ASSESSMENT AND REACTION OF SOFT RED WINTER WHEAT GENOTYPES TO *FUSARIUM GRAMINEARUM* AND EFFECTS ON TRAITS RELATED TO YIELD AND SEED QUALITY

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OBJECTIVES

1) To identify disease-assessment parameters that consistently differentiate resistant and susceptible host response to *Fusarium graminearum* and that are most predictive of subsequent effects on yield and quality related traits.
2) To discern the level of susceptibility or resistance among soft red winter wheat genotypes and the effectiveness of such resistance.

INTRODUCTION

Fusarium head blight (FHB) is responsible for large reductions in wheat yield and quality worldwide. In the 1997-98 growing season, Virginia growers suffered from epidemic levels of scab infection throughout the state. Yield losses for soft red winter wheat caused by scab were estimated to be as high as 33%. Epidemics likely will become more frequent in Virginia due to an increase in wheat acreage under conservation tillage. Virginia's climate is also quite favorable for scab epidemics, with cool moist conditions occurring frequently during flowering. Resistance levels are not high in current varieties grown in the state. However, it is apparent that there are differences among varieties not only in infection levels but also in yield response to the disease. From a grower's standpoint, it would be helpful to know which varieties grown in the region have the least yield and quality reduction under high disease pressure. From a breeder's standpoint, it would be helpful to know what parameters of disease assessment correlate best with losses in yield and quality related traits. Identification of the single most predictable, reliable and hopefully most feasible assessment parameter would allow breeders to focus and rely on a specific disease assessment parameter and, therefore, make field ratings less time consuming.

MATERIALS AND METHODS

Twenty (1997-98) and thirty (1998-99) soft red winter wheat genotypes were grown in replicated 100 ft^2 plots using a randomized complete block design with two treatments. Replications 1-3 comprised the inoculated block and replications 4-6 the non-inoculated control. Planting density was determined based on 1000 kernal weight and a target density of 24 seeds per row foot. All seed was treated prior to planting with Batanò (1.5 oz/100 lbs), Gauchoò (2 oz/100 lbs), and Captanò (3 oz/100 lbs). Baytanò was applied to control powdery mildew and Gauchoò to control aphids and, therefore, Barley Yellow Dwarf virus. Pre-plant fertilizer application included 25N-60P-90K (1997-98) and 25N-100P-100K (1998-99). Plots were planted on October 13, and Harmony Extraò herbicide (.5 oz/acre) was applied on Feburary 11. Spring nitrogen was applied at a rate of 60 lbs/acre along with another application of Harmony Extraò (.5 oz/acre) at growth stage 30, on March 31. Plots were harvested on July 5 with a small plot combine.

Treated plots were inoculated at flowering and again seven days post- anthesis using a conidial suspension of 1L/100 ft^2 at 50,000 spores/mL.

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After inoculation, all field plots received overhead mist irrigation from 8-9:30 A.M. and again from 6-7:30 P.M., unless conditions deemed irrigation not necessary (i.e. rain, heavy dew, or fog). Scab incidence and severity were measured at seven and fourteen days post-inoculation. Grain yield, test weight, 1000 kernel weight, DON toxin content, and percentage of scabby seeds were measured post-harvest. Barley Yellow Dwarf, *Staganospora* glume blotch, root rot, lodging, heading date, and height were also assessed in the field. All data was analyzed using Agrobase software (correlation analysis, LSD, and Anova).

RESULTS AND DISCUSSION

Parameters for Assessing Resistance

Correlations between traits for two growing seasons suggest that scab severity and percentage of scabby seed are the most useful parameters for assessing type IV and type V resistance in wheat varieties and are the main factors in the reduction of yield and test weight. Correlations for scabby seeds and scab severity with yield and test weight were significant over two growing seasons. In contrast, correlations for scab incidence and scab index with yield and test weight were not as significant as scab severity and percentage of scabby seed in 1997-98 and not significant in 1998-99. This indicates that scab incidence and scab index may be less effective tools for assessing resistance and loss. To confirm these findings, correlation values were obtained for yield loss and test weight loss (mean treatment differences calculated for inoculated vs. control) with scab severity, scab incidence, scab index, and percent scabby seeds. Again the correlation values were significant for percent scabby seed and scab severity, but not significant for scab incidence and scab index. This confirms that percent scabby seed and scab severity are the most useful parameters for assessing resistance and loss. DON toxin analysis can also be a useful tool in assessing type IV and V resistance. Correlation values among DON content with yield and test weight were significant. The problem with using DON content as a routine tool for assessing resistance and loss lies in the lack of test facilities and cost of analysis.

A concern in creating an environment that is ideal for *Fusarium* growth is the proliferation of other diseases of wheat, such as *Stagonospora nodorum* and root rot, that may affect similar traits and confound the effects due to scab. Excessive lodging can also occur in heavily irrigated plots. For this reason correlation of lodging with yield and test weight was also considered. The correlation values for root rot with yield and test weight were not significant, indicating that root rot had no significant impact on yield or test weight. Glume blotch and yield were significantly correlated, but glume blotch did not show a significant correlation with test weight. The significant correlation with yield infers that *Staganospora nodorum* can significantly impact and confound the assessment of type IV and V resistance to scab and, therefore must be precisely distinguished and accounted for in all field and lab assessments. Mesterhazy et. Al. have indicated that in field studies *Staganospora nodorum* control can be achieved with an application of Bayleton while plots were in the boot stage. This could be used to reduce or eliminate the correlation between yield and *Staganospora nodorum*, thereby focusing on yield losses due to Fusarium. Lodging also showed a significant correlation to yield and test weight. However, lodging effects can be controlled by application of a growth regulator (Cerone Ò), and should pose few problems in the future in assessing yield and test weight data.

Genotype Reaction and Response to *Fusarium*

Significant differences were observed among inoculated soft red winter wheat varieties with

respect to yield, test weight, scab severity, percentage of scabby seed, and DON concentration. Analysis of variance and LSD indicate that there is a continuum of resistance and yield mean values rather than easily definable group(s). For each parameter a statistically distinct grouping of cultivars was attained from the mean using LSD values and noted as statistically high or low.

In analyzing yield loss data it was found that Agripro Foster and Pioneer 2552, over two years, had lower yield loss than other varieties in the test. Coker 9835 and GA Gore showed consistent and high yield loss. Roane, Coker 9803, and Agripro Foster all showed consistently low test weight loss, whereas Coker 9835 and Madison consistantly had high test weight loss.

In analyzing parameters for assessing resistance, it was concluded that three measurements correlate well with type IV and V resistance. These assessment parameters are scab severity, percentage of scabby seed, and DON concentration. In analyzing scab severity data over two years, Ernie and Roane had the lowest statistically distinct scab severity values. This suggests that Ernie and Roane may be good sources of type IV and V resistance. GA Gore and Coker 9835, over two years, had the highest statistically distinct scab severity values and, therefore, it can be inferred that these cultivars do not possess type IV or V resistance to scab. In analyzing percentage of scabby seed data over two years, P92823A1-1-4-4-5 had a statistically lower percentage of scabby seed, and Agripro Foster, while not statistically lower than the mean, had a low percentage of scabby seed. This suggests that P92823A1-1-4-4-5 and Agripro Foster may also possess type IV and V resistance. As with scab severity, Coker 9835 had the highest statistically distinct percentage of scabby seed, indicating a lack of type IV and V resistance. In analyzing DON concentration over two years, Coker 9803 had statistically low DON values. This suggests that Coker 9803 may contain type IV and V resistance. GA Gore and Coker 9835 had statistically high DON values, which agrees with previous parameter analysis, suggesting that these varieties have little resistance.

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Table 1- SRWW Varieties in the Yield Loss Study and rankings with respect to Scab Severity and Scabby Seed Percentage. Ranks statistically higher or lower than the mean are indicated with a (*).

1997-98 Test			1998-99 Test		
Varieties	**Scab Sev.**	**Scabby Seed**	**Varieties**	**Scab Sev.**	**Scabby Seed**
ERNIE	1 *	4 *	ERNIE	1 *	28 *
P92823A1-1-4-4-5	2 *	1 *	IL94-1549	2	2 *
FREEDOM	3 *	5	IL94-1909	3	25
VA93-54-429	4 *	3 *	AGRIPRO PATTON	4	8
VA96-54-234	5	12	OH 552	5	17
COKER 9803	6	6	ROANE	6	11
PION 2552-B	7	11	P92823A1-1	7	3 *
JACKSON	8	18 *	PION 2552	8	14
PION 2580-B	9	17 *	VA96W-329	9	4
AGRIPRO FOSTER	10	2 *	VA96W-326	10	1 *
VA96-54-216	11	10	AGRIPRO FOSTER	11	5
AGRIPRO MASON	12	8	VA96W-348	12	22
WAKEFIELD	13	7	AGRIPRO MASON	13	6
PION 2684-B	14	15	NY87048W-7	14	7
PION 2643-B	15	9	PION 2643	15	9
FFR555W-B	16	13	VA96W-250	16	21
POCAHONTAS	17	16	COKER 9803	17	19
MADISON	18	14	QUANTUM 706	18	13
COKER 9835	19 *	19 *	FREEDOM	19	18
GORE	20 *	20 *	PION 2580	20	29 *
LSD	8.1	5.1	PION 2684	21	12
			WAKEFIELD	22	16
			FFR 555W	23	27
			MADISON:CH	24	20
			CAYUGA	25	23
			VA96W-247	26	26
			POCAHONTAS	27	10
			JACKSON:CH	28 *	24
			GA GORE	29 *	15
			COKER 9835	30 *	30 *
			LSD	0.07	8.1

HAPLOID PRODUCTION IN TWELVE WHEAT F_1 POPULATIONS VIA THE WHEAT X MAIZE HYBRIDIZATION METHOD

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ABSTRACT

Wheat x maize hybridization has proven to be efficient for haploid production in wheat. F_1 plants, from 12 crosses between six scab resistant sources and two susceptible soft red winter wheat (SRW) varieties, were pollinated with pollen of maize F_1 hybrid 'Seneca 60'. Mean frequencies of fertilization, embryo formation, embryo germination and haploid green plant regeneration were 83, 20, 45 and 8 %, respectively. Significant differences were found between two SRW parents in F_1 crosses for the efficiency of haploid production, based on the percentage of embryo germination and the percentage of haploid green plants regenerated. A total of 1024 haploids were regenerated from 2254 embryos derived from 13,527 florets pollinated. Improvement of haploid and doubled haploid production and potential use of the wheat x maize hybridization system for studying scab resistance is discussed.

INTRODUCTION

Doubled haploid techniques based on gamete selection provide the advantage of developing immediate homozygosity, which facilitates and improves the precision of genetic and mapping studies and provides greater efficiency of selection in plant breeding. The methods available for haploid production in cereals include anther culture, and wide-hybridization mediated chromosome elimination using *Hordeum bulbosum* or maize as the pollen sources. In wheat, use of the *Bulbosum* method is restricted to KrlKr2 genotypes. Anther culture in winter wheat is very much genotype dependent and offers very low success compared to barley, durum and spring wheat (Laurie and Reymondie, 1991; Fedak et al., 1997).

Since Laurie and Bennett (1986) first reported the method of haploid production in wheat mediated by maize chromosome elimination, this method has been used in wheat haploid production and applied with some success in generating genetic and mapping populations (Laurie and Reymondie, 1991; L. Shugar, K. Buerstmayr and G. Fedak, personal communication). The objectives of this study are: 1) To facilitate gene transfer and pyramiding of resistance to *Fusarium graminearum* from the six best sources screened in our program into soft red winter wheat varieties; 2) To generate the desired genetic and mapping populations for studying the inheritance of diverse resistance sources; 3) To study the feasibility and potential application in wheat breeding.

MATERIALS AND METHODS

Twelve wheat F_1 crosses were used in this study, which were derived from crosses between six scab resistance sources (Ernie, Roane, Shaan85-2, VR95B717, W14 and Freedom) evaluated in our breeding program (Griffey and Chen et al., 1998) and two commercial SRW varieties Pioneer 2684 and Madison. Wheat F_1 plants were vernalized and grown individually in 275 cm^3 pots in the greenhouse. Fertilizer was applied once a week to keep the plants healthy and promote tiller development. To ensure a continuous supply of pollen, maize seed were planted

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twice per week in 11,350 cm^3 pots with one to two seeds per pot. The maize variety Seneca 60 (Laurie, 1989) was supplied by Dr. Fedak of Plant Research Center of Research Agriculture Canada.

Wheat spikes were manually emasculated, with minimal cutting of the glume, one or two days prior to pollination. Fresh pollen was collected from maize and applied to emasculated wheat florets using a small brush. The intact pollinated heads were dipped in 100 mg/L of 2,4-D solution one or two days after pollination. Embryos were excised from fertilized seeds at 12 to 16 days after pollination and placed on Gamborg's B5 basal medium in test tubes. Embryos were maintained in the dark at 20 to 25°C in an incubator until coleoptiles were 1-2 cm long. The tubes were then transferred to a lab bench at 20 to 25°C with 12 hours of light per day provided by fluorescent lamps. Regenerated haploid plants were treated with a 0.1% colchicine solution for 5 hours to double the chromosomes, and then were planted in pots with soil in a growth chamber. Genotype effects were analyzed using Agrobase Software.

RESULTS AND DISCUSSION

Fertilization frequencies obtained for the wheat x maize (Seneca 60) hybridization system were high (mean 83 %) in all F_1 crosses used in this study. The average percentages of embryo formation (20 %), germination (45 %) and haploid production (8 %) were higher than those previously reported (Fedak et al., 1997; Kisana et al., 1993) for haploid production from wheat F_1's.

Less genotype-dependent response was reported for the wheat x maize hybridization system (Fedak, 1997) than for anther culture and the *Bulbosum* method. No significant difference was found for the percentage of seeds and embryos formed among 12 wheat F_1 crosses in the current study. This result agrees with that reported earlier by Matzk and Mahn (1994). However, large differences were observed among crosses for the percentage of embryo germination and haploid regeneration (Table 1). The frequency of embryo germination and haploid regeneration from crosses with Pioneer 2684 were significantly higher than those with Madison (Table 2). This suggests that the efficacy of haploid production could be improved through selection of more responsive parents, such as Pioneer 2684.

Compared to anther culture, the wheat x maize system has three advantages: higher efficacy, less variation and less time consuming. Based on the results previously reported by Kisana et al. (1993), the maize pollen method is about two to three times more efficient than anther culture (5 plants per 100 florets pollinated versus 3 plants per 100 anthers cultured). In the current study, twice as many green plants were regenerated (mean = 7.54%) using the maize pollen method. Kisana et al. (1993) reported that aneuploids or gross chromosomal abnormalities were not observed and confirmed that chromosome variations were not common in wheat x maize-derived plants. He also concluded that this technique could save four to six weeks in obtaining the same age haploid green plants.

The high efficiency of haploid production obtained by the wheat x maize hybridization system, likely will make it very useful for studying wheat scab resistance, as conventional methods of selection have given only incremental improvements to date. The reason for this limited success may be attributed to the complex inheritance of resistance and to limited sources of unique resistance. Results and data presented in this study were obtained by three people (2,400 hours) working over a six month period. The doubled haploid plants produced this spring already have been planted in the 1999-2000 field trails at two locations to evaluate scab resistance and agronomic traits. Type II resistance will be

evaluated in the winter of 2000 in greenhouse tests. This method theoretically will save about 3-5 years compared with the conventional breeding process. Pyramiding resistance genes by conventional selection likely would take a long time with limited success and precision. Producing doubled haploid populations will shorten the time to cultivar release, improve efficacy and efficiency in screening for resistance, and greatly facilitate genetic and mapping studies.

In order to use the wheat x maize system in practical breeding programs, further enhancement of embryo formation, germination, and green-plant regeneration and doubling is needed. Some green plants will die during colchicine-induced chromosome doubling and during transfer; therefore, the final population size may be too small to represent a sufficient number of possible genotypes to make selection effective. In the current study, an average of 20% of the green plants died during the chromosome doubling and then 20% of the surviving plants ceased during transfer stages. Approximately 80 % of the surviving green plants had normal seed set after doubling and transfering stages.

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Table1. Haploid production by wheat x maize hybridization in 12 wheat F1 crosses.								
	Florets	Seeds	Embryos	Green	%			
Pedigree	Pollinated	Developed	Rescued	Plants	B/A	C/B	D/C	D/A
	A	B	C	D				
MADISON/ERNIE	814	704	174	60	86.49	24.72	34.48	7.37
PION2684/ERNIE	1151	958	238	121	83.23	24.84	50.84	10.51
ROANE/MADISON	1569	1286	291	135	81.96	22.63	46.39	8.6
ROANE/PION2684	1730	1518	304	176	87.75	20.03	57.89	10.17
SHAAN85-2/MADISON	957	788	136	60	82.34	17.26	44.12	6.27
PION2684/SHAAN85-2	1040	717	130	76	68.94	18.13	58.46	7.31
VR95B717/MADISON	1300	1146	138	54	88.15	12.04	39.13	4.15
VR95B717/PION2684	1300	1015	159	77	78.08	15.67	48.43	5.92
MADISON/W14	612	452	48	12	73.86	10.62	25.00	1.96
PION2684/W14	729	587	140	61	80.52	23.85	43.57	8.37
ROANE / W14	616	502	109	63	81.49	21.71	57.80	10.23
FREEDOM / PION 2684	1709	1534	387	126	89.76	25.23	32.56	7.37
Total	13527	11207	2254	1021				
Mean					82.87	20.13	45.21	7.54
Crosses with PION2684	5950	4795	971	511	**79.50**	**20.50**	**51.84**	**8.46**
Crosses with MADISON	5252	4376	787	321	**82.56**	**17.45**	**37.82**	**5.67**
DIFFERENCES					**-3.06**	**3.05**	**14.02**	**2.79**
C.V.					7.93	22.76	5.84	21.71
HERITABILITY					0	0.05	0.93	0.59
LSD (0.05)					8.67	5.82	3.53	2.07

Table 2. Analysis of variance for frequency of embryo germination.			
Item	df	Mean Square	
		D/C	D/A
Between crosses with PION 2684 and Madison	1	490.980***	19.404*
Within crosses with either PION 2684 or Madison	4	106.088**	8.352
Error	4	6.864	2.352
Total	9		
* p< 0.05, ** p< 0.01, *** p< 0.001			

DISCOVERY AND DEPLOYMENT OF MOLECULAR MARKERS LINKED TO FUSARIUM HEAD BLIGHT RESISTANCE: AN INTEGRATED SYSTEM FOR WHEAT AND BARLEY

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ABSTRACT

Fusarium head blight (FHB) is a devastating disease of wheat and barley. Quantitative resistance and tremendous expense of screening indicate that molecular markers might expedite the search for resistance. Markers must be polymorphic and informative across populations to be used by breeders. Several groups are mapping genes for FHB resistance in wheat and barley. Although these markers may be validated and made breeder-friendly by labs that developed them, the urgency of the FHB situation requires efforts to accelerate this process. Prospects for a set of regional labs with high throughput characterization facilities, breeder-friendly markers, and doubled haploid technology will be discussed.

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DIALLEL ANALYSIS OF FHB AND TOMBSTONE KERNELS IN SPRING WHEAT EVALUATED UNDER GREENHOUSE AND FIELD CONDITIONS

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INTRODUCTION

Fusarium head blight (FHB) or head scab caused by *Fusarium graminearum* is one of the most serious diseases of wheat leading to wide spread yield losses worldwide. Provided the favorable weather conditions, this disease is capable of causing severe epidemics, particularly in the warm and humid/semihumid regions (3, 7). Moreover, FHB epidemics in wheat, in recent years, have been associated with above average precipitation, short rotation intervals, and increasing proportion of minimum- or no-till practices by the farmers (4). The losses from this disease are manifold. It not only causes direct loss in grain yield, but also severely affects grain quality by producing various toxic metabolites (1, 7). Under these situations, breeding resistant cultivars, undoubtedly, is the best and sustainable method of control in the long run. However, the limited information on the genetics and inheritance of the disease and the complex evaluation procedures, have slowed down progress. The objective of this study is, therefore, to see if the inheritance of resistance to FHB and tombstone kernels in spring wheat was similar in field and greenhouse environments.

MATERIALS AND METHODS

Five spring wheat genotypes/cultivars of diverse origin with varying degrees of tolerance to FHB (Table 1) were crossed in all possible combinations to produce ten populations. Using the single seed descent method, lines were derived at F5. Fifty $F_{5:6}$ lines, selected randomly, from each population were evaluated for FHB and tombstone kernels in both greenhouse and field experiments. Five hundred lines along with checks and parents were planted in individual hills (one set, replicated over time) in greenhouse in 1998 and 1999. Plants in each individual hill were tagged when they reached anthesis and were inoculated by spraying a conidial suspension (75000 conidia/ml) of isolates of *F. graminearum*. In addition, Fusarium-colonized corn seeds were spread on the ground. An automated misting system (30 seconds every hour from 6 PM-10 AM) was used to maintain a high level of humidity. The same set of materials was evaluated in a field FHB nursery in 1998 and 1999 each with two replications under similar automated misting system.

Visual scores of FHB on the inoculated spikes were recorded after 2-3 weeks using 0-9 scale (0=no infection, 9=100% infection). Plants were harvested at maturity and "tombstone" kernels were rated using 1-5 scale (1= 20%, resistant, 5=more than 80%, susceptible). These scales were expressed in terms of percentage and analyzed using Griffing's diallel method 4 (F1s only); model I (2).

RESULTS AND DISCUSSION

Analysis of variance for all the variables (FHB and tombstone kernels under greenhouse and field environments) revealed highly significant effects for genotypes (data not shown). The mean squares for general combining ability (GCA) were highly significant, and were 2-9 times higher than the mean squares for SCA (Table 2). This indicates GCA as the major

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source of genetic variation, and that the additive effects are of major importance, for the inheritance of resistance to FHB and tombstone kernels in these populations. Similar results were reported earlier (7). Highly significant SCA mean squares, on the other hand, does not rule out the possibility that some non-additive gene effects also exist (6).

CIMMYT 7 with the highest negative GCA effects is the best among five parents in terms of inheritance of resistance to FHB and tombstone kernels under both field and greenhouse conditions (Table 3). On the contrary, parents like Fang 60, with the highest positive GCA effects, appear to have factors for susceptibility. Parents showing highest negative GCA effects would be good sources for the improvement of resistance (5). The presence of highly significant GCA for susceptibility is an important consideration in a breeding program; therefore, highly susceptible parents should be avoided. Sonalika and Seri 82 both have similar and very high per se disease ratings, but the former has higher inheritance of susceptibility (highly significant positive GCA effects) than the latter.

Highly significant correlations (r=0.48-0.90) were observed between FHB and tombstone kernel estimates within field and greenhouse environments (Table 4). FHB and tombstone kernel estimates between field and greenhouse environments were also significantly correlated (r= 0.50).

CONCLUSIONS

- Our results indicate that GCA accounted for much of the genetic variations in these populations and hence the additive inheritance is of major importance.
- Resistance is heritable (e.g., CIMMYT 7 and 2375), and so is susceptibility (e.g., Fang 60). However, inheritance of susceptibility may not necessarily be the same even among the highly susceptible parents.
- Since FHB and tombstone kernels correlate well, any one of the two or both could be used as a tool for selection in the breeding program. Scab index, a weighted measure of FHB and tombstone kernels together, might be an even better method of estimation because of less environmental variation. Similarly, either greenhouse or field should be equally effective for FHB and tombstone kernel evaluation.

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Table 1. Spring wheat lines/cultivars used as parents in diallel cross along with their reaction to Fusarium head blight (FHB), tombstone kernels (TOMB), and maturity.

Lines/ cultivars	Pedigree	Origin	Reaction to		Maturity
			FHB	TOMB	
2375	Olaf/2/Era/Sugamuxi68/12/...	USA	MS-MR	MS-MR	ME
Fang60	Pitic62/Frondosa/3/Pitic62/...	Thailand	VS	VS	ME
Sonalika	II54-388/An/3/Yt54/N10B//Lr64	India	S	VS	E
Seri82	Kvz/Buho//Kal/Bb	Mexico	S	VS	M
CIMMYT7	CS/A.Curv//Glen/3/Ald/Pvn	Mexico	MS-MR	MR-MS	ML

MS-MR=Moderately resistant to moderately susceptible
MR-MS=Moderately Resistant to moderately susceptible
S=Susceptible
VS=Very Susceptible
E=Early
ME=Medium Early
M=medium
ML=Medium Late

Table 2. Analysis of variance for combining abilities (GCA and SCA), Griffing's method-4, model-1; Mean squares for Fusarium head blight and tombstone kernels under field and greenhouse environments.

Source	DF	FHB(FD)	FHB(GH)	TOM(FD)	TOM(GH)	Scab index
GCA	4	57.1**	178.3**	135.4**	96.9**	208.6**
SCA	5	6.4**	49.3**	29.2**	37.5**	43.0**
Residual	980	0.6	3.0	1.3	2.5	1.8

** Significant at 0.01 probability level

FHB(FD)=Fusarium head blight (field)
FHB(GH)=Fusarium head blight (greenhouse)
TOM(FD)=Tombstone (field)
TOM(GH)=Tombstone (greenhouse)

Table 3. GCA effects and mean disease scores (per se) of five spring wheat lines/cultivars for FHB and tombstone kernels under field and greenhouse conditions.

Lines/ Cultivars	GCA Effects (Per se)				
	FHB(FD)	**FHB(GH)**	**TOM(FD)**	**TOM(GH)**	**Scab index**
2375	-3.3**	-1.9	-6.5**	-2.5*	-4.9**
	(68.4)	(56.0)	(59.3)	(51.8)	(86.9)
Fang60	5.4**	11.7**	7.8**	4.1**	10.3**
	(86.2)	(82.1)	(88.6)	(80.8)	(125.3)
Sonalika	2.6**	1.6	4.2**	5.8**	5.1**
	(83.0)	(67.6)	(87.7)	(79.6)	(117.5)
Seri82	0.7	-2.1	2.0*	1.0	0.5
	(83.5)	(70.3)	(82.3)	(80.8)	(117.0)
CIMMYT7	-5.4**	-9.5**	-7.5**	-8.4**	-11.1**
	(65.1)	(56.1)	(53.0)	(44.0)	(81.1)

*, ** Significantly different from 0 at 0.05 and 0.01 probability levels, respectively.

Figures in parenthesis indicate per se disease ratings in percent.

Table 4. Correlation among FHB and tombstone kernels under field and greenhouse environments averaged over ten populations.

Variables	Correlation coefficient (r)
FHB vs Tombstone	
FHB (GH)-Tombstone (GH)	0.478**
FHB (FD)-Tombstone (FD)	0.899**
FHB-Tombstone (Overall)	0.709**
Field vs Greenhouse	
FHB (GH)-FHB (FD)	0.501**
Tombstone (GH)-Tombstone (FD)	0.502**
Scabindex (GH)-Scabindex (FD)	0.583**

** Significant at 0.01 probability level

TRANSFERRING FUSARIUM HEAD BLIGHT RESISTANCE FROM BREAD WHEAT SOURCES TO HIGH YIELDING ADVANCED LINES

L. Gilchrist*, S. Rajaram, M. van Ginkel, and C. Velazquez

INTRODUCTION

Fusarium head blight is a common disease in high rainfall environments, where it causes severe cereal yield losses. The causal pathogen produces toxins that are hazardous to human and animal health. This last issue is of particular concern in areas where farmers produce grain for food and feed without controlling the level of toxins in the grain.

Though scab resistant wheat germplasm is rather limited, resistance sources have been found in wheats from Brazil, Japan and China. Resistance from these diverse genetic sources has been incorporated into high yielding genotypes in the CIMMYT Bread Wheat Program. However, the sources are mostly tall, late maturing wheat landraces that are low yielding, highly susceptible to leaf, stem and stripe rust, and generally have undesirable agronomic traits. The process of transferring resistance from these sources to high yielding wheats is not an easy one, but over the years the Program has succeeded in developing high yielding lines that show excellent resistance to fusarium head blight.

In this poster we highlight the highest yielding lines with the best resistance to fusarium head blight developed by the Program.

MATERIALS AND METHODS

For many years, the CIMMYT Wheat Program has concentrated on screening and breeding bread wheat germplasm for type I (fungal penetration) and II (fungal spread through the rachis) resistance under field conditions. Before advanced germplasm is evaluated for its response to scab, it is screened for resistance to the three rusts (leaf, stem and stripe) and to foliar blights.

Selection for leaf and stem rust resistance is conducted in the Yaqui Valley, Sonora, in northwestern Mexico, while resistance to stripe rust, *Septoria* spp. and scab is evaluated in Toluca, Mexico State, under artificial inoculation. Additional scab resistance observations are made in Patzcuaro, in the State of Michoacan, and Guadalajara, in the Sierra of Jalisco (State of Jalisco), where natural infestation is very heavy (Fig. 1).

A variety's ability to fill grain properly is very important. To ensure adequate grainfilling, the advanced lines are evaluated for this trait by comparing seed from inoculated plots vs plots protected by fungicide (Folicur) applied every 10 days.

In response to concerns over grain toxicity, grain of advanced lines is screened for toxin content. In this past year levels of DON toxin in lines showing good field resistance were evaluated at CIMMYT using the fluoroQuant method (Romer Labs, Inc).

Details of the methods used by the Wheat Program for inoculum increase and other laboratory and field inoculation procedures are described in Gilchrist et al., 1997.

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RESULTS AND DISCUSSION

The best bread wheat lines obtained in the breeding program combine various resistance responses (lower levels of penetration, reduced spread, decreased DON content and well filled grain) (Table 1). These results are from the last two crop cycles. Data showing resistance reactions were obtained over four cycles.

Table 2 shows progress in the CIMMYT-Shanghai (China) shuttle breeding program, using Sumai 3 as a resistant check. The lines developed through this program show excellent levels of scab resistance (better than Sumai 3) as well as high yield potential and good agronomic type (Fig. 2).

Incorporating fusarium head blight resistance into high yielding wheats is an ongoing process that seeks to broaden the genetic base of resistance through the use of diverse sources. The next step envisioned for this process is to combine the resistance described here with resistance derived from wild relatives of wheat using derivative hexaploid wheats.

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Table 1. Fusarium head blight resistant bread wheat lines developed by CIMMYT s Bread Wheat Program.

Cross	Pedigree	% Damage 1998	% Damage 1999	1998	1999	DON ppm	Test weight losses (%)	Grain quality (0-5)[1]
CATBIRD	CM91045-5Y-0M-0Y-4M-4Y-2M-0M-4M-0Y-3SCM	2.41	0.94	10.34	15.73	1.3	0.85	1
SHA3/CBRD	CMSS92Y00595S-1SCM-0CHN 015Y-3SCM	1.00	0.30	8.95	8.70	0.47	3.06	11
NG8675/CBRD	CMSS92Y00639S-5-5SCM	0.0	0.34	4.40	8.75	0.24	4.40	0-1
MILAN/SHA7	CM97550-0M-2Y-030H-3Y-3Y-0Y-3M-010Y-1SCM-0FUS-1FUS	3.39	0.59	8.77	13.45	0.23	3.56	0-1
GOV/AZ//MUS/ 3/DODO/4/BOW	CM79515-044Y-1M-02Y-07M-3Y-3B-0Y-0PZ-2PZ-0Y-5PZ-010Y-0M-3M-0Y-1SCM	5.30	4.37	11.56	16.48	0.42	0.90	1-2
CHUM18//JUP/ BJY	CM91046-7Y-0M-0Y-4M-8Y -0B-0FC-2FUS-0Y-1SCM	3.29	0.84	10.86	10.63	0.85	18.39	3
CHECKS								
FLYCATCHER (Moderate suscept)	CM43598-II-8Y-1M-1Y-3M-0B-7B-0Y	25.57		21.28		3.2	3.64	4
FRONTANA (Moderate Resistant)		12.90		17.03		2.0	7.71	2

1 0 = Excellent (no differences in appearance with fungicide protected grain) 5 = Poor (shriveled, sunken, highly infected).
2 NG8675 = NIGMAI 4 /OLESON//ALONDRA/YANGMAI 3/3/08181.

Table 2. Fusarium head blight resistant bread wheat lines developed by the Shanghai/CIMMYT shuttle breeding program and tested two years in Atizapan, Toluca, State of Mexico.

Reintroduced lines selected in Shanghai, China	% Damage Type I 1998	Type I 1999	Type II 1998	Type II 1999	DON (ppm)	Grain quality (0-5)[1]
SHANGHAI -0SHG-13GH-0FGR-0FGR	3.97	2.4	3.62	3.33	0.0	0
SHA3/CBRD 0SHG-2GH-0FGR-0FGR	9.90	0.0	8.40	4.28	0.05	0-1
SHA3/CBRD 0SHG-3GH-0FGR-0FGR	10.00	1.13	12.90	1.10	0.13	0-1
SHA3/CBRD 0SHG-5GH-0FGR-0FGR	5.37	2.56	5.04	2.17	0.14	1
SHA3/CBRD 0SHG-1GH-0FGR-0FGR	3.72	0.0	12.41	6.59	0.02	1
SHA3/CBRD 0SHG-16GH-0FGR-OFGR	0.26	5.43	5.65	2.75	0.0	1
NS73/PCI//B143.241.2/3/NING8647 0SHG-8GH-0FGR-0FGR	7.97	2.17	8.33	7.16	NA[5]	4
MIAN YANG81-5[2]//PC B084.985/JIANZIMAI[3] 0SHG-14GH-0FGR-0FGR	6.16	2.45	11.33	4.30	NA	4
PC B084.985/JIANZIMAI//8744 0SHG-4GH-0FGR-0FRG	8.91	3.08	14.53	5.71	NA	2
Checks						
Sumai #3[4] (Resistant)	9.77	1.64	6.06	24.85	0.0	1-2
Flycatcher (Moderate Susceptible)	25.57	7.47	21.28	49.07	3.2	4

1 0 = Excellent (no differences in appearance with fungicide protected grain) 5 = Poor (shriveled, sunken highly infected)
2 MIAN YANG 81-5 = MIANYANG 20 (=70-5858/FAN 6)
3 JIANZIMAI = Landrace
4 SUMAI # 3 = FUNO/TAIWAN WHEAT
5 NA = Not Available

TRANSFERRING FUSARIUM HEAD BLIGHT RESISTANCE TO MALTING AND OTHER TYPES OF BARLEY

L. Gilchrist[1*], H. Vivar[2], P. Hayes[3], C. Velazquez[1], and J. Crossa[1]

INTRODUCTION

Fusarium head blight caused by *Fusarium graminearum* is a disease of barley that reduces grain yield and affects grain quality. The pathogen produces toxins in the grain that are harmful to human and animal health. Swine, in particular, are very sensitive to toxin-contaminated grain.

In the Andean Region (Bolivia, Ecuador, Peru and Southern Colombia) of South America, barley is used for food and feed. In contrast, Mexico, Argentina, Brazil and Uruguay are malting barley producers. Fusarium toxins can affect the quality of both malt and beer by increasing wort nitrogen and reducing rootlet growth, malt recovery and beer gas stability. Beer and malt produced with toxin-contaminated grain will in turn be contaminated and will have a higher propensity to overfoam or gush (Schwarz et al., 1996).

To provide farmers with effective protection against Fusarium head blight, the ICARDA/CIMMYT barley program in Mexico is evaluating new sources of resistance, transferring resistance genes into a malting background, and developing resistant hull-less barley for food and feed.

MATERIALS AND METHODS

Malting barley

A doubled haploid (DH) population (125 lines) was developed in a two-rowed background. Sources of Fusarium resistance (Gob DH 96, Gob DH 24 and CMB 643=Azafran) (Vivar et al., 1997) were crossed with malting quality barley lines [Orca from Oregon (USA), AF 9216 (Brazil) and NE 175 (Uruguay)].

The DH population and parents were evaluated in Atizapan, Toluca, State of Mexico, under field inoculation during the 1999 summer cycle (Fig. 1). The spray and cotton methods were used to screen for type I and type II resistance, respectively (Gilchrist et al., 1997). Twenty spikes at the same growth stage (flowering in the center of the spike) were labeled. Inoculation for type I resistance was done at sunset with a 20-ml suspension (50,000 spores/ml) sprayed over the spikes. The spikes were evaluated after 15 days based on total number of grains as related to the number of visible penetration points, expressed in percentages.

For type II resistance, 20 spikes were inoculated and covered with a glassine bag. The spikes were evaluated after 30 days by counting the total number of infected grains close to the inoculated point and the total number of grains/spike, expressed in percentages.

Statistical categorical data analysis was done to identify the lines with the best Fusarium resistance.

Barley for food and feed

A method similar to the one described above was used to inoculate six-rowed hull-less F6 lines derived from crosses with Chevron (type I

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resistance source). Hull-less barley was reported by Clear et al. (1996) to reduce toxin content in the grain.

RESULTS AND DISCUSSION

Malting barley

Information on parents of the doubled haploid population is presented in Table 1. The disease reaction of the best DH entries are shown in Table 2.

Table 1. Characterization of type I and type II resistance to fusarium head blight present in parents used to produce the doubled haploid population. Atizapan, Toluca, State of Mexico, 1999.

	% damaged grains	
Parents	Type I	Type II
GOB 96 DH	10.7	6.7
Azafran	0.9	10.9
GOB 24 DH	6.7	4.2
Orca	13.7	20.6
NE 175	2.5	38.5
AF9216	7.1	26.3

Table 2. Doubled haploid entries combining the best type I and II resistance to fusarium head blight. Atizapan, Toluca, State of Mexico, 1999.

	% of damage grains	
DH entry number	Type I	Type II
DH 5	7.0	8.9
DH 6	3.7	8.9
DH 24	0.7	9.8
DH 34	9.1	7.4
DH 43	3.7	10.1
DH 60	6.5	6.9
DH 66	8.1	9.2
DH 87	4.9	9.8
DH 112	2.7	9.0
DH 115	4.2	7.1

The statistical analysis gave the range of infection for the group with superior type I (0.7-6.1 % infected grains) and type II (6.9-10.6 %) resistance, with a 95% confidence interval.

When crosses were made, no information was available on fusarium resistance of the malting parents. After field testing, several parents (NE 175 and AF 216) showed a good level of type I resistance, but very high susceptibility to type II (Table 1). These results explain the distribution in the population, which showed 40% type I resistance, 11% type II, and only 8% combining both resistance types (Table 2). Figure 2 shows the range of type I and II infection obtained during the evaluation process.

Barley for food and feed

According to our previous field results, Chevron, a six-rowed barley variety, possesses type I resistance, but not type II. Results presented here show lines to which type I resistance was transferred (Fig. 3) and a susceptible sister line.Only a few lines in this population combine both types of resistance (Table 3).

Table 3. Chevron-derived food and feed barley lines combining type I and II resistance to fusarium head blight Atizapan, Toluca, State of Mexico, 1999.

	% damage	
Cross	Type I	Type II
Petunia 1/Chevron //Tocte CBSS96M00734T-C-1M-1Y-2M-0FGR	1.08 - 6.35	
Petunia 1/Chevron //Tocte CBSS96M00734T-C-2M-4Y-1M-0FGR	6.59 - 22.48	

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RESISTANCE TO FUSARIUM HEAD BLIGHT IN SYNTHETIC HEXAPLOID WHEATS (2n=6x=42, AABBDD)

L. Gilchrist*, C. Velazquez, and A. Mujeeb-Kazi

INTRODUCTION

Fusarium head blight resistance in bread wheat is thought to be controlled by two to three major genes and several minor genes as modifiers. Head blight resistance can be transferred from alien sources to reinforce and improve the resistance already present in bread wheat.

In general the transfer of alien genes from tertiary genepools is a long process. However, bridge crosses utilizing D-genome synthetic hexaploids (SH) (*Triticum turgidum/Aegilops tauschii*) are a powerful means of swiftly improving bread wheat (*T. aestivum*) for resistance to biotic stresses (Mujeeb-Kazi et al., 1998).

In the past few years, CIMMYT wheat wide crosses has worked in conjunction with wheat pathology on the identification of scab resistant synthetic hexaploid wheats and their bread wheat derivatives.

MATERIALS AND METHODS

Germplasm development

Elite durum wheat (*Triticum turgidum*) cultivars were crossed with several hundred *Aegilops tauschii* accessions following the scheme shown in Fig. 1.

Figure 1. Synthetic Hexaploid Generation

T. turgidum X *Ae. tauschii*

↓

Hybrid
(2n=3x=21 ABD)

↓

Synthetic Hexaploid
(2n=6x=42, AABBDD)

↓

Head Blight Screening

Germplasm screening

Preliminary screening for leaf and stem rust resistance is carried out in the Yaqui Valley, State of Sonora. During the summer cycle, germplasm found to possess leaf and stem rust resistance is inoculated with stripe rust and a mixture of five virulent isolates of *Septoria tritici* in Atizapan, Toluca, State of Mexico.

Lines found to be resistant to the three rusts (leaf, stem and stripe) and *Septoria tritici* are evaluated for head blight resistance. Five spikes from each line are inoculated using the cotton method (Gilchrist et al., 1997). All lines showing type II resistance in this preliminary screening are inoculated the following cycle to confirm the initial observations. In addition, a spore suspension (50,000 spores/ml) is sprayed on each plot to determine type I (penetration) resistance. The

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same plot is harvested and a sample is taken and analyzed for DON content using the fluoroQuant method (Romer Labs, Inc.) Lower DON levels suggest type III resistance.

Grain filling is assessed by comparing test plots inoculated using the spray method with a similar plot sprayed with fungicide (Folicur) every 10 days. Minimum losses in test weight indicate type IV resistance.

RESULTS AND DISCUSSION

Presented in Table 1 are lines showing good fusarium head blight resistance, as demonstrated by their scores for different the resistance components.

Some of the SHs produced so far possess desirable fusarium resistance (type II). These SHs were utilized in crosses with bread wheat cultivars and advanced derivatives that had been tested for resistance (types I, II, III, and IV). The SH diversity has been transferred to bread wheat plant types. The *Ae. tauschii* diversity is revealed by the presence of five accessions (Table 1) in the pedigrees of advanced lines 205, 222, 223, 369, and 447. Micro-satellites of the D-genome are being utilized to elucidate the uniqueness of these accessions.

The best resistance combination is present in the cross Mayoor// TK SN1081/*Ae. tauschii*(222), which has also been identified to possess multiple disease resistance (Mujeeb-Kazi et al., 1999). Studies are currently in progress to determine genetic control, as well as develop an F1-based doubled haploid population in which the bread wheat parent Flycatcher is susceptible to all stresses to which the cross Mayoor// TK SN1081/*Ae. tauschii*(222) is resistant.

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Table 1. Synthetic bread wheat hexaploids with fusarium head blight resistance under artificial inoculation, Atizapan, Toluca, State of Mexico, during the 1998 and 1999 summer cycles.

Lines	% Damage Type I 1998 1999	% Damage Type II 1998 1999	DON (ppm)	Test weight losses (%)	Grain (0-5)
TURACO/5/CHIR3/4/SIREN//ALTAR 84/*Ae. tauschii* (205)/3/3*BUC CASS94Y00034S-24PR-2B-0M- 0FGR-0FGR-0FGR	18.42 - 8.01	9.88 - 8.71	0.58	5.27	2+
BCN//DOY1/*Ae. tauschii*(447) CASS94Y00006S-53PR-2B-0M-0FRG-0FGR-0FRG-0FGR	10.34 - 9.59	10.34 10.07	1	2.64	1
MAYOOR//TK SN1081/*Ae. tauschii*(222) CASS94Y00009S-18PR-3M-0M-0FRG-0FRG-0FRG	7.26 - 0.0	5.81 - 13.96	1.2	6.06	1
MAYOOR//TK SN1081/*Ae. tauschii*(222) CASS94Y00009S-50PR-2B-0M-0FRG-0FRG-0FRG	4.14 - 0.0	9.67 - 14.19	1.2	6.50	0-1
OPATA/5/CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (223) CASS94Y00215S-5Y-1M-0M-0FRG-0FRG-0FRG	10.93 - 1.74	27.7 - 22.15	n.a.	6.18	3
MAYOOR/5/CS/*Thinopyrum curvifolium* //GLEN/3 /ALD/PVN/4/SC/ *Leymus racemosus* //2*CS /3/ CNO79 CIGM93.619-3Y-2B-0PR-1Y-0M-0FRG-0FRG-0FRG	10.17 - 0.0	29.46 - 22.58	n.a.	5.35	3
BCN/3/68112/WARD//*Ae. tauschii* (369) CASS94Y00125S-5Y-2M-0M-0FRG-0FRG-0FRG-0FR	12.66 - 11.21	20.60 - 3.76	n.a.	12.17	3-4
SUMAI#3 (resistant check)	4.41 0.86	15.49 10.59	0.27	38.59	3
FRONTANA (moderately resistant check)	12.90 - 11.56	17.03 - 27.85	2.0	7.71	2
MAYOOR (moderately resistant check)	16.94 3.4	17.04 - 27.85	4.5	5.09	3

+Grain 0= Excellent (no differences in appearance with fungicide protected grain).
Grain 5= Poor (shriveled, sunken, highly infected).
n.a. = Not available.

ADVANCES IN RESEARCH ON FUSARIUM HEAD BLIGHT IN THE VIRGINIA WHEAT BREEDING PROGRAM

Carl A. Griffey*, Jianli Chen**, Tom Pridgen, Matthew Chappell and Jane Shaw

OBJECTIVES

1) To optimize techniques for establishment and assessment of *Fusarium* head blight; 2) to identify resistance sources among both exotic germplasm and adapted wheat cultivars or lines; 3) to identify and select scab resistant wheat lines derived from crosses between type II and adapted resistance sources; and 4) to accelerate development of scab resistant cultivars or lines via use of back-crossing, wheat x maize mediated doubled haploid production and molecular marker assisted selection.

INTRODUCTION

Since the early 1990's *Fusarium* Head Blight (FHB) or scab has been one of the most devastating diseases in the U.S. and has increased in importance worldwide. The most tactical approach for controlling this disease is to utilize effective and diverse sources of resistance to develop cultivars possessing multiple types of defense mechanisms. However, success of this endeavor is greatly dependent on first obtaining knowledge of the amount of genetic diversity for resistance, identity of different mechanisms governing resistance, inheritance of resistance, and identifying selectable markers for incorporating and pyramiding resistance genes into wheat cultivars. Several type II resistance sources, such as Sumai 3 and its progeny, have been identified subsequent to the work of Shroeder and Christensen (1963). Recently, sources possessing other types of resistance such as type IV and V have been reported by Mesterhazy (1995 and 1999). Combining type II resistance with other types of resistance will likely lead to the development of cultivars possessing more effective and stable scab resistance.

MATERIALS AND METHODS

Optimization of Techniques for Disease Development and Assessment

Field Tests. Conidial suspension and scabby-seed dispersal methods of inoculation were used in 1997-99 field-tests. Conidial suspensions (378 L/Ac at 50,000 spores/ml) of *Fusarium graminearum* were sprayed on plots at both heading and flowering stages for homozygous genotypes. Segregating populations were sprayed with conidia once at heading and twice during flowering. Scabby seeds (20 Kg/Ac) were dispersed in plots at both the booting and heading stages for homozygous genotypes, and three times in segregating populations, once at booting and twice at heading stages. After inoculation, all field plots received overhead irrigation as a fine mist of water from 8-9 a.m. and again from 6-8 p.m. for 14 to 21 days or until symptoms were observed in most plots. Scab incidence, severity and infection type were assessed three times at 7 day intervals after inoculation. Grain yield, test weight, 1000 kernel weight, percentage of infected kernels and DON content were determined after harvest. All data analyses were performed as for a Randomized Complete Block Design using Agrobase software.

Greenhouse Tests. Single floret inoculation mainly was used in greenhouse tests. A drop

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(about 30ul) of conidial suspension (50,000 spores/ml) was placed into a single floret of the mid-spike using a SAMCO transfer pipette at the beginning of anthesis. Mist irrigation was applied immediately after inoculation at an interval of 45 seconds per 30 minutes for 3-5 days or until the first symptom was visible. Infection type (0-5; Chen, 1989) for each head was assessed 2-3 times at weekly intervals following inoculation.

Germplasm Evaluation and Breeding Tests

Germplasm has been evaluated in greenhouse tests from 1997 to 1999. Three sets of germplasm were used in these studies, which included 31 commercial cultivars or lines, 50 exotic resistance sources and 33 adapted resistant lines. The 31 commercial cultivars or lines were from the Virginia Official Variety Test and a mid-Atlantic Joint Test. The 50 scab resistant sources were from China (17), Italy (2), Brazil (1), Japan (3), Canada (12), France (3) and Austria (12). Three plants of each genotype were tested for type II resistance via the single floret inoculation method and one to two plants were tested for type I resistance via spraying a conidial suspension on the heads. Field tests included a Germplasm Screening Test (GP), Uniform Scab Test (UT) and Yield Loss Test (YT).

Breeding populations were first developed in 1994 with 20 crosses being made between SRW wheat genotypes and six Chinese resistance sources. From these populations, 2450 F_5 head rows were derived and evaluated in Advance Line Tests (AT) in 1999. Presently, more than 572 crosses are included in the Breeding Nursery, and consist of 290 F_1, 8 BC_2F_1, 24 BC_1F_1, 185 F_2, 41 F_3, 11 F_4 and 20 F_5 populations, respectively.

Three replicates of the UT test and one replicate of the GP and AT tests were grown in 20 ft^2 field plots (4-row headrow plots). Yield loss tests consisted of 100 ft^2 plots grown in six replicates, comprised of inoculated (3 reps) and non-inoculated (3 reps) treatments. Segregating populations were grown in individual 125 ft^2 plots.

Accelerated Development of Scab Resistant Germplasm and Populations

F_1 plants derived from 12 crosses between six of the best scab resistance sources and two susceptible cultivars, Madison and Pioneer 2684, were used to produce haploid plants by the wheat x maize hybridization method described by Chen et al. (1999). In addition, F_2 populations of 4 of the12 crosses were produced and are currently being used to study the inheritance and mechanism of resistance. An $F_{2:3}$ population of the cross Madison x W14 is being used to map the scab resistance genes in W14.

RESULTS AND DISCUSSION

Type of Resistance

Schroeder and Christensen (1963) first reported type I (penetration or invasion) and II resistance (spreading or extension) to FHB. Miller et al. (1985) reported type III (toxin accumulation) resistance, and Mesterhazy (1995) reported type IV and V resistance to kernel infection and tolerance, respectively. Few type I resistance sources have been reported, while several type II resistance sources have been identified and offer the most effective component of scab resistance (Bai, 1995; Buerstmayr, 1999; Masterhazy, 1995 and 1999). Results from our studies are in agreement with those of the aforementioned studies.

When conidia were sprayed onto wheat heads, no difference in infection type was found among genotypes, and 100% of the florets of the inoculated heads were infected in 1997 greenhouse tests. In contrast, when the single floret inocula-

tion method was applied, significant differences in resistance were found among genotypes (Tables 1 and 2) evaluated in 1997-1999 tests. While Mesterhazy defines type IV and V resistance independently, data from field tests conducted in 1997-1999 suggests that type IV and V resistance may result from a common mechanism. Significant positive correlations were observed between kernel infection with yield loss and test weight loss (Griffey and Chen, 1998 and 1999). DON content was significantly correlated with scab severity, kernel infection and yield loss. This finding is in agreement with results of most studies (Atlin et al. 1983; Wang and Miller 1988; Chelkowski 1989; Gilbert et al. 1993; Lemmens et al.; 1997; Buerstmayr et al. 1999) except for one by Mesterhazy (1999). Therefore, we conclude that selection of genotypes with a low percentage of scabby seeds is most likely to identify plants with relatively low DON levels. Severity, as a preharvest parameter and scabby seeds as a postharvest parameter are suggested as assessment parameters to evaluate resistance or tolerance to scab.

Techniques for Disease Development and Assessment

Use of precise inoculation methods and reliable assessment parameters are fundamental components for success in FHB research. Among a number of factors affecting disease development under field conditions, inoculum and free-moisture are controllable factors, whereas growth stage and temperature are non-controllable ones. The method used for disease establishment should correspond to specific research objectives and should reduce environmental and experimental variability to a minimum.

Scattering of scabby seeds as the primary source of inoculum is relatively easy, economical and not too laborious and, therefore, has been used in many breeding program to screen large numbers of pure lines and segregating populations. However, the incidence of disease resulting from this inoculation method can be affected greatly by plant height and growth stage (Griffey and Chen et al. 1997,1998) and, therefore, lacks precision in both uniformity and timing of infection. Spraying of conidial suspensions was found to be the most effective inoculation method under field conditions as it minimizes variability due to differences in plant height and growth stage and provides for precise application of uniform inoculum. Because infection rate and efficiency can be more precisely controlled and quantified, this method provides conditions for simultaneous assessment and selection of type II and other types of resistance, which likely represents field resistance.

Floret inoculation can be used effectively in both greenhouse and field evaluations for type II resistance (Griffey and Chen et al. 1997). This inoculation method is not affected by plant height or growth stage and can be conducted under controlled environmental conditions, which are critical for conducting genetic and mapping studies. Because floret inoculation is time consuming, it is not practical for screening large numbers of populations.

To evaluate field resistance, scab severity and percentage scabby seeds were found to be more effective and predictive than scab incidence for evaluating resistance or tolerance to scab. To evaluate type II resistance, mediated by floret inoculation, infection type assessment using a 1-5 incremental scale (Chen, 1989), was used in studies conducted in 1997-99. Infection type is assigned based on a qualified scale, with infection of the rachis being a critical component. Because type I resistance has not been identified in most currently used sources, all plants should produce infection symptoms upon inoculation. Differences among genotypes for type II resistance are expressed after initial infection, and are based on the length of extension time, the number of infected florets and the severity of infec-

tion. Results of our studies indicate that two to three ratings, made at 7-day intervals after inoculation, are necessary to accurately assess disease progress over time and, therefore, type II resistance. Differences between genotypes were observed after the first rating and 21 days after inoculation is usually the optimal time for the last rating. After 21 days, some susceptible spikes exhibit infection of the peduncle. Infection type ratings made 21 days post-inoculation are used in selection of genotypes with type II resistance.

Scab Resistance Sources

Assessments of type II resistance in 32 diverse sources are presented in Table 1. Significant differences were found among genotypes. Type II resistance identified in the germplasm was classified into three groups. Group I consists of highly resistant sources, which mainly includes the Chinese lines. Sumai 3, W14, Shaan 85, Futai 8944, Futai 9002 and Wuhan 1 were included in this group based on data from three years of testing. Based on replicated tests in a single year, Frontana, VR95B717, Shinchunaga, Saikai 165, H821, HC374 and H192 were also classified as highly resistant. Genotypes in this group usually have 1-3 infected florets per inoculated spike with little to no rachis infection, and do not exhibit significant change in infection type from 7-21 days after inoculation. W14, Shaan 85, Futai 8944 and VR95B717 are the four sources being used most extensively in our breeding program because of their resistance, superiority to Sumai 3 for other agronomic traits and/or their good combining ability. Approximately 552 crosses including type II resistant parents have been produced and are represented by $F_1 - F_4$ populations.

Group II includes moderately resistant sources, such as Ning 7840, Ning 9016, Yangmai 6, Yangmai 87158, Fan 1, Changjiang 8809 and VR95B295. The resistance of lines in this group is comparable to that of Funo, Mentana, and Nobeokabouzu-komugi, which are the primary parents of currently available type II resistance sources. Inoculated spikes of plants in this group have 3-5 florets infected, with increased rachis infection. Because some lines in this group have other favorable traits, such as early maturity, good head and/or plant type, these also may be useful sources of type II resistance. A total of 2470 F_5 head rows, with scab resistance derived from such sources, will be assessed for other agronomic traits and performance in field tests this year.

Group III includes sources with little or no type II resistance, such as Wuhan 3, Liang 10, VR95B063, FHB lines and most SRW wheat genotypes (Table 1 and 2). Susceptible genotypes have more than five infected florets per spike, with rachis infection, and exhibit either consistently high infection types or large changes in infection type between 7-21 days post-inoculation. Spikes of susceptible genotypes usually are bleached or white in color with infection of the peduncle in later stages of disease expression. Reaction response of genotypes in group I is more consistent and stable than that of genotypes in groups II and III. This is consistent with findings reported by Mesterhazy (1999) and Buerstmayr (1999).

Type II resistance was minimal or absent in the SRW wheat genotypes evaluated in these studies (Griffey and Chen et al. 1997); however, genotypes possessing other types of resistance or tolerance were identified. Genotypes such as Ernie, Roane, Freedom, P92823A1-1-4-4-5 and VA96W-326, had variable ratings for infection type, but had significantly low ratings for scab severity, yield and/or test weight loss, scabby kernels, and DON content (Table 2). Roane, released in 1998 by the Virginia Wheat Breeding Program, has consistently exhibited tolerance to scab in multi-year tests grown in Virginia and other states. VA96W-326 was found to be comparable to Ernie in maturity and level of resistance in 1999 tests. Roane and VA96W-326,

with high yield potential and resistance to other diseases, are scab-tolerant genotypes we are using in our program to incorporate type II resistance in to. Type II resistance derived from nine different sources was back crossed into 11 different SRW wheat backgrounds. Progeny from eight BC_2F_1 and 24 BC_1F_1 populations possessing type II resistance were crossed to their respective recurrent parents this year. Type II resistance is being transferred into acceptable SRW wheat backgrounds and combined with other types of resistance in some cases.

Future Plans

Because type II resistance has not been found in SRW wheat genotypes, identification and/or release of cultivars with tolerance to scab could provide for immediate reductions in yield and quality losses resulting from scab. SRW wheat genotypes with tolerance to scab will serve as ideal recurrent parents for incorporating type II resistance in to. In this way, type II resistance can be combined with other types of resistance in superior backgrounds. Tolerant SRW wheat genotypes, such as Roane, possess other favorable traits such as high test weight and yield potential, resistance to powdery mildew and Hessian fly, tolerance to Barley Yellow Dwarf Virus, and good general combining.

Development of adapted wheat cultivars with stable and improved levels of scab resistance can be best achieved by pyramiding diverse resistance genes that individually confer only partial resistance and /or act via different mechanisms. The most feasible, and perhaps only possible, means for achieving this goal is to identify molecular markers that are tightly linked to such resistance genes, which can be used to facilitate marker-assisted selection of scab resistance and gene pyramiding. Development of doubled haploid lines via the wheat x maze hybridization system may prove beneficial in accelerating the development of SRW wheat lines with higher levels of combined scab resistance, since pure lines can be obtained in one generation rather than six to eight (Chen and Griffey et al. 1999). These types of research projects are currently being implemented in the Virginia breeding program.

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Table 1. Identification of Type II Resistance to Scab in 32 Diverse Sources via Floret Inoculation in 1997-99 tests.

Genotype	Source	Infection Type (1-5)*		
		1997	1998	1999
Funo	Italy		3.2 - 4	3.0 - 4
Mentana	Italy		3.2 - 3.6	4
Frontana	Brazil		2 - 3.2	
Sumai 3	China	3	2 - 3.4	2 - 3.4
W14	China	1	2	3.4
Shaan 85	China	3	2	2
Futai 8944	China	3	2	2 - 3.4
Futai 8945	China		3.4 - 4	3.4 - 4
Futai 8946	China	3	3.0 - 4	3.8 - 4
Futai 9002	China	2	2	2 - 3.4
Ning 7840	China	2	2 - 3.6	2 - 3.8
Ning 9016	China	3	3.0 - 4	3.8 - 4
Yangmai 6	China		3.4 - 4	3.4 - 3.8
Yangmai 87158	China		3.2	3.6 - 4
Liangmai 10	China		4.0 - 5	4
Fan 1	China	4	3.4	2 - 3.8
Wuhan 1	China	1	3.4	3.2
Wuhan 3	China	5	3.6 - 4	4.0 - 5
Changjiang 8809	China		2 - 3.4	3.6 - 3.8
Nobeokabouzu-komugi	Japan			3.4 - 4
Shinchunaga	Japan			3.4
Saikai165	Japan			2 - 3.4
VR95B717	France			2 - 3.2
VR95B295	France			3.2 - 4
VR95B063	France			4.0 - 5
FHB143	Canada		3.6 - 4	
FHB147	Canada		3.8 - 4	
FHB148	Canada		4.0 - 5	
FHB161	Canada		4	
H821	Canada		2 - 3.2	
HC374	Canada		2	
H192	Canada		2 - 3.4	

* Infection type: (for evaluating type II resistance by floret inoculation)

1.0 = only inoculated floret infected; 2.0 = only inoculated spikelet infected;

3.0 = inoculated spikelet and rachis infected; 3.2 = inoculated and one adjacent spikelet infected;

3.4 = inoculated and two adjacent spikelets infected; 3.6 = inoculated and three adjacent spikelets infected;

3.8 = inoculated and four adjacent spikelets infected; 4.0 = half of spike or over six spikelets infected;

5.0 = whole spike infected.

According to the highest infection type of each individual, resistance can be subdivided into R (1-3), MR (3.2-

MS (3.6-3.8) and S (4-5) reaction classes.

Table 2. Evaluation of tolerance to scab among 18 soft red winter wheat genotypes tested at Blacksburg, Virginia in 1997-99.

LINE	Infection Type (1-5)		Test Weight Loss (%)		Scabby Seeds (%)		Scab Severity (%)		Scab Index		Scab Incidence (%)		Toxin (ppm)
	1997	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998
ERNIE		3.2-4	1.9	2.3	19	19	18	11	8	4	42	31	10.1
ROANE	3.6-3.8	3.0-4	1.8	2.2	20	8	23	14	18	9	79	61	10.6
P92823A1-1-4-4-5			1.8	1.1	14	4	21	15	15	7	67	49	8.1
FREEDOM		3.4-3.8	1.9	3.6	18	10	21	20	18	13	83	64	9.0
AGRIPRO FOSTER		3.6-4	3.6	0.6	19	6	32	17	27	7	83	43	9.0
COKER 9803	5	4.0-5	3.6	1.6	19	11	32	18	20	8	65	44	8.8
GA GORE		4.0-5	11.8	1.7	47	10	85	30	83	17	97	56	20.7
WAKEFIELD		4.0-5	3.8	1.7	23	10	32	21	23	14	71	62	12.3
AGRIPRO MASON	5	4.0-5	5.4	2.8	26	7	37	18	27	9	71	51	12.8
POCAHONTAS		3.8-5	5.7	2.8	29	8	42	26	38	18	93	69	14.2
JACKSON	5	4.0-5	5.6	2.3	39	13	32	29	25	24	77	79	12.8
PIONEER 2552	3.4-3.6	3.8-5	1.8	2.7	23	9	30	15	25	10	84	65	12.8
PIONEER 2684	5	4.0-5	3.6	2.8	32	9	42	21	36	14	87	65	14.1
PIONEER 2643	4	4.0-5	5.4	3.3	25	8	42	18	36	11	86	58	12.8
PION2580	5	4.0-5	3.7	3.4	37	22	29	21	20	11	69	57	13.3
FFR555		4.0-5	7.3	3.4	27	16	46	21	41	12	89	61	14.4
MADISON	5	4.0-5	9.1	5.2	31	12	45	22	34	14	73	63	15.4
COKER9835		4.0-5	7.8	9.1	42	27	52	37	50	33	95	89	17.6
LSD(0.05)					8	6	10	7	13	7	16	17	1.9
Mean					27	11	36	19	30	12	79	58	12.5

EVALUATION OF YUGOSLAVIAN WHEAT GERMPLASM FOR RESISTANCE TO HEAD SCAB OF WHEAT

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INTRODUCTION

Disease: Head Scab has been a huge threat to wheat population in the USA. Grain loss is primarily due to floret sterility, poor to no seed filling causing pink shriveled grains with low test weights. Loss is further amplified by the presence of the fungal mycotoxin, deoxynivalenol (DON).
Host: Wheat (*Triticum aestivum*) is the primary host for the pathogen.
Pathogen: The causal organism is *Fusarium graminearum* Schwabe. (teleomorph = *Gibberella zeae).*

OBJECTIVE

The main purpose of screening Yugoslavian wheat germplasm is to identify new sources of resistance and use these lines as parents in the Ohio Wheat Breeding Program to increase the level of resistance in the advanced breeding lines.

MATERIALS AND METHODS

Plant materials

Winter wheat genotypes were selected from GRIN database of the National Plant Germplasm System in 1998. Basis of selection was country of origin (Yugoslavia) and the improvement status (breeding lines and cultivar). 210 Yugoslavian winter wheat accessions were selected. 20 seeds per genotype were sown in flats of soil in 1998. Plants were vernalized for 60 days in a lighted cold room maintained day and night at 4^0C. Each germinated seed was transplanted individually into a styrofoam cup and filled with the soil. Plants were watered twice a day. The greenhouse temperature varied from 23^0C during the day, with a range of 19^0C to 30^0C and 19^0C at night, with a range of 17^0C to 21^0C.

Inoculum Preparation

Fungal cultures from four aggressive *Fusarium graminearum* isolates were grown in malt extract agarose media by a regular single spore transfer method (Stack, 1989). Cultures were grown at 25^0C under continuous fluorescent light. Inoculum was prepared from these plates as described by Mesterhazy (1964). Conidia were harvested by flooding plates with sterile distilled water followed by a gentle scraping of the top layer of the culture. The mixture was strained through sterile cheesecloth. The conidial suspensions from four different isolates were mixed in equal volume and concentration was adjusted to 10^5 conidia/ml.

Inoculation

The hypodermic syringe inoculation technique as described by Bai et al.(1986) was used. At anthesis, the center spikelet of each head was inoculated with a drop of freshly prepared conidial suspension (10^5 conidia/ml). Plants were maintained in a moist chamber at 100% relative humidity with temperatures ranging from 23^0C to 25^0C for three consecutive nights. Plants were then returned to the greenhouse bench. The greenhouse temperature varied from 23^0C during the day, with a range of 19^0C to 30^0C and 19^0C at night, with a range of 17^0C to 21^0C.

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Data

Inoculated heads were assessed as percentage of spikelet affected after 10 and 14 days. Disease severity was recorded using a visual assessment scale (Stack et al, 1994, NDSU Extension).

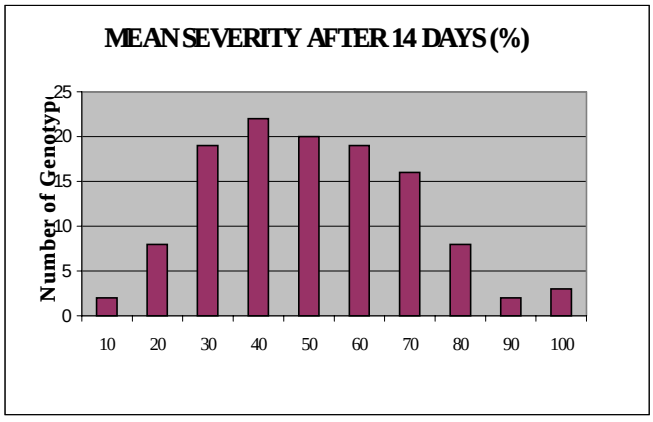
DATA ANALYSIS AND RESULTS

Mean severity for 20 plants for each genotype was calculated using SAS (Release 6.03). One way ANOVA was run over the entire population using general linear model procedure ('PROC GLM'). Genotypes with less than 10 plants were not used in the analysis.

Table I: One way Analysis of Variance for disease severity 14 days after inoculation for 119 genotypes (1148 plants) in the greenhouse.

Source	Degree of Freedom	Sum of Squares	Mean Square	F value	P value	Significance
Model	118	390503.89	3309.29	2.41	0.0001	***
Error	1029	1412829.3	1373.01			
Corrected Total	1147					

Fig. I: Distribution Curve for Mean vs. Frequency of the 119 Yugoslavian Wheat Accessions in the GH.



TEST OF SIGNIFICANCE

Dunnett's one tailed T test was performed for mean severity after 14 days to determine differences from a control mean. The control genotype selected had a mean severity of 91.6 and high number of plants (18) inoculated. Alpha=0.1, Confidence interval=90%, df=1029, Critical value of Dunnett's T=2.879.

CONCLUSION

· The mean severity after 14 days was distributed normally for the 119 genotypes which ranged from highly susceptible to moderately resistant (Fig. 1).

· The significant value of "F" (Table I) implies that there was sufficient evidence for significant differences between the genotype means.

· Dunnette's one tailed T test (<= 0.1) was used to select the genotypes that were statistically significant (more resistant) than the control.

A high alpha level (<= 0.1) was used to minimize the probability of mis- identifying the genotypes as susceptible when they were actually resistant (Type II error).

Table II: Genotypes with significant different mean severity than the control (% mean=91.6) with at least 10 plants inoculated in the greenhouse.

Accession no.	No. of plants per genotype	Mean severity after 10 days	Mean severity after 14 days	Significance, Critical value of Dunnett's T=2.879
17352	12	13.25	18.0	***
17353	10	8.7	21.3	***
184252	14	11.14	26.89	***
184253	12	4	21.58	***
221388	14	20.92	34.92	***
259879	18	14.83	31.88	***
259883	10	10	29.6	***
284657	19	18.05	32.26	***
346801	10	16.14	18.57	***
351263	11	8.72	12.18	***
358332	10	17.2	20.3	***
358337	12	9.25	23.16	***
367245	10	5.11	16.66	***
434650	10	19.37	23.25	***
470104	17	15.82	27.11	***
542442	14	20.14	28.14	***
542444	10	21	25.8	***

FIELD DATA ANALYSIS

These 200 Yugoslavian lines were screened for resistance to the fusarium head blight in the field at OARDC, Wooster, Ohio. Lines were planted in a completely randomized block design with two replications each. Experimental units were 1m long and 30cm apart (0.3 sq. feet). Patterson and Pioneer 2545 were included as susceptible check and Ernie and Freedom as resistant checks. The field was inoculated in the first week of May using colonized corn kernels (Campbell and Lipps, 1998). Heading dates were recorded as early, mid and late. 20 heads from each genotype were rated for %spikelet affected approximately 21 days after anthesis. Data collected were incidence, severity, visual kernel assessment, kernel test weight, % scabby seed and DON level within the harvested kernels. Data was analyzed and compared with the greenhouse data.

FUTURE WORK (1999-2000)

The selected genotypes from this generation with moderately high resistance were planted again in the greenhouse and in the field for evaluation in 2000. The same method for inoculation and disease screening will be conducted as it has been done in the year 1998/9. We will also make crosses between resistant and susceptible lines and with other sources of resistance that are currently available in different breeding program. A pedigree analysis for all the resistant genotypes will be done after completion of disease screening.

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Table III: One way analysis of variance performed on 149 genotypes with different dependent variables.

	DEPENDENT VARIABLE				
SOURCE	FHB incidence (%)	FHB severity (%)	FHB index	Visual kernel rating (%)	Tombstone Kernels (g)
df	148	148	148	100	100
Mean	93.13	32.42	31.08	46.49	23.66
Mean square	233.59	516.6	538.24	2217.09	249.3
F-value	1.86	3.49	3.52	3.19	3.15
P-value	0.0003	0.0001	0.0001	0.0001	0.0001
LSD	25.81	28.02	28.47	62.33	21.14

Table IV: Field data analysis of Yugoslavian wheat germplasm (1998-1999) for Fusarium Head Blight

Genotype	FHB Incidence (%)	FHB Severity (%)	FHB index	Visual Kernel (%)	DON (ppm)	Scabby seed (%)
OH 552	100	32.07	32.07	1.63	2.3	8.03
15893	45	4.1	1.7	30	2.9	18.6
* 17352	70	11.5	7.6	42.5	5.3	13.6
* 184253	94.5	9.7	9.2	6	1.3	12.2
* 184254	82.5	22.5	19.8	5	1.1	5.9
* 284657	85	24.9	24.5	7	3.6	7.5
284667	90	19.5	17.6	2	-	9
284668	80	17.3	14	2	-	6.5
316427	85	24.5	21	5	3.2	4.5
351260	85	16	13.9	7	2.7	8.1
434676	100	9.6	9.6	2	-	3.3
470103	100	19.9	19.9	1.5	0.9	9.6
* 470104	82.5	22.7	18.2	1	5.5	6.1
* 542444	100	22	22	1	-	5

* Genotypes showing strong resistance for head scab in the greenhouse screening.

OH 552 was used as the resistant check in the FHB

SCREENING FOR SCAB RESISTANCE OF WHEAT IN THE GREENHOUSE

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INTRODUCTION

Wheat scab or Fusarium head blight has become the first priority of the Minnesota wheat breeding program. During a 6-year period, greenhouse testing of new sources of scab resistance was conducted beginning in spring, 1994 to present (winter, 1999). About 10,000 wheat genotypes (70,000 plants), including 100 Chinese wheat varieties, have been inoculated and evaluated in the greenhouse.

In 1963, Schroeder (2) observed two types of resistance to scab in wheat cultivars: resistance to initial infection by the fungus (infection-resistance) and resistance to spread of the pathogen within the plant after infection (spread-resistance). Screening for resistance to FHB in the greenhouse is mainly focused on spread-resistance.

MATERIALS AND METHODS

Planting and management in the greenhouse

Greenhouse testing is conducted three times a year: spring (January to April), summer (May to August) and winter (September to December) tests. The days from planting to inoculation (heading) differ for each period. Based on 6-years average, the days from planting to inoculation are as follows: spring test - 42.6 days, summer test - 34 days, winter test - 38.6 days.

Plants are seeded in 14 cm pots, with 5 plants/pot used for evaluation. About 10 to 15 plants per line are used to obtain an accurate measure of spread resistance of advanced lines. In addition, 5 plants of each F5 line are evaluated for spread in the head while the F5 lines are under increase in the winter nursery prior to preliminary yield tests.

About 5 g of Nutricote (14-14-14) fertilizer are applied to each pot one week after planting. About 2 g of Peters (20-15-20) is also given to each pot before heading. Bayleton is used to control powdery mildew. Marathon was applied to the soil to control the aphids with Nicofume used for additional aphid control. Greenhouse temperature was controlled at 20° C with a 16 hour daylength.

Inoculum and inoculation

Pathogen - F. graminearum isolates were collected from the susceptible variety Wheaton from 4 locations in Minnesota: Crookston, Morris, Rosemount and St. Paul (4).
Isolates - Diseased kernels were isolated on PCNB agar first. > 30 isolates were mixed (4).
Inoculum - Inoculum was produced by shaking 4% mung bean soup with the isolates in 1994 and 1995 (3). Since 1996, 4 % mung bean agar has been used to produce conidia spores. Conidia suspension were diluted to 100 conidia per microscope field (100 X).
Inoculation - The tips of the glumes are removed by cutting one spikelet in the middle of the spike. Then 5 microliters of spore suspension

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are injected into a single floret in the middle of the cut spikelet using a microliter pipette (Pipetman).

Incubation - After inoculation, plants were incubated in a dew chamber at 20° C for 3 days, then returned in the greenhouse.

Evaluation of resistance

Two evaluations of disease spread are used:

(1) Scab index - Disease is evaluated 20 days after inoculation using four grades.

Grade 1. Only the inoculated spikelet was infected, **no spread**.
2. Diseases spread to the adjacent **1 or 2 spikelets**.
3. Disease spread to **one half of spike**.
4. Disease spread to the **whole spike**.

Evaluation of resistance:

1.0 - 1.4 = R 2.5 - 2.9 = MS
1.5 - 2.4 = MR 3.0 - 4.0 = S

(2) Severity - % of diseased spikelet.

$$\text{Severity} = \frac{\text{No. of spikelets infected}}{\text{Total No. of spikelets}} \times 100$$

Evaluation of resistance:

< 20 % = R 31-50% = MS
21-30% = MR > 50% = S

RESULTS

About 2500 to 3000 lines were screened each year in the greenhouse from 1994 to 1999. For example, in winter 1997, a total of 1084 F5 lines were evaluated and >50% lines were discarded because of their scab susceptibility. Sixty-five F5 lines from 1997 were selected (5 plants evaluated) based on R reaction and about 89% of the lines selected tested either R or MR in subsequent tests. Therefore, even testing only 5 plants provided reliable data to discard susceptible lines.

During the past 6 years, some Chinese genotypes with high resistance to scab were retested more than 10 times and have been used as resistance sources in the wheat breeding program (Table 1). New crosses with good resistance are presently being tested. Many still depend on Sumai 3 source resistance (Table 2, 3), but other sources are possibly present. For example, cross M5-212 has Yumai 7 as a principle source of resistance and 48 lines from this cross in spring 1999 greenhouse test had a mean scab grade similar to Sumai 3. Ten selected lines from this cross were retested in the summer greenhouse and had about 1/2 the % severity of Sumai 3. Ten lines from 1996-97 M5 have exhibited high resistance for the next three generations (Table 2). Even with high humidity in the summer of 1999, these lines remained similar to Sumai 3. Six advanced lines displayed high resistance in two greenhouse tests and in the field in 1999, with five of these six having Sumai source for resistance (Table 3).

The cooperative breeding and testing effort has released three new varieties with MR to R greenhouse reaction to scab: BacUp (1996), HJ 98 (1998), and McVey (1999).

Using percentage of diseased spikelets to evaluate scab resistance may be more precise than scab index. Percentage of diseased spikelets significantly correlated with greenhouse tests in different years (r = 0.73) and with severity in the field from 1994 to 1997 (r = 0.67-0.87), compared to lower correlations achieved with scab index (r = 0.5).

Table 1. Sources of resistance: (Chinese cultivars and lines)

Variety	Mean scab evaluation* 1995-97	Summer,99	Pedigree	Origin
Sumai 3	1.2(18)	54.4(18)**	Funo/Taiwan wheat	Suzhou, Jiangsu
Wang-shui-bai	1.1(12)	35.0(5)	Local variety	Liyang, Jiangsu
WZHHS	1.3(13)	47.3(4)	Local variety	Weizhou, Funjian
Ning 7840	1.2(9)	42.4(2)	Aurora/Anhui11//Sumai3	Nanjing, Jiangsu
Ning 8306	1.2(10)	35.3(3)	263/Fan5//Ning7302/3/Ning 7084/Yangmai5	Nanjing, Jiangsu
Ning 8331	1.1(5)	58.5(2)	Yangmai4/Ning 7071	Nanjing, Jiangsu
Fujian 5114	1.1(8)	29.0(2)	unknown	Fuzhou, Fujian
Fujian 5125	1.2(8)	37.5(2)	Fufan904/Ning8017	Fuzhou, Fujian
Fujian 60096	1.2(9)	39.2(2)	Jingzhou 2 X Sumai 2	Fuzhou, Fujian
Yumai 7	1.2(15)	39.1(4)	Npegrophar 2/Yanshi 4	Henan
Yan-shi 9	1.3(20)	50.0(2)	Npegrophar 2/Yanshi 4	Henan
Er-mai 9	1.2(19)	37.4(4)	unknown	Hubei
			(Ning7071, Ning7084 & Ning8017 all from Sumai 3)	

Table 2. New resistance lines after three greenhouse test.

Line	Mean scab evaluation* 1997	Summer,99	Pedigree	Sources
212		28.9(10)**	MN94151//Yumai 7/ND673	1998-99 M5
018-5	1.0	29.1(3)	MN90071/SBE0303-19	1996-97 M5
020-7	1.6	57.1(6)	MN90138/Kulm	1996-97 M5
061-2	1.1	54.4(4)	MN92192/SBF608-25	1996-97 M5
074-5	1.0	30.9(3)	MN92390/SBF608-25	1996-97 M5
274II-3	1.2	43.8(6)	SBF608-25/MN92045	1996-97 M5
301-14	1.0	29.5(1)	N90-0666/MN91227	1996-97 M5
307-1	1.4	38.5(3)	MN91227/SBE0303-18	1996-97 M5
308-25	1.3	49.5(3)	MN91227/MN86411	1996-97 M5
327-1	1.0	56.8(5)	MN92197/SBF608-25	1996-97 M5
333-3	1.2	53.0(2)	MN93413/SBE0303-10	1996-97 M5
Sumai 3 (CK)	1.2	54.4(18)		
Roblin (CK)	3.8	91.4(19)		

* 1995-97 scored by scab index, 1999 scored by % severity. ** No. of replicates in the parenthesis.

Table 3. Selected new advanced resistance lines (1999 PY s).

Line	Pedgree	Reaction to scab*			
		Greenhouse		Field,99	
		Winter,98	Spring,99	St. Paul	Crookston
MN 99051	MN91227/Ning8331-4	1.3	1.0	1.5	1.5
MN 99104	Hamer/Fujian60096	1.0	1.5	1.5	1.5
MN 99182	Ning7840-4/MN93505	1.0	1.3	1.5	1.0
MN 99190	Fujian5114-1/2375	1.0	1.0	1.0	1.5
MN 99201	BacUp/Ning7840-4	1.0	1.2	0.5	1.0
MN 99327	Fujian5125-11/MN93434	1.0	1.0	1.0	1.5
Sumai 3 (CK)		1.3	1.2	_	_
Roblin (CK)		3.9	3.8	5.0	5.0

* Scab grade: 1-4 (greenhouse), 0-5(field)

DISCUSSION

Severity, or resistance to spread, is repeatable in greenhouse screening of wheat germplasm and has good relationship to severity in the field. It increases our efficiency because of the ability to screen for resistance year around. Greenhouse severity readings are used as a basis of initial F5 line selection before preliminary yield trials. Susceptible lines are discarded during winter increase before harvest.

Advanced lines are evaluated for scab continuously throughout their development in both the greenhouse and field. Our greenhouse testing procedures do not permit evaluation of resistance to infection, or incidence. Incidence can be evaluated in field testing, but varies due to different environmental conditions (years, location, planting date, etc) (1, 4), as does severity, but severity seems to vary less than incidence.

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DEVELOPING FHB-RESISTANT WHEAT CULTIVARS FOR THE MIDSOUTH

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OBJECTIVE

To develop FHB-resistant wheat cultivars adapted to the Midsouth.

INTRODUCTION

In the Midsouth (Alabama, Arkansas, Louisiana, Mississippi, and Tennessee) soft red winter wheat is grown on over two million acres annually. Although the incidence of Fusarium head blight (FHB) on wheat in the region is sporadic, the disease can be severe if rainy weather occurs before, during, and after flowering. Environmental conditions were conducive for FHB development in 1990 and 1991 causing widespread, severe disease throughout the region. Currently, none of the wheat cultivars grown in the region have resistance to FHB. Having FHB-resistant cultivars would be an important component of any integrated management program for FHB.

MATERIALS AND METHODS

To develop FHB-resistant cultivars as quickly as possible, advanced Arkansas breeding lines with sources of FHB resistance in their pedigree were selected for FHB evaluation in 1999. Over 200 lines will be evaluated in 2000 for FHB resistance in an inoculated, irrigated screening nursery and for agronomic traits and resistance to other diseases in yield trials at several locations.

To develop cultivars with high levels of FHB resistance, a germplasm enhancement (parent building) program was begun in 1997. Agripro Mason and Pioneer variety 2684 were selected as the adapted parents because of their short vernalization requirements and photoperiod sensitivities which permit more rapid advancement of generations and wide adaptation, respectively. Various sources of FHB resistance (mostly CIMMYT spring wheat lines) were crossed, backcrossed or topcrossed with the adapted parents. Seventy-eight F_3, backcross F_2, or topcross F_2 populations were screened in 1999 for maturity, plant height, yield potential, FHB resistance, and Septoria tritici blotch resistance. Two hundred heads were selected from the best plants in each of the 76 populations that had both good agronomic and resistance characteristics. Heads were threshed individually, and seed from the best 120 heads in each population were planted to the field as headrows and will be screened for FHB resistance in 2000.

RESULTS AND DISCUSSION

Progress is being made toward developing FHB-resistant wheat cultivars for the Midsouth. Arkansas breeding lines with FHB resistance will be advanced to the Wheat Strains Nursery and tested at several locations for yield, resistance to other diseases, and milling and baking quality. Germplasm lines that are selected will be sorted by pedigree, and the best lines within each pedigree will be advanced to a Regional Observation Nursery to be planted by volunteer cooperators who are interested in utilizing the germplasm. Some of the best lines from populations with different sources of FHB resistance will be intercrossed within the Agripro Mason and Pioneer 2684 genepools in order to obtain lines with higher levels of FHB resistance.

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'WINTER NURSERY' FOR WINTER WHEAT

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OBJECTIVE

Accelerate generation advance under field conditions in breeding for resistance to Fusarium head blight.

INTRODUCTION

A primary objective of the U.S. Wheat and Barley Scab Initiative is to develop commercial cultivars (inbred lines) with resistance to Fusarium head blight (FHB) as soon as possible. A major limitation to achieve this objective is the time required to develop inbred lines and to select, with confidence, for FHB resistance. Given that at least certain FHB resistance alleles are codominant (Drake, 1997) and the low heritability of FHB resistance (Van Ginkel et al., 1996), selection for resistance is expected to be more effective with inbreeding.

One can produce two generations of spring wheat, wheat that does not require vernalization, per year seeded in the field and four generations per year if grown in a greenhouse or growth chamber.

At a given location one can produce one generation of winter wheat seeded in the field per year. In southern areas of the U.S. two generations can be grown per year: one seeded in the field and one vernalized in a controlled temperature chamber and transplanted to a greenhouse. In northern locations in the U.S. two generations can be produced per year, but both would need to be vernalized in a controlled temperature chamber and grown in a greenhouse. One could also grow one generation in a greenhouse and one generation transplanted to the field after vernalization in a controlled temperature chamber.

Seed production of wheat plants grown in a greenhouse or transplanted to the field is generally much less than that of plants seeded in the field, seriously limiting the number of progeny. Thus, devising a procedure to increase the number of generations of winter wheat per year under field conditions and maximize seed production per plant, particularly in early generations after a cross, would be valuable to accelerate the development of wheat cultivars with resistance to FHB.

MATERIALS AND METHODS

Wheat crosses were carried out in a greenhouse at Lafayette, IN in March to early April and harvested by 8 May, 1998. F_1 seeds were seeded at Colon, Argentina on 14 May. The seeding rate was approximately 4 seeds per 30 cm in rows 30 cm apart. Approximately 80% of plants had flowered by 23 September at Colon. Plants were harvested by 25 November and carried to Indiana on 27 November. F_2 populations were seeded at Evansville, Indiana on 28 November. A portion of the F_2 seeds were seeded at Kinston, North Carolina on 3 December as a backup. Selected plants were harvested at Kinston by 1 June, 1999 and at Evansville on 16 June. F3 populations were seeded at Lafayette on 1 October, the normal time of seeding.

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RESULTS AND DISCUSSION

All aspects of the generation advance/seed production scheme were successful. The F_1 and F_2 generations were successfully grown, seeding under field conditions. The thinly seeded F_1 plants typically produced 6+ spikes with 60+ well-developed seeds per spike. The F_3 generation was seeded at the normal time of seeding at Lafayette, Indiana. Based on experience in the 1998-1999 seasons, times of seeding and harvest are being shifted to allow earlier seeding of the F_2 generation at Evansville and Kinston. In 1999, the F_1 seeds were seeded at Colon on 7 May. Harvest at Colon is expected to be completed by 18 November, allowing seeding of the F_2 generation at Evansville by 20 November, at least one week earlier than in 1998. Seeding one week earlier at Evansville is important for the establishment of the seedlings for winter survival. The winter of 1998-1999 fortunately was milder than normal.

It is critical to choose a location in the southern hemisphere for generation advance that has sufficient cool temperatures for adequate vernalization, but that has a short wheat dormancy period, so that one can seed the next generation as early as possible in the area of adaptation in the U.S.—especially northern areas like Indiana. The general seasonal temperatures and the latitude near Colon is similar to southern Tennessee, which is at the southern edge of the climate in which wheat adapted to Indiana can be successfully grown.

A benefit of generation advance of winter wheat at Colon is that selection for resistance to certain diseases can be carried out, depending on the season. In 1998, Septoria leaf blotch was moderately severe and a low infection of Fusarium head blight was present. Stem rust was also moderately severe. However, one must take into account differences in pathogen populations in different regions.

One would need to identify a collaborator at the location of the winter nursery. It is important to apply for and obtain all necessary official permits prior to transporting seed into and out of countries. When done on a timely basis, obtaining permission to transport seeds need not be a hindrance to the operation of a program that requires the transport of seed between countries.

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YIELD AND FUSARIUM HEAD BLIGHT RESISTANCE OF HARD RED SPRING WHEAT CULTIVARS

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INTRODUCTION

Fusarium head blight (scab) has increased to epidemic proportions in many regions of the world in recent years. The 1993 epidemic in the North Central wheat-growing belt of the United States stimulated a major research effort to fight this devastating disease. It has been theorized that modern cultivars may be more susceptible than their predecessors and this may have contributed to recent epidemics. This study includes modern and historically significant hard spring wheat cultivars from the Northern Great Plains. Both yield trial data and reaction to scab in an inoculated nursery is included.

MATERIAL AND METHODS

Plant Materials

A set of spring wheat cultivars of historical significance is routinely included in the yield trials of the South Dakota State University spring wheat breeding program. This is generally a non-replicated trial grown at several locations. These historical cultivars serve a dual purpose of demonstrating the evolution of spring wheat cultivars to producers and since they are well characterized, they provide the breeder with a probe of the environment. The plots are planted, maintained, and harvested in the same manor as the breeding yield trial plots. Yield data (bu/a) from 1996 to 1999 were used in this study. These cultivars were also evaluated in 1997-1999 for scab resistance in an inoculated FHB nursery; planted as single row plots (1m) with two replications.

Inoculations and Disease Evaluation

In the mist-irrigated FHB inoculated nursery the rows were tagged when 50 to 75% of tillers in the row were in anthesis. Macroconidical spore suspensions, containing approximately 75,000 to 100,000 conidia/ml, were produced by scraping Fusarium graminearum cultured on acidic potato dextrose agar plates and suspending into water. Approximately 50 ml per row was applied. The first inoculation was done at anthesis and the second one week later. After inoculation the rows were mist-irrigated for 2 min. every 30 min. for a 12-hour period during the night (approximately 24 times per night). The nightly misting continued until readings were taken on the latest maturing cultivar. Percent-infected spikelets (FHB) of 20 spikes per replication were visually estimated 14 days after the first inoculation. After harvest, % tombstone kernels (scab) was determined and grain yield (Inoc Yield) was measured as grams/row.

RESULTS AND DISCUSSION

Table 1 shows the means of the 32 cultivars used in the study. Although the cultivars do differ in their reaction to scab, none are highly resistant. BacUp was the only cultivar with less than 50% FHB. The table illustrates that breeders have made progress for yield, but little progress for scab resistance until recent years. It was not until 1993 that spring wheat breeders began to consciously include reaction to scab into their evaluation criteria.

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Table 2 shows the correlations between the year of release and the four measurements that were taken. All three of the scab assessment measurements were highly correlated with each other. Grain yield and year of release were highly correlated, but there was no relation between year of release and FHB or tombstone kernels. There was no correlation between scab resistance and yield potential, indicating that breeders should be able to make progress on scab resistance and grain yield simultaneously.

Table 1. Means of spring wheat cultivars for three FHB disease assessment measurements in an inoculated FHB nursery and grain yield from non-inoculated yield trials.
Scab= tombstone kernels, FHB= % infected spikelets, Inoc yield= grain yield in the inoculated nursery.

			----------FHB Nursery---------			--Yield Trial--
			Inoc yield	Scab	FHB	Yield
Entry	Name	Release	g/plot	%	%	bu/acre
1	Baart	1900	18.2	51.0	66.1	22.1
2	Marquis	1903	15.2	58.3	64.0	24.7
3	Thatcher	1921	8.3	58.3	73.7	22.9
4	Chris	1965	12.9	69.2	72.1	30.4
5	Era	1971	12.7	64.2	83.3	34.3
6	WS1809	1975	12.4	69.2	66.2	35.9
7	Len	1978	12.8	60.0	80.3	34.2
8	Alex	1981	15.2	60.0	72.6	35.7
9	Marshall	1982	14.7	69.2	75.4	40.1
10	Wheaton	1983	11.4	88.3	84.3	42.9
11	Guard	1983	16.0	67.5	80.1	40.0
12	Stoa	1983	15.2	73.3	72.3	41.9
13	Nordic	1986	15.6	64.2	74.4	40.1
14	Butte 86	1986	23.9	57.5	62.4	41.5
15	2375	1987	24.0	50.8	66.5	39.1
16	Amidon	1988	15.5	65.0	81.3	39.1
17	Prospect	1988	17.8	56.7	78.9	36.7
18	Grandin	1989	27.2	56.7	57.0	40.2
19	Sharp	1990	20.3	56.7	66.5	40.3
20	Kulm	1994	20.8	54.2	68.0	40.0
21	Russ	1995	32.5	50.0	58.3	44.7
22	Norlander	1995	26.1	53.3	55.0	41.5
23	Lars	1995	15.3	70.0	74.7	41.6
24	Hamer	1995	22.3	62.5	69.3	42.8
25	Verde	1995	21.5	55.8	74.9	42.7
26	2398	1995	11.8	80.0	86.6	40.2
27	Trenton	1995	25.6	57.5	69.5	41.2
28	BacUp	1996	26.8	41.7	48.8	31.7
29	Oxen	1996	26.0	60.0	70.2	47.4
30	Keene	1996	17.5	55.0	79.3	42.2
31	Forge	1997	24.7	49.2	65.8	46.2
32	Ingot	1998	36.0	46.7	60.0	45.9
	AVERAGE		19.2	60.4	70.6	38.4
	CV%		28.3	12.6	12.4	6.7
	LSD(.05)		8.9	12.4	14.3	3.1

Table 2. Correlations between year of release, yield in multi-location yield trials (Yield), and three FHB disease assessment measurements in an inoculated FHB nursery. Scab= tombstone kernels, FHB= % infected spikelets, Inoc yield= grain yield in the inoculated nursery.

	Year of Release	Inoc yield	Scab	FHB
Inoc yield	0.40*			
Scab	-0.07ns	-0.79**		
FHB	0.04ns	-0.74**	0.69**	
Yield	0.64**	0.47**	-0.16ns	-0.16ns

*,** significant at 0.01 and 0.05, respectively

RESISTANCE ASSESSMENT IN FIELD AND GREENHOUSE SCREENING OF THE UNIFORM WINTER WHEAT FUSARIUM HEAD BLIGHT NURSERY AT BLACKSBURG, VIRGINIA, 1997-1999

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ABSTRACT

Epidemics of Fusarium head blight in Virginia have been intermittent, but the severity with which they affect the winter wheat crop and subsequently, the Virginia farmer, necessitate rapid discovery of mitigating factors. In the year 1998 alone, farmers saw losses of over 12 million dollars. Research in Virginia has focused on identification and development of resistant cultivars and germplasm, among other aspects of this disease. In the past two years and continuing in this year, Virginia has participated in the Uniform Winter Wheat Fusarium Head Blight Nursery. 1999-2000 will also see the inclusion of a Southern Uniform Winter Wheat Fusarium Head Blight Nursery. In 1997-1998, entries were screened in the field with a conidial suspension and in the greenhouse by the single floret method. Disease was rated by the following parameters: incidence, severity, fhb index (a composite of incidence and severity), percent scabby seed, 1000 kernel weight and DON level. Yield, heading date and flowering date were also recorded. In 1998-1999, Uniform Nursery lines were screened in the field, again with a conidial suspension, and fusarium infection was characterized by incidence, severity, fhb index, percent scabby seed and DON levels. Yield, heading date, flowering date, barley yellow dwarf virus presence and *Stagonospora nodorum* infection were also noted.

Resistance assessment of the seventeen entries included in the Uniform Nursery both years was analyzed in order to show repeatability of types of resistance or relative resistance among entries. There was no correlation between the rankings of entries for any of the disease parameters between the years. In addition, although in each year various disease ratings were correlated to each other or to agronomic traits such as flowering date, not one of these trends was significant in both years. Relative resistance rankings of Uniform Nursery entries in the field and in the greenhouse for 1997-1998 were likewise not correlated. Disease pressure varied greatly between the two growing seasons, as precipitation in 1997-1998 was far above normal and in 1998-1999 was extremely low. This along with variations in inoculation and irrigation methods (as protocols are being refined) may help to explain the lack of similar resistance levels between the years. More Type II and IV/V screening results from this and following years will allow for a more complete analysis. However, the lack of same relationships of disease resistance between lines over years suggests that there is more to be discovered about their various escape mechanisms. In a similar vein, non-repeatable correlations of disease parameters and significant differences between single floret and field disease assessments caution careful identification of quality and quantity in *Fusarium* resistant sources.

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GREENHOUSE AND FIELD EVALUATION OF RESISTANCE TO FUSARIUM HEAD BLIGHT IN SOFT RED WINTER WHEAT

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ABSTRACT

Entries in the Uniform Winter Wheat Scab Nursery along with numerous SRW cultivars and breeding lines were evaluated for resistance to Fusarium head blight in the field and greenhouse in 1999. Type II resistance was assessed in the greenhouse via single floret injections. Type I resistance was measured in a mist irrigated inoculated field nursery, and, to a limited extent in the greenhouse. Seeds from the greenhouse evaluations were plated on selective media to confirm visual evaluation. Field incidence and severity were low, but may have been closer to natural infection levels, since the resistant checks showed some resistance. Visual assessment of seed was misleading in that apparently normal seed, in many cases, were infected with *Fusarium graminearum*.

OBJECTIVES

1) To identify resistance to FHB in greenhouse and field screening trials.
2) To evaluate apparent vs. actual FHB infection by plating out seeds on selective media.

INTRODUCTION

Fusarium head blight 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 non - 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

Entries in the 1999 Uniform Winter Scab Nursery along with a number of advanced breeding lines were planted in the field in a randomized complete block design with four replications on 29 October 1998. Each plot consisted of a single 4ft. row. The previous crop was corn (*Zea mays* L.) and the seedbed had been chisel plowed and disked. Entries in the greenhouse were planted in a completely randomized design with a variable number of replications.

Isolation of *Fusarium graminearum*

A culture of *F. graminearum* was obtained from scabby wheat seed by surface sterilization and plating onto acidified potato dextrose agar (APDA). To induce sporulation, mycelium from this culture was plated onto carnation leaf agar (CLA). Plating a single-spore onto APDA ensured culture purity. This culture was then increased on PDA to use as the inoculum source for our field and greenhouse screens.

Field Inoculation

The field inoculation protocol was modeled after the method by Fauzi and Paulitz (1994) with some modifications. Fifty-three mason jars containing approximately 500 g of autoclaved corn seed were inoculated with 6 mycelial plugs of *F. graminearum* on April 5, 1999. Ten days later, twenty-five of the mycelium inoculated corn jars were also inoculated with a 10-ml macroconidia solution (400,000 spores/ml). Inoculated corn was maintained at room tem-

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perature for 22 days; jars were shaken daily to help disperse inoculum throughout each jar. Corn kernels were sampled from jars at random and plated onto PDA to verify presence of the pathogen. On April 27, wheat plots were inoculated just prior to heading by spreading 35-40g of the inoculated corn mixture per plot. Plots were mist irrigated daily beginning May 7 for approximately one hour during the early part of the morning, mid-day, and late evening throughout anthesis into early grain fill. Because of extremely dry weather and a delay in irrigation, wheat plots were inoculated a second time with more corn inoculum on May 17.

Disease evaluations were initiated on June 3 when scab symptoms were detected on several of the susceptible cultivars. Incidence was reported as the percentage of scab-infected heads per total number of heads per 2 ft. of row. Disease severity was assessed according to the Visual Scale for Estimating Head Blight in Wheat (Stack and McMullen, 1998). All heads in each row were tagged. Severity was determined by counting the number of infected spikelets and dividing by the total number of spikelets on diseased heads only.

Greenhouse Inoculations

Several advanced breeding lines were evaluated in the greenhouse for Type I (preventing initial penetration and Type II (reducing fungal penetration or spread within the head) resistance. Wheat entries were vernalized on Nov. 13-14, 1998 and potted in the greenhouse on Jan . 8, 1999.

Type II screen (Injections): Macroconidial suspensions were prepared by placing two mycelial plugs from a culture of *F. graminearum* in carboxymethylcellulose (CMC) liquid media. Flasks were placed on a shaker (115 rpm) for 2 weeks at 24 C. Spore suspensions were prepared by filtering the culture through a 3.0-mm Millipore filter system. Macroconidia were resuspended in sterile water and spore concentration was calculated with the aid of a hemacytometer. Inoculation and disease assessment was modeled after the method by Bai et al. (1996). At time of anthesis, a central floret of each spike was marked with a sharpie and inoculated by pipetting 3 µl containing approximately 1,200 spores. After inoculation, plants were placed directly into humidity chambers for three consecutive nights. Chambers were constructed of PVC-pipe tented with plastic, which sat on benches in the greenhouse. A cold-mist humidifier was placed inside each chamber to ensure high humidity. During the day at least one side of chamber was open for ventilation. Plants were moved out of chambers on day 4 and monitored for disease development. The number of diseased spikelets per spike was counted beginning one week after injection and continued every 3 days. The final percentage of infected spikelets per spike was recorded on day 21.

Type I screen (Sprays): Macroconidial suspensions were prepared in sterile water and conidial concentration was adjusted to 400,000 sp/ml. Wheat spikes were sprayed to run-off with a hand–held atomizer and placed in humidity chambers for three consecutive nights as previously described. Twenty-one days after inoculation plants were rated for disease development using a 0-4 scale: 0 = no disease, 1 = 1-25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76-100% of spikelets infected.

Seed Assessment: Wheat seed was collected from both injection and spray test entries. Seeds were surface sterilized for 5 minutes in 0.5% sodium hypochlorite, rinsed three times in sterile distilled water, blotted on sterile filter paper and plated on APDA or PDA containing pentachloronitrobenzene (PCNB) and chloramphenicol. Seed collected from the spray tests were screened first. Total seed number plus the number of visually scabby seed were recorded

for each seed lot. Plates were incubated for 7-10 days at 20 C. Each plate was visually inspected for *F. graminearum* contaminated seed. Seed from the injection test was also assessed for the presence of *F. graminearum*. In this particular test, seed was visually inspected and placed in the following three categories according to appearance: l) normal, 2) small, wrinkled and 3) tombstone. The location and category of each seed was recorded on the top of each petri plate. After incubation, those seed that were positive for the presence of *F. graminearum* were recorded.

RESULTS AND DISCUSSION

Field Screening

For the first time in three years of field screening, incidence and severity in our inoculated, irrigated nursery were rather low (Table 1). This was due to a rare spring drought in Kentucky that we did not fully overcome with irrigation. Nonetheless, the reportedly resistant checks, Ernie and Freedom, actually showed some signs of resistance in our nursery, which was not the case in the previous two years. Thus, the scab pressure that we observed this year may have been closer to what would be expected under a natural infection.

Greenhouse Screening

Even with a large number of replications (15 plants), repeatability of assessment of type II resistance was low (Table 2). The most promising entry, KY 91C-022-36, ranged from 6 to 26% scabby spikelets. With only three plants, however, it is difficult to have confidence in this estimate.

Selective Media

We tend to regard an evaluation of scabby seed after harvest as a confirmation of our assessment of scab on the intact spike. Although visual assessment of seed seems straightforward, plating out the seed on a selective medium revealed some surprises (Tables 3, 4). Seed of Freedom, for example, was visually rated at 17 % scabby, yet plating the seed revealed that 82% was actually infected with *F. graminearum.*

REFERENCES

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Table 1. Uniform Winter Wheat Scab Screening Nursery, Lexington, KY 1999.

Cultivar	Average Severity %	Average Incidence %	FHB Index	Height (in)	Yield (bu/a)	Heading Date (Julian)	DON Levels (ppm)
NY87048-7387	1.75	0.65	0.46	40	61.25	134	0.60
Ernie	3.50	1.34	0.09	32	57.82	125	0.78
IL94-1909	4.38	0.68	0.06	41	89.05	129	0.55
OH 544	6.42	1.10	0.10	41	93.68	134	0.55
NY86003-106	8.50	1.75	0.19	37	71.89	133	0.80
M94-1069	11.13	5.63	0.91	35	75.49	128	0.20
Freedom	15.95	4.11	0.72	35	77.90	129	0.63
Geneva	17.08	5.02	1.71	36	56.62	131	0.93
OH 522	17.19	9.92	1.88	35	93.51	127	0.80
NY6003-27	18.79	4.50	2.23	41	68.29	132	1.88
Foster	19.25	2.18	0.93	36	77.90	128	0.50
2545	22.95	9.02	2.00	35	57.99	132	1.70
OH 657	23.38	3.55	1.03	41	80.64	132	0.65
IL96-24078	27.08	1.73	0.49	34	63.48	126	0.75
NY87048W-7405	27.08	8.16	0.98	34	66.23	129	1.00
P92823A1	27.50	5.39	2.58	35	91.28	128	0.55
VA96-54-216	29.45	8.63	2.68	33	102.60	126	0.45
Goldfield	30.21	2.36	0.88	38	107.75	129	0.33
OH 609	30.40	4.98	1.63	36	97.11	127	0.33
Cayuga	31.48	3.97	1.72	42	78.75	135	1.00
Patterson	33.25	5.92	2.15	38	82.36	126	0.88
P88288C1	34.49	7.57	2.94	34	72.23	129	0.55
VA96W-348	38.71	15.27	5.75	32	71.72	127	0.90
M95-3349	43.13	3.15	1.11	37	109.12	128	0.53
IL95-4162	47.50	2.74	1.26	37	101.06	127	0.48
P86958RC2	47.54	3.92	2.35	36	81.33	129	0.95
KY89-895-14	51.20	11.51	5.78	34	88.88	129	0.58
Roane	53.45	18.44	10.06	33	81.84	126	2.38
Location Mean	16.68	4.27	1.06	37	82.29	130	0.80
L.S.D.	21.90	4.24	2.06	2.00	23.63	1.35	0.62
C.V.	71.90	65.99	89.81	4.69	24.91	0.89	59.20

Table 2. Evaluation of fourteen advanced breeding lines in the greenhouse for Type II[a] resistance to scab.

		AUDPC[b]			% Diseased spikelets[c]		
Entry	N	Min	Max	Mean	Min	Max	Mean
91C-092-3	5	0.7	2.3	1.7	6	32	22
91C-092-5	12	0.5	6.5	1.6	5	100	18
91C-092-7	3	0.7	2.8	1.9	6	36	16
91C-092-72	14	0.1	9.1	3.9	5	100	53
91C-092-105	6	0.7	8.2	3.9	7	100	37
91C-092-111	3	1.2	5.3	4.5	17	94	48
91C-019-17	4	0.6	3.2	2.9	5	39	24
91C-022-34	4	0.7	3.2	2.1	6	41	16
91C-022-36	3	0.3	2.5	2.2	6	26	17
91C-022-42	4	0.3	5.0	4.2	6	100	53
91C-046-2	4	0.9	4.9	4.4	7	64	43
91C-261-13	6	0.2	3.4	2.6	5	100	35
91C-261-24	15	0.7	8.8	3.3	6	100	46
92C-432-62	14	0.7	7.4	3.3	6	100	46

[a]Reduction of spread within the spike.[b]Area under the disease progress curve.
[c]Percent of infected spikelets per spike recorded 21 days after injection.

Table 3. Mean number of seed collected and percent of seed infected with *F.graminearum* from fourteen advanced breeding lines screened in the greenhouse for Type II resistance to scab.

	Mean number of seed[a]			Percentage of infected seed		
Entry	Normal	Small/ Wrinkled	Tombstone	Normal	Small/ wrinkled	Tombstone
91C-092-3	32.8	3.8	4.2	5.7	7.7	41.7
91C-092-5	26.6	3.6	1.5	0.3	1.6	33.3
91C-092-7	18.3	0	0	0	0	0
91C-092-72	15.2	15.4	5.5	5.2	14.8	26.1
91C-092-105	15.8	5.5	2.8	5.7	12.2	47.0
91C-092-111	1.0	24.3	7.3	0	3.0	34.2
91C-019-17	24.5	0	4.3	1.8	0	61.9
91C-022-34	12.5	14.3	0.3	4.4	0	0
91C-022-36	8.8	19.6	4.4	2.3	1.0	30.5
91C-022-42	18.8	3.0	3.0	1.0	0	12.5
91C-046-2	12.8	9.0	1.8	0	7.4	11.1
91C-261-13	15.2	5.0	3.1	15.4	7.2	48.4
91C-261-24	10.2	8.3	9.2	9.2	10.2	33.4
92C-432-62	12.0	5.4	2.9	5.1	13.9	50.3

[a] Visual assessment of seed by appearance. [b] Percent of *F. graminearum* contaminated seed per total number of seed by category, recorded 7-10 days after plating on selective media.

Table 4. Evaluation of fifteen advanced breeding lines screened in the greenhouse for Type I resistance to scab.

Entry	N	Disease Score[a]	Total Seed	Percent Visual scabby seed[b]	Percent of seed infected with *F. graminearum*[c]
Glory	3	2.7	19	77	87
KAS EX 108	1	4	6	100	83
FFR 555	5	3.6	21.2	66	70
Foster+Gaucho	4	2.8	16.3	40	92
2552	3	1.7	13.3	34	76
KY 89C-744-44	3	2.3	19.3	37	60
92C-432-62	3	0.3	13	30	18
92C-433-77	1	1	32	19	72
91C-261-3	5	1.2	38.2	15	43
91C-261-3	3	2.7	3	99	100
90C-383-18	1	1	27	100	81
91C-260-6	3	0.7	34.6	6	15
91C271-74	3	1.7	18	51	53
Freedom	2	2	29	17	82
Ernie	3	1	13.4	25	17

[a]Twenty-one days after inoculation plants were rated for disease development using a 0-4 scale: 0 = no disease, 1 = 0-25%, 2 = 26-50%, 3 = 51-75%, and 4 = 76-100% of spikelets infected.
[b]Visual assessment of seed by appearance.
[c] Percent of *F. graminearum* contaminated seed per total number of seed, recorded 7-10 days after plating on selective media.