.40 | 59.4 |

# RESULTS IN BREEDING FOR RESISTANCE AGAINST FUSARIUM HEAD BLIGHT (FHB) IN WHEAT

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## OBJECTIVES

To show the novel results of breeding for FHB resistance in Szeged and the usefulness of AUDPC as way of evaluation.

## INTRODUCTION

The *Fusarium* susceptibility of most European cultivars is the basic cause of the irregularly occurring severe epidemics. Beside yield and quality loss the toxin contamination is the major threat. Besides clinical consequences their insidious effect at very low concentration may cause immunology problems also for man as shown for DON inhibiting the activity of human T cells at 50 ppb up to 80 % Berek et al. (2000).

Knowledge on FHB resistance and genetics increased significantly (Gilbert and Tekauz 1999, Mesterházy 1997, 2001, Miedaner 1996, Parry et al. 1995) and significant information was published about the breeding experiences in China and the USA (National FHBI Forums, 1998, 1999, 2000). As in our winter wheat gene pool the resistance level is not high, we started with the crosses of the well-known Sumey-3 and the seldom-used Nobeoka Bozu. Their $F_1$ plants were crossed with another adapted cultivars or the two single crosses were crossed again as it happened with Sgv-Nobeoka Bozu/Mini Manó-Sumey-3. By this way adapted lines with high resistance were achieved. The resistance of the best lines was equal or sometimes better than that of Nobeoka Bozu or Sumey-3. Then we started the crosses with adapted materials, now to breed commercial cultivars. The lines of the first crosses are also maintained up to now and many of them are in the regular testing program as control lines. Of course, resistance to other diseases, yielding ability and quality played an important role in the choice of parents.

## MATERIALS AND METHODS

The isolates used, increase of inoculum, inoculation technique, its evaluation and deoxynivalenol analysis are described by Mesterházy et al. (1999). Every entry was sown on a 5 $m^2$ plot, within this 4 isolates (two *F. graminearum* and two *F. culmorum* was used in three replicates. One replicate was represented by a group of heads consisting of 15-20 heads. Every entry was inoculated once at flowering by spraying the heads from every side. Until 2000 only 24 hrs wet period, in 2001 we changed it for 48 hrs. Disease evaluation was made 10, 14, 18, and 22 and 26 days after inoculation, in the cooler 2001 season a $6^{th}$ reading was also made. After harvest from each group 10 heads were randomly separated, threshed by low wind, weighted and the ratio of FDK kernels was estimated as a percentage. DON was measured from the mean grain sample of the three replicates.

Recently the use of AUDPC became very popular. It seems that to be scientific, it should be used. As we have back for 20 years 5, sometimes 6 readings we calculated AUDPC and the long used arithmetical means to compare the two ways of evaluation. As we have data not only of FHB values, but also for yield loss, FDK and deoxynivalenol contamination, the possibility was used to evaluate the data for 1998-2001. FHB values are given as a mean of five readings, and also AUDPC was calculated. We did not use the integral function, but it was assumed that disease development between readings is linear, therefore the area between the two points can be calculated simply according to the function $10\ x_1/2+4*(x_1+x_2)/2+ 4*(x_2+x_3)/2+4*(x_3+x_4)/2+4*(x_4+x_5)/2$, where $x_{1-5}$ are the percentages of FHB infected spikelets within the group of heads inoculated, 10 and 4 are the number of days between ratings.

Resistance evaluation to other diseases was also made. In the tables all data represent the mean performance to four isolates.

## RESULTS AND DISCUSSION

Table 1 presents the results of those entries that were evaluated also for DON. Also the resistance sources are listed to see the development of the breeding work. Between the best lines no significant difference exists, all traits tend to have very low values. The check cultivars, however, have very high infection severity, FDK value and DON contamination. It seems that high resistance helps to prevent toxin contamination, even at isolates with high aggressiveness. The closer correlation of DON with FDK shows that for toxin production the amount of kernel infection seems to be more important than the FHB value. The AUDPC and mean FHB values correlated very closely, r = 0.9752. From this number it is clear that they seem to say the same thing. When we see their relations with FDK ratio, yield or DON production, the numbers are nearly the same. From the 1999 and 2000 results only the correlations are shown (Table 2). They show no significant difference between relations of means or AUDPCs with other traits like FDK, DON response or yield loss.

In 2001 two populations were tested, 143 cultivars and breeding lines and 45 from the breeding material. The correlations are between AUDPC and arithmetical means r = 0.9973 and 0,9997, e. g. they describe the same phenomenon. From the 2001 tests a selection is given (Table 3) where the most resistant genotypes (resistance sources, advanced lines (with pedigree) and cultivars are listed with three highly susceptible entries.

In the Szeged breeding program highly resistant winter wheat lines were bred whose resistance exceeds most of the resistance sources and equal with Sumey 3. In many cases the resistance to other diseases is also good or excellent. The resistance to FHB seems to be durable as the resistance is the same for a number of *Fusarium* species like *F. graminearum, F. culmorum, F. avenaceum, F. sporotrichioides, F. nivale, F. poae, F. moniliforme.* There are no vertical races within the fungal population. Resistance to invasion cannot be detected, as there is no invasion or only in traces. Resistance to toxin contamination falls apart as not infected grains do not contain toxins or only in traces. This means that the highly resistant materials automatically consider and solve all problems of the resistance components meaning that there is no need to breed separately for them. However, the significance of these and other components is in less resistant, moderately susceptible or susceptible genotypes considerable.

The four years data clearly show that AUDPC is not better than the mean of the readings. No difference does exist between them in value. I have the impression that AUDPC seems to be more elegant, but less informative than the simple mean values. The mean value has the advantage that the reader understands better the means than the more complicated AUDPC. I could summarize shortly: the understandable is beautiful.

In the Szeged breeding program a number of highly resistant lines were produced, some of them with good or excellent resistance to powdery mildew, leaf rust, yellow rust and stem rust. New data were gained for the identity of FHB resistance to a number of. The FHB resistance is durable. By comparing AUDPC and mean data of rating no difference was found, but the means of the infection data are more informative. So AUDPC does not give new information to the mean infection values.

**Table 1.** Resistance and toxin accumulation of wheat genotypes (n=54), 1998.(only the most resistant ones with controls are shown)

| Plot No | Genotípus | Traits | | | |
|---|---|---|---|---|---|
| | | FHB % | AUDPC | Ker.inf.% | DON ppm |
| 397 | Sum3-81.60/Kõ | 0.33 | 6.46 | 0.33 | 0.05 |
| 173 | Sgv-NB*MM-Sum3 | 0.96 | 13.33 | 0.42 | 0.79 |
| 157 | Sgv-NB*MM-Sum3 | 1.00 | 15.00 | 0.75 | 1.17 |
| 169 | Sgv-NB*MM-Sum3 | 1.25 | 90.42 | 6.88 | 0.94 |
| 162 | Sgv-NB*MM-Sum3 | 1.50 | 22.50 | 1.75 | 0.74 |
| 187 | **Sumey3** | 1.50 | 25.83 | 0.10 | 0.05 |
| 139 | RStxMM-NB | 2.26 | 32.25 | 6.00 | 0.57 |
| 136 | RSt*MM-NB | 2.42 | 38.33 | 1.42 | 0.72 |
| 175 | Sgv-NB*MM-Sum3 | 2.63 | 33.75 | 3.75 | 1.54 |
| 160 | Sgv-NB*MM-Sum3 | 2.97 | 45.79 | 2.42 | 0.84 |
| 156 | Sgv-NB*MM-Sum3 | 4.30 | 67.75 | 10.33 | 2.43 |
| 179 | Kõ-Krp/Sgv-mm.. | 5.13 | 78.75 | 2.25 | 3.16 |
| 185 | **Wuhan-2** | 5.96 | 104.79 | 4.50 | 2.15 |
| 113 | B 1201 | 7.63 | 125.00 | 58.33 | 11.97 |
| 144 | Sum3-81.60*Kõ | 7.92 | 133.33 | 1.67 | 1.25 |
| 190 | **Nobeoka Bozu** | 8.29 | 138.96 | 0.83 | 0.05 |
| 145 | Sum3-81.60/Kõ | 8.50 | 146.04 | 1.33 | 0.69 |
| 124 | RStxMM-NB | 8.88 | 149.38 | 0.75 | 1.67 |
| 143 | Sum3-81.60*Kõ | 10.08 | 172.92 | 2.08 | 2.39 |
| 141 | RStxMM-NB | 10.13 | 149.58 | 20.85 | 2.35 |
| 135 | RStxMM-NB | 12.38 | 183.13 | 7.50 | 3.86 |
| | ..... | | | | |
| 84 | Garaboly | 61.71 | 1122.29 | 67.50 | 13.24 |
| 88 | Mérõ | 61.88 | 1175.00 | 45.00 | 15.56 |
| 204 | Kalász | 73.96 | 1335.42 | 87.08 | 24.72 |
| | Mean | 5.14 | 300.42 | 5.66 | 1.57 |
| | LSD 5 % | 2.8 | 31.13 | 7.37 | 3.38 |

Correlations

| Traits | FHB % | AUDPC | Ker.inf.% |
|---|---|---|---|
| AUDPC | 0.9752*** | 1 | |
| Ker.inf.% | **0.8238*** ** | **0.8182*** ** | 1 |
| DON ppm | **0.8180*** ** | **0.8185*** ** | 0.9206*** |

***P = 0.1 %

**Table 2.** Correlations for the 1999 and 2000 FHB resistance tests.

| 1999 n = 72 | | | | |
|---|---|---|---|---|
| Traits | FHB % | AUDPC | FDK % | Yield loss % |
| AUDPC | 0.9962*** | | | |
| FDK % | **0.7069*** ** | **0.7005*** ** | | |
| Yield loss % | **0.8467*** ** | **0.8381*** ** | 0.6952*** | |
| DON ppm | **0.7339*** ** | **0.7208*** ** | 0.8926*** | 0.71666*** |
| 2000 n = 56 | | | | |
| AUDPC | 0.9991*** | | | |
| FDK | **0.7302*** ** | **0.7252*** ** | | |
| Yield loss | **0.5159*** ** | **0.5127*** ** | 0.5976*** | |
| DON | **0.5233*** ** | **0.5228*** ** | 0.6341*** | 0.5255*** |

*** P = 0.1 %

**Table 3.** Resistance to FHB and leaf diseases of genotypes of FHB program, 2001

| Plot | Genotype | FHB | | E. graminis | P. triticina | | P. striiformis |
|---|---|---|---|---|---|---|---|
| No. | | AUDPC | Mean % | 29 May | 14 June | 22 June | 14 June |
| 149 | Sumey-3 | 0.0 | 0.0 | S60,7 | S30 | n | MRt |
| 194 | Sum3/81.60//kő | 0.0 | 0.0 | MS5,5-7 | MS10 | MS50 | MS5 |
| **243** | **Sgv/NB//MM/Sum3** | **0.0** | **0.0** | **MS5,5** | **MS5** | **MS5** | **MRt** |
| 161 | Nobeoka Bozu, NB | 1.3 | 0.1 | S10,3 | MS10 | MS30 | MS30 |
| 145 | Wuhan2 | 3.0 | 0.2 | S40,5-7 | MS30 | S40 | MS20 |
| 237 | FHB R | 9.0 | 0.6 | MS30,7 | 0 | 0 | MRt |
| **159** | **Sum3/81.61//Kő** | **12.3** | **0.6** | **MRt,3** | **S5** | **S5** | **MRt** |
| 147 | Wuhan 6B | 10.0 | 0.8 | S70,7 | S30 | S50 | MS10 |
| 192 | Wuhan42B | 37.7 | 2.1 | S60,7 | S10 | n | S70 |
| **242** | **Sgv/NB//MM/Sum3** | **65.0** | **3.4** | **MSt,5** | **MS20** | **MS20** | **MRt** |
| **151** | **SgvNB/MMSum3** | **72.7** | **3.7** | **MRt,3** | **MSt** | **MS5** | **MS5** |
| **30** | **81.60//NB/Kő** | **51.4** | **4.5** | **MSt,3** | **0** | **MR5** | **MR10** |
| 97 | Véka | 59.7 | 4.8 | S30,5 | S30 | S40 | MS30 |
| **208** | **FHB143** | **95.0** | **5.8** | **MS5,5** | **0** | **0** | **MR10** |
| **153** | **SgvNB/MMSum3** | **99.8** | **6.0** | **0** | **MR5** | **MS10** | **0** |
| 29 | 81.61//RSt/NB | 29.4 | 6.0 | MSt,5 | 0 | MS5 | MS5 |
| **107** | **Várkony** | **64.3** | **9.9** | **MSt,3** | **MS5?** | **MS20** | **MS5** |
| 209 | Bence | 192.2 | 10.6 | MS10,5 | S10 | S30 | MR5 |
| **121** | **Kapos** | **78.5** | **12.3** | **MS5,3** | **MR5** | **MR5** | **MRt** |
| **26** | **85.92-Zu** | **95.1** | **13.9** | **0** | **MRt** | **MRt** | **MRt** |
| 173 | Frontana | 243.0 | 14.3 | MS10,5 | MS30 | MS40 | MR10 |
| **100** | **Csalogány** | **153.1** | **17.3** | **0** | **MSr** | **MS10** | **MR5** |
| **199** | **Zu/RSt** | **317.5** | **18.4** | **MRt,4** | **MSt** | **MR5** | **MRt** |
| | .... | | | | | | |
| 218 | P8635 | 1064.1 | 52.6 | MS5,3 | MS10 | MS40 | MSt |
| 142 | Selyem dur | 970.8 | 60.7 | MSt,5 | S60 | n | MS20 |

n = the leaves died by leaf rust infection, bold printed: good or excellent overall resistance

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# STEM RUST RESISTANCE IN SPRING WHEAT GERMPLASM RESISTANT TO FUSARIUM HEAD BLIGHT

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## ABSTRACT

Since 1993, Fusarium head blight (FHB), caused mainly by *Fusarium graminearum*, has caused serious loss of yield and quality in the spring wheat region of the USA. The only real solution to FHB appears to be to breed resistant wheats. Fortunately, sources of resistance to FHB are available but some sources of good resistance to FHB are in wheats susceptible to wheat stem rust (WSR), caused by *Puccinia graminis*, a disease threat potentially as serious as FHB. The widespread use, in several major spring wheat breeding programs, of germplasm resistant to FHB but highly susceptible to WSR raises the risk that a WSR susceptible wheat might become widely planted, invoking the specter of a future major rust epidemic.

To gauge this risk, we tested 14 FHB resistance source lines for reaction to 14 pathotypes of WSR including past and present prevalent pathotypes and several potentially threatening ones. This SR test was done after previous results showed that one source line “Sumai 3” was highly susceptible to many races of SR. Seedlings of each breeding line were tested for WSR reaction using standard methods of inoculation and scoring for infection type (IT). Resistant checks and a universal susceptible “Little Club” were always included. Most of the FHB source lines were of an intermediate type — susceptible to some WSR cultures and resistant to others. Four lines were resistant to all 14 WSR cultures in the test; three of these lines were from China (W9207, Ning 7840, and Busch CG-29) and one was from Brazil (BR19). The strain of Sumai 3 that has been widely used for FHB resistance breeding in the spring wheat region was susceptible to 13 of the WSR cultures and showed a mixture of resistant and susceptible reactions to the fourteenth WSR culture. Interestingly, an authentic strain of this line from another source was susceptible to only 8 WSR pathotypes. Wheat breeders need to be aware that lines derived from FHB resistant parent sources should be thoroughly screened for WSR reaction prior to release.

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# TRANSFERRING FHB RESISTANCE TO SOUTHERN SOFT RED WINTER WHEAT

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## OBJECTIVES

1) To transfer genes for Fusarium head blight (FHB) resistance to soft red winter wheat adapted to the Midsouth and 2) to develop a recurrent selection program designed to obtain higher levels of FHB resistance and to combine FHB resistance with resistance to other diseases.

## INTRODUCTION

Resistant cultivars are likely to be an important component of any integrated management program for FHB. FHB resistance generally has been found in genotypes unadapted to the Midsouth and has been based on additive interactions among minor genes that are quantitatively inherited (Singh and van Ginkel 1996). The breeding strategies commonly used for developing adapted cultivars with high levels of resistance have been designed to accumulate minor genes from diverse sources into suitable backgrounds. In the spring wheat region of the United States, a recurrent selection and intercrossing program has been used to make step-wise progress toward developing resistant cultivars (Rudd 1996). In the region of China that is prone to severe FHB epidemics, Yao et al. (2000) concluded that combining resistance genes via recurrent selection programs was necessary to develop high-yielding, resistant cultivars, and Jiang et al (1994) advocated using genetic male sterility to expedite recurrent selection that was done at multiple locations each year. Singh and van Ginkel (1996) recommended 1000 to 2000 hybrid plants per topcross population in order to identify adapted lines with four or five minor genes. For programs unable to handle such large populations, they proposed breeding in two steps: first, parent building; and second, transferring resistance to adapted wheats.

## MATERIALS AND METHODS

Agripro Mason and Pioneer variety 2684 were selected as adapted parents because of their photoperiod sensitivity (confers wide adaptation across the Midsouth) and two-week vernalization requirement (facilitates growing plants in the greenhouse). Sources of resistance available in 1996 (mostly from CIMMYT) were crossed to each adapted parent, and some backcrosses (with permission) or topcrosses were made to increase the probability of recovering lines with acceptable quality. Selections for FHB resistance were made in inoculated, irrigated field plots at Fayetteville and Kibler, AR, and at Baton Rouge, LA and in the greenhouse at Fayetteville. Lines also were evaluated for resistance to other diseases in field plots. Selection criteria for each generation are summarized in Table 1. Lines were derived from headrows selected in 1999 and 2001.

## RESULTS AND DISCUSSION

Eighty-four lines from 16 different sources of FHB resistance (Table 2) have been selected during the 2001 season. These $F_7$, $BCF_6$, and $TCF_6$ lines are currently planted in the field in Arkansas and Louisiana and in the greenhouse at Fayetteville for further evaluations to select the best lines for release to breeders as adapted sources of FHB resistance. All lines have acceptable plant type, winter hardiness (survived record cold November and December temperatures at three locations), maturity, yield potential, and visual grain quality for the Midsouth. In addition to FHB resistance, all lines are resistant to contemporary races of the leaf rust, stripe rust, and leaf blotch pathogens. Based on a sample of 20 lines that had enough seed for preliminary quality evaluations, 13 were soft, four were hard, and three were intermediate. Lines within the soft and hard groups had acceptable quality for their market class.

To form the foundation for a recurrent selection program to combine resistance genes and obtain lines with higher levels of FHB resistance, the 84 selected lines are being used as males in a crossing block with a heterogeneous adapted population of male-sterile plants with the Ms 3 gene that was developed by Steve Harrison. Additional lines with different sources of FHB, leaf rust, stripe rust, leaf blotch, Stagonospora (glume) blotch, and barley yellow dwarf resistances are being developed in Agripro Mason and Pioneer variety 2684 backgrounds, thereby providing the potential to facilitate combining FHB resistance with resistance to other diseases. Resistance to Stagonospora blotch may be especially important in the Midsouth because conditions that favor FHB epidemics also favor Stagonospora blotch.

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**Table 1.** Selection history of the lines developed for *Fusarium* head blight resistance.

| | Year and Generations | | | | | |
|---|---|---|---|---|---|---|
| Selection criteria | 1997 F2, BCF1, TCF1 | 1998 F3, BCF2, TCF2 | 1999 F4, BCF3, TCF3 | 2000 F5, BCF4, TCF4 | 2001 F6, BCF5, TCF5 | 2002 F7, BCF6, TCF6 |
| Heading date | x | x | x | x | x | P |
| Plant type | x | x | x | x | x | P |
| Yield potential | x | x | x | x | x | P |
| Visual grain quality | x | x | x | x | x | P |
| Adaptation to eastern Arkansas | | | | | x | P |
| Winter hardiness | | | | | x | |
| Milling and baking quality | | | | | x* | P |
| Fusarium head blight: Field | | | x | - | x | P |
| Fusarium head blight: Greenhouse | | | | | x | P |
| Leaf rust | | x | x | - | x | P |
| Stripe rust | | | | x | x | P |
| Septoria leaf blotch | | x | x | - | x | P |
| Spindle streak mosaic | | | | | | P |
| Spindle streak + soilborne mosaic | | | | | | P |

x = Meaningful selections made
- = No meaningful selections made
P = Planned for coming year
* = only for lines with enough seed

**Table 2.** Parentage of F7, BCF6, and TCF6 lines developed for *Fusarium* head blight resistance.

| Parentage | No. of lines |
|---|---|
| Mason/Catbird (G49) | 1 |
| Mason/Catbird (G52) | 2 |
| Mason/Catbird (G90) | 1 |
| Mason*2/Catbird(G90) | 7 |
| Mason/Catbird (G93) | 4 |
| Mason/Catbird (G95) | 3 |
| Mason/Catbird (G98) | 2 |
| P2684//Mason/Catbird(G52) | 1 |
| P2684//Mason/Catbird(G95) | 2 |
| P2684//Mason/Catbird(G98) | 1 |
| Freedom/Catbird (G82) | 4 |
| Freedom/Catbird (G97Lr resistant) | 2 |
| Mason/3/Freedom//NG8675/Catbird | 6 |
| P2684*2//NG8675/Catbird | 2 |
| Mason//Sha 3/Catbird | 2 |
| P2684/3/Mason//Sha 3/Catbird | 1 |
| Mason/Freedom | 2 |
| Mason//Freedom/Super Zlatna | 5 |
| Mason*2//Sha3/Super Kauz | 5 |
| Mason/Er-Mai 9 | 3 |
| P2684/Er-Mai 9 | 2 |
| Mason/Yu-Mai 7 | 2 |
| Mason/3/Chil//Ald/Pvn | 3 |
| P2684/3/Mason//Chil/Chum18 | 1 |
| Mason//Chum 18/Seri | 3 |
| Mason*2//Alucan/YMI 6 | 3 |
| Mason//Clark*4/N7840 | 3 |
| P2684/3/Mason//Clark*4/N7840 | 1 |
| Mason/3/Freedom//Clark*4/N7840 | 3 |
| Clark*4/N7840/5/Gov/Az//Mus/3/Dodo/4/Bow | 2 |
| P2684/3/N7840//Parula/Veery#6 | 3 |
| N895004-1/P2684 | 2 |
| **Total** | **84** |

# RESISTANCE BREEDING OF FUSARIUM HEAD BLIGHT IN WINTER WHEAT BY INTRODUCING RESISTANCE FROM SPRING WHEAT

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## OBJECTIVES

The aim of our study is the improvement of FHB resistance and winter tolerance in winter wheat. We conducted screening of these resistances, and evaluated their genetic modes using two winter/spring-crossed populations.

## INTRODUCTION

Fusarium head blight caused by *Fusarium* spp. is a devastating disease in Japan. In 1996, nearly 30% of Japanese wheat field were damaged by FHB. Especially in Hokkaido, in where they produce over 60% amount of domestic wheat, more than 40% of wheat fields infected FHB and yield was reduced by 20%. As the resistance of FHB is quantitative traits and the assessing of resistance is variable by the environmental conditions, the breeding of FHB resistant variety is still a challenging objective. The narrow genetic diversity for FHB resistance also makes it difficult to improve the resistance in winter wheat. Therefore, we have to introduce the resistance to winter wheat from spring wheat such as Sumai 3. Nevertheless these difficulties, the improvement of FHB resistance in winter wheat in Japan is strongly encouraged for increasing of yield with winter tolerance and controlling harmful mycotoxin contamination.

In this study, we conducted screening of the winter wheat lines with FHB resistance and winter tolerance, and also analyzed the segregation of them to evaluate the number of resistance genes using two winter/spring crossed populations.

## MATELIALS AND METHODS

***Plant materials for assessment of reaction to FHB*** - Two populations of 250 and 240 $F_6$-derived recombinant inbred lines (RILs) developed from crosses Hokushin (major winter wheat variety in Hokkaido, very susceptible to FHB)/ Norin-PL4 (developed from the cross Nobeokabouzu-komugi/ Sumai 3, moderate resistant) and Norin-PL33 (Sumai 3/ Asakaze-komugi, moderately resistant), and other 70 breeding lines including standard varieties were evaluated for their reaction to inoculation of FHB in the field and greenhouse.

***Inoculation methods of FHB and disease assessment*** - A strain of *Fusarium graminearum* 'S1' (kindly provided by Tokachi Agr. Exp. Sta., Japan) was used to assess the reaction of plants material to FHB. The isolates were cultured for 7days on a PDA medium (Wako Co. Ltd., Japan) and conidia were prepared in Mung Beans Medium (20g mung

beans, 1l water) through suspension culture. The concentration of conidia was adjusted to $5x10^5$/ml as inoculum. The inoculation was conducted in the field and the greenhouse equipped with a sprinkler system in 2001. The plant materials were provided 72cm row and 1m length with two replications. From the heading of early cultivar, simulated rainfall was provided for 60 seconds every 5 minutes in a sunny day or every 10 minutes in a cloudy day to keep the spikes wet until maturity. At the anthesis day of each line, 10 ul of the inoculum was injected into a single spikelet at the center of spike. Disease severity was scored with the average of 10 spikes based on the total area of lesions per inoculated spike [disease severity index (DSI), 0-9] about 14 days and 21 days after inoculation, when typical lesions appeared on the spike of susceptible plants.

Mean values of FHB severity were analyzed using ANOVA and LSD test. The FHB severity of each RIL were classified into three groups of reactions: resistant(R; DSI= 0-5), moderately resistant (MR; DSI= 6-7) and susceptible (S; DSI= 8-9).

***Evaluation of winter tolerance***- The winter tolerance of each RIL was assessed at the regrowth stage in spring by the snow mold disease severity. (0 to 5; 0 = no damage (Hokushin), 1 = less than half of leaves were dead, 2 = half of the stems or more were dead, 3 = less than half of the stems were dead, 4 = half of the stems or more were dead, 5 = totally dead (Norin-PL4 and Norin-PL33)).

## RESULTS AND DISCUSSION

The correlation coefficient for FHB severity of each line scored in the field and greenhouse was highly significant, indicating that the condition of inoculation was relatively relevant and reliable (Figure 1, Table1 and 2). A frequent spraying for every 5 or 10 minutes was supposed to be effective to compare the resistance level of FHB for variable heading date. The reactions of four varieties improved in Hokkaido were stable both in the field and greenhouse then they were selected as FHB resistance standard varieties in Hokkaido. Horoshiri-komugi and Takune-komugi were resistant (R), Chihoku-komugi was moderately resistant (MR), and Hokushin that has been cultivated over 90% fields in Hokkaido was susceptible (S). The scores of FHB severity among four varieties were compared using the LSD test (Table 3). The results indicated that the reactions classified into three groups were distinguishable.

In the RILs developed from the crosses Hokushin/ Norin-PL-4 and Hokushin/ Norin-PL-33, heading dates ranged similarly from 5 to 14 June, from 4 to 14 June respectively. The frequency distribution of FHB severities was continuous but transgressive segregation of resistant lines was observed in both populations (Figure 2). That indicated the number of resistance genes was assumed to be not so many. The segregation of FHB severity of two RILs combinations was classified into as R, MR, and S as subscribed and tested for fitting the tri-modal distribution. The segregation ratio of the reaction to FHB in the population Hokushin/ Norin-PL-4 was 47R: 105M: 89S. Chi-square tests indicated it was not fitted ($P<0.05$) a two genes model for 1R: 2M: 1S ratio. Observed data of the segregation ratio in another RIL population, Hokushin/ Norin-PL-33, could be explained by two different models, 12R: 208 (M+S) or 102 (R+M): 118S. Chi-square tests indicated that they were fitted 2 susceptible gene model 1R: 7(M+S) ($P=0.62$) or 1 resistance gene model 1(R+M): 1S ($P=0.28$).

From these results, Norin-PL4 might have at least one major gene and possible to have one more resistance gene derived from Sumai 3 and Nobeokabouzu-komugi which are the famous FHB resistance sources said to possess two major dominant resistance genes respectively. Norin-PL33 would have one moderate resistance gene derived from Sumai 3 but from Asakaze-komugi which expressed weak or no additive effect on FHB resistance (Ban and Suenaga, 2000). Meanwhile, Hokushin would have one or two susceptible genes supposed to distort the FHB severity distribution to susceptible.

The distribution of snow resistance as winter tolerance of the two population were varied from very susceptible (Norin-PL4, Norin PL33) to very resistant (Hokushin) as a normal distribution, and the correlation coefficient between the snow resistance and FHB resistance was very low (r=0.085). It is indicated there is high possibility to breed high FHB resistant variety with snow tolerant. As these results, we are starting to screen FHB resistance among the breeding lines with winter tolerance.

***Prospects of FHB resistant breeding in winter wheat*** - FHB resistance is caused many resistance genes working for complex interaction between plants and pathogens(Pritsch *et al*., 2000), and the resistance is classified into five types. In these types, resistance to invasion (type I) and resistance to elongation (type II) seemed to be important. And it is suggested the numbers of resistance genes are not so many in other and this studies (Snijders, 1990; Ban, 2000). To identify the gene for resistance, the expression of some pathogenesis-related proteins (PR1-5) are going to be cleared and the key of resistance might be how fast of the resistance related genes expression (Pritsch *et al*., 2000). Toward for the accumulation of resistance genes, more improvement for the reliable resistance assessment is indispensable and the identification of the resistance gene function and mapping for the marker selection will be important for the acceleration of the resistance breeding. The introducing of modified resistance gene by genetically engineering to winter wheat without crossing with spring wheat is also one possible strategy for the worldwide menace of FHB.

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**Table 1.** Correlation coefficient of the FHB severities in the field and greenhouse.

| | G.H.14d | G.H.21d | Field 14d | Field 21d |
|---|---|---|---|---|
| G.H.14d | - | | | |
| G.H.21d | 0.85** | - | | |
| Field 14d | 0.85** | 0.75** | - | |
| Field 21d | 0.84** | 0.80** | 0.91** | - |

*G.H.=greenhouse d= days after inoculation
**P<0.01

**Table 2.** Analysis of varianxe for FHB severities of the 70 varieties and breeding lines.

| Source | d.f. | Mean square | F-value |
|---|---|---|---|
| Cultivars | 69 | 0.341 | 33.69** |
| Environment | 1 | 0.104 | 10.29** |
| Error | 140 | 0.01 | |

**P<0.01

**Table 3.** FHB severities and range for 4 standard cultivars tested in the field. And greenhouse.

| Cultivar | Level of resistance | Days to heading | FHB severity | Range | LSD | Year of registration |
|---|---|---|---|---|---|---|
| Horoshiri-komugi | R | 269 | 3.0 | 2-4 | a | 1974 |
| Takune-komugi | R | 264 | 4.0 | 3-5 | a | 1974 |
| Chihoku-komugi | M | 270 | 5.5 | 4-6 | b | 1981 |
| Hokushin | S | 267 | 9.0 | 8-10 | c | 1994 |

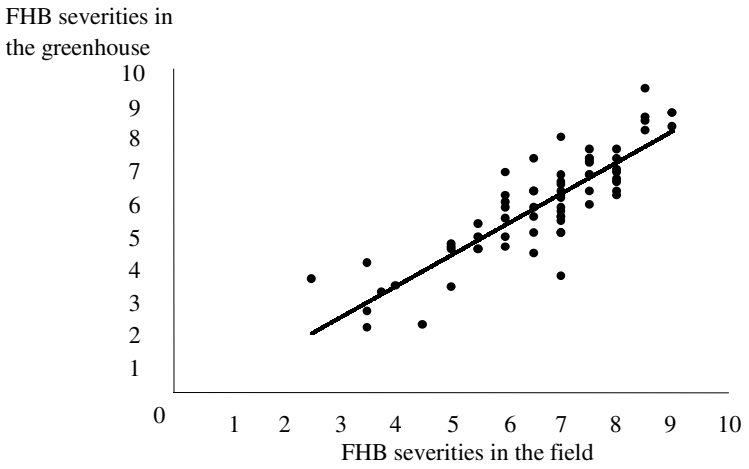


**Figure 1**. Scatter plot of the FHB severities among 70 varieties and breeding lines evaluated in the field and greenhouse.

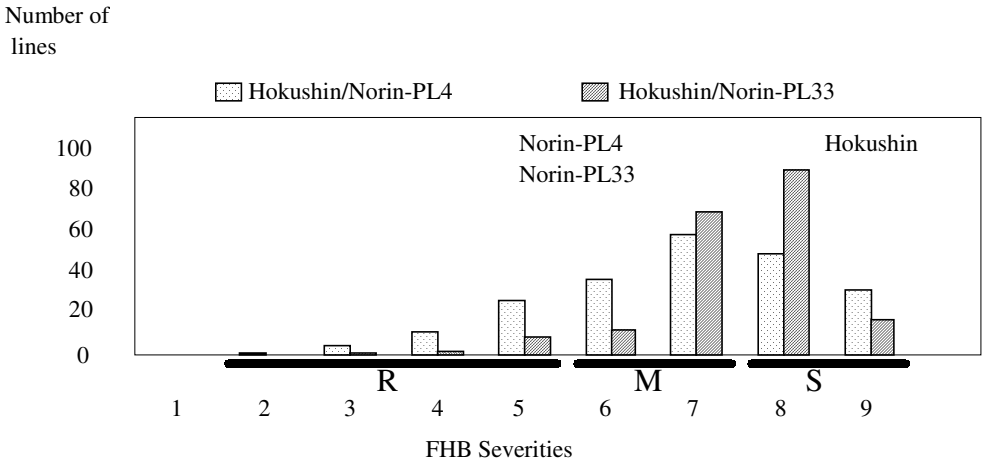


**Figure2.** Frequency distribution of FHB severity and reaction to FHB in recombinant inbred lines (RIL) developed from Hokushin/Norin-PL4 and Hokushin/Norin PL-33.

# VARIETY DEVELOPMENT AND UNIFORM NURSERIES: WINTER WHEAT RESEARCH PROGRESS

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Acknowledgments: This report is a synthesis of research progress provided by researchers of all cooperating winter wheat breeding programs of the U.S. Wheat & Barley Scab Initiative (USWBSI).

Current cultivars throughout the winter wheat growing regions have been characterized for Fusarium head blight disease severity in various tests. Thus, growers can avoid cultivars on which the disease generally develops more severely than on others, taking into account other factors of agronomic performance and resistance to other important diseases and pests. Losses to Fusarium head blight will be reduced as current cultivars that have resistance become more widely grown and as new cultivars that have more effective resistance are developed. In the hard winter wheat region, the cultivars Hondo, Heyne, and the newly released cultivar, Lakin, have been identified as having resistance to Fusarium head blight compared to other current cultivars grown in Kansas. Previously, the soft wheat public cultivars Freedom and Ernie, private cultivars 25R18 and Patton and the licensed cultivar INW9824 were released as having Fusarium head blight resistance, and the public cultivar Goldfield and licensed cultivar INW9853 were released as having reduced incidence of the disease compared to other varieties.

Significant progress for the objective of breeding for winter wheat lines that have resistance to Fusarium head blight has been made, as evidenced by performance of entries in the 2001 Southern and Northern Uniform Winter Wheat *Fusarium* Nurseries. Unreleased entries in these nurseries generally are advanced lines from various breeding programs, some lines of which are or soon will be on seed increase for release (for example, MO980525). It is apparent that several entries from various breeding programs reflect significant breeding progress for Fusarium head blight resistance compared to current cultivars as checks. Several entries in the Northern nursery developed disease indexes ranging from 7 to 18%, compared to 40% for susceptible checks. It is clear that as certain of these Fusarium head blight resistant lines are commercialized in the next one to several years they will significantly reduce production and grain utilization losses due to this disease.

Until now, the most commonly used sources of resistance have been various Chinese lines. However, as other unrelated sources of resistance (and hopefully different resistance genes) are identified, wheat breeders are focusing on combining different sources of resistance, both for presumably more durable resistance and for higher expression of resistance.

Given that winter wheat requires a cool period to complete its life cycle, limiting the number of generations per year, remarkable progress has been made in the various winter wheat breeding programs since the beginning of the USWBSI. Essentially all winter wheat breeding programs have developed efficient protocols for testing and selection for *Fusarium*

resistance in breeding nursery populations; these include point inoculation under controlled conditions, spray inoculation, combinations of misting systems, scattering Fusarium-colonized maize seed as a source of inoculum, and seeding nurseries after corn with minimum tillage. Researchers are utilizing various approaches to minimize the time for incorporation of Fusarium resistance into new adapted winter wheat lines: production of doubled haploids (IL, OH, VA), 'winter' nurseries to reduce generation time (IN, OH, MD), utilizing transgenic sources of resistance (NE). Most breeding programs are maximizing use of greenhouse testing and generation advance as well as bulk breeding and backcross breeding methods to accelerate the breeding and selection process. There is ongoing extensive reciprocal exchange of *Fusarium* resistant germplasm and partially improved lines among breeding programs to maximize breeding progress for improved resistant cultivars. Cultivars that were developed with FHB resistance as the primary focus, but that also are competitive for productivity and resistance to the many other important diseases and pests of wheat in the great plains and eastern U.S., are already beginning to be released as a result of funding by the USWBSI.

# DEVELOPMENT AND CHARACTERIZATION OF WHEAT LINES NEAR ISOGENIC FOR A FUSARIUM HEAD BLIGHT QTL

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## ABSTRACT

We are investigating the usefulness of a molecular marker-based approach to complement the selection of lines with resistance to FHB. Results of two quantitative trait locus (QTL) analysis experiments in two spring wheat populations indicate a major QTL (*Qfhs.ndsu-3BS*) for FHB resistance is located on chromosome 3BS of Sumai 3 (Anderson et al., Theor. Appl. Genet. 102:1164-1168, 2001). The consistent ability to detect this major QTL and the magnitude of effect in each population imply that it should be useful for marker-assisted selection (MAS). However, to justify breeding program-scale MAS for the 3BS QTL region, increased levels of resistance due to this QTL should be observed in multiple genetic backgrounds.

Multiple SSR markers flanking the 3BS QTL region and strongly associated with FHB resistance in both QTL studies were selected to develop QTL near-isogenic lines (NILs) in adapted genetic backgrounds. A total of 10 families were chosen based on desirable marker alleles, with one parent in each family having Sumai 3-derived resistance. In the summer of 2000, $F_{3:4}$ lines from each family were genotyped with two co-dominant markers to identify heterogeneous $F_4$ lines. Three plants were harvested from lines segregating for both markers, and the progeny of each was genotyped as a bulk to identify single $F_4$ heterozygous plants. Using the same markers, homozygous types ($F_{4:5}$ sib lines) with alternate marker alleles at the 3BS QTL were identified. Selfing of heterozygous lines and subsequent marker analysis to identify homozygous types has produced 27 QTL-NILs from the 10 families at various inbreeding generations ($F_4$, $F_5$, and $F_6$ derived homozygous types). Five out of twelve NIL pairs tested in our 2001 field inoculated nursery showed significant ($P<0.05$) reduction in FHB severity in the presence of the resistance QTL. The remaining seven NIL pairs did not show a significant difference in FHB severity. Data from fall 2001 greenhouse point inoculation screens will also be presented. $F_6$ or $F_7$ derived NILs showing significant differences in FHB severity will be used to create fine mapping population(s) to further define this QTL region.

# QTLS OF FHB RESISTANCE IN WHEAT LINE NING 894037

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## ABSTRACT

Fusarium head blight (FHB) is a destructive disease in wheat production worldwide. Diseased heads have reduced kernel number and test weight, which result in the yield loss. Diseased kernels are also contaminated with mycotoxins such as deoxynivalenol, which lowers its commercial use and results in large price discounts. Breeding resistant wheat cultivars is an effective way to control the disease. However, the quantitative expression of resistance and scarcity of resistant germplasm have hampered breeding progress. More FHB resistance genes need to be discovered and characterized for being incorporated into adapted cultivars.

Here we characterized a resistant wheat line Ning 894037, derived from tissue culture of the cultivar Yangmai 3. There is no Sumai 3 in its pedigree. It was thought that the resistance came from somaclonal variation.

A recombinant inbred line (RIL) population derived from the cross Ning 894037/Alondra was used in this study. Each of the 218 lines, together with the two parents, was tested for FHB resistance in F8, F9, F10 and F11 generations. Three tests were conducted in greenhouse conditions and one was done in the field at West Lafayette, IN. The distribution of the disease severity is normal with two major peaks, indicating that there is a major gene responsible for the resistance. Broad sense heritability is 0.65. With the strategy of bulked segregant analysis, 250 SSR markers were assayed and 58 showed polymorphism between the two parents. Six of them are detected polymorphism between the bulks. Analysis on the whole population showed that there is a gene with large effect in Ning 894037. The marker Xgwm533 and Xgwm493, which are 14 cM apart on chromosome 3B, each explain 29.1% and 27.1% phenotypic variation, respectively. A couple of other SSR markers associated with FHB resistance are also identified. Xgwm644 and Xgwm518, 11 cM apart in chromosome 6B, each explain 4.7% and 4.6% phenotypic variation, respectively. Xgwm566 on chromosome 3B explains 2% phenotypic variation. Xgwm261 on chromosome 2D showed a QTL contributed by the susceptible parent Alondra. This marker explains 5.5% phenotypic variation. Multiple regressions of the 4 markers in different chromosomes totally explain 40% of the phenotypic variation. The resistance alleles of these 4 loci decrease disease severity at the amount of 19%, 11%, 6%, 6%, respectively.

# MANAGEMENT OF FUSARIUM HEAD BLIGHT IN NORTHERN NEW SOUTH WALES OF AUSTRALIA

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## ABSTRACT

Serious outbreaks of Fusarium head blight (FHB) occurred in wheat crops on the Liverpool Plains in northern New South Wales (NSW) of Australia in 1999 and 2000. Yield losses ranged from between 20-100% with associated major downgrading in quality and was most severe in durum wheat crops sown into maize residues. Head blight also occurred in a localised area around Cowra in southern NSW in 2000. *Fusarium graminearum* was the main species associated with head blight in northern NSW while in southern NSW infection appears to have been caused by the splash of macroconidia of *F. pseudograminearum* into heads during rainfall events. Climatic conditions were a major factor resulting in these epidemics with unusually prolonged wet weather occurring over the flowering period in 1999 and 2000. In northern NSW, FHB generally occurs at low levels in durum wheat crops in most seasons. A low incidence of infection (<1% of heads) was evident in many durum crops in the 2001 season that had little rainfall during flowering.

A disease nursery was established at Tamworth in 2001 using screenhouses with overhead mist irrigation to provide high humidity conducive to disease development. Macroconidial spore suspensions were prepared by harvesting sporodochia produced on sterile durum wheat seeds placed on water agar plates following 7-days incubation at 20°C with a 12 hour U.V. light/12 hour darkness cycle. Macroconidia were suspended in sterile distilled water with the spore suspension being an equal mixture of four isolates of *F. graminearum* to provide a final concentration of around 50,000 conidia/mL. Approximately 20 mL per row (150 cm length) was sprayed onto heads at anthesis with a second inoculation one week later. After inoculation, all rows were mist-irrigated for 2 min. every 30 min. for a 12-hour period during the night. The incidence of infected heads and percent-infected spikelets were counted 14 and 21 days after the first inoculation. Grain yield, percentage infected grains and 1000 grain weight were determined after harvest. Twenty bread wheat, 9 barley and 3 durum wheat varieties commercially grown in northern NSW were assessed for resistance to FHB in the disease nursery in 2001. In a separate experiment, chemical and biological control of FHB were investigated. Two fungicide (tebuconazole or carbendazim) and 4 *Bacillus* spp. treatments were evaluated for control of FHB in two bread wheat (cvv. Sunvale and Kennedy) and two durum wheat (cvv. Yallaroi and Kamillaroi) varieties. Treatments were applied 4 hours prior to spray inoculation with macroconidia of *F. graminearum* at anthesis and one week later. Preliminary results from these experiments will be discussed. Future research will also focus on incorporating FHB resistance into Australian durum varieties. Initial crosses with Sumai 3 have been made and will be evaluated for resistance to FHB in 2002. Funding for this research is being provided by the Grains Research and Development Corporation and through the provision of an Agriculture Fisheries and Forestry Australia research award.

# VARIETY DEVELOPMENT AND UNIFORM NURSERIES: FHB RESISTANCE IN BARLEY

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## ABSTRACT

Acknowledgments: This report is an overview of research progress made by researchers from cooperating barley breeding programs in the upper Midwest working on FHB resistance.

All of the barley varieties currently grown in the Midwest are susceptible to FHB. Barley grain surveys indicate that in the years 1993 - 2000 between 16% and 42% of the harvested crop in the upper Midwest was acceptable on the basis of having no detectable levels (< 0.5 ppm) of deoxynivalenol (DON), the toxin that is associated with Fusarium head blight (FHB). Since 1993, the four major barley breeding programs in this region have been actively working to identify FHB resistant sources and introgress resistance genes into germplasm adapted to the upper Midwest. These programs have investigated approaches to breeding for resistance, screened and utilized different sources of resistance, and worked to understand the genetics of FHB resistance.

A cooperative regional FHB nursery (MinnDak) has been in place since 1995. This nursery includes six-rowed and two-rowed resistant and susceptible checks and entries from each barley breeding program. There are seven sites in Minnesota and North Dakota consisting of four misted and inoculated nurseries and three dryland nurseries where natural infection occurs. Currently, the entries in MinnDak are promising breeding lines, but initially the nursery was used to screen new and often “exotic” sources of resistance. The data from the past few years of the MinnDak nursery indicate that slowly progress is being made and in the next few years we expect to see variety candidates with improved FHB resistance entering industry malting evaluation trials.

Most sources of resistance to FHB in barley are two-rowed presenting a significant problem for the six-rowed breeding programs. Segregation and mapping studies indicate that several QTL for FHB resistance are located on chromosome two and are linked to the locus that determines two rowed or six-rowed spike type. In addition, there are several other genes affecting morphological traits such that several recombination events in this region of chromosome 2 will be necessary to break up the undesirable linkages and recover acceptable two-rowed or six-rowed resistant lines.

Studies evaluating early generation selection for FHB resistance conclude minimal gains can be made by visual selection based on individual F2 plants or selection of bulked F2 seed from misted and inoculated nurseries. However, marker assisted selection (MAS) among F2 plants for resistant alleles at FHB QTL does show some promise, particularly if undesirable linkages between resistance genes and genes for other traits can broken. Early

generation selection of bulked populations for low DON is underway and will be evaluated in 2002. Many different sources of resistance are being used in breeding programs and in mapping studies. Analysis using simple sequence repeat data suggests that these sources are reasonably diverse and will hopefully provide different FHB resistance genes.

# BREEDING FOR FHB RESISTANCE AT THE OHIO STATE UNIVERSITY

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OSU has an ongoing program to improve FHB resistance in soft new winter wheat germplasm adapted to Ohio. The program routinely screens breeding lines for FHB resistance. The sources of resistance in these lines are not always known. Many are likely to derive resistance from Freedom and some Virginia material. We screened 103 such lines for reaction in 2001 (Figure 1). Over 40% of the lines had an FHB index that was less than that observed for Freedom, with 14.5% with index values less than 10%. Of the 42 lines with good FHB resistance, 11 were advanced to 2001-02 trials based on FHB resistance and other traits.

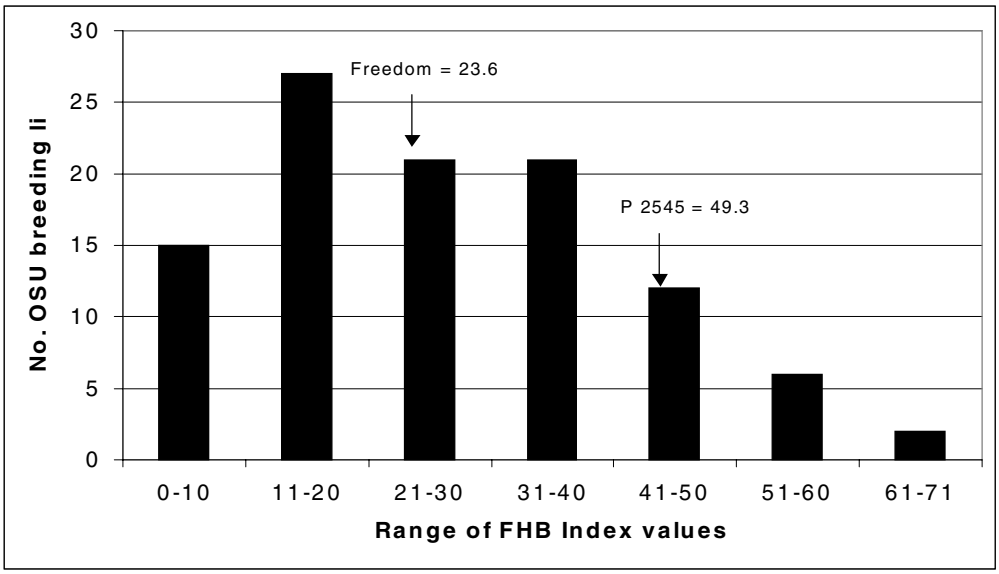


**Figure 1.** Distribution of disease index values for 103 Ohio State University breeding lines screened for resistance to FHB in 2001

The OSU program is incorporating multiple sources of resistance in our early generation populations. The sources include: Ning 7840, Sumai 3, Nobeaka Bosus, Frontana (from FHB161, 143, 147, 148), Szeged, Zombar, Ringo Starr, Saguari, and Sagu.

Through multiple years of selection, we have developed a set of lines with high levels of resistance to FHB and moderate to good resistance to Stagonospora leaf and glume blotch (SLGB). The lines were derived from crosses of 3-way or 4-way crosses and are at most 25% exotic. The FHB resistance sources were Ning 7840, Ning 8331, Sumai 3, Freedom, or ZM10782. The SLGB resistance sources were Ohio breeding material. Forty-four of these lines (Table 1) are entered in 2001-02 yield trials and their SGLB reaction will also be evaluated in 2002. The best of these lines will be used as parents this winter.

**Table 1.** Summary of data on 44 Ohio State University breeding lines selected for resistance to FHB and Stagonospora leaf and glume blotch (SLB, SGB).

| | | | FHB | | | Kernel | Percent | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Heading | Severity | Incidence | Index | rating | scabby | SLB | SGB |
| PEDIGREE | OH Code | date | % | % | % | % | Seed | (0-10) | (%) |
| NING7840/FREEDOM//OH528/VA91-54-219 | OH917 | 141 | 4.4 | 55.0 | 3.3 | 3.7 | 2.0 | 9.0 | 20.0 |
| NING7840/FREEDOM//OH528/VA91-54-219 | OH920 | 143 | 5.0 | 63.3 | 3.7 | 3.0 | 1.0 | 9.0 | 20.0 |
| NING7840/FREEDOM//OH528/VA91-54-219 | OH921 | 143 | 4.7 | 66.7 | 3.8 | 3.7 | 1.2 | 9.0 | 30.0 |
| NING7840/FREEDOM//OH528/VA91-54-219 | OH931 | 141 | 8.7 | 63.3 | 7.0 | 3.7 | 1.4 | 9.0 | 30.0 |
| NING7840/FREEDOM//OH528/VA91-54-219 | OH932 | 143 | 9.0 | 60.0 | 7.0 | 3.7 | 0.9 | 9.0 | 18.0 |
| NING7840/GLORY//OH526 | OH903 | 145 | 0.3 | 13.3 | 0.1 | 6.7 | 0.9 | 8.0 | 17.0 |
| NING7840/GLORY//OH526 | OH905 | 145 | 1.2 | 20.0 | 0.5 | 2.3 | 1.4 | 9.0 | 33.0 |
| NING7840/GLORY//OH526 | OH906 | 145 | 1.2 | 25.0 | 0.5 | 5.3 | 1.5 | 8.0 | 22.0 |
| NING7840/GLORY//OH526 | OH907 | 145 | 1.3 | 35.0 | 0.6 | 1.7 | 2.0 | 8.0 | 23.0 |
| NING7840/GLORY//OH526 | OH908 | 146 | 1.8 | 38.3 | 1.1 | 2.3 | 2.6 | 9.0 | 19.0 |
| NING7840/GLORY//OH526 | OH911 | 147 | 2.6 | 40.0 | 1.7 | 4.3 | 2.5 | 8.0 | 25.0 |
| NING7840/GLORY//OH526 | OH901 | 144 | 5.1 | 51.7 | 2.4 | 5.7 | 1.0 | 10.0 | 30.0 |
| NING7840/GLORY//OH526 | OH913 | 146 | 4.4 | 41.7 | 2.4 | 1.7 | 10.1 | 8.0 | 23.0 |
| NING7840/GLORY//OH526 | OH916 | 145 | 4.4 | 50.0 | 2.9 | 9.0 | 2.2 | 8.0 | 18.0 |
| NING7840/GLORY//OH526 | OH919 | 147 | 4.7 | 51.7 | 3.6 | 1.3 | 2.2 | 8.0 | 22.0 |
| NING7840/GLORY//OH526 | OH923 | 145 | 6.1 | 73.3 | 4.6 | 3.7 | 1.5 | 7.0 | 11.0 |
| NING7840/GLORY//OH526 | OH924 | 147 | 8.0 | 63.3 | 5.0 | 7.7 | 0.8 | 9.0 | 12.0 |
| NING7840/GLORY//OH526 | OH928 | 146 | 9.7 | 70.0 | 6.4 | 16.7 | 3.7 | 8.0 | 27.0 |
| NING7840/GLORY//OH526 | OH936 | 144 | 11.0 | 75.0 | 8.9 | 10.3 | 1.7 | 9.0 | 25.0 |
| NING7840/GLORY//OH526 | OH937 | 145 | 10.7 | 81.7 | 9.0 | 12.0 | 1.2 | 9.0 | 27.0 |
| NING7840/GLORY//OH526 | OH941 | 144 | 13.8 | 61.7 | 13.3 | 5.3 | 1.0 | 8.0 | 13.0 |
| NING8331/FREEDOM//OH519/10584-08-1 | OH910 | 144 | 2.8 | 51.7 | 1.4 | 9.3 | 1.9 | 7.0 | 22.0 |
| NING8331/FREEDOM//OH519/10584-08-1 | OH915 | 144 | 3.6 | 55.0 | 2.6 | 8.3 | 3.0 | 8.0 | 16.0 |
| NING8331/FREEDOM//OH519/10584-08-1 | OH926 | 143 | 7.4 | 71.7 | 5.5 | 11.0 | 2.6 | 8.0 | 18.0 |
| NING8331/FREEDOM//OH519/10584-08-1 | OH927 | 144 | 7.1 | 76.7 | 5.8 | 13.3 | 2.4 | 9.0 | 30.0 |
| NING8331/FREEDOM//OH519/10584-08-1 | OH943 | 145 | 17.6 | 81.7 | 15.0 | 16.7 | 3.8 | 8.0 | 20.0 |
| SUMAI 3/OH542//OH528/MO9965-52 | OH934 | 143 | 8.9 | 60.0 | 8.1 | 10.3 | 2.0 | 9.0 | 23.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH902 | 145 | 0.1 | 1.7 | 0.0 | 2.0 | 2.8 | 8.0 | 20.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH904 | 147 | 1.3 | 21.7 | 0.2 | 2.0 | 1.6 | 8.0 | 20.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH909 | 144 | 2.8 | 48.3 | 1.3 | 6.0 | 2.4 | 9.0 | 25.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH912 | 145 | 3.2 | 40.0 | 1.9 | 5.7 | 4.7 | 7.0 | 7.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH914 | 146 | 4.4 | 55.0 | 2.5 | 5.0 | 2.3 | 9.0 | 25.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH918 | 145 | 5.1 | 51.7 | 3.5 | 3.3 | 2.5 | 9.0 | 24.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH922 | 145 | 6.4 | 53.3 | 4.6 | 12.7 | 3.3 | 8.0 | 28.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH925 | 146 | 7.1 | 66.7 | 5.1 | 6.0 | 3.5 | 7.0 | 18.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH929 | 144 | 8.0 | 73.3 | 6.4 | 7.7 | 2.2 | 8.0 | 27.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH930 | 146 | 8.2 | 55.0 | 6.7 | 21.7 | 2.6 | 8.0 | 25.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH933 | 146 | 9.0 | 61.7 | 7.6 | 10.7 | 2.9 | 7.0 | 18.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH935 | 144 | 10.8 | 68.3 | 8.6 | 3.3 | 4.5 | 9.0 | 25.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH938 | 144 | 10.9 | 81.7 | 9.2 | 21.7 | 4.6 | 8.0 | 20.0 |
| ZM10782/FREEDOM//30584-37-2/VA91-54-219 | OH939 | 144 | 14.1 | 76.7 | 12.5 | 5.3 | 3.8 | 8.0 | 23.0 |
| Resistant Check | Freedom | 146 | 23 | 88 | 21 | 58 | 4 | | |
| Susceptible Check | P 2545 | 145 | 50 | 98 | 49 | 88 | 11 | | |
| Susceptible Check | GR863 | | | | | | | 10.0 | 57.0 |

# SUMMARY REPORT ON THE 2001 NORTHERN UNIFORM WINTER WHEAT SCAB NURSERY (NUWWSN)

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## INTRODUCTION

This report is a compilation and analysis of data from the cooperative assessment of resistance to Fusarium head blight (scab) in winter wheat adapted to the northern regions of the US and funded by the USWBSI. This report contains preliminary data that has not been confirmed and thus is not suitable for general release to the public. Interpretation of the presented results may be modified with additional research. Confirmed results should be published through established channels. This report is to be used as a tool for the cooperators in the NUWWSN, their staff, and persons having direct interest in the development of wheat germplasm and agricultural research programs. This report and data is not intended for unrestricted publication or distribution and should not be used in or referred to in publicity or advertising. Use of this data may be granted for certain purposes upon written request to the agency or agencies involved.

The report can be accessed in its entirety on the USWBSI and OSU wheat breeding web sites. As such, this report is a brief summary of main findings and not a complete report on methods, location, cooperators, and results. These facets are reported in the final report.

## METHODS

The 2001 test consisted of 49 entries that were evaluated in eight field locations and three greenhouse tests (Table 1). The traits measured were heading date (HD), disease incidence (IND), severity (SEV), index (IND), kernel rating (KR), percent scabby seed (%SS), and vomitoxin concentration (DON) (Table 1).

Most cooperators sent entry means that were then were used to calculate entry means over tests. ANOVAs were conducted for each trait and the entry x test mean square was used as the error term to calculate a LSD for entry means over tests. $R^2$ values in the tables indicate the proportion of total sum of squares accounted for by entry and test effects while $1-R^2$ is the proportion of total sum of squares due to the entry x test interaction (ETI) effect.

Based on $1-R^2$, ETI appeared quite large for DON, IND and SEV from the field trials, so multivariate statistics (Yan et al., 2000 Crop Science 40:597-605) were used to analyze ETI and group those tests that produced similar results for DON, IND, and SEV. Entry means were then calculated over the tests that produced similar rankings (Tables 2, 3). A group of tests that produced similar rankings and results was called a megaenvironment.

## RESULTS

Entry was a significant source of variance for all traits. There was little ETI for HD, IND, SEV from greenhouse tests, KR, and %SS. Thus, entry means over all tests are appropriate estimators of genetic value for these traits (Table 1). ETI seemed to be an important source of variation of SEV from field trials, IND, and DON.

ETI accounted for 46% of the treatment sum of squares for field SEV. Analyses indicated that most of the ETI among the eight tests was due to differences between three groups of tests, called megaenvironments: (AR+IL+KY+MO+VA) versus (IN+OH+ONT) versus MI (Table 4). Correlations among entry means from tests within the same megaenvironment were mostly greater than 0.5. The correlations between entry means from different megaenvironments were less than 0.36, with the lowest correlation between the MI and AR+IL+KY+MO+VA groups (r = 0.02). The ETI would appear to have little effect on selection. Assuming selection of the best (or worst) six entries, five entries would be selected in all megaenvironments (Fig. 1). MO981020 would be selected for resistance in two megaenvironments, but not in MI. Two lines (IL97-1828 and Harding) would be selected in only one megaenvironment each (Fig. 1). Five entries (OH684, OH669, Patterson, P 2545, and MDV71-19) would be selected as susceptible in all three megaenvironments (Fig. 1).

The ETI pattern for IND was strongly associated with the ETI pattern for SEV. This is logical as IND is a function of SEV and INC and there was little ETI for INC. ETI accounted for 53% of the treatment sum of squares for disease IND. The tests were placed in three megaenvironments: (IL+KY+MO+VA) versus (KS+OH+ONT) versus MI (Table 5). Tests that were in the same megaenvironment for SEV were in the same megaenvironment for IND and the MI site was an outlier again. Correlations among entry means from tests within the same megaenvironment were mostly greater than 0.55. The correlation between entry means from the IL+KY+MO+VA and KS+OH+ONT megaenvironments was high (r=0.78), indicating that these two produce similar results (KY could really have been put in either set). The lowest correlation was between the MI and KS+OH+ONT group (r = 0.04).

The ETI had a greater affect on selection for index than for severity. Assuming selection of the six most resistant (or susceptible) entries, only one entry (MO980525) would be selected for resistance using data from each of the three megaenvironments (Fig. 2). The lack of concordance between selections in the megaenvironments arises primarily from the results from MI as five entries would be selected as resistance in both KS+OH+ONT or IL+KY+MO+VA. Two entries (MDV71-19, OH669) would be considered susceptible using data from any megaenvironment (Figure 2). One entry (97463A1-17-1) would be selected for resistance using IL+KY+MO+VA data, but would be considered susceptible using MI data.

ETI accounted for 35% of the treatment sum of squares for DON. The VA and OH locations gave similar results (r = 0.60 between them) while the AR site gave different rankings from the other two sites (r = 0.38 between AR and other two sites). Only one genotype ranked 5$^{th}$ or lower in AR was similarly ranked in VA or OH. P 2545 was ranked last (most DON) in OH but ranked 1$^{st}$ (lowest DON) in AR.

Correlations were calculated among entry means for all traits including disease severity in the greenhouse. HD was not highly correlated to any other trait, but was moderately correlated to DON (r = 0.42). There was a high correlation among head traits (incidence, severity, index) from the field (r = 0.74 to 0.96). These traits were moderately correlated to severity from the greenhouse (r = 0.43 to 0.59). Kernel traits (kernel rating, % scabby seed, DON) were highly correlated to one another (r = 0.70 to 0.79). Kernel rating and % scabby seed were highly correlated to the field head traits (r = 0.65 to 0.75), while DON was only moderately correlated to the field head traits (r = 0.48 to 0.51). All kernel traits were only moderately correlated to greenhouse severity (r = 0.27 to 0.43).

Entries were rated for seven disease traits by comparing the each entry means to the best and worst entry mean for each of the seven traits (Tables 1, 2). Only two lines (MO980525, MO981020) were not significantly different from the most resistant entry for all seven traits. These entries also had low IND and SEV (Table 4, 5) in all three megaenvironments, indicating stable resistance. They were also the most resistant in the 2000 NUWWSN greenhouse tests and had low IND scores in 2000 field tests.

Six entries appeared quite resistant based on six of seven traits, often having moderate SEV in the greenhouse tests as their weakness (Table 2). Five other entries appeared resistant based on five of seven traits, generally having moderate SEV in greenhouse tests and moderate to high INC as their weaknesses. NY97048W-7388 also had low SEV (field and greenhouse) in 2000. The probable source of resistance for these lines in presented in Table 3.

Two entries (OH669, NY88005-6035) were not significantly different from the most susceptible lines for six disease traits (Table 2). Two other entries were susceptible based on five of seven traits.

**Table 1.** Entry means for 2001 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 | | SEV | | INC | | IND | | KR | | %SS | | DON | | SEV-GH | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | # of test: | 6 | | 9 | | 8 | | 8 | | 4 | | 3 | | 3 | | 5 | | # low | # High |
| | Units: | Days | | % | | % | | % | | 0-100 | | % | | PPM | | % | | scores | scores |
| 1 | Patterson | 134 | l | 38.4 | h | 61.6 | h | 34.1 | h | 31.0 | l | 14.7 | l | 6.9 | l | 52.4 | | 3 | 3 |
| 2 | Freedom | 138 | | 21.4 | | 62.8 | h | 21.8 | | 50.1 | | 17.5 | l | 12.6 | l | 30.5 | | 2 | 1 |
| 3 | P2545 | 136 | | 39.8 | h | 71.4 | h | 40.7 | h | 66.5 | h | 26.8 | h | 16.2 | l | 55.8 | | 1 | 5 |
| 4 | Ernie | 134 | l | 20.1 | l | 51.4 | | 19.4 | | 29.9 | l | 16.9 | l | 7.9 | l | 28.7 | | 4 | 0 |
| 5 | Hondo | | | 16.7 | l | 48.4 | l | 13.0 | l | 33.1 | l | 17.8 | l | 4.9 | l | 35.6 | | 6 | 0 |
| 6 | KS96HW115 | 135 | | 22.5 | | 61.5 | h | 24.1 | | 38.6 | | 19.1 | h | 14.6 | l | 65.5 | | 2 | 2 |
| 7 | Heyne | 138 | | 18.0 | l | 57.7 | h | 14.9 | l | 24.6 | l | 13.0 | l | 15.1 | l | 31.0 | | 5 | 1 |
| 8 | MDV71-19 | 137 | | 38.4 | h | 72.4 | h | 42.4 | h | 60.6 | h | 23.9 | h | 9.7 | l | 60.0 | | 1 | 5 |
| 9 | MO980525 | 141 | | 11.8 | l | 34.6 | l | 7.5 | l | 23.0 | l | 5.4 | l | 5.3 | l | 14.3 | l | 7 | 0 |
| 10 | MO960827 | 135 | | 30.7 | | 68.5 | h | 30.5 | | 55.9 | | 28.7 | h | 14.6 | l | 36.1 | | 1 | 2 |
| 11 | MO981020 | 137 | | 13.6 | l | 41.3 | l | 9.5 | l | 27.3 | l | 11.8 | l | 5.8 | l | 16.8 | l | 7 | 0 |
| 12 | MO980429 | 135 | | 22.3 | | 49.9 | | 19.9 | | 33.7 | l | 14.4 | l | 6.3 | l | 37.8 | | 3 | 0 |
| 13 | IL96-3514 | 136 | | 23.1 | | 52.1 | | 21.2 | | 27.4 | l | 15.5 | l | 3.2 | l | 36.9 | | 3 | 0 |
| 14 | IL96-6472 | 133 | l | 20.9 | l | 48.2 | l | 17.3 | l | 20.6 | l | 10.2 | l | 8.4 | l | 40.6 | | 6 | 0 |
| 15 | IL97-1828 | 135 | | 17.6 | l | 45.8 | l | 14.2 | l | 19.8 | l | 11.8 | l | 4.6 | l | 46.0 | | 6 | 0 |
| 16 | IL97-4228 | 134 | l | 22.8 | | 45.4 | l | 19.5 | | 29.8 | l | 12.5 | l | 4.2 | l | 48.9 | | 4 | 0 |
| 17 | IL97-6268 | 137 | | 19.7 | l | 47.1 | l | 15.8 | l | 32.6 | l | 11.6 | l | 5.6 | l | 33.6 | | 6 | 0 |
| 18 | Roane | 136 | | 20.0 | l | 60.3 | h | 19.9 | | 32.0 | l | 16.3 | l | 5.4 | l | 27.3 | | 4 | 1 |
| 19 | VA96-54-326 | 136 | | 22.8 | | 54.1 | | 21.0 | | 49.0 | | 12.5 | l | 7.3 | l | 94.1 | h | 2 | 1 |
| 20 | VA98W-591 | 137 | | 20.4 | l | 56.4 | | 16.6 | l | 34.5 | l | 9.7 | l | 7.4 | l | 47.1 | | 5 | 0 |
| 21 | VA98W-593 | 136 | | 27.4 | | 59.8 | h | 21.6 | | 36.3 | l | 7.2 | l | 5.3 | l | 58.8 | | 3 | 1 |
| 22 | VA99W-553 | 134 | l | 23.8 | | 59.2 | h | 23.8 | | 40.3 | | 19.9 | h | 10.4 | l | 61.1 | | 2 | 2 |
| 23 | VA99W-562 | 137 | | 26.0 | | 60.7 | h | 25.9 | | 50.3 | | 19.1 | h | 11.1 | l | 54.7 | | 2 | 2 |
| 24 | VA99W-567 | 138 | | 19.9 | l | 59.4 | h | 19.4 | | 50.8 | | 31.1 | h | 19.5 | h | 63.7 | | 1 | 3 |
| 25 | 25R18 | 139 | | 13.2 | l | 59.4 | h | 13.2 | l | 48.8 | | 14.3 | l | 16.3 | l | 9.3 | l | 5 | 1 |
| 26 | OH669 | 137 | | 42.2 | h | 64.6 | h | 37.6 | h | 53.8 | | 27.0 | h | 21.3 | h | 92.2 | h | 0 | 6 |
| 27 | OH684 | 137 | | 36.0 | h | 61.5 | h | 27.9 | | 50.5 | | 25.8 | h | 13.5 | l | 76.2 | h | 1 | 4 |
| 28 | OH699 | 138 | | 26.0 | | 62.9 | h | 21.2 | | 50.3 | | 21.9 | h | 9.9 | l | 63.9 | | 1 | 2 |
| 29 | NY87048W-7388 | 142 | | 17.0 | l | 50.3 | | 11.9 | l | 24.0 | l | 9.0 | l | 8.4 | l | 23.6 | | 5 | 0 |
| 30 | NY87047W-6048 | 142 | | 31.1 | | 64.6 | h | 28.6 | | 77.5 | h | 30.5 | h | 32.2 | h | 39.8 | | 0 | 4 |
| 31 | NY89052SP-9232 | 143 | h | 27.4 | | 61.1 | h | 24.6 | | 38.1 | | 25.0 | h | 14.8 | l | 55.8 | | 1 | 2 |
| 32 | NY88024-117 | 142 | | 29.1 | | 61.6 | h | 27.8 | | 49.7 | | 18.6 | h | 19.5 | h | 46.8 | | 1 | 3 |
| 33 | NY88005-6035 | 143 | h | 36.1 | h | 61.7 | h | 32.3 | h | 70.3 | h | 33.0 | h | 29.5 | h | 53.2 | | 0 | 6 |
| 34 | NY89103-9149 | 144 | h | 24.8 | | 59.7 | h | 22.0 | | 62.3 | h | 28.8 | h | 22.6 | h | 35.0 | | 0 | 4 |
| 35 | 961331A46-1-6 | 139 | | 29.9 | | 61.7 | h | 28.4 | | 57.2 | | 27.2 | h | 15.0 | l | 38.4 | | 1 | 2 |
| 36 | 9793A1-5 | 134 | l | 17.8 | l | 47.3 | l | 14.2 | l | 24.2 | l | 14.9 | l | 5.4 | l | 33.6 | | 6 | 0 |
| 37 | 97397B1-4-5 | 135 | | 18.4 | l | 55.4 | | 18.6 | | 28.9 | l | 11.2 | l | 6.8 | l | 23.7 | | 4 | 0 |
| 38 | 97398C1-5-3 | 138 | | 21.9 | | 66.9 | h | 22.3 | | 45.5 | | 20.1 | h | 8.5 | l | 34.9 | | 2 | 2 |
| 39 | 97417A1-3-4 | 136 | | 18.7 | l | 52.1 | | 15.9 | l | 30.8 | l | 11.6 | l | 4.5 | l | 47.9 | | 5 | 0 |
| 40 | 97463A1-17-1 | 133 | l | 22.3 | | 50.7 | | 19.0 | | 21.0 | l | 19.0 | h | 9.9 | l | 25.0 | | 3 | 1 |
| 41 | GA901146 E 15 | 134 | l | 33.8 | h | 68.2 | h | 35.6 | h | 56.9 | | 23.8 | h | 10.9 | l | 69.8 | | 1 | 4 |
| 42 | KY92C-491-18-1 | 136 | | 27.6 | | 61.7 | h | 28.8 | | 47.8 | | 18.1 | h | 8.5 | l | 66.1 | | 2 | 2 |
| 43 | KY92C-432-62 | 137 | | 26.2 | | 66.6 | h | 27.9 | | 46.5 | | 27.5 | h | 8.5 | l | 37.3 | | 1 | 2 |
| 44 | KY91C-170-3 | 136 | | 28.9 | | 65.3 | h | 28.8 | | 51.7 | | 23.0 | h | 18.1 | h | 64.9 | | 0 | 3 |
| 45 | KY91C-170-4-1 | 137 | | 26.5 | | 55.2 | | 26.2 | | 44.8 | | 22.2 | h | 21.7 | h | 70.0 | | 0 | 2 |
| 46 | Harding | 143 | h | 17.9 | l | 50.6 | | 13.3 | l | 41.5 | | 19.1 | h | 11.4 | l | 47.0 | | 4 | 1 |
| 47 | SD97060 | 144 | h | 14.7 | l | 45.5 | l | 10.5 | l | 35.8 | l | 9.2 | l | 9.5 | l | 35.5 | | 6 | 0 |
| 48 | D6234 | 139 | | 25.3 | | 66.8 | h | 24.6 | | 41.3 | | 11.9 | l | 15.2 | l | 43.7 | | 2 | 1 |
| 49 | D8006 | 136 | | 32.5 | | 65.4 | h | 31.1 | | 59.3 | | 21.4 | h | 26.9 | h | 61.2 | | 0 | 3 |
| | Average | 138 | | 24.6 | | 57.5 | | 22.6 | | 42.0 | | 18.4 | | 11.9 | | 46.3 | | | |
| | LSD (0.05) | 1.9 | | 9.3 | | 15.0 | | 10.5 | | 17.1 | | 15.0 | | 14.2 | | 18.9 | | | |

†Indicates a mean that is not different from the low est (l) or highest (h) mean in the column based on LSD(0.05).

**Table 2.** Entry means for the most resistant and susceptible entries in the 2001 NUWWSN.

| | Trait: | HD | | SEV | INC | IND | KR | %SS | DON | SEV-GH | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | # of test: | 6 | | 9 | 8 | 8 | 4 | 3 | 3 | 5 | # low | # High |
| | Units: | Days | | % | % | % | 0-100 | % | PPM | % | scores | scores |
| 9 | MO980525 | 141 | | 11.8 l | 34.6 l | 7.5 l | 23.0 l | 5.4 l | 5.3 l | 14.3 l | 7 | 0 |
| 11 | MO981020 | 137 | | 13.6 l | 41.3 l | 9.5 l | 27.3 l | 11.8 l | 5.8 l | 16.8 l | 7 | 0 |
| 5 | Hondo | 140 | | 16.7 l | 48.4 l | 13.0 l | 33.1 l | 17.8 l | 4.9 l | 35.6 | 6 | 0 |
| 14 | IL96-6472 | 133 | l | 20.9 l | 48.2 l | 17.3 l | 20.6 l | 10.2 l | 8.4 l | 40.6 | 6 | 0 |
| 15 | IL97-1828 | 135 | | 17.6 l | 45.8 l | 14.2 l | 19.8 l | 11.8 l | 4.6 l | 46.0 | 6 | 0 |
| 17 | IL97-6268 | 137 | | 19.7 l | 47.1 l | 15.8 l | 32.6 l | 11.6 l | 5.6 l | 33.6 | 6 | 0 |
| 36 | 9793A1-5 | 134 | l | 17.8 l | 47.3 l | 14.2 l | 24.2 l | 14.9 l | 5.4 l | 33.6 | 6 | 0 |
| 47 | SD97060 | 144 | h | 14.7 l | 45.5 l | 10.5 l | 35.8 l | 9.2 l | 9.5 l | 35.5 | 6 | 0 |
| 20 | VA98W-591 | 137 | | 20.4 l | 56.4 | 16.6 l | 34.5 l | 9.7 l | 7.4 l | 47.1 | 5 | 0 |
| 29 | NY87048W-7388 | 142 | | 17.0 l | 50.3 | 11.9 l | 24.0 l | 9.0 l | 8.4 l | 23.6 | 5 | 0 |
| 39 | 97417A1-3-4 | 136 | | 18.7 l | 52.1 | 15.9 l | 30.8 l | 11.6 l | 4.5 l | 47.9 | 5 | 0 |
| 7 | Heyne | 138 | | 18.0 l | 57.7 h | 14.9 l | 24.6 l | 13.0 l | 15.1 l | 31.0 | 5 | 1 |
| 25 | 25R18 | 139 | | 13.2 l | 59.4 h | 13.2 l | 48.8 | 14.3 l | 16.3 l | 9.3 l | 5 | 1 |
| 41 | GA901146 E 15 | 134 | l | 33.8 h | 68.2 h | 35.6 h | 56.9 | 23.8 h | 10.9 l | 69.8 | 1 | 4 |
| 30 | NY87047W-6048 | 142 | | 31.1 | 64.6 h | 28.6 | 77.5 h | 30.5 h | 32.2 h | 39.8 | 0 | 4 |
| 34 | NY89103-9149 | 144 | h | 24.8 | 59.7 h | 22.0 | 62.3 h | 28.8 h | 22.6 h | 35.0 | 0 | 4 |
| 27 | OH684 | 137 | | 36.0 h | 61.5 h | 27.9 | 50.5 | 25.8 h | 13.5 l | 76.2 h | 1 | 4 |
| 8 | MDV71-19 | 137 | | 38.4 h | 72.4 h | 42.4 h | 60.6 h | 23.9 h | 9.7 l | 60.0 | 1 | 5 |
| 3 | P2545 | 136 | | 39.8 h | 71.4 h | 40.7 h | 66.5 h | 26.8 h | 16.2 l | 55.8 | 1 | 5 |
| 33 | NY88005-6035 | 143 | h | 36.1 h | 61.7 h | 32.3 h | 70.3 h | 33.0 h | 29.5 h | 53.2 | 0 | 6 |
| 26 | OH669 | 137 | | 42.2 h | 64.6 h | 37.6 h | 53.8 | 27.0 h | 21.3 h | 92.2 h | 0 | 6 |
| | Average | 138 | | 24.6 | 57.5 | 22.6 | 42.0 | 18.4 | 11.9 | 46.3 | | |
| | CV (%) | 1.2 | | 41.0 | 26.5 | 47.7 | 29.2 | 50.4 | 73.8 | 32.7 | | |
| | LSD (0.05) | 1.9 | | 9.3 | 15.0 | 10.5 | 17.1 | 15.0 | 14.2 | 18.9 | | |
| | R2 | 0.98 | | 0.54 | 0.85 | 0.47 | 0.72 | 0.84 | 0.65 | 0.77 | | |

† 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.

| NAME | Possible sources of resistance |
|---|---|
| 97397B1-4-5 | Freedom, Ning7840, and/or from the moderate resistant cultivar Goldfield |
| 9793A1-5 | Ernie, INW 9853 |
| Hondo | Not known |
| IL97-1828 | Not known |
| IL97-6268 | Not known |
| IL96-6742 | Not known |
| MO980525 | MO 11769, which is not a descendent of Ernie, Sumai 3, or Ning 7840 |
| MO981020 | MO 11769, which is not a descendent of Ernie, Sumai 3, or Ning 7840 |
| NY87048W-7388 | Su Mei, and/or from the moderate resistant cultivars Howser and Harus |

**Table 4.** Field disease severity (% infected spikelets) for entries in 2001 NUWWSN

| | NAME | ALL | IN+OH +ONT | IN | ON | OH | AR+IL+ KY+MO +VA | AR | IL | KY | MO | VA | MI | NE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Patterson | 38.4 h | 40.1 h† | 41 | 35.0 | 44.4 | 33.6 | 7 | 43.8 | 39.0 | 43 | 35 | 57.1 | 80 |
| 2 | Freedom | 21.4 | 13.4 l | 11 | 7.3 | 22.0 | 23.4 | 8 | 23.3 | 34.6 | 32 | 19 | 35.7 | 20 |
| 3 | P2545 | 39.8 h | 40.4 h | 44 | 20.2 | 56.9 | 39.4 h | 15 | 55.0 | 42.0 | 42 | 43 | 40.0 | 30,100 |
| 4 | Ernie | 20.1 l | 19.6 l | 10 | 18.7 | 30.1 | 12.9 l | 5 | 8.5 | 16.1 | 16 | 19 | 57.1 | 100 |
| 5 | Hondo | 16.7 l | 9.3 l | 16 | 4.8 | 7.2 | 21.3 | 7 | 15.8 | 21.8 | 35 | 27 | 15.4 | 30 |
| 6 | KS96HW115 | 22.5 | 17.1 l | 16 | 4.1 | 31.2 | 18.5 l | 5 | 25.0 | 18.6 | 21 | 23 | 58.3 | 100 |
| 7 | Heyne | 18.0 l | 16.4 l | 29 | 3.3 | 17.0 | 16.9 l | 7 | 19.3 | 17.2 | 22 | 19 | 28.6 | 20,100 |
| 8 | MDV71-19 | 38.4 h | 30.3 | 18 | 24.4 | 48.5 | 43.3 h | 22 | 66.3 | 44.0 | 44 | 40 | 38.5 | 70 |
| 9 | MO980525 | 11.8 l | 7.4 l | 12 | 6.9 | 3.4 | 14.1 l | 7 | 12.5 | 17.9 | 19 | 14 | 13.3 | 80 |
| 10 | MO960827 | 30.7 | 25.9 | 22 | 26.5 | 29.1 | 31.7 | 13 | 36.8 | 34.6 | 38 | 36 | 40.0 | 80 |
| 11 | MO981020 | 13.6 l | 10.4 l | 9 | 13.1 | 9.2 | 14.3 l | 5 | 10.3 | 13.2 | 14 | 29 | 20.0 | 80 |
| 12 | MO980429 | 22.3 | 23.7 | 10 | 21.9 | 39.3 | 16.6 l | 5 | 10.5 | 23.4 | 25 | 19 | 46.7 | 100 |
| 13 | IL96-3514 | 23.1 | 24.7 | 14 | 14.9 | 45.1 | 16.1 l | 7 | 13.8 | 25.9 | 21 | 13 | 53.3 | 100 |
| 14 | IL96-6472 | 20.9 l | 24.3 | 20 | 23.3 | 29.6 | 12.5 l | 3 | 7.5 | 13.8 | 14 | 24 | 53.3 | 100 |
| 15 | IL97-1828 | 17.6 l | 16.0 l | 10 | 13.6 | 24.3 | 12.0 l | 7 | 9.0 | 12.2 | 11 | 21 | 50.0 | 100 |
| 16 | IL97-4228 | 22.8 | 29.7 | 26 | 18.9 | 44.1 | 15.2 l | 5 | 8.3 | 24.7 | 14 | 24 | 40.0 | 100 |
| 17 | IL97-6268 | 19.7 l | 23.3 | 17 | 22.8 | 30.2 | 14.4 l | 5 | 16.0 | 21.0 | 16 | 14 | 35.7 | 100 |
| 18 | Roane | 20.0 l | 19.2 l | 18 | 19.4 | 20.2 | 20.2 l | 5 | 19.5 | 33.3 | 31 | 12 | 21.4 | 70 |
| 19 | VA96-54-326 | 22.8 | 24.2 | 28 | 16.7 | 28.0 | 23.5 | 8 | 24.3 | 30.2 | 34 | 21 | 15.4 | 80 |
| 20 | VA98W-591 | 20.4 l | 22.8 | 32 | 12.6 | 23.9 | 20.0 l | 10 | 19.3 | 23.6 | 19 | 28 | 15.4 | 70 |
| 21 | VA98W-593 | 27.4 | 41.5 h | 61 | 16.9 | 46.5 | 21.4 | 7 | 21.3 | 25.5 | 28 | 25 | 15.4 | 80 |
| 22 | VA99W-553 | 23.8 | 29.1 | 18 | 27.1 | 42.2 | 20.8 l | 5 | 21.3 | 28.7 | 30 | 19 | 23.1 | |
| 23 | VA99W-562 | 26.0 | 26.5 | 30 | 10.3 | 39.2 | 27.1 | 7 | 35.8 | 28.7 | 38 | 26 | 18.8 | 60 |
| 24 | VA99W-567 | 19.9 l | 27.2 | 29 | 11.7 | 41.0 | 17.1 l | 5 | 23.8 | 17.9 | 17 | 22 | 11.8 | 80 |
| 25 | 25R18 | 13.2 l | 12.1 l | 7 | 7.1 | 22.2 | 13.8 l | 5 | 10.8 | 17.1 | 12 | 24 | 13.3 | 80 |
| 26 | OH669 | 42.2 h | 47.6 h | 56 | 19.2 | 67.7 | 38.1 h | 10 | 62.5 | 47.8 | 27 | 43 | 46.2 | 70 |
| 27 | OH684 | 36.0 h | 43.2 h | 63 | 27.9 | 38.8 | 31.2 | 15 | 48.8 | 36.1 | 24 | 32 | 38.5 | 80 |
| 28 | OH699 | 26.0 | 23.0 | 28 | 14.9 | 26.1 | 27.3 | 15 | 32.5 | 22.1 | 19 | 48 | 28.6 | 90 |
| 29 | NY87048W-7388 | 17.0 l | 11.6 l | 23 | 3.0 | 8.8 | 20.7 l | 10 | 22.3 | 11.4 | 28 | 32 | 14.3 | 0 |
| 30 | NY87047W-6048 | 31.1 | 25.2 | 21 | 9.1 | 45.5 | 32.8 | 15 | 50.0 | 27.0 | 35 | 37 | 40.0 | 20,80 |
| 31 | NY89052SP-9232 | 27.4 | 14.0 l | 19 | 7.4 | 15.6 | 35.1 | 15 | 52.5 | 29.8 | 42 | 36 | 29.4 | 20 |
| 32 | NY88024-117 | 29.1 | 16.6 l | 15 | 4.0 | 30.9 | 38.9 h | 15 | 62.5 | 39.0 | 38 | 40 | 17.6 | 0,60 |
| 33 | NY88005-6035 | 36.1 h | 14.1 l | 13 | 7.2 | 22.2 | 45.9 h | 25 | 72.5 | 47.9 | 48 | 36 | 53.3 | 20 |
| 34 | NY89103-9149 | 24.8 | 12.9 l | 22 | 9.5 | 7.3 | 31.9 | 15 | 52.5 | 24.1 | 33 | 35 | 25.0 | 20 |
| 35 | 961331A46-1-6 | 29.9 | 23.8 | 16 | 26.6 | 28.7 | 36.1 | 15 | 41.3 | 36.3 | 47 | 41 | 17.6 | 30,80 |
| 36 | 9793A1-5 | 17.8 l | 22.9 | 20 | 15.8 | 33.0 | 15.2 l | 5 | 11.0 | 14.9 | 18 | 27 | 15.4 | 40 ? |
| 37 | 97397B1-4-5 | 18.4 l | 13.3 l | 6 | 10.1 | 23.8 | 19.8 l | 5 | 14.8 | 21.3 | 32 | 26 | 26.7 | |
| 38 | 97398C1-5-3 | 21.9 | 20.5 l | 13 | 10.2 | 38.4 | 21.7 | 5 | 23.5 | 27.2 | 16 | 37 | 26.7 | |
| 39 | 97417A1-3-4 | 18.7 l | 15.3 l | 20 | 13.4 | 12.5 | 16.5 l | 5 | 14.3 | 19.0 | 24 | 20 | 40.0 | |
| 40 | 97463A1-17-1 | 22.3 | 25.1 | 28 | 22.3 | 25.1 | 11.6 l | 5 | 9.8 | 14.4 | 14 | 15 | 66.7 | |
| 41 | GA901146 E 15 | 33.8 h | 41.0 h | 46 | 27.7 | 49.2 | 32.0 | 10 | 55.0 | 36.0 | 24 | 35 | 21.4 | 20,60 |
| 42 | KY92C-491-18-1 | 27.6 | 27.1 | 17 | 15.3 | 49.1 | 24.8 | 5 | 30.8 | 27.3 | 34 | 27 | 42.9 | 90 |
| 43 | KY92C-432-62 | 26.2 | 21.4 l | 18 | 17.8 | 28.4 | 28.9 | 7 | 48.8 | 27.9 | 28 | 33 | 27.3 | 100 |
| 44 | KY91C-170-3 | 28.9 | 28.4 | 26 | 20.5 | 38.8 | 27.8 | 7 | 31.0 | 40.9 | 25 | 35 | 35.7 | 90 |
| 45 | KY91C-170-4-1 | 26.5 | 23.3 | 20 | 17.3 | 32.5 | 28.0 | 8 | 35.0 | 37.1 | 28 | 32 | 28.6 | 30 |
| 46 | Harding | 17.9 l | 9.5 l | 20 | 0.7 | 7.9 | 23.7 | 10 | 25.8 | 29.8 | 25 | 28 | 14.3 | 100 |
| 47 | SD97060 | 14.7 l | 8.7 l | 14 | 1.3 | 10.7 | 18.2 l | 5 | 20.0 | 19.0 | 25 | 22 | 15.4 | 100 |
| 48 | D6234 | 25.3 | 14.6 l | 13 | 9.9 | 21.0 | 30.1 | 13 | 31.3 | 31.0 | 23 | 52 | 33.3 | 30,70 |
| 49 | D8006 | 32.5 | 32.1 | 30 | 13.8 | 52.6 | 34.2 | 15 | 37.5 | 35.6 | 37 | 46 | 25.0 | 60 |
| | Average | 24.6 | 22.7 | 22.8 | 14.8 | 30.4 | 24.3 | 9.0 | 29.5 | 27.1 | 27.1 | 28.8 | 31.6 | |
| | CV (%) | 41.0 | 39.7 | 39.0 | 25.9 | | 30.4 | 21.8 | 29.6 | 25.5 | 32 | | 46.8 | |
| | LSD (0.05) | 9.3 | 14.60 | 16.0 | | 24.5 | 9.2 | 3.2 | 12.1 | 9.7 | 14.0 | | | |
| | R2 | 0.54 | 0.71 | | | | 0.77 | | | | | | | |

†Indicates a mean that is not different from the lowest (l) or highest (h) mean in the column based on $LSD_{(0.05)}$.

**Table 5.** Disease index ([severity% x incidence%]/100) for entries in 2001 NUWWSN

| | NAME | ALL | IN+OH +ONT | IN | ON | OH | AR+IL+ KY+MO +VA | AR | IL | KY | MO | VA | MI | NE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Patterson | 38.4 h | 40.1 h† | 41 | 35.0 | 44.4 | 33.6 | 7 | 43.8 | 39.0 | 43 | 35 | 57.1 | 80 |
| 2 | Freedom | 21.4 | 13.4 l | 11 | 7.3 | 22.0 | 23.4 | 8 | 23.3 | 34.6 | 32 | 19 | 35.7 | 20 |
| 3 | P2545 | 39.8 h | 40.4 h | 44 | 20.2 | 56.9 | 39.4 h | 15 | 55.0 | 42.0 | 42 | 43 | 40.0 | 30,100 |
| 4 | Ernie | 20.1 l | 19.6 l | 10 | 18.7 | 30.1 | 12.9 l | 5 | 8.5 | 16.1 | 16 | 19 | 57.1 | 100 |
| 5 | Hondo | 16.7 l | 9.3 l | 16 | 4.8 | 7.2 | 21.3 | 7 | 15.8 | 21.8 | 35 | 27 | 15.4 | 30 |
| 6 | KS96HW115 | 22.5 | 17.1 l | 16 | 4.1 | 31.2 | 18.5 l | 5 | 25.0 | 18.6 | 21 | 23 | 58.3 | 100 |
| 7 | Heyne | 18.0 l | 16.4 l | 29 | 3.3 | 17.0 | 16.9 l | 7 | 19.3 | 17.2 | 22 | 19 | 28.6 | 20,100 |
| 8 | MDV71-19 | 38.4 h | 30.3 | 18 | 24.4 | 48.5 | 43.3 h | 22 | 66.3 | 44.0 | 44 | 40 | 38.5 | 70 |
| 9 | MO980525 | 11.8 l | 7.4 l | 12 | 6.9 | 3.4 | 14.1 l | 7 | 12.5 | 17.9 | 19 | 14 | 13.3 | 80 |
| 10 | MO960827 | 30.7 | 25.9 | 22 | 26.5 | 29.1 | 31.7 | 13 | 36.8 | 34.6 | 38 | 36 | 40.0 | 80 |
| 11 | MO981020 | 13.6 l | 10.4 l | 9 | 13.1 | 9.2 | 14.3 l | 5 | 10.3 | 13.2 | 14 | 29 | 20.0 | 80 |
| 12 | MO980429 | 22.3 | 23.7 | 10 | 21.9 | 39.3 | 16.6 l | 5 | 10.5 | 23.4 | 25 | 19 | 46.7 | 100 |
| 13 | IL96-3514 | 23.1 | 24.7 | 14 | 14.9 | 45.1 | 16.1 l | 7 | 13.8 | 25.9 | 21 | 13 | 53.3 | 100 |
| 14 | IL96-6472 | 20.9 l | 24.3 | 20 | 23.3 | 29.6 | 12.5 l | 3 | 7.5 | 13.8 | 14 | 24 | 53.3 | 100 |
| 15 | IL97-1828 | 17.6 l | 16.0 l | 10 | 13.6 | 24.3 | 12.0 l | 7 | 9.0 | 12.2 | 11 | 21 | 50.0 | 100 |
| 16 | IL97-4228 | 22.8 | 29.7 | 26 | 18.9 | 44.1 | 15.2 l | 5 | 8.3 | 24.7 | 14 | 24 | 40.0 | 100 |
| 17 | IL97-6268 | 19.7 l | 23.3 | 17 | 22.8 | 30.2 | 14.4 l | 5 | 16.0 | 21.0 | 16 | 14 | 35.7 | 100 |
| 18 | Roane | 20.0 l | 19.2 l | 18 | 19.4 | 20.2 | 20.2 l | 5 | 19.5 | 33.3 | 31 | 12 | 21.4 | 70 |
| 19 | VA96-54-326 | 22.8 | 24.2 | 28 | 16.7 | 28.0 | 23.5 | 8 | 24.3 | 30.2 | 34 | 21 | 15.4 | 80 |
| 20 | VA98W-591 | 20.4 l | 22.8 | 32 | 12.6 | 23.9 | 20.0 l | 10 | 19.3 | 23.6 | 19 | 28 | 15.4 | 70 |
| 21 | VA98W-593 | 27.4 | 41.5 h | 61 | 16.9 | 46.5 | 21.4 | 7 | 21.3 | 25.5 | 28 | 25 | 15.4 | 80 |
| 22 | VA99W-553 | 23.8 | 29.1 | 18 | 27.1 | 42.2 | 20.8 l | 5 | 21.3 | 28.7 | 30 | 19 | 23.1 | |
| 23 | VA99W-562 | 26.0 | 26.5 | 30 | 10.3 | 39.2 | 27.1 | 7 | 35.8 | 28.7 | 38 | 26 | 18.8 | 60 |
| 24 | VA99W-567 | 19.9 l | 27.2 | 29 | 11.7 | 41.0 | 17.1 l | 5 | 23.8 | 17.9 | 17 | 22 | 11.8 | 80 |
| 25 | 25R18 | 13.2 l | 12.1 l | 7 | 7.1 | 22.2 | 13.8 l | 5 | 10.8 | 17.1 | 12 | 24 | 13.3 | 80 |
| 26 | OH669 | 42.2 h | 47.6 h | 56 | 19.2 | 67.7 | 38.1 h | 10 | 62.5 | 47.8 | 27 | 43 | 46.2 | 70 |
| 27 | OH684 | 36.0 h | 43.2 h | 63 | 27.9 | 38.8 | 31.2 | 15 | 48.8 | 36.1 | 24 | 32 | 38.5 | 80 |
| 28 | OH699 | 26.0 | 23.0 | 28 | 14.9 | 26.1 | 27.3 | 15 | 32.5 | 22.1 | 19 | 48 | 28.6 | 90 |
| 29 | NY87048W-7388 | 17.0 l | 11.6 l | 23 | 3.0 | 8.8 | 20.7 l | 10 | 22.3 | 11.4 | 28 | 32 | 14.3 | 0 |
| 30 | NY87047W-6048 | 31.1 | 25.2 | 21 | 9.1 | 45.5 | 32.8 | 15 | 50.0 | 27.0 | 35 | 37 | 40.0 | 20,80 |
| 31 | NY89052SP-9232 | 27.4 | 14.0 l | 19 | 7.4 | 15.6 | 35.1 | 15 | 52.5 | 29.8 | 42 | 36 | 29.4 | 20 |
| 32 | NY88024-117 | 29.1 | 16.6 l | 15 | 4.0 | 30.9 | 38.9 h | 15 | 62.5 | 39.0 | 38 | 40 | 17.6 | 0,60 |
| 33 | NY88005-6035 | 36.1 h | 14.1 l | 13 | 7.2 | 22.2 | 45.9 h | 25 | 72.5 | 47.9 | 48 | 36 | 53.3 | 20 |
| 34 | NY89103-9149 | 24.8 | 12.9 l | 22 | 9.5 | 7.3 | 31.9 | 15 | 52.5 | 24.1 | 33 | 35 | 25.0 | 20 |
| 35 | 961331A46-1-6 | 29.9 | 23.8 | 16 | 26.6 | 28.7 | 36.1 | 15 | 41.3 | 36.3 | 47 | 41 | 17.6 | 30,80 |
| 36 | 9793A1-5 | 17.8 l | 22.9 | 20 | 15.8 | 33.0 | 15.2 l | 5 | 11.0 | 14.9 | 18 | 27 | 15.4 | 40 ? |
| 37 | 97397B1-4-5 | 18.4 l | 13.3 l | 6 | 10.1 | 23.8 | 19.8 l | 5 | 14.8 | 21.3 | 32 | 26 | 26.7 | |
| 38 | 97398C1-5-3 | 21.9 | 20.5 l | 13 | 10.2 | 38.4 | 21.7 | 5 | 23.5 | 27.2 | 16 | 37 | 26.7 | |
| 39 | 97417A1-3-4 | 18.7 l | 15.3 l | 20 | 13.4 | 12.5 | 16.5 l | 5 | 14.3 | 19.0 | 24 | 20 | 40.0 | |
| 40 | 97463A1-17-1 | 22.3 | 25.1 | 28 | 22.3 | 25.1 | 11.6 l | 5 | 9.8 | 14.4 | 14 | 15 | 66.7 | |
| 41 | GA901146 E 15 | 33.8 h | 41.0 h | 46 | 27.7 | 49.2 | 32.0 | 10 | 55.0 | 36.0 | 24 | 35 | 21.4 | 20,60 |
| 42 | KY92C-491-18-1 | 27.6 | 27.1 | 17 | 15.3 | 49.1 | 24.8 | 5 | 30.8 | 27.3 | 34 | 27 | 42.9 | 90 |
| 43 | KY92C-432-62 | 26.2 | 21.4 l | 18 | 17.8 | 28.4 | 28.9 | 7 | 48.8 | 27.9 | 28 | 33 | 27.3 | 100 |
| 44 | KY91C-170-3 | 28.9 | 28.4 | 26 | 20.5 | 38.8 | 27.8 | 7 | 31.0 | 40.9 | 25 | 35 | 35.7 | 90 |
| 45 | KY91C-170-4-1 | 26.5 | 23.3 | 20 | 17.3 | 32.5 | 28.0 | 8 | 35.0 | 37.1 | 28 | 32 | 28.6 | 30 |
| 46 | Harding | 17.9 l | 9.5 l | 20 | 0.7 | 7.9 | 23.7 | 10 | 25.8 | 29.8 | 25 | 28 | 14.3 | 100 |
| 47 | SD97060 | 14.7 l | 8.7 l | 14 | 1.3 | 10.7 | 18.2 l | 5 | 20.0 | 19.0 | 25 | 22 | 15.4 | 100 |
| 48 | D6234 | 25.3 | 14.6 l | 13 | 9.9 | 21.0 | 30.1 | 13 | 31.3 | 31.0 | 23 | 52 | 33.3 | 30,70 |
| 49 | D8006 | 32.5 | 32.1 | 30 | 13.8 | 52.6 | 34.2 | 15 | 37.5 | 35.6 | 37 | 46 | 25.0 | 60 |
| | Average | 24.6 | 22.7 | 22.8 | 14.8 | 30.4 | 24.3 | 9.0 | 29.5 | 27.1 | 27.1 | 28.8 | 31.6 | |
| | CV (%) | 41.0 | 39.7 | 39.0 | 25.9 | | 30.4 | 21.8 | 29.6 | 25.5 | 32 | | 46.8 | |
| | LSD (0.05) | 9.3 | 14.60 | 16.0 | | 24.5 | 9.2 | 3.2 | 12.1 | 9.7 | 14.0 | | | |
| | R2 | 0.54 | 0.71 | | | | 0.77 | | | | | | | |

†Indicates a mean that is not different from the lowest (l) or highest (h) mean in the column based on $LSD_{(0.05)}$

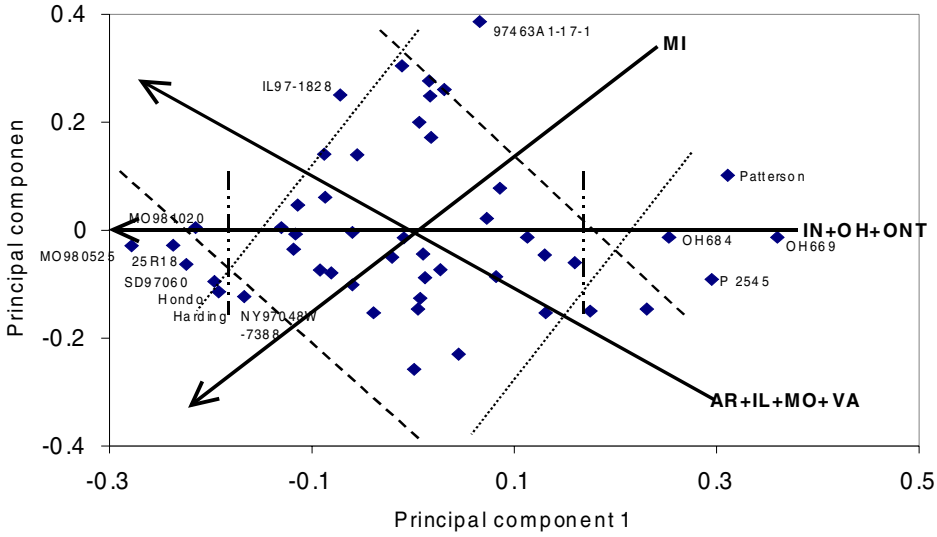


**Figure 1.** Biplot of entry, and entry x megaenvironment effects using three sets of disease severity means. Each set was the mean severity across tests that formed a single megaenvironment: (AR+IL+MO+VA), (IN+OH+ONT), and MI. Entries are represented by points (some are labeled). Megaenvironments are represented by character codes. Vectors are drawn from each megaenvironment through the origin with arrows pointing to decreasing severity values. The cosine of the angle between two vectors estimates the correlation between means in those two groups. For example, the angle between the MI and (AR+IL+MO+VA) vectors is close to $90^0$, suggesting a correlation of nearly zero between these two sets of means (actual r is 0.00). The other two angles suggest correlations near 0.25. The relative performance of an entry in a megaenvironment is estimated by its position perpendicular to the vector for that megaenvironment. For example, the analysis estimates that OH669 has the highest severity score in the AR+IL+MO+VA and IN+OH+ONT megaenvironments, while Patterson had the highest severity in the MI test. Light lines perpendicular to each vector delineate the six best and six worst entries for each megaenvironment.

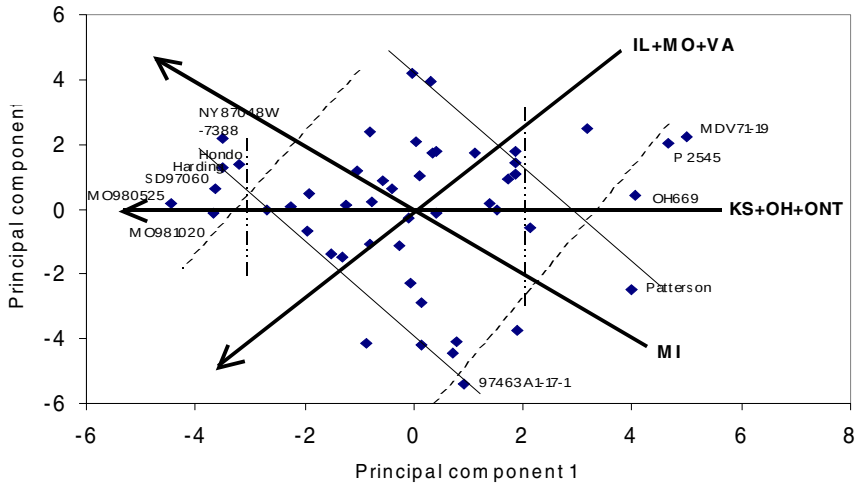


**Figure 2.** Biplot of entry, and entry x megaenvironment effects using three sets of disease index means. Each set was the mean index across tests that formed a single megaenvironment: (IL+MO+VA), (KS+OH+ONT), and MI. Entries are represented by points (some are labeled). Megaenvironments are represented by character codes. Light lines perpendicular to each vector delineate the six best and six worst entries for each megaenvironment.

# RELATIONSHIP BETWEEN GREENHOUSE ESTIMATES OF FHB SPIKELET INFECTION AND LABORATORY SEED INFECTION BY *F. GRAMINEARUM*

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## OBJECTIVE

To determine the relationship between visual estimates of spikelet infection following point inoculation in the greenhouse and seed infection by *Fusarium graminearum* of the same spikes

## INTRODUCTION

Shi and Wang (2000) described several techniques for screening for resistance to FHB and summarized that the injection inoculation method was precise and the most suitable for screening for disease spread in the greenhouse or field. Greenhouse studies in our laboratory following point inoculation of a middle spikelet have identified different trends for the movement of the *Fusarium graminearum* (Schwabe) in the rachis, glume, lemma, palea and seed of all spikelets of each spike (TeKrony et al., 2000). For the resistant cultivars,' Ernie, Roane,' the fungus was primarily localized around the point of inoculation (PI), while for three susceptible breeding lines infection and movement occurred primarily downward from the PI to the base of the spike with little movement above the PI. Infection levels in the rachis were consistently higher than in the glumes, lemma, palea and seed, however none were closely related to visual ratings of spikelet infection after 28 days in the greenhouse. A recent study of DON movement following point inoculation of a middle spikelet in a susceptible cultivar (Savard et al., 2000) also reported high levels of DON at all spikelets below the point of inoculation, but little DON accumulation above the PI. This study evaluated the relationship of FHB spikelet infection following green house point inoculation to movement of *F. graminearum* in wheat spikes (seed) in a wide range of genotypes in the Uniform Northern and Southern FHB nurseries.

## MATERIALS AND METHODS

Plants of wheat cultivars and breeding lines [Uniform FHB Northern ( n = 49) and Southern (n = 29) Screening Nurseries] with variable levels of Type II resistance to FHB were established in pots in the greenhouse. At flowering, macroconidia spores were injected into a single floret (between lemma and palea) of a middle spikelet of five spikes (plants) of each genotype. Injections were made from a composite of 12 different isolates of *Fusarium graminearum.* After misting the inoculated spikes for three nights in a high humidity chamber to encourage fungal growth, the pots were moved to the greenhouse. Spikes and individual spikelets were visually rated for disease incidence and severity at 7, 14, 21 and 28 days post inoculation (dpi). At maturity spikes were harvested, dissected into individual spikelets and each seed of the lowest (left) floret of all spikelets on the spike was removed and plated

on a modified PCNB agar and grown at 25 °C for 14 d. The spikelets were numbered in relation to their position relative to the inoculated spikelet (0), (positive numbers representing the spikelets above the point of inoculation (PI) and negative numbers representing the spikelets below the PI). The seeds on each plate were identified by spikelet location on each spike and examined for *F. graminearum* infection, which was confirmed by randomly comparing the morphological identity of the fungus to the inoculum source.

Average visual rating of FHB spikelet infection across five spikes in the greenhouse was determined for each genotype, and the genotypes were grouped into the following three categories; resistant (<25% spikelet infection), susceptible (50 to 75 % spikelet infection) and highly susceptible (> 75% spikelet infection). Within each group all spikes were evaluated for *F. graminearum* seed infection by spikelet location (as described above) and the mean seed infection above (+) and below (-) the PI was determined for each resistant/ susceptible genotype group.

## RESULTS

Visual estimates of FHB spikelet infection (21 days) in each spike following point inoculation in the greenhouse ranged from 5% (infection at PI only) to 100% across all genotypes in the Northern and Southern FHB nurseries (Fig.1). Although the individual spikes of many genotypes showed Type II resistance to FHB with spikelet infection levels of d•10% (n =110), the mean level of *F. graminearum* infection of seeds from the same spikelets ranged from 0 to 92 % (mean = 25%). Likewise, those susceptible spikes which showed 100% FHB infection of spikelets (n = 92) in the greenhouse averaged from 4 to 92 % (mean = 56%) seed infection in the laboratory. Even those spikes showing moderate levels (40 to 60%) of spikelet FHB infection in the greenhouse had a wide range in seed infection (5 to 90%). There was little difference between the two nurseries in the range of FHB spikelet infection in the greenhouse vs. the range *F. graminearum* seed infection in the laboratory.

Previous studies of *F. graminearum* movement in floral components of spikelets following greenhouse inoculation have shown variable patterns of movement depending on the Type II resistance of each cultivar (TeKrony et al.,2000). Thus, for a moderately resistant cultivar, Roane, the fungus was primarily localized around the point of inoculation (PI), while for a susceptible breeding line (VA96-54-326) infection and movement occurred primarily downward from the PI to the base of the spike with little movement above the PI. When all genotypes in the Uniform Northern and Southern FHB nurseries were grouped by resistance and susceptibility to FHB type II infection, similar trends in *F. graminearum* seed infection and movement in the spike was observed (Fig. 2). For those highly susceptible genotypes (75-100% spikelet infection in the greenhouse) 100% seed infection was shown at the PI and remained near this level down the spike. High average levels of infection (92%) were shown at only the first spikelet (+1) above the PI and declined rapidly at the next five upper spikelets to 11% infection at +5. Those genotypes classified as susceptible also exhibited much higher seed infection at spikelets below the PI than for those spikelets above the PI (Fig. 2). As expected, those genotypes classified as resistant to Type II FHB spikelet infection ($\leq$ 25% spikelet infection in the greenhouse), had lower levels of seed infection in the laboratory. Seed infection at the PI was 77% and gradually declined to 34% at the -5 spikelet below the PI. As for susceptible genotypes, the seed infection above (+) the PI declined

sharply at the first spikelet to 29% and continued to decline up the spike. It was somewhat surprising, that the same trends occurred for both resistant and susceptible cultivars (Fig. 2).

## DISCUSSION

These results show a poor relationship between visual ratings of FHB spikelet infection following point inoculation in the greenhouse and *F. graminearum* infection of seed from the same spikelets (Fig. 1). Although DON was not measured in individual seeds in this study, Pandeya and Sinha, 1998, (in Savard et. al., 2000) also suggest that there may not be a definitive association between the visual FHB disease ratings following point inoculation and DON concentration in wheat kernels. Both studies would appear to question the accuracy of greenhouse disease ratings when visually evaluating genotypes for FHB Type II resistance.

Our studies of movement of *F. graminearum* into floral components following greenhouse inoculation show highest levels of infection in the rachis (TeKrony et al., 2000), which implies movement in vascular tissue. We observed little difference in the levels of *F. graminearum* among other floral components, except that infection in seeds and glumes was slightly higher than in lemma and palea. We consistently find greater seed infection, however below the point of greenhouse inoculation than above this point (Fig. 2). A recent study of DON movement following point inoculation of a middle spikelet in a susceptible cultivar (Savard et al., 2000) also reported high levels of DON at all spikelets below the point of inoculation, but little DON accumulation above the PI. The authors suggested that the fungus impedes circulation at the point of entry into the rachis preventing the movement of water and nutrients to the top of the spike and limiting movement of water soluble toxins, including DON. Schroeder and Christensen (1963) also reported that seeds in the spikelets above the point of infection are unable to obtain the nutrients and water needed for full development, leading to the white head symptom associated with infection, which was especially prevalent in susceptible cultivars. These reports would tend to explain why levels of *F. graminearum* were consistently higher below the point of inoculation than above this point for susceptible cultivars, however they do not explain the same trends that occurred for resistant cultivars.

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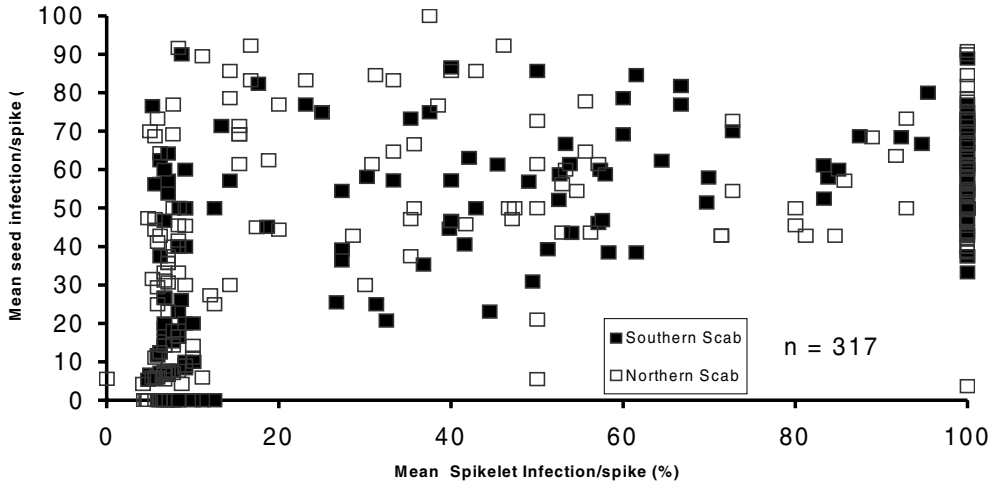


**Figure 1**. Relationship between visual estimate of FHB spikelet infection for each spike following point inoculation and bioassay of *F. graminearum* seed infection for same spikes for all entries in Uniform Northern and Southern Scab Nursery 2001.

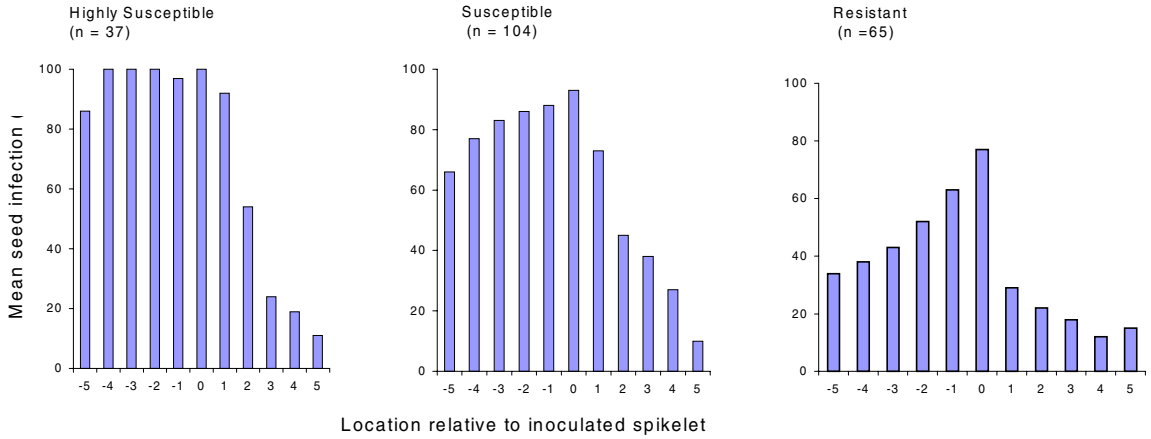


**Figure 2 .** *F. graminearum* seed infection of wheat spikes following point inoculation of a middle spikelet. Each point is an average of all wheat spikes evaluated in each resistant/ susceptible genotype category for both the Uniform Northern and Southern FHB nurseries, 2001. (spikelet location is shown above (+) and below (-) point of inoculation (0).

# TESTING METHODS FOR RESISTANCE TO FUSARIUM HEAD BLIGHT AND THE EFFECT OF SPIKE TRAITS IN BARLEY

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## ABSTRACT

Resistant reaction of barley to Fusarium head blight (FHB) is influenced by the hygrothermal condition and the stage of inoculated plants. In order to estimate the resistance, two testing methods, namely "pot-plant" method and "cut-spike" method, were employed, and barley varieties previously reported with wide range of resistance were tested in 2000 and 2001. Barley varieties planted in plastic pots were grown in a greenhouse. Spikes exactly at anthesis were sprayed with macroconidia suspension ($5 \times 10^5$ conidia per ml) of *Fusarium graminearum* H-3 strain in both testing methods. On the "pot-plant" method, five to seven spikes in each pot were inoculated. After the inoculation, the plants were nursed in a greenhouse at 20±5°C with 100% humidity overnight. After that, the spikes were kept wet by mist-sprinkler during the test. The "cut-spike" test was performed according to the procedure developed by Takeda and Heta (1989) with some modifications. Three spikes cut off at third internodes were inoculated together. The inoculated spike sets were cultured in running water and infection was promoted in a humidistat at 100% humidity and 25°C for a day. After that, the spike sets were placed in a growth chamber at 18-25°C with 80-100% humidity. In both testing methods, the reaction to FHB was observed one and two weeks after inoculation and scored from 0 to 9 according to the percentage of infected spikelets. Barley varieties showed wide range of symptom scores in both testing methods. The scores of each method highly correlated in both years (r=0.72-0.83 and 0.51-0.66 at one and two weeks after inoculation, respectively), and year-to-year correlation was also high (r=0.78, 0.74 for "pot-plant" method and r=0.83, 0.62 for "cut-spike" method at one and two weeks after inoculation, respectively). It suggests that both testing methods are available for accurate and stable evaluation of FHB resistance. Two-rowed varieties were more resistant than six-rowed ones significantly, and cleistogamous varieties were evidently more resistant than chasmogamous ones. Almost all of Japanese two-rowed varieties are cleistogamous and showed high resistance to FHB, while six-rowed varieties are chasmogamous and were moderately resistant or susceptible to FHB. Cleistogamy appears to be an important characteristic for FHB resistance, and also, two-rowed characteristic or genetic background of Japanese two-rowed varieties may be important.

# VALIDATION AND MARKER-ASSISTED SELECTION OF A MAJOR SCAB RESISTANCE QTL WITH SSR MARKERS IN WHEAT

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## ABSTRACT

Objectives in this study were to validate a major quantitative trait locus (QTL) for scab resistance on chromosome 3BS in hexaploid wheat and to isolate near-isogenic lines for this QTL using flanking simple sequence repeat (SSR) markers. Using Ning 7840 as the resistant parent, we developed two resistant by susceptible populations to examine the 3BS QTL in different genetic backgrounds. Data for scab resistance and markers linked to the resistance QTL were analyzed in the $F_{2:3}$ generation of one population and in the $F_{3:4}$ generation of the other population. Selected SSR markers on chromosome 3BS were closely associated with scab resistance in both populations. Selection with the aid of SSR markers was more efficient in selecting homozygotes for the 3BS QTL than was selection based on phenotypic evaluation of scab resistance. Using two flanking markers, Xgwm389 and XBARC147, near-isogenic lines with this major QTL were identified in the $F_{6:7}$ generation of one population. Two lines were identified with scab resistance similar to Ning 7840. Strategies using SSR marker-assisted selection for the 3BS QTL are discussed.

# APPLYING SIMPLE SEQUENCE REPEAT (SSR) MARKER IN SCREENING FUSARIUM HEAD BLIGHT RESISTANT PARENTS

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## ABSTRACT

A QTL on chromosome arm 3BS of cultivar Sumai 3 is a major contributor to Fusarium head blight (FHB) resistance. Three SSR markers (*Qfhs.ndsu-3BS-Xgwm533, Qfhs.ndsu-3BS-Xgwm493, and Qfhs.ndsu-3BS-Xgwm389*) have been found closely linked to the resistant QTL. This project aimed at screening FHB resistant parents used in the South Dakota spring wheat improvement program for these SSR molecular markers. This is the first step toward implementation of marker-assisted selection (MAS) for FHB resistance. Fifty lines in our Fall Crossing Block (FCB00), 24 lines from the Preliminary Yield Experiment (PPY) in the year of 2000, and three FHB resistant lines with unknown pedigrees were analyzed with the SSR primer sets Xgwm533 and Xgwm493. Our results showed that out of 78 lines assayed, eight lines (10%) were found to possess the Xgwm533 marker. All the lines possessing this marker had at least moderate FHB resistance and were derived from Sumai 3 or its derivative except N99-0107, whose pedigree information is unknown. However, about 70% of assayed FHB resistant lines do not have the Xgwm533 marker even thought they were derived from Sumai 3. The linkage between this marker and the 3BS FHB resistant lines seemed easily broken through crosses. Thirty-six assayed lines (46%), including some susceptible lines without Sumai 3 in their pedigree, have the Xgwm 493 marker. Therefore this marker did not show much polymorphism between resistant and susceptible lines and thus have to be used with limits. This information will be applied to MAS for FHB resistance.

# PRE-ANTHESIS DROUGHT AND HEAT STRESS ON FUSARIUM HEAD BLIGHT DEVELOPMENT IN SPRING WHEAT

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## ABSTRACT

It has been observed that less Fusarium head blight (FHB) developed in wheat plants when plants were subjected to early season drought and/or heat stress. The objective of this study was to investigate the response of wheat plants to pre-anthesis drought and/or heat stress in FHB development. Four hard red spring wheat lines, ND2710, Wheaton, Russ and 2375 were used in this study. Three simultaneous experiments were conducted under controlled conditions in the greenhouse and growth chamber to evaluate the effect of different levels of pre-anthesis drought stress, heat stress and their combination on FHB development. Drought stress was applied by withholding water from the plants until the soil moisture content reached the desired level (35% pot capacity, 30% pot capacity, and 3 days after 30% pot capacity). Heat stress was applied by transferring the plants from a growth chamber at 20/15°C (day/night temperature) to a chamber at 30/20°C. FHB was induced by point inoculation. The results of the experiments indicated that drought stress, either separate or combined with heat stress reduced the level of FHB in spring wheat. Drought stress reduced FHB severity across all the four lines. Disease severity seemed to be affected by the level of drought stress rather than the stage of development at which stress was imposed. The severe and intermediate levels of drought stress caused more reduction in FHB severity than the mild level of stress. The reduction in FHB severity induced by the pre-anthesis drought stress was higher in the moderately susceptible lines 2375 and Russ than the resistant line ND2710 and susceptible line Wheaton. Drought stress also reduced kernel damage. The reduction of the kernel damage appeared to vary among trials and lines. Heat stress did not affect FHB severity when continuously imposed from booting stage until anthesis when inoculation was applied. However, heat stress applied for only three days after the start of booting tended to reduce FHB severity. Among the four lines, Russ was most sensitive to heat stress in response to FHB development. Heat stress combined with drought also reduced the level of FHB development in the susceptible line Wheaton. Although all levels of drought, heat stress, and their combination affected wheat plants adversely, the intermediate level of pre-anthesis drought stress did not reduce spike weight significantly when the plants were inoculated. Compared to non-stressed control plants, drought stressed plants had less reduction in spike weight under FHB infection due to the lower level of FHB severity. In conclusion, pre-anthesis drought and heat stress could reduce FHB development through their effects on plants and may confound results of FHB resistance screening in both field and greenhouse. Therefore, breeders have to be aware of the effects of modifying of pre-anthesis environments on the FHB development of their breeding materials, especially for the lines with moderate resistance or susceptibility.

# NCR-184 2001 ARKANSAS STATE REPORT

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## WHEAT PRODUCTION

Arkansas growers harvested 970,000 acres of soft red winter wheat with a average yield of 52 bu/acre. Growing conditions were extremely cold during November and December and extremely dry during April and May. The major constraints to production were the cold conditions that hampered stand establishment and the dry conditions that hastened maturity. The dry conditions favored Fusarium root and crown rot, and this was the most prevalent disease during the season.

## FHB SITUATION

FHB was non-existent because of the extremely dry conditions before, during, and after flowering. However, the widespread occurrence of *Fusarium* root and crown rot demonstrates the ubiquitous nature of *Fusarium* species in the wheat production areas of Arkansas.

## CURRENT FHB PROJECTS

Personnel: Robert Bacon and John Kelly are working on the development of scab-resistant varieties. Gene Milus, Peter Rohman, and Chris Weight are working on transferring genes for scab resistance to southern soft red winter wheat, screening lines for scab resistance, and evaluating fungicides and biocontrol agents for efficacy against scab.

Breeding: The breeding program has several advanced lines that appear to have FHB resistance from early CIMMYT spring wheat selections and Chinese lines, and several of these lines have been entered in the Uniform Southern Winter Wheat Scab Nursery. Additional crosses were made using adapted Arkansas breeding lines and resistant soft red winter wheats and eastern European winter wheats.

Germplasm Enhancement: Eighty-four $F_7$, $BCF_6$, or $TCF_6$ lines from populations derived primarily from recent CIMMYT spring wheat cultivars and lines were selected during 2001. All of these lines also are resistant to contemporary races of the leaf rust, stripe rust, and leaf blotch pathogens. Steve Harrison, wheat breeder at Louisiana State University, is collaborating with this project by screening lines at two locations in Louisiana. Selections made in Louisiana and Arkansas have been exchanged each year. A recurrent selection program was begun to combine resistances.

Screening: The Uniform Winter Wheat Scab Nursery, Uniform Southern Winter Wheat Scab Nursery, Bacon's scab-resistant selections, and lines from the germplasm enhancement program were evaluated for scab resistance in field screening nurseries at two locations and in the greenhouse for type 2 evaluations.

Fungicide Evaluations: Treatments in the Uniform Fungicide and Biocontrol Trial and additional treatments were evaluated for efficacy against scab. Scab developed later than normal, and the biocontrol agent, OH 182.9, was the most efficacious.

# NCR-184 MANAGEMENT OF HEAD SCAB IN SMALL GRAINS ILLINOIS REPORT - DECEMBER, 2001

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**Illinois Wheat Production:**

The estimated wheat yield in Illinois in 2001 was 61 bushels per acre. This was four bushels per acre greater than last year's average of 57 bushels per acre, and equal to the record state average of 61 bushels per acre set in 1997. Acreage harvested was about 720 thousand acres, down about 21 % from 2000. Wheat production in Illinois in 2001 was about 43.9 million bushels. This was a 16 % decrease from the 2000 production of 52.4 million bushels. In general, conditions for wheat production were excellent in 2001. Wheat came through the winter in excellent condition, and spring weather conditions were very favorable. Some leaf rust and crown rust were observed, but, in general, diseases did not limit yields. The most serious production problem in 2001 in Illinois was a severe armyworm infestation. Armyworms completely stripped leaves from wheat in some areas and a significant percentage of the wheat acreage was sprayed with insecticide for armyworm control. Scab damage was very limited in 2001 in Illinois due to dry weather at flowering and below normal temperatures for about a week during early grainfill. In spite of excellent yields for many farmers in 2001, the number of wheat acres planted for 2002 is projected to be about the same as in 2001 or somewhat reduced. Excessive rainfall in much of southern Illinois at the optimal planting time most likely limited the amount of wheat planted for 2002.

**University of Illinois Research:**

Breeding for Scab Resistance in Soft Red Winter Wheat: Development of scab resistant germplasm and varieties is a major research emphasis in the wheat breeding program. The long-term objective is the development of soft red winter wheat genotypes with excellent resistance to scab combined with resistance to other diseases, high yield potential, and acceptable winter hardiness and milling and baking quality. Our short-term objectives are: 1) to combine genes for resistance to scab from diverse sources; 2) to evaluate the genotypes produced from crosses and identify those with resistance to scab; 3) to identify molecular markers associated with genes for resistance to scab; and 4) to work toward using molecular markers to assist in breeding for scab resistance.

About 228 single and 161 three-way crosses were made with one or more scab resistant parents in each cross. In addition, about 36 crosses were made with the objective of combining scab resistance from 6 new sources with other sources of scab resistance and with adapted lines. Many of the crosses in the second set involve parents with excellent scab resistance, but many of these parents are unadapted. In 2001 about 550 breeding lines were evaluated in replicated rows in the misted, inoculated scab evaluation field nursery. In addition, about 700 entries from single plots, and about 800 headrows were also evaluated in the field inoculated nursery. Heads were selected from 36 $F_3$ bulk populations grown in

the field scab nursery, and about 1700 headrows resulting from these selections were planted this fall (2001-02 season). Plants from four segregating populations were screened in the greenhouse. A total of 1707 plants were evaluated, and 567 plants (33.2%) were selected (most with Type II resistance better than Ernie). Scab resistant lines were evaluated for many traits in the field. Many of the lines with good scab resistance are poor for other traits such as grain yield, milling and baking quality, standability, or resistance to other diseases. We are working on combining different sources of resistance and combining resistance with other desirable traits. Five lines from the Illinois program were entered into the 2001 Cooperative Eastern Winter Wheat Scab Screening Nursery. These lines were made available to other breeders by entering them into the Cooperative Eastern Winter Wheat Fusarium Head Blight Screening Nursery. Seed increases of 213 doubled haploid lines originating from 26 crosses were grown.

**Research on Molecular Markers:**

Research is continuing on identification of molecular markers associated with genes for scab resistance. A number of markers associated with one quantitative trait locus (QTL) on 3BS have been identified. These markers should be useful for marker-assisted selection for scab resistance. We are beginning to use some of these markers as an aid for selection of scab resistant breeding lines, but additional research needs to be done before marker assisted selection can be used routinely in the breeding program.

Six microsatellites on chromosome 3BS, Xgwm389, Xgwm533, XBARC147, Xgwm493, XBARC102, and XBARC131 were integrated into an amplified fragment length polymorphism (AFLP) linkage group containing a major QTL for scab resistance in a mapping population of 133 recombinant inbred lines (RILs) derived from Ning7840/Clark. Based on single factor variance analysis of scab infection data from four experiments, Xgwm533 and XBARC147 were the two microsatellite markers most tightly associated with the major scab resistance QTL. Interval analysis based on the integrated map of AFLP and microsatellite markers showed that the major QTL was located in a chromosome region of about eight cM in length around Xgwm533 and XBARC147. Mapping of six microsatellite markers on eight 3BS deletion lines showed that the major QTL was located distal to breakage point 3BS-8. In total, eighteen microsatellites were physically located on different sub-arm regions on 3BS. Two microsatellites, Xgwm120 and Xgwm614, were significantly associated with QTL for scab resistance on chromosome 2BL and 2AS, respectively. Significant interaction between the major QTL on 3BS and QTL on 2BL was detected based on microsatellite markers linked to them.

To further validate a major QTL for scab resistance on chromosome 3BS in hexaploid wheat we identified near-isogenic lines for this QTL using flanking simple sequence repeat (SSR) markers. We developed two resistant by susceptible populations, both using Ning 7840 as the resistant parent to examine the 3BS QTL in different genetic backgrounds. Data for scab resistance and markers linked to the resistance QTL were analyzed in the $F_{2:3}$ generation of one population and in the $F_{3:4}$ generation of the other population. Selected SSR markers on chromosome 3BS were closely associated with scab resistance in both populations. Selection with the aid of SSR markers was more efficient in selecting homozygotes for the 3BS

QTL than was selection based on phenotypic evaluation of scab resistance. Near-isogenic lines with this major QTL were isolated in the $F_{6:7}$ generation of one population using two flanking markers, Xgwm389 and XBARC147. Two lines were identified with scab resistance similar to Ning 7840. This research is in cooperation with Guihua Bai, Oklahoma State University; Greg Shaner, Purdue University; and Les Domier, USDA-ARS at Urbana, Illinois.

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# MANAGEMENT OF SCAB OF SMALL GRAINS NCR-184
# 2001 INDIANA STATE REPORT

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Scab was sporadic and generally not severe in Indiana in 2001, although some rain fell during flowering. Where scab was found, incidence was generally low.

**Current research programs include:**

Host resistance: development of resistant varieties of soft red winter wheat, germplasm enhancement, genetic studies of resistance, and identification of molecular markers associated with resistance.

Pathogen genomics: Additional ESTs (expression sequence tag) were generated from the nitrogen-starved cDNA library. Gene replacement mutants were generated from two *F. graminearum* genes. One of them, CHV1, was found to be important for plant infection. In addition, subtraction libraries enriched for genes specifically or highly expressed during plant infection were also sequenced. All sequence information is available at http://www.genomics.purdue.edu/~jxu/Fgr.

Epidemiology: the relation between weather variables, quantity of airborne inoculum of *Gibberella zeae*, and development of head blight symptoms.

**Biological and chemical control:** evaluation of fungicides and biocontrol agents for efficacy against head blight of wheat.

Reports on these various projects are available on the USWBSI Web site and in the Forum proceedings.

**Cereal classes and acreage in Indiana:** Indiana produces soft red winter wheat. In 2001, Indiana farmers harvested 380,000 acres of wheat, substantially down from the production during 2000, but continuing a trend of declining production. Average yield was 66 bu/A, for a total production of 25.08 million bushels.

# ANNUAL REPORT FOR 2001 NCR-184 - IOWA

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Wheat production and head blight in Iowa in 1999. Iowa's winter wheat yield of 54 bushels per acre was a record high level and seven bushels above the yield of 47 bushels per acre in 2000. The state's production of 972,000 bushels was up nearly 15 percent from the previous year. Acres harvested for grain was 18,000 acres, equal to last year's record low harvested acreage. Although rainfall was abundant during April and early May, dry weather followed and there were few reports of scab problems.

Fusarium head blight research. In 2001 we participated in the uniform scab nursery for spring and winter wheat. Winter wheat plots were completely destroyed during the winter of 200-2001. In the spring wheat, scab levels were very low in spite of frequent irrigation and infestation of the plots with *F. graminearum* inoculum. We do not have DON results at this time. We also participated in the uniform fungicide trials with a winter wheat variety, but scab levels again were very low. There were some statistically significant differences in scab severity. Only the TrigoCor and BAS 505 treatments were significantly different from the control. The BAS 505 treatment had the highest test weight, the lowest incidence and severity of scab, and the highest 100-kernel weight. We do not have DON results at this time. This fall we planted the uniform scab nursery for winter wheat.

# NCR-184 STATE REPORT FOR KANSAS 2001

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## FHB Situation in Kansas in 2001

Kansas produced approximately 328 million bushels of wheat in 2001, which was down from 348 million bushels the previous year. The major disease problem in the state was stripe rust, which was promoted by unusually cool weather in May. There were only traces of Fusarium head blight reported in the state in 2001.

## Breeding for Resistance

Bill Bockus is in charge of the scab field nursery. Additional members on the team include Mark Davis, Allan Fritz, Joe Martin, Gina Brown-Guidera, and Bob Bowden Because of the scab screening efforts, a new column for reaction to Head Scab has been added to the popular extension publication *Wheat Variety Disease and Insect Ratings* for the 2001 issue. For the first time, this will allow producers in Kansas to use the reaction to scab to help select cultivars for planting. Additionally two commercial cultivars in Kansas (Hondo and Heyne) were identified in 2000 (and confirmed in 2001) as having good levels of resistance (3 and 4 on the 1-9 scale where 1=immune and 9=highly susceptible). During 2001, these cultivars had an average of 12 and 20% scab, respectively compared with over 50% in highly susceptible cultivars. Similarly, the advanced breeding line KS96HW115 (released in August, 2000 as Lakin) showed moderate levels of resistance with 26-34% scab in 2001. Five other commercial cultivars displayed some level of resistance in the 2001 nursery; however, these results need to be confirmed. Therefore, there is scab resistance already present in cultivars adapted to Kansas that can potentially be used in the development of future cultivars.

Inheritance of resistance and allelism tests are currently underway with those cultivars. Additionally, several advanced breeding lines (mostly in the Kansas Intrastate Nursery) have been identified with improved levels of resistance. Both KSU wheat breeders (T. J. Martin, and A. K. Fritz) and the USDA wheat geneticist (G. Brown-Guedira) have been having material screened.

## Relationship of PPO and Resistance to FHB

Andre Rosa is working on an interesting question about white wheat. The enzyme polyphenol oxidase (PPO) confers a detrimental discoloration to noodles and other wheat products. To offset this problem, breeders select wheats with lower levels of PPO in the kernels. PPO is known to be involved in plant resistance to disease. Two studies were designed to determine the relationship between levels of PPO in wheat kernels and plant response to diseases in Kansas. Plant response to disease was evaluated directly by visual scoring or, indirectly, by evaluating traits affected by diseases. Ninety-two F4 and 206 F5 lines selected

for their contrasting PPO levels and representing three crosses were evaluated for two years in a replicated head-scab nursery. Lines were scored for heading date (HD), green leaf duration (GLD), and scab resistance (scab). The second study involved 16 F6 lines with contrasting PPO levels representing five crosses. A split-plot design with four replications was used to test these lines for two years in up to four locations for HD, GLD, scab, test weight, and yield. The means for the evaluated traits were similar for lines with high and low levels of PPO indicating that plant response to diseases was not affected by the levels of PPO in the kernels. Therefore, selecting for low levels of PPO should not hamper disease resistance in Kansas breeding programs.

**Transgenic resistance to FHB**

Subbaratnam Muthukrishnan, Harold Trick, and Bikram Gill generated several transgenic wheat plants containing different combinations of genes for pathogenesis-related proteins by a biolistic transformation protocol. Ten of these were found have high level expression of the transgenes. The inheritance and expression of these genes have been followed up to the T4 generation in some cases. Two transgenic lines that are homozygous for the transgenic loci have been tested for resistance to scab by the single floret inoculation assay. One of them was found to be significantly more resistant to scab compared to the control. Crosses involving some transgenic lines and Heyne are in progress.

**Can we debilitate the scab fungus with a virus?**

Lou Heaton screened over 100 *F. proliferatum* isolates collected in Kansas and found four isolates harboring dsRNAs. The dsRNAs range in size from approximately 700 bp to approximately 3,300 bp, and each of the four isolates harbors a distinct set of dsRNAs. The dsRNAs of isolates D-720, D-591, and D-599 are transmitted to approximately 97% of single-conidiospore cultures, while the dsRNAs of D-890 are only rarely transmitted to single-conidiospore cultures (approximately 3%). None of the dsRNAs were transmitted to single-ascospore cultures resulting from crosses in which the isolates were males. Attempted crosses in which the isolates were the female parent are thus far sterile. Since the transmission patterns were consistent with a mitochondrial location, sub-cellular fractions were examined for the presence of dsRNAs. The dsRNAs of all four of the *F. proliferatum* isolates were found in mitochondrial fractions. We are in the process of screening our e:*F. graminearum* isolates. Thus far, we have identified one isolate that contains dsRNAs. Additionally, we have obtained a Korean *F. graminearum* isolate with a dsRNA that renders the isolate hypovirulent on wheat.

**Pathogen Variability and Genetics**

People working on this project include; K. A. Zeller, J. I. Vargas, Y.-W. Lee, R. L. Bowden, and J. F. Leslie. We isolated populations of *Gibberella zeae* (*Fusarium graminearum*) from field samples of wheat, barley, maize or sorghum from North and South America, and from South Korea. We compared the phylogenetic lineage composition from these sources using AFLP markers produced by three standard primer combinations. United States populations of *G. zeae* from wheat are composed of a single phylogenetic lineage (lineage VII) and are diverse but relatively homogeneous across the country. South Korean populations from barley

were dominated by a single lineage (lineage VI). South Korean populations from maize are dominated by lineage VII, but lineage III is a relatively common component. Populations of *G. zeae* from wheat in Brazil also appear to be dominated by lineage VII, but at least one other lineage is present. We have also examined *G. zeae* populations from wheat and sorghum in Uruguay.

# NCR-184 2001 KENTUCKY STATE REPORT

Donald Hershman[1]*, Paul Bachi[1], David VanSanford[2], Dennis TeKrony[2], Marla Hall[2], Brenda Kennedy[2] and Liu Hua[2]

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## FUSARIUM HEAD BLIGHT STATUS DURING 2001

Fusarium head blight (FHB) levels were extremely low throughout Kentucky during the spring of 2001. Nonetheless some fields experienced significant problems with unacceptable levels of deoxynivalenol (DON), in spite of the fact that FHB was not evident. This is thought to be the result of delayed harvest due to untimely rain events following crop maturity. Most impacted fields were seriously lodged.

## CURRENT RESEARCH PROJECTS

### Field and Greenhouse Screening

Brenda Kennedy, Marla Hall and David VanSanford

Numerous soft red wheat cultivars, breeding lines, entries in the Uniform Northern and Southern Scab Nurseries, and approximately 1400 exotic accessions were evaluated under mist irrigation in two field nurserys: one near Lexington, KY and the second at Princeton, KY. Cracked corn infested with various pathogenic isolates of *Fusarium graminearum* was used as inoculum in both nurseries. Most of the lines evaluated in field nurseries were also evaluated in the greenhouse for Type II resistance. Variation was observed, with some breeding lines showing good Type II resistance as well as apparent combining ability for this trait.

### Inheritance Studies

Marla Hall, Liu Hua, and David VanSanford

A number of populations were synthesized from wheat parents with reportedly different sources of resistance to FHB. $S_1$ lines in three populations were evaluated under mist irrigation in an inoculated nursery to elucidate inheritance of resistance. Highly significant variation for Type I/Type II resistance was observed in the populations. Two diallel series of crosses were made for Type II resistance and DON accumulation in the field and greenhouse. Significant genetic variation was observed for severity of infection and accumulation of DON. While much of the variation appears to be additive, there was evidence of non-additive variation in the form of significant specific combining ability. It appears that significant progress could be made within the soft red wheat market class without excessive reliance on exotic sources of resistance. S2 lines from several populations were evaluated in the greenhouse for Type I resistance using an air brush to apply spores of the causal organism. Populations had been previously characterized in the field for open vs. closed flower types, head length, and other morphological traits. In one of the populations, there

appeared to be a significant difference among open vs. closed flower lines in terms of severity of infection. The S3 progeny of these lines will be evaluated in the field in 2001.

**Breeding Program**
David VanSanford

Numerous crosses have been made to various sources of resistance, within and outside the soft red wheat market class. Several elite breeding lines look promising in terms of reduced severity of infection at two field locations and in the greenhouse. One of these lines is being increased for possible release.

**FHB Fungicide and Biocontrol Test**
Donald Hershman, Paul Bachi, Dennis TeKrony and David VanSanford

Ten foliar treatments, eight involving foliar fungicides and two involving biocontrol agents (BCA's) were evaluated for efficacy against FHB at a mist-irrigated, inoculated test site in Princeton, KY during 2001. Seven of the ten treatments were part of the National FHB Uniform Fungicide and Biocontrol Test. Chemical products evaluated were Folicur, AMS 12619, BAS 505, and Tilt; the BCA's tested were a yeast (OH 182.9) and a bacterium (TrigoCor 1448). All treatments involved single applications applied at early flowering. Moderate disease levels were produced as a result of the inoculation and misting protocol used in the test. BAS 500 alone and in combination with Folicur and AMS 12619 resulted in significantly reduced disease incidence compared with the control. No treatment significantly reduced severity of FHB on individual heads. However, BAS 500 alone, at the highest tested rate (0.2 lb a.i.), and AMS 12619 significantly reduced field severity of FHB. Yields of plots treated with AMS 12619 yielded significantly higher than check plots. This difference was thought to be the result of partial FHB control in that other diseases were not a factor in the test. All treatments except Folicur applied and by itself and the yeast BCA significantly lowered levels of DON compared to the non-treated check. Percent visually scabby kernels and standard germination levels were significantly lower and higher, respectively, than the check plots for treatments involving AMS 12619 and BAS 500 alone at the highest test rated. Overall the most consistent performer in the test was AMS 12619 applied at 5.7 fl oz/A plus 0.125% Induce.

# NCR-184 2001 MICHIGAN STATE REPORT

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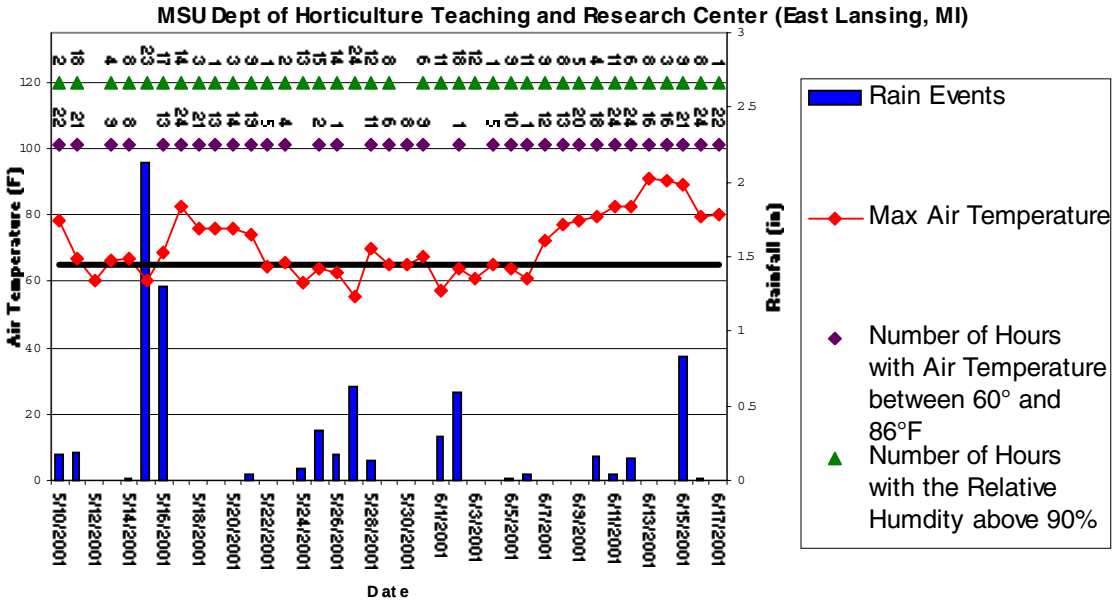
## STATE SITUATION:

FHB occurred at epidemic proportions in Michigan in 2001. The incidence varied across the state. Mild to moderate field symptoms occurred in the southern counties, and in the thumb. Moderate to severe symptoms occurred across the central counties. DON levels were low to moderate in the central counties, and low to very low in the rest of the state. Generally, the incidence and severity of head symptoms suggested that DON levels on the average should have been higher. The chart below shows rain events and temperature during flowering. The FHB prediction models indicated FHB was unlikely to occur based on these data. Flowering started about May 20-25 across the state, but because of the cool temperatures flowering progressed slowly and didn't end until the middle of June. It appears that the rain events of June 10-12th may have contributed significantly to the FHB epidemic, and because the infection period was late in flowering development this may explain why DON levels were low. DON data shown at http://www.cips.msu.edu/wheat/Vomitoxin analysis July 2001.htm was collected by analyzing grain threshed from heads collected pre-harvest and submitted to MSU by extension agents and agri-business dealers. Processors of white wheat were affected by the DON levels, and remain concerned about future FHB problems.



## RESEARCH REPORTS

A study was conducted in 2000 and 2001 to determine if wheat fields could be sampled before harvest to estimate DON levels of the harvested grain. The results of the two-year study can be found in the Proceedings of the 2001 National Fusarium Head Blight Forum.

Fungicide trials were conducted on wheat to determine if FHB and DON levels could be lowered. Specific information on the trials can be found in the Proceedings of the 2001 National Fusarium Head Blight Forum. None of the fungicide treatments increased yield. Disease severity and DON were reduced in a few treatments.

### DON Diagnostic Services Laboratory

The National FHB Initiative provided funding for regional DON testing. A complete report can be found in the Proceedings of the 2001 National Fusarium Head Blight Forum. The Neogen 5/5 test was used analyzer all samples for DON. Samples ranged in size from 25 g to 1000g. The majority of samples required milling and sub-sampling prior to analysis. From 8-1-00 through 7-30-01 there were 2,480 samples analyzed, and from 8-1-01 through 11-6-01 there were 3,371 samples analyzed.

## USEFULNESS OF FINDINGS:

The year 2001 FHB epidemic was unusual in that the visual expression of symptoms, both incidence and severity, would have predicted higher than detected levels of DON. This suggested the importance of estimating DON in individual fields. This is important not only to growers and processors, but estimates of DON variability in research plots has not been reported or studied.

## WORK PLANNED FOR NEXT YEAR:

The statistical study on sampling will be continued. Work will also continue on the development of recombinant antibody to DON, and identification of peptide mimics that may be useful in elucidating the receptor ligands associated with DON toxicity.

# NCR-184 MANAGEMENT OF FUSARIUM HEAD BLIGHT OF SMALL GRAINS MINNESOTA STATE REPORT –2001

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## Small Grains Crops and the FHB situation in Minnesota in 2001

The 2001 season was difficult for small grains production with state wheat yields averaging 44 bu/A, 5 b/A below last year. A cold spring and near-record rainfall in April delayed planting of most cereal crops into late May and contributed to reductions in the acreage of small grains. Minnesota's barley acreage continued to fall with only 145,000 acres harvested in 2001, making the year the smallest crop since 1881. Similarly, the oat acreage at 210,000 was the smallest crop since 1867. As much as 250,000 acres were not planted in Minnesota in the 2001 cropping season. Continued cold and wet weather in May slowed the establishment of crops. In June weather conditions favored small grains and crops that had gotten off to a late start developed well with few disease problems. By late June the cereal crops were looking great. In July temperatures above 85°F stressed crops and hastened crop development. Harvest was completed ahead of the 5-year average and the rapid maturing of crops likely contributed to the reduced yields. Rainfall in combination with the high temperatures in July promoted the development of some foliar diseases and Fusarium head blight. The late development of disease mitigated much of their impact and losses were generally below 5%. While Fusarium levels were low and yield losses minor in 2001, levels of deoxynivalenol in barley may prevent some of the crop from being sold for malt production.

## Overview of Present Research Programs

The Fusarium head blight research effort in Minnesota continues as a large collaborative. Faculty from the four departments of the College of Agriculture, Food and Environmental Sciences, three University of Minnesota Research and Outreach Centers and two USDA-ARS units (Cereal Disease Laboratory & Plant Science Research Unit) are involved in FHB research. While many researchers in Minnesota have projects funded by the USWBSI, researchers have also been supported by state funding.

The research being conducted in Minnesota includes breeding for resistance to FHB in wheat and barley utilizing classical and molecular techniques, studies aimed at improving the efficiency of breeding methodologies and selection of resistance, investigations on the pathogenic variation in *Fusarium graminearum*, examinations into the pathways of entry by Fusarium head blight, and the chemical and cultural control of FHB.

# NCR-184 COMMITTEE - MANAGEMENT OF HEAD SCAB IN SMALL GRAINS: 2001 MISSOURI REPORT

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**Winter Wheat Production in Missouri and the 2001 FHB Situation in Missouri:**

Most of the Missouri wheat acreage is soft red winter wheat with a minimal number of hard red winter wheat acres. Fall seedings for the 2001 winter wheat crop in Missouri totaled 900,000 acres, down 14 percent from the 2000 crop seeded acreage. Of the 900,000 acres planted, 760,000 acres were harvested. Missouri wheat production in 2001 totaled 41,040 million bushels, down 17 percent from last year's production of 49,400 million bushels. Missouri yields averaged 54 bushels per acre, up 2 bushels from last year's average yield of 52 bushels per acre.

2000-2001 was a good year for wheat production and a fairly poor year for Fusarium head blight in much of Missouri. Winter wheat planting began in southeastern Missouri in mid-September but planting was slow throughout the state as farmers waited for rain. Both planting and emergence were behind normal for most of the fall due to moisture shortages. Planting was completed by early November, and rains in November led to significant improvement in the crop. Record low temperatures during December and the first week of January were tempered by heavy snows and record snow cover duration. Winter survival was not a problem and spring stands were good. Wheat in southeastern Missouri began heading around April 15. Rainfall was normal during most of April and May but temperatures fluctuated from 7 to 8 degrees above normal during the weeks ending May 14 and May 21 to 6 to 10 degrees below normal during the week ending May 27 and 2-8 degrees below normal for the week ending June 3. Scab did occur in localized areas of the state, especially in central and northern Missouri.

The most serious pest problem on wheat during the 2001 season was true armyworm larvae. Although larvae fed heavily on foliage causing severe damage in fields in southern and central Missouri, little head clipping occurred. Leaf rust and Septoria leaf blotch came in late in the season and did not move up to the flag leaves until well past heading. For the second year in a row stripe rust was found in low levels throughout the state. Losses from foliage diseases were low for most of Missouri. Missouri did have a Special Local Need Registration (Section 24c Registration) for Tilt, which extended the time of application to Feeke's Growth Stage 10.5. However, because of the low level of foliage diseases few growers took advantage of the Tilt label change or the new federal label for Quadris on wheat.

Wheat harvest began around June 6 roughly 10 days behind 2000 and 4 days behind normal. However, harvest continued at a fast pace and was completed by about July 9, 6 days ahead of 2000 and 8 days ahead of normal.

**Current Scab Research at the University of Missouri:**

**Uniform Scab Fungicide Trial**: The University of Missouri did participate in the Uniform Scab Fungicide Trial coordinated by Dr. Marcia McMullen, NDSU. Six fungicide treatments were evaluated on Elkhart. FHB incidence ranged from 57.5 % on the untreated check to a low of 39.5 % in the AMS 21619 + Induce treatment. Scab severity on heads showing scab ranged from 28.4% on the untreated check to a low of 15.6 % on the Folicur + Induce treatment. In spite of differences in both incidence and severity between treatments in the trial there were no statistically significant differences in yield between the untreated control and any of the six fungicide treatments. Results of this trial are given in more detail in the report for this initiative project.

**Breeding Program:** Since scab resistance was introduced as an objective within the Missouri wheat breeding program in1993 we have successfully identified resistance in a number of pedigrees within the program. Among the first scab resistant varieties released was 'Ernie'. Its functional levels of type II resistance and excellent kernel quality under natural infection have resulted in invaluable economic return to Missouri growers particularly in years when scab has been a concern. Field and greenhouse screening programs at Missouri, have resulted in the identification of several pedigrees, different from Ernie by descent, that are potentially useful as sources of scab resistance. Results from evaluations of ~ 300 entries being advanced into advanced yield trials in 2001 identified 68 lines from 49 pedigrees that had field indices (incidence x severity) and Type II reactions from greenhouse evaluations of $\leq$30%. Of these pedigrees, 37 differ from Ernie by descent and are not derived from Chinese sources of resistance. Lines were evaluated relative to the resistant check Ernie (greenhouse FHBI = 14% and field index = 11.5%) and the susceptible check MO 94-317 (greenhouse FHBI = 92% and field index = 60%). Of the 68 lines that will be retained, 32 had greenhouse scores $\leq$Ernie while 38 had field scoress $\leq$Ernie. They provide either different sources or different types of resistance in adapted genetic backgrounds and their combination with other exotic sources and types of resistance should enable accelerated development and release of scab resistant varieties that possess either more effective or more stable scab resistance.

One of these pedigrees, MO 11769/Madison has yielded at least 4 lines that have levels of type II resistance that are < Ernie. We expect to release one of these, MO 980525, in 2002. After being verified in Missouri, MO 980525 was entered in both the 2001 and 2000 Uniform Winter Wheat Scab Nurseries where its type II resistance was confirmed by several programs. In the 2000 Northern Winter Wheat Scab Nursery, this line and an earlier maturing sister line MO 980725 ranked 1$^{st}$ and 2$^{nd}$ of 29 entries for type II resistance, 6$^{th}$ and 7$^{th}$ for field index and MO 980525 ranked 6$^{th}$ for low DON. In its first year of testing in the Eastern Soft Red Winter Wheat Cooperative Nursery, MO 980525 performed well across the US corn belt finishing 1$^{st}$ at Woodburn, IN 3$^{rd}$ at Urbana IL, and 6$^{th}$ at Wooster, OH. It was in the top yield group at other corn-belt locations suggesting it could be a valuable line, meeting the USWBSI objective of providing effective scab control.

**Germplasm Evaluation Center:** Research funded by the National Wheat and Barley Scab Initiative has led to the systematic evaluation of types I, and II resistance to scab and kernel quality under inoculation of accessions from targeted geographical regions of the world.

Approximately 3800 accessions from geographical areas in Asia, South America and Eastern Europe where resistance has been identified or where environmental conditions are conducive to scab development have now been screened at Missouri. From initial screens of Asian and Italian germplasm, 50 accessions that possess good to excellent levels of type II resistance, reduced incidence and good kernel quality under field inoculation have been identified, purified and verified through 2 generations of progeny testing. Twenty of these accessions were distributed to 15 collaborating programs within the initiative in 2000 and some have been shown by other researchers to possess resistance. The remaining 30 lines will be distributed to interested breeders following the scab forum in Cincinnati in December 2001. Approximately 2000 Yugoslavian accessions have also been screened for resistance. Sixty-eight accessions have held up well under two cycles of greenhouse and field evaluation at Missouri. An additional 246 accessions will be evaluated for the second cycle in the 2001/2002 crop season. Dr. Paul Murphy at North Carolina State University concurrently screened a sub-sample of this second group of lines and 70 lines of the 246 lines identified as promising at Missouri were also identified in the NC State program as having good to excellent levels of type II resistance. These lines will be purified and re-screened prior to distribution to interested breeders. The majority of resistant accessions (>90%) are landraces. Many are tall and late and significant pre-breeding will be required to transfer their otherwise excellent levels of resistance into adapted backgrounds. During the fall and winter of 2001/2002, 784 accessions from Romania (182), Hungary (283) and Bulgaria (319) will be evaluated for the first time under greenhouse inoculation. The lines will be planted in the field and the first data on resistance under spray inoculation will be collected in 2002. Based on the frequency of resistant accessions identified to date, it is anticipated that resistance will also be identified in this collection of germplasm.

**CIMMYT Germplasm Introduction Partnership:** Approximately 57 wheat lines introduced into the US through the National Scab Initiative's partnership with CIMMYT were distributed to interested breeders in the spring of 2001. In November 2001 wheat germplasm from Argentina (107 lines), Brazil (19 lines), Japan (15 lines) and CIMMYT (51 lines) will be introduced. In addition to genes for scab resistance, CIMMYT germplasm also contains resistance to other important US pathogens (e.g. Septoria spp., leaf rust, and barley yellow dwarf virus). Lines will be quarantined and distributed to interested breeders in the spring of 2002.

**Genetic Studies**: Studies investigating the inheritance of resistance in MO 980505 have been initiated and those characterizing resistance in Ernie are nearing completion. Both conventional and molecular genetic analyses of these sources of resistance are being conducted.

# NCR-184 REPORT 2001 - NORTH DAKOTA

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**The FHB situation in North Dakota in 2001 and its impact on small grain crops.** Results provided by Marcia McMullen, extension plant pathologist, who conducted a survey of 1500 grain crops across ND in 2001. Statewide, Fusarium head blight (FHB) occurrence was about the same in 2001 as in 2000. Individual severely affected fields of spring wheat could be found in parts of central ND and fields of durum in north central, west central and northwest ND. In northwest ND there were many late planted durum crops which may have increased the problem in that region. FHB was noted in 70% of spring wheat and durum and 42% of barley fields statewide. Overall, wheat losses to FHB in 2001 were moderate, averaging about 4% (range 0.1 to 34.2%) in spring wheat, and 7.2% (range 0.1 to 80.%) in durum. Yield loss in barley averaged less than 1% (range 0.1 - 2.3%).

**Overview of present research programs.** The FHB research effort at NDSU continued to be a large one in 2001. Six NDSU departments, three NDSU Research & Extension Centers, and the USDA-ARS Northern Crop Sciences Laboratory located on the NDSU campus, all were involved in research on FHB. Many of the projects received funding from the scab initiative and reports from those investigators are included in the forum proceedings. Several of the projects are cooperative efforts between state and federal scientists.

While the principal research emphasis at North Dakota State Univ. continues to be on breeding for resistance to FHB, and classical and molecular genetics of resistance, there is active research in several other areas including epidemiology, soil microbial ecology, physiology and biochemistry, grain quality, food science, disease survey, and chemical control.

FHB resistance is being sought in breeding programs for spring wheat, durum wheat, and barley. Methods to obtain resistant varieties include both conventional and molecular plant breeding methods. These efforts utilize combinations of inoculated-irrigated field nurseries and greenhouse testing. Durum and barley breeding programs are also using screening nurseries in China.

As reported previously, the highlight of year 2000 was the release of ‘Alsen’, a hard red spring wheat combining moderately high resistance to FHB with excellent grain quality, good agronomics and resistance to leaf rust and stem rust. Over 450,000 A of Alsen were produced in ND in 2001 and there is every expection that it will be planted on more than 2 million production acres in 2002.

Units involved in FHB Research.

NDSU:

*Dept. of Plant Pathology

*Dept. of Plant Sciences.

*Dept. of Soil Science.

*Dept. of Cereal and Food Sciences.

*Dept. of Agricultural Engineering.

*Dept. of Veterinary Science and Microbiology.

*NDSU Extension Service

*NDAES Research-Extension Centers
at Langdon, ND, Carrington, ND, Minot, ND.

USDA

USDA-ARS Northern Crop Sciences Laboratory, Fargo

# NCR-184 STATE REPORT NEW YORK 2001

Gary C. Bergstrom

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## FHB situation in 2001 in New York

There was only minor occurrence of FHB in the soft winter wheat production area of New York in 2001. Although moisture was present during the early flowering period, conditions were not favorable for inoculum production in the period preceding flowering. Also, night temperatures during flowering were lower than optimal. Dry conditions prevailed during grain maturation. In general, the disease had no impact on grain yields or test weights. Vomitoxin contamination above 1 ppm was uncommon, though a few pockets of higher vomitoxin were observed. A cooperative survey between Cornell and the Star of the West Flour Mill in Churchville, NY was continued in 2001 to assess associations among vomitoxin level, incidence of Fusarium infected grains, and geographic location of production fields.

## Programs and personnel involved in FHB research

Winter wheat cultivar evaluation

One site of the northern uniform winter wheat scab nursery is located at Ithaca, NY. Despite provision of grain spawn inoculum and overhead irrigation in the plots, only modest levels of scab occurred. See the report by Sneller, Lipps and Herald for a summary of results at all locations. One New York line, NY87047W-7388, was for the second year among the top entries for reduced FHB severity and lowered DON content. In addition to the standard 45 cooperative lines, an additional 50 regionally-adapted varieties and lines are also being evaluated. Also, scab reaction of over 75 lines derived from crosses of New York-adapted winter wheat cultivars with Chinese sources of resistance is being assessed.
*Personnel: Mark Sorrells and David Benscher (CU Plant Breeding); Gary Bergstrom and Stan Kawamoto (CU Plant Pathology)*

Fungicide evaluation

One site of the uniform fungicide trial is located at Aurora, NY. See the summary report by McMullen and Milus for multistate results. Fungicides and biocontrols were applied by foliar spray utilizing the dual (forward-backward) flat fan nozzle system configured by North Dakota researchers. Grain spawn inoculum was spread in the border areas and macroconidial suspension was also applied to flowering spikes. The plots were not irrigated. Only modest levels of FHB developed though harvested grain from nontreated plots averaged 4.6 ppm of DON. Leaf blights were not significant at this location. No treatment resulted in a significant increase in yield, though plots treated with Folicurplus the biocontrol TrigoCor 1448 had the highest yields (Table 1). Various treatments induced moderate reductions in scab incidence, Fusarium damaged kernels, DON contamination, and im-

provement of test weight, but only AMS21616 reduced DON to below 2 ppm.
*Personnel: Stanley Kawamoto, Christine Stockwell, Gary Bergstrom (CU Plant Pathology); William Cox and Dilwyn Otis (CU Crop and Soil Sciences)*

Biological control

Microbial antagonists of *Fusarium graminearum* are being isolated, characterized, and tested for for potential application to wheat spikes, seed, and crop residue. See the report by Stockwell et al in this volume for a summary of tests on field application to spikes.
*Personnel: Christine Stockwell, Stanley Kawamoto, Gary Bergstrom (CU Plant Pathology); Wilmar da Luz (Embrapa Trigo, Passo Fundo, Brazil)*

Aerobiology/epidemiology

Sandra Maldonado-Ramirez completed a Cornell University Ph.D. Dissertation entitled, "*Aerobiology of the Wheat Scab Fungus, Gibberella zeae:Discharge, Atmospheric Dispersal, and Deposition of Ascospores*" in August 2001. Ascospores were discharged from corn stalk substrates primarily during daylight hours. There appeared to be an association of major ascospore discharge with moderately warm temperatures. Using remote piloted aircraft we found that viable propagules of *Gibberella zeae :* are present in the planetary boundary layer of the lower atmosphere under a wide range of environmental conditions at the time of wheat flowering, suggesting a potential for regional dispersal of airborne inoculum. Gravitational settling of spores onto wheat spikes appeared to occur principally during late evening to early morning hours. See the report by Shah and Bergstrom in this volume on spatial aspects of FHB epidemics in New York that also points to the contribution of inoculum from outside of wheat fields. David Schmale has begun a Ph.D. project on the aerobiology of *G. zeae* relative to sources of inoculum for FHB.
*Personnel: Gary Bergstrom, David Schmale (CU Plant Pathology); Elson Shields (CU Entomology); Sandra Maldonado-Ramirez (University of Puerto Rico, Mayaguez); Denis A. Shah ( Private Consultant); David Gadoury (CU Plant Pathology, Geneva campus); Don Aylor (Connecticut Ag Experiment Station); Robert Bowden, Kurt Zeller (Kansas State University)*

**Table 1.** Effect of foliar treatment at anthesis on scab incidence, *Fusarium*-damaged kernels, yield, test weight, and DON contamination in Caledonia winter wheat in Aurora, NY in 2001.

| Treatment and amount | Scab (spike) incidence on 1 Jul (%) | *Fusarium* damaged kernels (%) | Test weight @ 13.5% moisture (lb/bu) | Yield @13.5% moisture (bu/A) | DON ppm |
|---|---|---|---|---|---|
| Nontreated | 4.8 | 10.0 | 57.0 | 75.4 | 4.6 |
| AMS 21616 (5.7 fl oz/A) + Induce (0.125% v/v) | 3.1 | 2.7 | 59.0 | 76.2 | 1.2 |
| BAS 500 (0.2 lb a.i./A) + Induce (0.125% v/v) | 4.2 | 7.1 | 58.3 | 75.2 | 3.6 |
| BAS 500 (0.1 lb a.i./A) + Induce (0.125% v/v) + Folicur 3.6F (2 fl oz/A) | 4.8 | 3.9 | 59.2 | 72.7 | 3.2 |
| Folicur 3.6F (4 fl oz/A) + Induce (0.125% v/v) | 2.2 | 5.3 | 58.8 | 75.7 | 2.0 |
| Messenger (2.25 oz/A) 3 applications* | 3.7 | 6.6 | 58.4 | 76.2 | 3.2 |
| Serenade (6 lb/A) biocontrol | 7.0 | 8.3 | 57.5 | 73.4 | 5.6 |
| TrigoCor 1448 biocontrol | 4.8 | 7.7 | 58.0 | 66.8 | 4.0 |
| TrigoCor 1448 biocontrol + Folicur 3.6F (4 fl oz/A) + Induce (0.125% v/v) | 3.5 | 6.8 | 59.5 | 86.5 | 3.0 |
| TrigoCor 4712 biocontrol | 4.3 | 7.0 | 57.9 | 67.5 | 3.1 |
| TrigoCor 4712 biocontrol + Folicur 3.6F (4 fl oz/A) + Induce (0.125% v/v) | 4.0 | 5.6 | 59.0 | 77.3 | 3.0 |
| **LSD (*P*=0.05)** | 2.1 | NS | NS | NS | NS |
| **CV (%)** | 101.9 | 202.9 | 1.7 | 14.1 | 68.7 |

* Messenger was applied at Feekes growth stages 4.2, 10, and 10.5.

# NCR-184 MANAGEMENT OF HEAD SCAB OF SMALL GRAINS: 2001 OHIO REPORT

Patrick E. Lipps[1]*, Laurence V. Madden[1], Samia El-Allaf[1], Jessica Engle[1],Clay Sneller[2] and Anju Gupta[2]

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1,110,000 acres of soft red winter wheat were planted in Ohio in the fall of 2000, but due to winter injury and the more favorable price of soybeans only 900,000 acres were harvested in 2001. Frost heaving reduced stands in some fields that were shallow planted, but mild spring conditions favored tiller development. Weather conditions in April and the first week of May were relatively dry and cool which limited perithecial development on corn residues. Precipitation events became more frequent during mid to late May in many regions of the state with most locations reporting from 2 to 4 days of measurable precipitation during the 7 days prior to anthesis. However, average daily temperatures for most locations in the state were generally below 15C between 22 May and 4 June when most of the wheat was in anthesis.

There was moderate levels of head scab in some central and north west counties, though most counties had only trace levels of disease. Fields with the highest level of scab did not exceed 20% incidence. Yield losses probably range from 0 to 5%, but state wide the average yield loss was less than 1%. Dry temperatures during and after anthesis probably limited scab severity and DON accumulation in the grain.

## Research

Research efforts at OSU were focused on: Disease forecasting, screening germ plasm, breeding for disease resistance, and evaluation of fungicide efficacy.

**Disease forecasting:** De Wolf, Madden, Lipps

A) Erick De Wolf and Larry Madden have developed a scab risk assessment model based on historical weather information and scab severities. The model was constructed from hourly temperature, relative humidity and precipitation data from 50 location years from Ohio, North Dakota, Kansas and Missouri. Stepwise regression identified two time periods in which three environmental parameters were critical to reasonably accurate prediction: 1) (Model 1) duration of precipitation and duration of temperature between 15 and 30°C for 7 days prior to crop anthesis and 2) (Model II) duration of temperature between 15 and 30°C and corresponding relative humidity above 90% for the 10 days post anthesis.

These two models were used to estimate the risk of head scab during the 2001 wheat growing season using hourly weather data from six weather stations in Ohio. Calculated head scab risk probabilities ranged from 32% to 84% for Model 1 and from 4% to 78% for Model II. In no case did both models predict a high risk for head scab at any location. Based on these results the head scab risk prediction was reported to be low to moderate

depending on the location in the state. Head scab risk predictions were posted on the Ohio State University Oho Field Crop Disease web page (www.oardc.ohio_state.edu/ohiofieldcropdisease/) during the critical time of disease development through harvest. Weekly reports were provided to wheat growers through the Ohio State University Extension electronic newsletter Crop Observation and Recommendation Network (C.O.R.N.) (www.ag.ohio_state.edu/~corn/agcrops.html/).

B)We are participating in a cooperative program with North Dakota, South Dakota, Indiana, Manitoba and Pennsylvania to monitor inoculum levels, environmental parameters and disease severity in replicated plots. Information from multiple sites is being used to validate and improve the head scab risk assessment models and to determine the importance of pathogen inoculum level in predicting disease occurrence. The cooperative effort is necessary to assess the effect of regional variation in cropping practices, tillage and climate on inoculum levels and subsequent disease level across the wheat producing regions. Volumetric air sampling and a wheat head bioassay are being used to monitor fluctuations in the levels of inoculum reaching heads. Automated environmental monitoring instrumentation is used to measure temperature, relative humidity, precipitation, solar radiation, wind speed, and moisture status of the crop. This is the third year of our monitoring project.

C) Samia El-Allaf is examining the spatial and temporal distribution of scab in fields in order to have a better understanding of the disease epidemiology. Disease assessments were recorded from four research plots in two locations (Wooster and Hoytville). Data is currently being analyzed for the 2001 season.

**Breeding for scab resistance:** Sneller, Lipps, Gupta, Engle

A) The departments of Hort and Crop Science and Plant Pathology are cooperating to develop varieties with resistance to head scab. Four avenues of research are being followed; 1) evaluation of varieties and advanced lines for resistance, 2) evaluate and select lines with combined resistance to FHB and Stagonospora blotch, 3) incorporate resistance from sources identified within the breeding program into elite lines and 4) increase the level of resistance above current levels by incorporating new genes and gene combinations from diverse germplasm sources. During the year the following germ plasm were screened for resistance in field nurseries: Advanced breeding lines, scab resistant by Stagonospora resistant crosses, and early generation head row selections.

B) Anju Gupta completed a molecular and pedigree analysis of sources of resistance to Fusarium head scab. Twenty-three lines were evaluated based on pedigree relationships and resistance reactions. The analysis indicated that Ning 7840 and Sumai 3 possess unique alleles at 3BS which are not shared by other resistant genotypes. Based on geneetic studies, Ning 7840 and Frontana and Ning 7840 and Freedom have unique genes for resistance. Marker studies indicate that Freedom and Ning 7840 have different resistance alleles at 3BS. Results indicate that SSR genotyping of ancestral lines may help establish the degree of relatedness in resistant germplasm. Markers on 3BS and 2AS may be useful for identifying new sources of resistance with unique resistance genes.

C) Jessica Engle is evaluating inoculation procedures for identifying different reaction types and possible of resistance. Six lines previously screened for resistance in the 1999 Northern Uniform Winter Wheat Scab Nursery were selected based on their different reactions (incidence and severity ). Plants were inoculated in the greenhouse using four different techniques: single floret syringe inoculation, atomizing conidia on the head, placing ascospores on glume juncture and placing ascospores onto extruded anthers. Results indicated that highest disease levels occurred from atomizer and anther inoculation procedures. Results indicated that ranking of wheat lines according to severity assessment varied with inoculation technique and did not necessarily predict reaction in the field.

**Fungicide efficacy:** Lipps, El-Allaf

We are participating in the cooperative effort of the US Wheat and Barley Scab Initiative Chemical and Biological Control research area headed by M. McMullen and G. Bergstrom. Five fungicide treatments and two biologicals were evaluated in 2001 using procedures and rates outlined by the project leaders. Plots were inoculated by spreading infested corn kernels on the soil surface. Mist irrigation was used to provide moisture levels favorable for infection and disease development during anthesis. The biological agents (TrigoCor 1448 and USDA OH182.9) did not limit scab development. AMS21619 and BAS 505 reduced the severity of scab and the level of DON to about 50% of the untreated control. BAS 505, BAS 505 plus Folicur and AMS21619 treated plots had significantly higher yield (LSD 7.2 bu/A) than the untreated control.

**Evaluation of commercial wheat varieties:** Lipps

Commercial wheat varieties submitted to the Ohio Wheat Performance Test were evaluated for Fusarium head blight in the test planted near Circleville, Oh. Severity assessments for the 46 soft red winter wheat lines ranged from 5% to 63%. Based on an LSD (P=0.05) of 11.5%, the varieties with the lowest scab severities were Valor, Patton, Dynasty, Hopewell, Classic RW1517, Coker 94774, Thompson TS 6020, Sabbe, and Wellman W9830. The four triticale lines in the test had scab severity levels less than 2%. Data for this trial can be obtained online (http://www.oardc.ohio-state.edu/wheat2001/).

# NCR-184, MANAGEMENT OF HEAD SCAB OF SMALL GRAINS 2001 SOUTH DAKOTA STATE REPORT

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## 2001 SCAB DEVELOPMENT IN SOUTH DAKOTA

Minimal scab on wheat occurred in most part of South Dakota in 2001. The statewide average of scab indices was estimated at 1%. Higher scab on spring wheat was observed in the north central region. In some spring wheat fields near Selby (Walworth County), in excess of 40% scab index was observed. (M. Draper and Y. Jin).

## CURRENT RESEARCH PROJECTS

**Germplasm introduction and evaluation:** The overall project goal is to identify new sources of scab resistance in spring wheat and to introgress the resistances into adapted materials. Spring wheat accessions from targeted regions of the world and relatives of wheat were evaluated in inoculated field nurseries and in the greenhouse. A germplasm screening system utilizing three nurseries was implemented in the germplasm screening project. In 2001, 1262 accessions of spring wheat were evaluated in the Preliminary Screening Nursery (PSN) and 141 accessions were selected based on scab index and seed infection. These selections from PSN will be evaluated in the greenhouse to derive entries for Elite Germplasm Nursery (EGN) of 2002. One hundred thirty-one accessions selected from the 2000 PSN were evaluated in the 2001 EGN. These selections will continue to be evaluated in the 2002 EGN. Five accessions of resistant selections were entered into the Uniform Regional Scab Nursery for spring wheat for testing at multiple environments and for direct access by researchers. Elite selections were used for crossing to introgress the resistance into adapted germplasm. (X. Zhang and Y. Jin)

**Epidemiology:** The overall goal of our research in scab epidemiology is to understand the sources, production, and survival of scab inoculum to provide a knowledge base for the development of accurate disease forecasting systems and comprehensive disease management strategies. We initiated research to address questions concerning scab inoculum production and survival, specifically effects of environmental conditions on inoculum production, and inoculum (ascospores) survival and accumulation on plant surface. As part of a multi-state collaboration, environmental conditions and disease development were monitored in plots in eastern South Dakota to relate certain environmental factors to inoculum production and disease progress. We continue to develop and utilize the soil surface wetness sensor to monitor moisture at the soil/air interface and its effects on inoculum development. (L. Osborne and Y. Jin)

**Breeding for scab resistance in spring wheat:** The project emphasizes on simultaneous improvement of scab resistance and agronomic traits in breeding materials. Established off-season nurseries and mist-irrigated greenhouse and field screening nurseries are being

utilized to accelerate breeding efforts. Three generations of breeding materials are evaluated for scab resistance: two generations in the greenhouse and one generation in the field. Approximately 8,000 individual hills are evaluated in the greenhouse nurseries and 3,000 rows are screened in the field nurseries. Both the field and greenhouse nurseries are inoculated with infected corn and conidial suspensions. The breeding population contains sources of resistance that can be traced back to Sumai 3, from other introduced sources, and advanced breeding lines that have various “field tolerance” qualities. The off-season nursery aids in the simultaneous selection for resistance and desirable agronomic characteristics. We have seen a continued increase in the number of lines that have good agronomic performance along with good scab resistance. A new hard red spring wheat variety, "Walworth" (SD3348) was released in 2001. Walworth has high grain yield and good bread-making characteristics and has improved scab resistance. (D. Gustafson, R. Devkota, Y. Jin)

**Breeding for scab resistance in winter wheat:** The objective is to use traditional breeding techniques to develop scab resistant hard winter wheat cultivars. Breeding efforts for improved head scab resistance in winter wheat have been focused on i) characterizing scab resistance or tolerance among commercially grown cultivars and elite and preliminary lines from SDSU and regional breeding programs, ii) identifying winter wheat germplasm sources that show a high level of scab resistance, and iii) developing populations segregating for scab resistance and desirable agronomic traits. Mist-irrigated greenhouse and field screening nurseries have been used to evaluate the material. The following nurseries were screened for scab resistance in 2001: Northern Regional Performance Nursery; Winter Wheat Regional Scab Nursery; South Dakota Crop Performance Trials; SDSU Advanced Hard Red and Hard White Yield Trials; SDSU Preliminary Hard Red and Hard White Yield Trials. (A. Ibrahim, D. Gustafson, Y. Jin)

**Fungicide efficacy studies:** South Dakota participated in the uniform fungicide trial for scab suppression. Two hard red spring wheat cultivars were planted at three locations each and treated at anthesis with fifteen treatments. The treatments were also applied to two hard red winter wheat cultivars planted at a single location. One spring wheat location was lost due to glyphosate drift injury. Erratic stand in the winter wheat added variability to the data. Plots were evaluated for protection of the flag leaf against leaf diseases as well as for average incidence of scab infected heads, average head severity of scab, average plot severity of scab, Fusarium damaged kernels (FDK), deoxynivalenol (DON) content in the harvested grain, grain yield, protein and test weight of harvested grain. Under ambient conditions, scab was not severe at any of the study locations. Leaf diseases, predominantly leaf rust, occurred late in the test locations and no significant reduction in disease was realized. (M. Draper and K. Ruden)

**Molecular biology of scab resistance:** One of big obstacles in fighting scab epidemics is that little is known about the pathogen-host interaction, particularly at molecular level. Our research aims at addressing this problem by getting insight into the molecular mechanism of *Fusarium graminearum*-wheat interaction. Our goal is to identify, clone and study the essential genes that control the initiation of FHB pathogenesis in wheat spikes, so that their expressions can be manipulated in favor of controlling FHB. Comparing differential expression of genes at the early stages of FHB pathogenesis between the FHB-inoculated and the

health control wheat spikes should lead to the identification and cloning of, at least some of such essential genes. So far, seven expressed sequence tags (ESTs) have been observed to be specific to the FHB-inoculated Sumai 3 spikes in our repeated experiments. Four such ESTs were cloned and sequenced. Blasting Genebank with these ESTs as query sequences has revealed no homologue with any known R or PR gene. Northern and Southern Blottings revealed that two of the four cloned ESTs belong to pathogen *F. graminearum* and the rest are wheat. We are currently cloning the other three. (Y. Yen and D. Xing)

**Implementation of marker-assistant selection in South Dakota wheat breeding programs:** The goal of this project is to implement marker-assisted selection (MAS) in the SDSU spring and winter wheat breeding programs and the USWBSI spring wheat germplasm program. To reach our goal, we will adopt useful markers from other programs while incorporating new marker selection into our breeding routine. As the first step toward our goal, we have screened 78 elite breeding materials from SD spring wheat breeding program and 87 elite selections from USWBSI spring wheat germplasm program for SSR markers with primer sets gwm533, gwm493 and gwm389. Sumai 3 and Wheaton were used as the controls. The results showed that 38 of the 78 elite breeding lines screened have the Qfhs.ndsu-3BS-gwm493 marker identified by Anderson et al. (2001) but only five also have the Qfhs.ndsu-3BS-gwm533 marker. Of the 87 elite germplasm selections screened, 27 lines have the Qfhs.ndsu-3BS-Xgwm493 markers; 31 lines have the Qfhs.ndsu-3BS-Xgwm533 marker; and 26 lines have the Qfhs.ndsu-3BS-Xgwm389 marker. The Xgwm533-120bp, the Xgwm493-140bp and the Xgwm493-160-bp markers observed in our elite breeding lines were also observed among the elite germplasm selections. In addition, new markers Xgwm389-130bp, Xdwm533-300bp and Xgwm533-165bp were also observed among the selections. (Y. Yen, X. Zhang and Y. Weng)

**Biological control studies:** Screening trials of biological control agents to control FHB were continued in 2001. During the summer of 2001 two bacterial biocontrol agents from the SDSU collection were applied in field plots equipped with misting systems, together with biocontrol agents from other research groups. However, initial plantings in Brookings suffered from Roundup drift, and replantings experienced extensive insect damage. Two ground bed trials in the greenhouse were carried out. Folicur significantly reduced FHB, but none of the biocontrol agents employed (two from South Dakota and two from Brazil) significantly reduced FHB. However, some of these biocontrol agents reduced FHB, even though it was not statistically significant. The BCAs may have been washed off by the overhead misting apparatus that covered the plants in the greenhouse, and heavy pathogen inoculum may have overwhelmed the biocontrol agent inoculum. These problems will be corrected in next year's trials. Based on partial sequencing of ribosomal RNA, membrane fatty acid analysis, and standard physiologic testing, the four South Dakota biocontrol agents have been verified as members of the genus *Bacillus*, with an as yet uncertain species affiliation. (B. Bleakley and M. Draper)

## PERSONNEL INVOLVED IN SCAB RESEARCH

**Project and Researchers:**

Small Grain Pathology: Y. Jin, X. Zhang, L. Osborne, Y. Weng
Extension Plant Pathology: M. Draper, K. Ruden
Winter Wheat Breeding: I. Ibrahim, S. Kalsbeck, R. Little, D. Gustafson
Spring Wheat Breeding: D. Gallenberg, R. Devkota, D. Gustafson, L. Peterson, G. Lammers
Cytogenetics-Molecular Biology: Y. Yen
Soil Microbiology: B. Bleakley

# VIRGINIA 2001 NCR 184 REPORT ON FUSARIUM HEAD BLIGHT

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## Production and Fusarium Head Blight Development

In Virginia, 175,000 acres of soft red winter (SRW) wheat were harvested in 2001, with a state average yield of 57 Bu/A. Annual declines in the price of wheat since 1996 have resulted in corresponding declines in harvested acres, which currently are 100,000 less than in 1996. Widespread epidemics of Fusarium head blight (FHB) have not occurred in Virginia since 1998, when the crop was devastated. The incidence of FHB was low, yet noticeable, in the 2000-2001 season due to dry conditions prevailing throughout much of the flowering stage. One seed grower in Hanover County suffered severe damage from FHB in an irrigated wheat-production field, and the seed failed to pass certification standards due to a high incidence of scabby seed.

## Evaluation of Four Fungicides and Two Biological Agents for the Control of Fusarium Head Blight in the Soft Red Winter Wheat Cultivar Roane in Virginia in 2001.

Erik L. Stromberg, Department of Plant Pathology, Physiology, and Weed Science.

The soft red winter wheat cultivar Roane was no-tillage seeded into corn residues in the fall of 2000 to establish FHB control plots. Four fungicides (Folicur 3.6F, AMS 21619 F, BAS 505 G, Stratego 250E) and two biological agents (a yeast isolate and a *Bacillus subtilus* isolate) where applied to the wheat at Zadoks' Growth Stage 59 (9 May). The plots were scored for incidence and severity of FHB on 5 Jun at Zadoks' GS 85. The plots were harvested on 28 Jun. Seed samples were taken to determine 1000 kernel and bushel weights and sample were sent to Michigan State University for DON determinations for each treatment. The incidence and severity of FHB in the non-treated control was 9% and 10.5%, respectively. All treatments provided significantly lower incidences and severity ($P \leq 0.05$) over the non-treated control. The fungicide treatments had ranges of FHB incidences of 3.8-5.3% and severities of 2.3-2.8% while the two biological agents had incidences from 3.8-4.3% and severities from 3.0-3.8%. All treatments had significantly higher 1000 kernel weights (range: 31.5-32.9 g) ($P \leq 0.05$) over the non-treated control's 1000-kernel weight (30.7 g). Grain yield was significantly greater ($P \leq 0.05$) for three of the seven treatments (range: 7085-7267 kg/ha) over the non-treated control (6537 kg/ha) at a standard 13.5% moisture.

## Breeding and Mapping Research on Fusarium Head Blight

Our program continues to identify and verify wheat genotypes possessing resistance to FHB through collaboration with colleagues in the Uniform FHB Winter Wheat Screening Nurser-

ies. Incorporation and pyramiding genes conferring scab resistance into high-yielding SRW wheat genotypes continues to be a major objective of our breeding program. This is being accomplished through the development and selection of top-cross, backcross and doubled haploid populations. Results from our initial mapping studies indicate that QTLs in addition to the one located on chromosome 3BS confer resistance to FHB in Chinese line W14. Mapping studies will be intensified via use of doubled haploid populations and concurrent mapping in populations with type II and other types of resistance. This research has the potential to identify new QTLs associated with scab resistance, provide additional markers linked to previously reported QTLs, and to identify markers that are effective across genetic backgrounds; all of which are essential for successful exploitation of marker-assisted selection.

# NOTES

U.S. Wheat & Barley
Scab Initiative

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