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Heritability Estimates and Response to Selection for Fusarium Head Blight Resistance in Soft Red Winter Wheat

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum* (Schwabe), is an economically important disease of wheat (*Triticum aestivum* L.). After epidemics in the USA during the 1990s, a resistance-breeding effort was undertaken focusing initially on the transfer of Type II resistance from unadapted Chinese cultivars. The objective of this study was to determine the magnitude and heritability of resistance in populations derived from adapted parents. Three soft red winter (SRW) wheat populations of 40 families each were artificially inoculated with *Fusarium graminearum* under mist irrigation in 2003 and 2004 at Lexington and Princeton, KY. Traits measured included anthesis date, plant height, disease severity, Fusarium-damaged kernels (FDK) and deoxynivalenol (DON) concentration. Broad sense heritability (BSH) estimates were generated from entry means over the four environments. Heritability of severity was approximately 0.30 in all populations; heritability of FDK ranged from 0.16 to 0.20. In 2003, a selection intensity of 20% was imposed on all populations, and the eight lowest severity families were advanced and evaluated at Lexington and Princeton in 2004. Direct selection response, averaged over both locations, ranged from 1.9 to 4.1% reduction in severity. Correlated reduction in FDK ranged from 0.4 to 6.5%; there was also a correlated increase in plant height of 1.7 to 4.1 cm after one cycle of selection. Progress in FHB resistance breeding in the absence of major QTL is likely to be constrained by low heritability and genotype × environment (G × E) interaction.

Fusarium head blight (FHB), or head scab, is a historically devastating disease of wheat and barley (*Hordeum vulgare* L.) around the world (Schroder and Christensen, 1963). Since 1991, scab outbreaks of varying intensity have been common and widespread across much of the eastern half of the United States, affecting the yield and quality of wheat produced. During FHB epidemics, the infected grain is often contaminated with DON, a mycotoxin produced by *F. graminearum* that has become a major concern for animal production and human health (McMullen et al., 1997).

The spring wheat cultivar Sumai 3 and its derivatives such as 'Ning 7840', are the most widely used and best characterized sources of resistance in the world. Because of its high general combining ability for scab resistance, Sumai 3 was used as a resistant parent with success in China (Bai and Shaner, 1994). After its introduction in USA it has been used extensively for both spring wheat and winter wheat breeding programs (Wilcoxson, 1993; Bai and Shaner, 1994).

Resistance to FHB is usually reported to be quantitatively inherited with a continuous distribution among the progeny (Bai and Shaner, 1994; Buerstmayr et al., 1999). Different studies indicate that resistance is mainly controlled by additive genetic effects (Snijders 1990a, 1990b; Bai et al., 2000), but dominance effects (Hall and Van Sanford, 2003) and G × E interaction (Miedaner et al., 2001) have also been reported.

Several highly resistant cultivars with the Sumai 3 type of resistance have been released ('Alsen' [www.ag.ndsu.nodak.edu/alsen.htm; verified 18 Mar. 2006]; 'Pioneer Brand 25R18' [Greg Marshall, personal communication, 2005]), but breeders continue to search for other sources of resistance. There is a reluctance to rely exclusively on one source of resistance, and there is a preference to work within one's own market class when possible, to comply with end use quality constraints. Breeders have also tried to find the best strategies for successful selection of resistant materials and optimization of resources. Selecting in early generations is a possible strategy, but the optimal generation of selection is unknown especially when a large amount of genetic variation within a population is present. Bernardo (2003) stated that when dominance effects were absent, the expected genetic correlation between the line performance in segregating generations and at homozygosity was 0.70 for F_2–derived lines in self-pollinated crops. Unfortunately, environmental effects often impede this goal and limit the success of early generation selection.

Heritability estimates for FHB resistance traits in segregating populations have been reported in a few studies. In European winter wheat populations, Snijders (1990b) calculated heritabilities ranging from 0.05 to 0.89 among 23 F_2 populations for head blight rating. Miedaner et al. (2003) working in four environments, calculated heritabilities of 0.83 for head blight rating and 0.71 for DON content in one F_3 population. They also reported a strong correlation (0.8) between these traits. Buerstmayr et al. (2000) reported heritabilities greater than 0.7 for severity in $F_{4:5}$ and $F_{4:6}$ populations in a 2-yr study at one location.

It has been suggested that selection for FHB resistance can be effectively started in the F_3 generation (Snijders, 1990c; Miedaner et al., 2003) in as many environments as possible, and a few authors report success in a selection

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Abbreviations: BSH, broad sense heritability; daa, d after anthesis; DON, deoxynivalenol; FDK, Fusarium-damaged kernels; FHB, Fusarium head blight; G × E, genotype × environment; QTL, quantitative trait locus or loci; SRW, soft red winter.

scheme for FHB resistance, such as Jiang et al. (1994), who reported on three cycles of a phenotypic recurrent selection scheme. We are not aware of any reports on among-family selection for FHB resistance in wheat, and the possible success of this methodology in early generations has not been analyzed in depth. In barley, Urrea et al. (2002) selected for FHB resistance and reported a reduction of 6.9% in mean severity of the selected $F_{4:6}$ families compared with the unselected families. They also found a reduction in DON accumulation.

This study had two objectives: (i) to evaluate three SRW wheat populations in two locations during two consecutive years and estimate the heritability of FHB resistance traits and (ii) to conduct one cycle of among-family selection based on FHB severity and evaluate the response of the three populations in two locations.

MATERIALS AND METHODS

Genetic Material

Three SRW wheat populations of 40 lines each composed the experimental material used in this study. Preliminary research with these populations was described by Hall (2001). The pedigree of Population 1 was 'Ning 7840'/'2691'//'2684'/3/'Elkhart'. Population 2's pedigree was Purdue 5/'Agripro Foster'*2 and Population 3 was derived from the following cross: 'Ning 7840'/'2691'//'2684'/3/'25R57'. Initial single-cross F_1 seeds of Populations 1 and 3 were kindly provided by Dr. Carl Griffey. Purdue 5 was a germplasm line derived from crosses with Ning 7840. Seed was kindly provided by Dr. Herb Ohm. The F_2 populations were grown in 2000 and heads were selected at random to develop families. $F_{2:3}$ families were grown in 2001 and evaluated for Type II resistance in the field at one location (Hall, 2001).

Total genomic DNA was isolated from seedlings of the parents and the $F_{2:4}$ lines developed from each population. The DNA isolations were from a bulk of 10 to 20 seedlings of each line according to the method of Riede and Anderson (1996). Lines were evaluated with microsatellite markers *Xgwm533* and *Xgwm493* that flank the major FHB resistance QTL *Qfhs.ndsu-3BS* found in Sumai 3 and other Asian lines, including Ning 7840 (Anderson et al., 2001; Buerstmayr et al., 2001; Zhou et al., 2002). Genomic DNA of Sumai 3 was included as a control. The polymerase chain reaction assays were performed in 25 μL volumes and included 50 ng of template DNA, 1.0 U of *Taq* DNA polymerase (Promega, Madison, WI), 1× PCR buffer, 2.0 m*M* $MgCl_2$, 200 μ*M* each dNTP, 12.5 pmol of each primer. The forward primer was modified with a 5′ 6-FAM or HEX label (Applied Biosystems, Foster City, CA). Reactions were amplified in an MJ Research PTC200 thermocycler (Watertown, MA). Amplicons were separated by capillary electrophoresis on an ABI Prism 3100 DNA Analyzer (Applied Biosystems) with GeneScan software and GeneScan-500 ROX (Applied Biosystems) as an internal size standard.

Field Experiment

The three populations were evaluated during 2003 ($F_{2:4}$) and 2004 ($F_{2:5}$) on the Kentucky Agricultural Experiment Station research farms near Lexington and Princeton, KY. The $F_{2:4}$ populations were planted in two-row plots 1 m long with 0.20-m row spacing on 24 Oct. 2002 at Lexington and 4 Nov. 2002 at Princeton. Seeding rate was approximately 9.8 g m^{-2}. The experimental design was a randomized complete block with three replications at Lexington and two at Princeton. In the 2004 crop year, the $F_{2:5}$ populations were planted in hill plots on 30-cm centers at a seeding rate of 20 seeds per plot. The plots were planted in a randomized complete block design with three replications at Lexington on 22 Oct. 2003 and two replications at Princeton on 20 Oct. 2003. In both years the previous crop was corn (*Zea mays* L.) and the seedbed had been chisel-plowed and disked.

Among-Family Selection

In 2003, the top eight (20%) $F_{2:4}$ families of each population, based on the lowest FHB severity score, were selected to evaluate the response to selection. Seeds from the selected families and a bulked seed sample of all 40 families were planted in four-row plots on 20 Oct. 2003 at Lexington and Princeton. Plots 1 m long with a 0.20-m row spacing were arranged in a randomized complete block design with three replications at Lexington and two at Princeton. Seeding rate was approximately 9.8 g m^{-2}.

Grain Spawn Inoculation

The field inoculation protocol was modeled after the method of Fauzi and Paulitz (1994) with some modification. Eleven local *F. graminearum* isolates were obtained from wheat fields throughout Kentucky. Pure cultures of each isolate were obtained by transferring mycelium to carnation leaf agar to induce sporulation, and then single spores were transferred to and increased on potato dextrose agar (PDA). Inoculation was initiated by combining 1 kg corn and 769 mL water to provide adequate moisture for the pathogen to grow in grain spawn bags (Stewart et al., 2004). The corn imbibed water overnight, then the bags were autoclaved on two consecutive days. Inoculum was introduced to the bags by placing several PDA plugs of a single isolate in a bag. The bags were sealed, set upright on a shelf for incubation, and shaken daily to ensure a uniform colonization of the grain. After 3 wk at room temperature when the fungus had adequately colonized the corn, the grain spawn was thoroughly mixed to incorporate the 11 isolates into one mixture. The plots were inoculated before heading (GS 7, Feeke's Scale) by spreading 35.4 g m^{-2} of the inoculated corn mixture within each plot. Inoculation dates were: 16 Apr. 2003 and 16 Apr. 2004 at Lexington and 9 Apr. 2003 and 11 Apr. 2004 at Princeton.

An overhead mist irrigation system on an automatic timer was installed to provide adequate moisture and humidity to create an FHB epidemic. Between 0600 and 0800 h the irrigation ran for 5 min in 15-min intervals. The irrigation schedule also included a 10-min misting every 20 min between 0800 and 1000 h.

Disease Evaluation

Anthesis notes were taken daily; plots that reached anthesis (Feeke's 10.5) first were scored first. Disease severity was assessed approximately 21 to 24 d after anthesis. In each plot 10 spikes were chosen at random, the number of visually diseased spikelets on each spike was recorded and that number was divided by the total number of spikelets to yield percentage of severity of infection (Stewart et al., 2004). Plant height (cm) was measured during the seed filling period.

Seed Quality Evaluation

In 2003, at harvest maturity, each plot was harvested with a small plot combine on which the forced-air setting was at a minimum to avoid scabby seed loss. In 2004 the hill plots were

Table 1. Means (±SE) for Fusarium head blight severity (%), Fusarium-damaged kernels (FDK), and deoxynivalenol (DON) in three wheat populations at Lexington and Princeton, KY, 2003- 2004.

	Population 1			Population 2			Population 3		
	Severity	FDK	DON	Severity	FDK	DON	Severity	FDK	DON
	%		mg kg^{-1}	%		mg kg^{-1}	%		mg kg^{-1}
2003 ($F_{2:4}$)									
Lexington	29.5 ± 0.9	12.2 ± 0.6	8.02 ± 1.3	30.1 ± 0.9	10.4 ± 0.5	10.2 ± 1.3	31.2 ± 0.9	13.0 ± 1.1	12.2 ± 1.2
Princeton	38.9 ± 1.4	18.6 ± 1.1		35.2 ± 1.3	27.0 ± 1.1		40.7 ± 1.3	27.7 ± 1.1	
2004 ($F_{2:5}$)									
Lexington	50.8 ± 1.5	65.8 ± 2.1		38.9 ± 1.4	43.4 ± 2.3		41.6 ± 1.2	57.4 ± 2.5	
Princeton	32.9 ± 1.2	26.6 ± 2.0		35.9 ± 1.1	21.1 ± 1.4		39.4 ± 1.2	20.2 ± 1.3	

cut with a sickle and the spikes threshed in a stationary thresher with a minimum of forced air to reduce loss of scabby seed.

A 200-seed sample was randomly taken from each yield bag and visually inspected for damage from *F. graminearum* infection. The seeds were sorted into two classes: healthy seeds with little or no discoloration and shriveling, and nonhealthy seeds which were moderately to severely shriveled and discolored. Many of the seeds in the nonhealthy class were "tombstone" kernels with white or light pink coloring and extreme shriveling. The number of seeds in each class was counted and the percentage of scabby seed was used as the estimate of FDK.

Deoxynivalenol Test Procedure

In the among-family selection study, a 5-g sample of grain from each plot at Lexington was analyzed for DON using the EZ-Quant Vomitoxin Test Kit from the Diagnostix Company (Mississauga, ON) (Hall and Van Sanford, 2003). Each sample was ground in a coffee grinder for 15 s. The coffee grinder was vacuumed between samples to protect against any cross contamination. Twenty-five milliliters of distilled water was added to each ground sample and the remainder of the test was completed following the kit protocols. Two replications from each population were sampled for DON analysis.

Statistical Analysis

Data from the individual locations were analyzed using the following model for all traits:

$$Y_{ij} = \mu + \beta_i + G_j + E_{ij}$$

where Y_{ij} = the observation on the ith block and the jth genotype, μ = the overall mean, β_i = the effect of ith block, G_j = the effect of jth genotype, E_{ij} = the residual error.

Because the 2 yr of the study were extremely different and we observed significant interaction of genotypes and locations with years, data from individual years were analyzed separately according to the following model for all traits:

$$Y_{ij} = \mu + L_i + B_{j(i)} + G_k + (GL)_{ik} + E_{ijk}$$

where Y_{ij} = the observation on the kth genotype in the jth block in the ith location, μ = the overall mean, L_i = the effect of ith location, B_j = the effect of the jth block, G_k = the effect of the kth genotype, $(GL)_{ik}$ = the effect of the interaction of the ith location and the kth genotype, E_{ijk} = the residual error.

Severity data taken on 10 individual heads per plot were averaged to give a mean severity for each plot. The other traits were measured on a plot basis and plot means were used in all analyses. Broad sense heritabilities and their standard errors were estimated on an entry-mean basis over the four environments using Proc MIXED (SAS Institute, 1990) and the methods presented by Holland et al. (2003). This method relies on restricted maximum likelihood estimates of variance components which are more precise than ANOVA-based estimates when the data are unbalanced. Correlations among traits were estimated from genotype means using Proc CORR (SAS Institute, 1990). Degrees of freedom ranged from 37 to 39, depending on year and population.

RESULTS

Severity of infection was significantly higher in 2004 than in 2003 across all populations grown in Lexington (Table 1) where mean severity was 43.8% in 2004, compared with 30.3% in 2003. FDK percentages were also higher in 2004 than in 2003 (Table 1), at Lexington, although the reverse was true at Princeton. Population 2 had the lowest FDK ratings at Lexington in both years. Genetic variance for severity was observed in each population, with significant ($P < 0.01$) differences among the $F_{2:4}$ families in 2003 and among $F_{2:5}$ families in 2004 (Table 2). Similarly, significant genetic variation ($P < 0.01$) was also observed among the $F_{2:4}$ families and among $F_{2:5}$ families for FDK (Table 2). Deoxynivalenol was measured in all 40 families in the three populations in 2003 at Lexington only, and there was significant ($P < 0.05$) genetic variation in Populations 1 and 3 (Verges, 2004). The effect of location was significant ($P < 0.05$)

Table 2. Mean squares from the combined ANOVA for Fusarium head blight severity and Fusarium-damaged kernels (FDK) in three wheat populations at Lexington and Princeton, KY, 2003–2004.

Source of variation	Population 1	Population 2	Population 3	Population 1	Population 2	Population 3
	Severity			FDK		
$F_{2:4}$ (2003)						
Location (L)	2686.0**	1235.0**	3638.7**	420.6**	12927.5**	9470.5**
Genotype (G)	184.2**	227.6**	204.1**	69.9**	121.7**	80.95**
L × G	123.0*	89.0	83.6	460.0*	52.9	62.9*
$F_{2:5}$(2004)						
L	16425**	433.71*	221.3	347.8**	19466.1**	52418.3**
G	386.5**	289.5**	234.9**	293.0**	425.6**	596.2**
L × G	139.0	187.7**	61.3	210.0**	349.8**	255.7

* $P < 0.05$.
** $P < 0.01$.

Table 3. Broad sense heritability estimates (standard errors in parentheses) for severity, Fusarium-damaged kernels (FDK) and height estimated from family means in three wheat populations averaged over Lexington and Princeton, KY, 2003–2004.

	Severity	FDK	Height
	%		cm
Population 1	0.32 (0.09)	0.16 (0.11)	0.46 (0.12)
Population 2	0.30 (0.09)	0.20 (0.09)	0.71 (0.07)
Population 3	0.33 (0.08)	0.19 (0.11)	0.69 (0.07)

for FDK and severity in 2003 and 2004, except for severity in Population 3 in 2004. Genotype × location interaction was not significant for severity (Table 2) except for Population 2 in 2004 ($P < 0.01$) and Population 1 in 2003 ($P < 0.05$). In the case of FDK (Table 2), genotype × location interaction was significant in 2004 except for Population 3 and in 2003 except for Population 2.

Broad sense heritabilities were estimated on a family mean basis for three traits over four environments. Estimates were moderately low for severity (Table 3). FDK, a highly variable trait had low BSH estimates in the three populations (0.16–0.20) while height was highly heritable (~0.70) in Populations 2 and 3. Heritability of DON accumulation was not estimated because this trait was only measured in 2003 on all families in all populations. In 2004, DON measurements were made on a composite of all families as part of the selection experiment.

Association between Severity and Fusarium-Damaged Kernels

To investigate the relationship between two potential indicators of resistance, severity, and FDK, we first distilled the data down to 12 observations: three populations in four environments where each of the data points was a mean of either 80 or 120 observations. When FDK was regressed on severity using these means, the relationship was positive and significant. (Fig. 1; $R^2 = 0.70$). However, when we looked at entry means (~40 per population) and evaluated the relationship between FDK and severity by population and year, a different story emerged (Fig. 2). In Fig. 1, the three points above the 1:1 line correspond to the three populations at Lexington in 2004; it is evident that this was a high FDK environment. In Population 1, for example, the correlation between severity and FDK went from $r = 0.43$ ($P < 0.01$) in 2003 to $r = 0.27$ (NS) in 2004 (df = 37–39). In Population 2, on the other hand, the relationship between the two traits was stable over 2003 and 2004 ($r = 0.60$ and 0.67, respectively; $P < 0.01$). Population 3 was intermediate in that the correlation in 2004 was lower ($r = 0.54$, $P < 0.01$) than in 2003 ($r = 0.64$, $P < 0.01$) but still highly

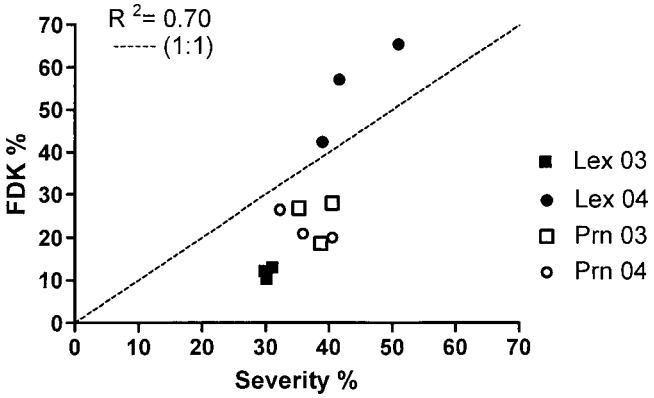

Fig. 1. Regression of mean Fusarium-damaged kernels (FDK) on mean Fusarium head blight severity in three wheat populations and the four environments, Lexington (Lex) and Princeton (Prn), KY, 2003–2004.

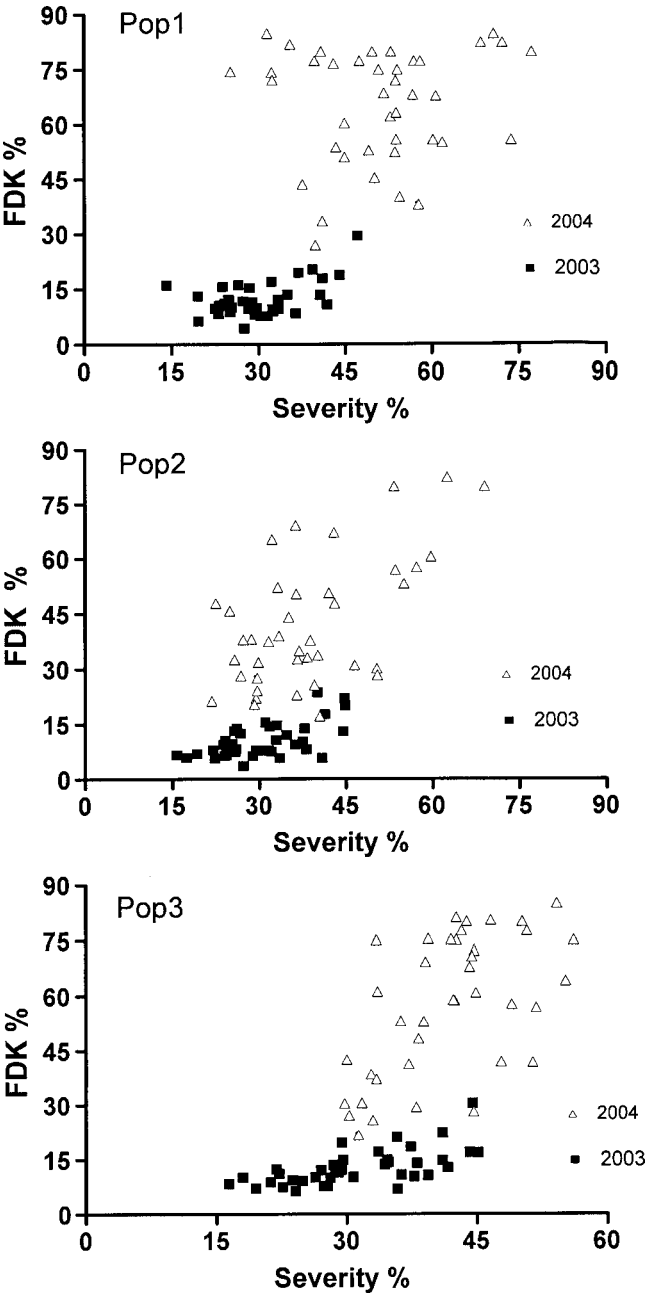

Fig. 2. Scatter plot of severity vs. Fusarium-damaged kernels (FDK) in 40 wheat families in three populations after inoculation with *Fusarium graminearum*, Lexington and Princeton, KY, 2003–2004.

Table 4. Selection response for Fusarium head blight severity, Fusarium-damaged kernels, plant height, and deoxynivalenol (DON) of top 20% of $F_{4:5}$ SRW wheat families selected for low FHB severity from $F_{2:4}$ families evaluated at Lexington (Lex) and Princeton (Prn), KY, 2004.

	Severity			FDK			Plant height†	DON†
	%			%			cm	mg kg^{-1}
Population 1	Lex	Prn	Mean	Lex	Prn	Mean	Lex	Lex
Mean of selected $F_{4:5}$	50.5 ± 3.0	29.8 ± 1.8	40.2	52.1 ± 3.0	26.8 ± 3.0	39.5	93.2 ± 1.2	12.6 ± 0.8
Population mean $F_{2:5}$	50.8 ± 1.5	33.4 ± 1.2	42.1	65.8 ± 2.1	26.2 ± 2.0	46.0	89.1 ± 0.3	13.2 ± 3.1
Response to selection	−0.3	−3.5	−1.9	−13.7	0.6	−6.5	4.1	−0.6
Population 2								
Mean of selected $F_{4:5}$	33.9 ± 2.1	32.7 ± 2.2	33.3	39.5 ± 5.7	23.4 ± 3.4	31.5	102.8 ± 1.3	10.8 ± 0.9
Population mean $F_{2:5}$	38.9 ± 1.4	35.9 ± 1.1	37.4	42.6 ± 2.3	21.1 ± 1.4	31.8	100.4 ± 0.4	18.8 ± 1.5
Response to selection	−5.0	−3.2	−4.1	−3.1	2.3	−0.4	2.4	−8.0
Population 3								
Mean of selected $F_{4:5}$	36.5 ± 1.8	37.1 ± 1.5	36.8	54.2 ± 6.2	21.6 ± 2.5	37.9	88.7 ± 1.2	11.7 ± 1.1
Population mean $F_{2:5}$	41.6 ± 1.2	39.3 ± 1.2	40.5	57.2 ± 2.5	20.1 ± 1.3	38.6	87.0 ± 0.3	13.6 ± 3.0
Response to selection	−5.1	−2.2	−3.7	−3.0	1.5	−0.7	1.7	−1.9

† Height and DON measured at Lexington only.

significant. It is clear from Fig. 2 that the magnitude of the variation was smaller in 2003 than in 2004, especially for FDK.

To evaluate the effectiveness of among-family selection for low severity in early generations the top 20% (eight lowest severity) families were selected from each population. Correlated responses of FDK, DON, and height were also evaluated. In the selection study, the overall population mean was based on a composite of all families, while in the heritability study the overall population mean was the actual mean of all families. Thus, the values from the selection study (Table 4) differ from the actual means reported in Table 1. At both locations in all three populations we observed a reduction in the mean severity in response to selection (Table 4). Selection response was higher in Lexington than in Princeton except in Population 1, in which we observed a surprisingly high response at Princeton. Averaged over both locations, Population 2 had the highest response to selection with a 4.1% reduction in severity. Population 2 also presented the highest reduction in DON content (8 mg kg^{-1}; Table 4) compared with the population mean. Population 1 had the highest reduction in FDK (Table 4) at Lexington (13.7%) but there was little reduction in FDK at Princeton. A slight increase in mean height was observed for the three populations (Table 4). Population 1 had the greatest increase; the selected families were, on average, 4 cm taller than the population mean. Correlations between height and severity or FDK were generally negative (data not shown). However, the magnitude of the correlations varied among populations from 0.05 (severity, height, NS) to 0.63 (severity, height, $P < 0.01$) (Verges, 2004).

Figure 3 shows the performance of the eight families relative to the population mean when the family ratings are averaged over Lexington and Princeton. In Population 1 there were four families with significantly ($P < 0.05$) lower FDK, and two families with significantly lower severity than the population mean. In Population 2, there were two families with significantly lower FDK and three families with significantly lower severity, while in Population 3 there were three families with significantly lower FDK and two families with significantly lower severity than the population mean.

Genotypic Data

DNA fragments of 192 and 142 bp were amplified for markers *gwm493* and *gwm533*, respectively, in the FHB resistant Sumai 3 and its derivative Ning7840. Nineteen lines of Population 2 had 192- and 142-bp alleles derived from these resistance sources for these marker loci that flank the major resistance QTL *Qfhs.ndsu-3BS*. The other two populations did not possess the Sumai 3 type alleles at these loci. This is likely due to the complex nature of these crosses where the resistance QTL may have been lost during population development.

DISCUSSION

Environmental factors have a significant impact on screening for FHB resistance and, therefore, an accurate assessment of resistance requires multiple tests over years and locations (Parry et al., 1995). Our results confirm the need for multiple years and locations, because the environmental characteristics of this study had a substantial impact on its results. Although artificial inoculation resulted in an epidemic both years of the study, the nature and intensity of the epidemic differed significantly between years. Higher temperatures and rainfall in May 2004 compared with the same period in 2003 may have led to higher levels of disease. This period corresponds to flowering and early grain fill when the wheat plant is vulnerable to infection (Schroder and Christensen, 1963).

In 2003, a moderate FHB level was achieved; the rate at which the epidemic developed met the expectation based on our previous experience (Stewart et al., 2004). In 2004, however, the disease was slow to develop, so that by 21 d after anthesis (daa), the normal time at which severity is assessed, the epidemic had not yet reached its peak (Stewart et al., 2004). The most important implication of the difference in the developmental profiles of the epidemics of 2003 and 2004 is that severity ratings, based on chaff symptoms, seriously underestimated the magnitude of the damage caused by the disease in 2004. Thus, the severe seed damage observed in 2004 was not predicted by or correlated with the chaff severity that was recorded relatively early in the development of the epidemic (Fig. 2). There are excellent reasons for reading severity at 21 daa; for ex-

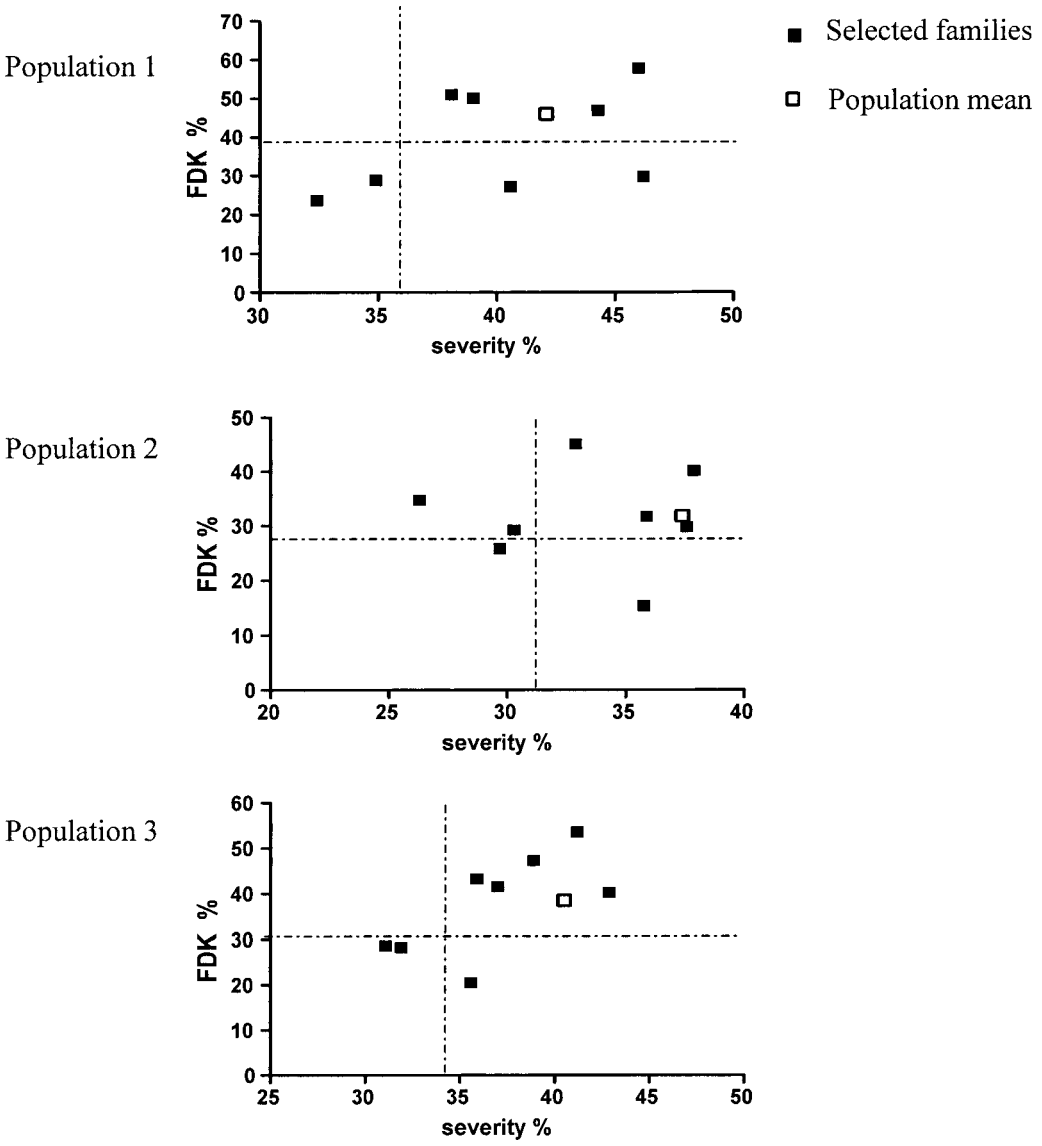

Fig. 3. Relationship between Fusarium-damaged kernels (FDK) and Fusarium head blight severity in three wheat populations after one cycle of family selection. Family means averaged over Lexington and Princeton, KY, 2004. Population mean minus one LSD (0.05) represented by dotted lines.

ample, if this rating is delayed, there is a strong probability that normal senescence and chaff bleaching will occur and be mistaken for scab symptoms, leading to an overestimate of severity. However, in 2004, 21 daa proved to be too early to adequately assess disease severity on the basis of chaff symptoms.

In this study four testing environments were used to estimate heritability in three clearly defined reference populations. Therefore, the combined heritability estimates presented in Table 3 are not inflated by genotype × environment interaction, and should provide a reasonable picture of the relative importance of genetic variation in these populations. Our heritability estimates are, for the most part, lower than most estimates in the literature. Miedaner et al. (2003), analyzing one wheat population, suggested that selecting for low DON content and severity could be successfully started in the F_3 when testing is done in many environments. They found high heritabilities (0.70–0.80) for both traits. In another study, Snijders (1990b) calculated heritabilities ranging from 0.05 to 0.89 among 23 F_2 populations for head blight rating. The magnitude of our heritability estimates, though relatively low, is in line with real world experiences of breeders. Once major resistance QTLs are eliminated from consideration, genetic progress in breeding for scab resistance is likely to be slow and incremental; current scab-resistant variety release patterns support this notion. In this study only Population 2 had the Sumai 3 type alleles associated with marker loci *gwm*493 and *gwm*533 (Bai et al., 1999; Waldron et al., 1999; Zhou et al., 2002). Interestingly, the heritability estimate for this population was lowest but the response to selection for reduced severity and DON was highest (Tables 3, 4). However, the 19 families that were genotyped for the Sumai 3 type alleles are only known to be segregating at these marker loci; the frequency of these

alleles within the families was not ascertained. Thus, the resistance gene could be present at a very low frequency and have little impact on the overall low heritability in the population. On the other hand, as noted by Bai and Shaner (2004) if a high proportion of diseased spikelets are infected because of a heavy inoculum pressure and favorable environment, severe FHB will result regardless of Type II resistance. In fact, our resistant check, Pioneer Brand 25R18, known to carry the Sumai 3 resistance gene (Greg Marshall, personal communication, 2005) had severity and FDK ratings of 43 and 65%, respectively, at Lexington in 2004 (Verges, 2004).

One cycle of selection resulted in lower severity in every population, although the magnitude of the selection response varied across populations and locations. Given these results, what conclusions might we draw about family-based selection for scab resistance? There is not a great deal in the literature that addresses this question. Some authors (Snijders, 1990b; Miedaner et al., 2003) suggest that at least two locations are critical for early generation screening. Our data underscores the need for multi-location screening, given the differences observed between Lexington and Princeton. In addition to genotype × location interaction, genotype × year interaction is a major consideration in designing scab resistance breeding strategies. Material that appears promising one year must be screened for several more years and at multiple locations to confirm scab resistance. In this respect, the trait is very similar to grain yield.

The other question that surfaced in this study is one that many breeders wrestle with: which trait or traits should be measured when screening for scab resistance and how much weight should be assigned to each? It is customary for breeders to screen material on the basis of chaff symptoms because data collection is relatively quick and painless. However, we have observed numerous cases where chaff symptoms belie actual seed damage (Hall, 2001; Argyris et al., 2005). In this study, the correlation between severity and FDK varied between 2003 and 2004. A primary reason for this disparity was that the late peak of the 2004 epidemic produced higher FDK than it did severity measured at 21 daa, especially at Lexington (Fig. 1). Measuring FDK is a daunting and time-consuming process, but it provides an estimate of seed damage and attendant losses in grain yield and quality that the growers and end users suffer. Scab researchers recognize the importance of FDK; it is frequently measured in uniform nurseries (Sneller et al., 2004) and has been incorporated into a weighted index (Kolb and Boze, 2003). Most researchers would agree, though, that DON concentration is ultimately the most critical trait because it affects all sectors of the wheat industry and it has such serious food safety implications. However, DON measurement is more expensive and time consuming than FDK, so it is typically measured only on the most elite breeding material. In the present study, DON was significantly correlated with FDK, in agreement with other researchers (Bai et al., 2001). The correlation varied among populations, (r = 0.48, 0.71, and 0.41 in Populations 1, 2, and 3, respectively) but was always higher than the correlation between severity and DON (Verges, 2004). Miedaner et al. (2003) suggested that it is possible to begin DON screening in the F_3 generation. While there is enough seed in this generation, it is not practical to sample enough plants in a large number of populations to account for within-family genetic variation.

Although DON and FDK are recognized as critically important FHB traits, an additional problem with measuring them is sampling variance. In sampling grain for DON analysis, for example, the current protocol used by the U.S. Wheat and Barley Scab Initiative (http://www.scabusa.org) calls for 5 g of grain to be ground and assayed. In this study we based FDK estimates on 200 kernels per family, which is a fraction (<10%) of the grain produced by an experimental unit such as a headrow. If an efficient method were devised for separating scabby kernels, then all of the grain produced in a headrow or hill plot could be screened for FDK. This would likely result in more accurate phenotyping and increased progress from selection.

In summary, in the absence of major QTL, progress in breeding for scab resistance is likely to be constrained by the same factors that limit breeding for yield and other quantitative traits: low heritability, high cost of data collection, and G × E interaction. Nonetheless, there is evidence to support the existence of quantitative, incremental resistance to head scab within the SRW market class and its role in the positive response to selection. This sort of resistance can be used either as an alternative or a supplement to the Sumai 3 resistance and should allow breeders to develop resistant SRW cultivars without deviating from quality requirements of end users.

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