# 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

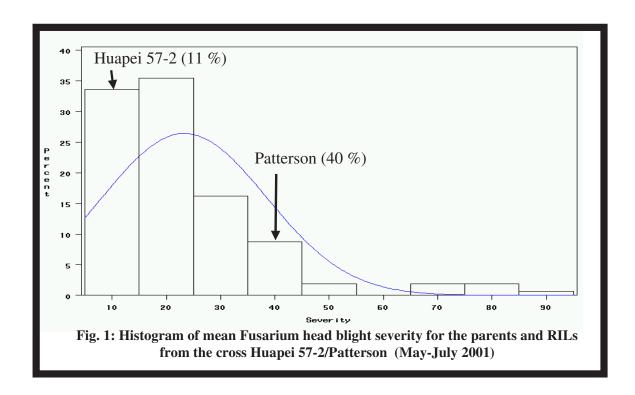
Primers for the SSRs published by Röder et al. (1998) were screened for polymorphism among the parents. Using polymorphic markers, bulked segregant analysis (Michelmore et al., 1991) using 8 putative resistant and 8 putative susceptible RILs was initiated

# **RESULTS AND DISCUSSION**

### FHB evaluation

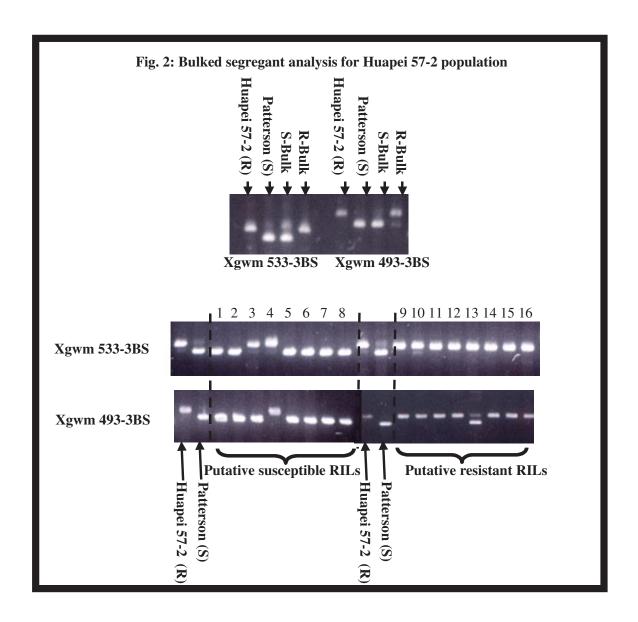
There was significant variation among RILs for FHB severity (data not shown). Broad-sense heritability and heritability on an entry mean basis were 0.43 and 0.87.

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.



# **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.



# 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<sub>3</sub> 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<sub>3</sub> 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<sup>4</sup> conidia /ml) with a hypodermic syringe into a single central floret on the spike, 2) atomizing a macroconidial suspension (6 x 104 conidia/ml) onto the entire spike surface to the point of run-off, 3) applying 1 µ1 drops of ascospore suspensions (6 x 106 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 Fðl drop of ascospore suspensions (6 x 106 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. Mistirrigation 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: nonmisted, 2, 4, and 8 mm of water per day. Split-block treatments were inoculated versus noninoculated. Plots were inoculated with macroconidia in an aqueous suspension adjusted to 100,000 spores per ml using a CO<sub>2</sub> 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<sub>5</sub> lines, derived from 53 F<sub>4</sub> 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<sub>g</sub> lines and 13 doubled haploid (DH) lines were evaluated simultaneously for scab resistance in a mistirrigated 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<sub>6</sub> 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

2001 National Fusarium Head Blight Forum	
between resistant progeny derived from three BC <sub>3</sub> F <sub>1</sub> and 16 BC <sub>2</sub> F <sub>1</sub> populations and their recurrent parents. Near-isogenic lines with type II resistance incorporated into adapted SF 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. 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> 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:	ANOV	/A for FH	В
severity in	1 a 9x	diallel cr	ross of
SRWW so	creene	d in the gr	reenhouse
at Lexingt	ton, K	Y 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 18.4	47%	CV 101.2	9

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<sub>1</sub> 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:	ANO'	VA for FI	łВ
severity in	a 8x	8 diallel c	ross of
SRWW so	creene	ed in the f	ield at
Lexington	, KY	2001.	
Source	df	SS	MS
Crosses	27	93533.4	3464.2**
GCA	7	28388.3	1055.5**
SCA	20	65145.1	3257.3**
Error	791	280102	354.1
**P<0.01			
Mean 67.0	)5%	CV 28.07	7

Table 5:	ANOVA	for DON	levels			
in a 8x8 d	liallel cro	oss of SRV	VW			
screened	in the fie	eld at				
Lexingtor	n, KY 20	01.				
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	1				
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<sub>1</sub> 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	6.6	5.3	0.9	10.54	-4.13
CK 9663	#	40.7	16.6	58.0	29.0	21.0	9.6	6.7	26.45	*2 <del>7</del> .7
CK 9474	#	28.2	19.8	22.6	8.9	29.5	14.2	14.4	9.52	-2.07
Roane	24.6	49.4	8.62	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	6.7	17.4	6.2	5.3	5.61	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
										ı

#  $F_1$  was not made. \*Exceeds standard error

Table 3: Mean FHB Severity (%) (above diagonal) and deoxynivalenol production (ppm) (below diagonal) for parents (diagonal) and F. crosses and GCA effects in a 8x8 diallel cross of SRWW screened in the field at Lexington. KY 2001.

and F <sub>1</sub> crosses and GCA el	and GC/	verrects in	a 8x8 chall	lei cross o	Hects in a 8x8 dialiel cross of SKW W screened in the field at Lexington, KT	ned in the neic	ı at Lexingu	on, KY 2	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 31.6	7:99	2.95	46.1	66.1	6.27	54.2	68.7	-5.93	-0.13
CK 9663	38.5	71.6 30.7	71.2	70.3	78.8	6.69	81.4	67.4	5.99	-0.14
CK 9474	30.4	29.9	73.8 32.7	54.4	86.7	5.99	81.6	6.69	2.93	0.59
Roane	22.3	28.4	24.3	56.8 21.2	6.99	50.3	66.3	65.6	-8.24*	-2.82
KY 89C 804	20.4	36.5	38.3	42.1	78.1 34.4	47.1	9.79	59.4	0.56	0.72
KY 86C 127	45.0	30.4	28.8	22.9	32.1	81.7 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 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 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. Thirtyone 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 °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 °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 ( $h_{\rm g}^2$ ) depended on the source of materials. In the experiment with China-introduced materials and Norm,  $h_{\rm g}^2$  was 83.4%. Among the entries from different trials of MSU Wheat Breeding Program, the  $h_{\rm g}^2$  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,  $h_{\rm g}^2$  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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Entry	Origin	No Scahl	oy spikelets	Resistance	1999/2000	
<u> </u>	Origin	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

Entry	No. scabl	y spikelets	Percentage of	Resistance	1999/2000	)
,	Average	Range	scabby spikelets		No of SS	-
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  $F_2$  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: <a href="http://www.uky.edu/Ag/Wheat/wheat\_breeding/scabpage.html">http://www.uky.edu/Ag/Wheat/wheat\_breeding/scabpage.html</a>

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Table 1. FHB symptoms in entries from the 2001 Kentucky variety trial at Lexington, Princeton, and in the Greenhouse.

		EXINGTON		F	RINCETON		GH
	% Disease	Mean		% Disease	Mean		Mean
Entry	Incidence	Severity	FHB Index	Incidence	Severity	FHB Index	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
	88.03	32.76	28.84	42.86	15.92	6.82	61.1
Exsegen Rebekah  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			39.29	13.10		10.2
		30.33	29.55 14.14		10.28	5.15	•
Ernie	81.20	17.41		32.14		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	•
						ļ	
Mean	80.41	36.25	30.98	45.86	20.59	11.58	49.9
LSD <sub>(0.05)</sub>	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<sub>1</sub> 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 m² 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<sup>th</sup> 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  $10 \text{ x}_1/2+4^*(\text{x}_1+\text{x}_2)/2+4^*(\text{x}_2+\text{x}_3)/2+4^*(\text{x}_3+\text{x}_4)/2+4^*(\text{x}_4+\text{x}_5)/2$ , where  $\text{x}_{1.5}$  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/S gv-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
	LSD5 %	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	F HB		E. graminis	P. tritic in a		P. striiform is
Νo.		AUDPC Mean %		29 M ay	14 June	22 June	14 June
149	Sumey-3	0.0	0.0	S60,7	S 3 0	n	MRt
194	Sum3/81.60//kő	0.0	0.0	MS5,5-7	MS10	MS50	MS5
243	Sgv/NB//M M/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	1 2.3	0.6	MRt,3	S5	S 5	MRt
147	Wuhan 6B	1 0.0	0.8	S70,7	S 30	S50	MS10
192	W uhan42B	37.7	2.1	S60,7	S10	n	S70
242	Sgv/NB//M M/Sum3	65.0	3.4	MSt,5	MS20	MS20	MRt
151	Sg vN B/M M Su m3	72.7	3.7	MRt,3	M St	MS5	M S5
30	81.60//NB/Kő	51.4	4.5	MSt,3	0	MR5	MR10
97	Vé ka	59.7	4.8	S30,5	S30	S40	MS30
208	FHB143	95.0	5.8	MS5,5	0	0	MR10
153	Sg vN B/M M Su m3	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	1 3.9	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/R St	317.5	18.4	MRt,4	MSt	MR5	MRt
218	P8635	1064.1	52.6	M S5,3	MS10	MS40	MSt
142	S elyem dur	970.8	60.7	MSt,5	S 60	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<sub>7</sub>, BCF<sub>6</sub>, and TCF<sub>6</sub> 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.

		Yea	ar and G	enerati	ons	
	1997	1998	1999	2000	2001	2002
	F2,	F3,	F4,	F5,	F6,	F7,
	BCF1,	,	•	BCF4,	•	BCF6,
Selection criteria	TCF1	TCF2	TCF3	TCF4	TCF5	TCF6
Heading date	Χ	X	X	X	X	Р
Plant type	Χ	X	X	X	X	Р
Yield potential	Χ	X	X	X	X	Р
Visual grain quality	Χ	X	Х	X	X	Р
Adaptation to eastern Arkansas					X	Р
Winter hardiness					X	
Milling and baking quality					<b>X</b> *	Р
Fusarium head blight: Field			Х	-	Х	Р
Fusarium head blight: Greenhouse					Х	Р
Leaf rust		X	X	-	X	Р
Stripe rust				X	X	Р
Septoria leaf blotch		X	Х	-	X	Р
Spindle streak mosaic						Р
Spindle streak + soilborne mosaic						Р

x = Meaningful selections made

<sup>- =</sup> No meaningful selections made

P = Planned for coming year

<sup>\* =</sup> only for lines with enough seed

**Table 2.** Parentage of F7, BCF6, and TCF6 lines developed for *Fusarium* head blight resistance.

Parentage  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<sub>6</sub> - 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, 1I water) through suspension culture. The concentration of conidia was adjusted to 5x10<sup>5</sup>/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**	-

<sup>\*</sup>G.H.=greenhouse d= days after inoculation

**Table 2.** Analysis of varianxe for FHB severities of the 70 varieties and breeding lines.

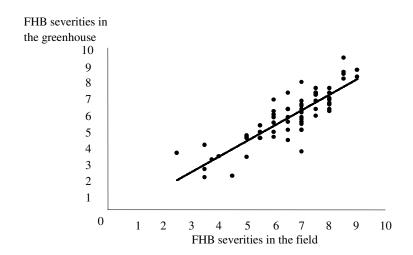
Source	d.f.	Mean square	F-value
Cultivars	69	0.341	33.69**
Environment	1	0.104	10.29**
Error	140	0.01	
			ded D 0 0 1

\*\*P < 0.01

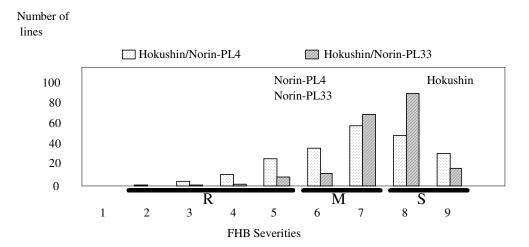
**Table 3.** FHB severities and range for 4 standard cultivars tested in the field. And greenhouse.

Cultivar	Level of	Days to	FHB	Range	LSD	Year of
	resistance	heading	severity			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

<sup>\*\*</sup>P<0.01



**Figure 1**. Scatter plot of the FHB severities among 70 varieties and breeding lines evaluated in the field and greenhouse.



**Figure 2.** 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<sub>3:4</sub> lines from each family were genotyped with two co-dominant markers to identify heterogeneous F, 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<sub>4</sub> heterozygous plants. Using the same markers, homozygous types (F<sub>4.5</sub> 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<sub>4</sub>, F<sub>5</sub>, and F<sub>6</sub> 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<sub>6</sub> or F<sub>7</sub> 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

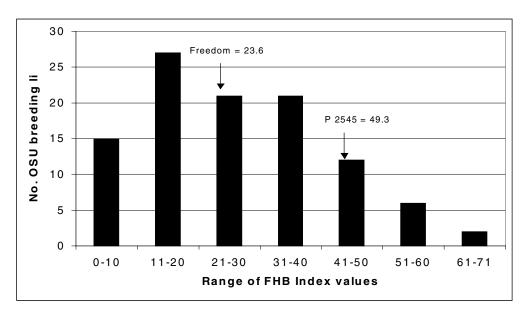
generation selection of bulked populations for low DON is underway and will be evaluated in 2002. Many different sources of resistance are being used in breeding programs and in mapping studies. Analysis using simple sequence repeat data suggests that these sources are reasonably diverse and will hopefully 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).

• .	Ū		•	,					
				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	400	F 7 0
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. R² values in the tables indicate the proportion of total sum of squares accounted for by entry and test effects while 1-R² is the proportion of total sum of squares due to the entry x test interaction (ETI) effect.

Based on 1-R<sup>2</sup>, 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<sup>th</sup> or lower in AR was similarly ranked in VA or OH. P 2545 was ranked last (most DON) in OH but ranked 1<sup>st</sup> (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 (I) 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	# I -···	и 111-1
	# of test:	6		9	8		8	4	3	3 DDM	5	# low	# High
	Units:	Days		%	%	ı.	%	0-100	%	PPM	%	scores	scores
1	Patterson	134	ı	38.4 h	61.6 h		34.1 h	31.0 I	14.7	6.9		3	3
2	Freedom	1 38		21.4	62.8 h		21.8	50.1	17.5 I	12.6		2	1
3	P 25 45	1 36		39.8 h	71.4 h	n	40.7 h	66.5 h	26.8 h	16.2		1	5
4	Ernie	1 34	- 1	20.1	51.4		19.4	29.9	16.9	7.9		4	0
5	Hondo	4.05		16.7 I	48.4		13.0 I	33.1 I	17.8 I	4.9		6	0
6	KS96HW115	1 35		22.5	61.5 h		24.1	38.6	19.1 h	14.6		2	2
7	Heyne	1 38		18.0 I	57.7 h		14.9 I	24.6 I	13.0 l	15.1		5	1
8	MDV71-19	1 37		38.4 h	72.4 h		42.4 h	60.6 h	23.9 h	9.7		1	5
9	MO 98 05 25	141		11.8 I	34.6		7.5 I	23.0 I	5.4	5.3		7	0
10	MO 96 08 27	1 35		30.7	68.5 h		30.5	55.9	28.7 h	14.6		1	2
11	MO981020	137		13.6 I	41.3	I	9.5 I	27.3 I	11.8 I	5.8		7	0
12	MO 98 04 29	1 35		22.3	49.9		19.9	33.7 I	14.4 I	6.3		3	0
13	IL 96 - 35 14	1 36		23.1	52.1		21.2	27.4 I	15.5 I	3.2		3	0
14	IL 96 - 64 72	1 33	- 1	20.9 I		I	17.3 I	20.6 I	10.2 I	8.4		6	0
15	IL 97 - 18 28	1 35		17.6 l	45.8	l	14.2 I	19.8 I	11.8 l	4.6	46.0	6	0
16	IL 97 -42 28	134	I	22.8	45.4		19.5	29.8 I	12.5 I	4.2		4	0
17	IL 97 -62 68	137		19.7 l	47.1	I	15.8 I	32.6 I	11.6 I	5.6	33.6	6	0
18	Roane	1 36		20.0 I	60.3 h	h	19.9	32.0 I	16.3 I	5.4		4	1
19	VA 96 -54 -3 26	1 36		22.8	54.1		21.0	49.0	12.5 I	7.3	94.1 h	2	1
20	VA98W-591	1 37		20.4 l	56.4		16.6 I	34.5 I	9.7	7.4	47.1	5	0
21	VA98W-593	1 36		27.4	59.8 h	h	21.6	36.3 I	7.2 I	5.3	58.8	3	1
22	VA99W-553	134	- 1	23.8	59.2 h	h	23.8	40.3	19.9 h	10.4	61.1	2	2
23	VA99W-562	1 37		26.0	60.7 h	h	25.9	50.3	19.1 h	11.1	54.7	2	2
24	VA99W-567	1 38		19.9 l	59.4 h	h	19.4	50.8	31.1 h	19.5 h	63.7	1	3
25	2 5R 18	1 39		13.2 l	59.4 h	h	13.2 I	48.8	14.3 l	16.3	9.3	5	1
26	O H6 69	1 37		42.2 h	64.6 h	h	37.6 h	53.8	27.0 h	21.3 h	92.2 h	0	6
27	O H6 84	137		36.0 h	61.5 h	h	27.9	50.5	25.8 h	13.5	76.2 h	1	4
28	O H6 99	1 38		26.0	62.9 h	h	21.2	50.3	21.9 h	9.9	63.9	1	2
29	NY87048W-7388	1 42		17.0 l	50.3		11.9 l	24.0 I	9.0	8.4	23.6	5	0
30	NY87047W-6048	1 42		31.1	64.6 h	h	28.6	77.5 h	30.5 h	32.2 h	39.8	0	4
31	N Y89052SP-9232	1 43	h	27.4	61.1 h	h	24.6	38.1	25.0 h	14.8	55.8	1	2
32	NY88024-117	1 42		29.1	61.6 h	h	27.8	49.7	18.6 h	19.5 h	46.8	1	3
33	N Y 8 8 0 0 5 - 6 0 3 5	1 43	h	36.1 h	61.7 h	h	32.3 h	70.3 h	33.0 h	29.5 h	53.2	0	6
34	NY89103-9149	1 44	h	24.8	59.7 h	h	22.0	62.3 h	28.8 h	22.6 h	35.0	0	4
35	961331A46-1-6	1 39		29.9	61.7 h	h	28.4	57.2	27.2 h	15.0 l	38.4	1	2
36	97 93 A1 -5	1 34	-1	17.8 I	47.3	l	14.2 I	24.2 I	14.9 I	5.4	33.6	6	0
37	97397B1-4-5	1 35		18.4 I	55.4		18.6	28.9 I	11.2 l	6.8	23.7	4	0
38	97398C1-5-3	138		21.9	66.9 h	h	22.3	45.5	20.1 h	8.5	34.9	2	2
39	97417A1-3-4	1 36		18.7 I	52.1		15.9 I	30.8 I	11.6 I	4.5	47.9	5	0
40	97463A1-17-1	1 33	-1	22.3	50.7		19.0	21.0 I	19.0 h	9.9	25.0	3	1
41	GA901146 E 15	134	-1	33.8 h	68.2 h	h	35.6 h	56.9	23.8 h	10.9		1	4
42	KY92C-491-18-1	136		27.6	61.7 h		28.8	47.8	18.1 h	8.5		2	2
43	KY92C-432-62	137		26.2	66.6 h		27.9	46.5	27.5 h	8.5		1	2
44	KY91C-170-3	1 36		28.9	65.3 h		28.8	51.7	23.0 h	18.1 h		0	3
45	KY91C-170-4-1	137		26.5	55.2		26.2	44.8	22.2 h	21.7 h		0	2
46	Harding	1 43	h	17.9 I	50.6		13.3 I	41.5	19.1 h	11.4		4	1
47	S D9 70 60	1 44	h	14.7 I	45.5	ı	10.5 I	35.8 I	9.2	9.5		6	0
48	D6234	139		25.3	66.8		24.6	41.3	11.9	15.2		2	1
49	D8006	136		32.5	65.4 I		31.1	59.3	21.4 h	26.9 1		0	3
	Average	138		24.6	57.5	-	22.6	42.0	18.4	11.9	46.3		
	Avoiage	. 50		L 7.0	01.0		0	12.0			+ ∪ .∪		

 $<sup>^\</sup>dagger$ Indicates a mean that is not different from the low est (I) 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		
	# oftest:	6		9	8	8		4	3	3	5	# low	# High
	Units:	Days		%	%	%		0-100	%	PPM	%	scores	scores
9	MO980525	141		11.8 I	34.6 I	7.5 I		23.0 I	5.4 I	5.3 I	14.3 I	7	0
11	MO981020	137		13.6 I	41.3 l	9.5 I		27.3 I	11.8 I	5.8 I	16.8 I	7	0
5	Hondo	140		16.7 I	48.4 I	13.0 I		33.1 I	17.8 I	4.9 I	35.6	6	0
14	IL96-6472	133	I	20.9 I	48.2 I	17.3 I		20.6 I	10.2 l	8.4 I	40.6	6	0
15	IL97-1828	135		17.6 I	45.8 I	14.2 I		19.8 I	11.8 I	4.6 I	46.0	6	0
17	IL97-6268	137		19.7 I	47.1 l	15.8 I	l	32.6 I	11.6 l	5.6 I	33.6	6	0
36	9793A1-5	134	1	17.8 I	47.3 I	14.2 I	l	24.2 I	14.9 I	5.4 I	33.6	6	0
47	SD97060	144	h	14.7 I	45.5 l	10.5 I	l	35.8 I	9.21	9.5 I	35.5	6	0
20	VA98W-591	137		20.4 I	56.4	16.6 I		34.5 I	9.7	7.4 I	47.1	5	0
29	NY87048W-7388	142		17.0 I	50.3	11.9 l		24.0 I	9.01	8.4 I	23.6	5	0
39	97417A1-3-4	136		18.7 I	52.1	15.9 I	l	30.8 I	11.6 l	4.5 I	47.9	5	0
7	Heyne	138		18.0 I	57.7 h	14.9 I	l	24.6 I	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 I	9.31	5	1
41	GA901146 E 15	134	1	33.8 h	68.2 h	35.6 h	า	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 I	76.2 h	1	4
8	MDV71-19	137		38.4 h	72.4 h	42.4 h	ı	60.6 h	23.9 h	9.7 I	60.0	1	5
3	P2545	136		39.8 h	71.4 h	40.7 h	า	66.5 h	26.8 h	16.2 I	55.8	1	5
33	NY88005-6035	143	h	36.1 h	61.7 h	32.3 h	า	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	1	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		

 $<sup>^{\</sup>dagger}$  Indicates a mean that is not different from the lowest (I) or highest (h) mean in the corresponding column in Table 1 based on LSD<sub>(0.05)</sub>.

**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

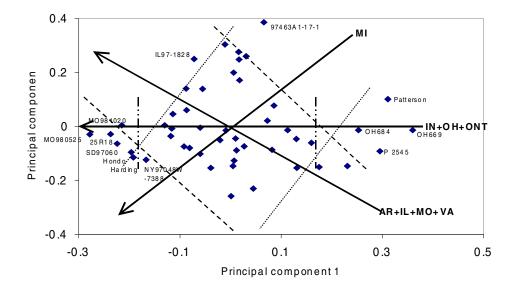
			IN 611				AR+IL+							
	N AM E	ALL	IN +O H +O N T	IN	ON	ОН	K Y +M O + V A	AR	IL	ΚY	МО	VA	МΙ	ΝE
1	Patterson	38.4 h	40.1 h <sup>†</sup>	41	35.0	44.4	33.6	7	43.8	39.0	43	35	57.1	80
2	Freedom	21.4	13.4 I	11	7.3	22.0	23.4	8	23.3	34.6	32	19	35.7	20
3	P2545	39.8 h	40.4 h	44	20.2	56.9	39.4 h		55.0	42.0	42	43		30,100
4	Ernie	20.1 I	19.6 I	10	18.7	30.1	12.9 I	5	8.5	16.1	16	19	57.1	100
5	Hondo	16.7 I	9.3	16	4.8	7.2	21.3	7	15.8	21.8	35	27	15.4	30
6	KS 96HW115	22.5	17.1 I	16	4.1	31.2	18.5 I	5	25.0	18.6	21	23	58.3	100
7	Heyne	18.0 I	16.4 I	29	3.3	17.0	16.9 I	7	19.3	17.2	22	19		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 I	7.4 I	12	6.9	3.4	14.1 I	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 I	10.4 I	9	13.1	9.2	14.3 I	5	10.3	13.2	14	29	20.0	80
12	MO980429	22.3	23.7	10	21.9	39.3	16.6 I	5	10.5	23.4	25	19	46.7	100
13	IL 96 -35 14	23.1	24.7	14	14.9	45.1	16.1 l	7	13.8	25.9	21	13	53.3	100
14	IL 96 -6472	20.9 I	24.3	20	23.3	29.6	12.5 I	3	7.5	13.8	14	24	53.3	100
15	IL 97 -1828	17.6 I	16.0 I	10	13.6	24.3	12.0 I	7	9.0	12.2	11	21	50.0	100
16	IL 97 -4228	22.8	29.7	26	18.9	44.1	15.2 I	5	8.3	24.7	14	24	40.0	100
17	IL 97 -62 68	19.7 I	23.3	17	22.8	30.2	14.4 l	5	16.0	21.0	16	14	35.7	100
18	Roane	20.0 I	19.2 l	18	19.4	20.2	20.2 I	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 I	22.8	32	12.6	23.9	20.0 l	10	19.3	23.6	19	28	15.4	70
21	VA 98 W -593	27.4	41.5 h	61	16.9	46.5	21.4	7	21.3	25.5	28	25	15.4	80
22	VA 99 W -553	23.8	29.1	18	27.1	42.2	20.8 I	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	VA 99 W -5 67	19.9 I	27.2	29	11.7	41.0	17.1 l	5	23.8	17.9	17	22	11.8	80
25	25R18	13.2 I	12.1 I	7	7.1	22.2	13.8 I	5	10.8	17.1	12	24	13.3	80
26	O H6 69	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	O H6 84	36.0 h	43.2 h	63	27 .9	38.8	31.2	15	48.8	36.1	24	32	38.5	80
28	O H6 99	26.0	23.0	28	14.9	26.1	27.3	15	32.5	22.1	19	48	28.6	90
29	N Y87048W-7388	17.0 I	11.6 I	23	3.0	8.8	20.7 I	10	22.3	11.4	28	32	14.3	0
30	N Y87047 W-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 I	19	7 .4	15.6	35.1	15	52.5	29.8	42	36	29.4	20
32	NY 88 024-117	29.1	16.6 l	15	4 .0	30.9	38.9 h		62.5	39.0	38	40	17.6	0,60
33	NY88005-6035	36.1 h	14.1 I	13	7.2	22.2	45.9 h		72.5	47.9	48	36	53.3	20
34	NY89103-9149	24.8	12.9 I	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	97 93 A1 -5	17.8 I	22.9	20	15.8	33.0 23.8	15.2 I	5	11.0	14.9	18	27	15.4	40 ?
37	973 97 B1 - 4 - 5		13.3 I	6	. •	_0.0	19.8 I		14.8	21.3	32	26	26.7	
38	973 98C1 -5-3	21.9	20.5	13	10.2	38.4	21.7	5	23.5	27.2	16	37	26.7	
39	97417A1-3-4	18.7 I	15.3 I	20	13.4	12.5	16.5 I	5	14.3	19.0	24	20	40.0	
40	97463A1-17-1	22.3	25.1	28	22.3	25.1	11.6 I	5	9.8	14.4	14	15	66.7 21.4	20.60
41	GA 90 11 46 E 15	33.8 h	41.0 h	46	27.7	49.2	32.0	10	55.0	36.0	24	35		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 44	KY 92 C - 432 - 62	26.2	21.4	18 26	17.8 20.5	28.4	28.9	7 7	48.8	27.9	28 25	33	27 .3 35 .7	100
	KY91C-170-3 KY91C-170-4-1	28.9	28.4			38.8	27.8		31.0	40.9		35		90
45 46	Harding	26.5 17.9 l	23.3 9.5 I	20 20	17.3	32.5 7.9	28.0 23.7	10	35.0 25.8	37.1 29.8	28 25	32 28	28.6 14.3	30 100
46	SD97060	17.9 I	8.7 I	14	1.3	10.7	23.7 18.2 l	5	20.0	19.0	25 25	22	15.4	100
47	D6234	25.3	14.6 I	13	9.9	21.0		13	31.3	31.0	23 23	52	33.3	30,70
49	D8006	32.5	32.1	30	13.8	-		15	37.5	35.6		46	25.0	60
+3	Average	24.6	22.7	22.8	14.8			9.0	29.5		27.1		31.6	00
	CV (%)	24.6 41.0	39.7	39.0	25.9	30.4	30.4	21.8	29.5	25.5		28.8	46.8	
	LSD (0.05)	9.3	14.60	16.0	20.8	24.5		3.2			14.0		-0.0	
	LSD (0.05) R2		0.71	10.0		24.0	0.77	٥.۷	14.1	J./	14.0			
_	nz	0.04	J. / I				0.77						L	

 $<sup>^{\</sup>dagger}$ Indicates a mean that is not different from the lowest (I) or highest (h) mean in the column based on LSD $_{(0.05)}$ .

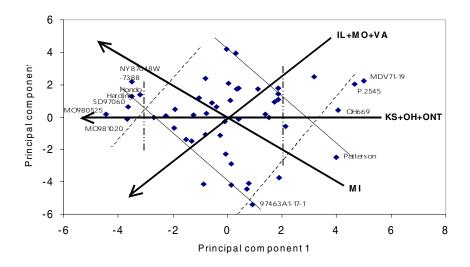
Table 5. Disease index ([severity% x incidence%]/100) for entries in 2001 NUWWSN

			1				4.5. !!						1	
			IN+OH				AR+IL+ KY+MO							
	NAME	ALL	+ONT	IN	ON	ОН	+ V A	AR	IL	KY	МО	VA	MI	NE
1	Patterson	38.4 h	40.1 h <sup>†</sup>	41	35.0	44.4	33.6	7	43.8	39.0	43	35	57.1	80
2	Freedom	21.4	13.4 I	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 I	19.6 I	10	18.7	30.1	12.9 I	5	8.5	16.1	16	19	57.1	100
5	Hondo	16.7 I	9.3	16	4.8	7.2	21.3	7	15.8	21.8	35	27	15.4	30
6	KS 96HW115	22.5	17.1 I	16	4.1	31.2	18.5 I	5	25.0	18.6	21	23	58.3	100
7	Heyne	18.0 l	16.4 I	29	3.3	17.0		7	19.3	17.2	22	19	28.6	20,100
8	M D V 71-19	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 I	7.4	12	6.9	3.4	14.1 I	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 I	10.4 I	9	13.1	9.2	14.3 I	5	10.3	13.2	14	29	20.0	80
12	MO980429	22.3	23.7	10	21.9	39.3	16.6 I	5	10.5	23.4	25	19	46.7	100
13	IL96-3514	23.1	24.7	14	14.9	45.1	16.1 I	7	13.8	25.9	21	13	53.3	100
14	IL96-6472	20.9 I	24.3	20	23.3	29.6	12.5 I	3	7.5	13.8	14	24	53.3	100
15	IL97-1828	17.6 l	16.0 I	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 I	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 I	5	16.0	21.0	16	14	35.7	100
18	Roane	20.0 I	19.2 I	18	19.4	20.2	20.2	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 I	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 I	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 I	27.2	29	11.7	41.0	17.1 l	5	23.8	17.9	17	22	11.8	80
25	25R18	13.2 I	12.1 I	7	7.1	22.2	13.8 I	5	10.8	17.1	12	24	13.3	80
26	O H669	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	O H684	36.0 h	43.2 h	63	27.9	38.8	31.2	15	48.8	36.1	24	32	38.5	80
28	O H699	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 I	11.6 I	23	3.0	8.8	20.7 I	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 I	19	7.4	15.6	35.1	15	52.5	29.8	42	36	29.4	20
32	NY88024-117	29.1	16.6 I	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 I	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 I	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 I	22.9	20	15.8	33.0	15.2 I	5	11.0	14.9	18	27	15.4	40 ?
37	97397B1-4-5	18.4 I	13.3 I	6	10.1	23.8	19.8 I	5	14.8	21.3	32	26	26.7	
38	97398C1-5-3	21.9	20.5 I	13	10.2	38.4		5	23.5	27.2	16	37	26.7	
39	97417A1-3-4	18.7 I	15.3 I	20	13.4	12.5		5	14.3	19.0	24	20	40.0	
40	97463A1-17-1	22.3	25.1	28	22.3	25.1	11.6 I	5	9.8	14.4	14	15	66.7	
41	GA 901146 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	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		7	31.0	40.9	25	35	35.7	90
45	KY91C-170-4-1	26.5	23.3	20	17.3	32.5		8	35.0	37.1	28	32	28.6	30
46	Harding	17.9 I	9.5 I	20	0.7	7.9		10	25.8	29.8	25	28	14.3	100
47	SD97060	14.7 I	8.7 I	14	1.3	10.7		5	20.0	19.0		22	15.4	100
48	D6234	25.3	14.6 I	13	9.9	21.0		13	31.3	31.0		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		9.0	29.5		27.1	28.8	31.6	
	CV (%)	41.0	39.7	39.0	25.9		30.4	21.8	29.6	25.5			46.8	
	LSD (0.05)	9.3	14.60	16.0		24.5		3.2	12.1	9.7	14.0			
	R2	0.54	0.71				0.77							

 $<sup>^\</sup>dagger$ 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°, 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 (< 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.

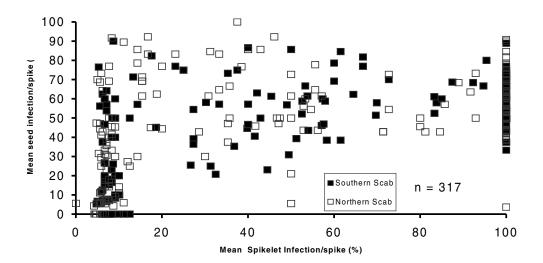
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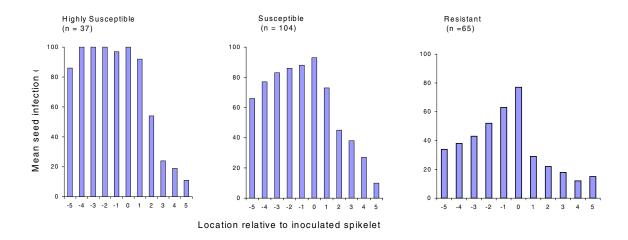
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**Figure 1**. Relationship between visual estimate of FHB spikelet infection for each spike following point inoculation and bioassay of *F. graminearum* seed infection for same spikes for all entries in Uniform Northern and Southern Scab Nursery 2001.



**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 ×10<sup>5</sup> 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.