

FUSARIUM HEAD BLIGHT RESISTANCE IN F₁ HYBRID HARD RED SPRING WHEAT

A Thesis
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Justin James Leier

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

October 2019

Fargo, North Dakota

North Dakota State University
Graduate School

Title

FUSARIUM HEAD BLIGHT RESISTANCE IN F₁ HYBRID HARD RED
SPRING WHEAT

By

Justin James Leier

The Supervisory Committee certifies that this *disquisition* complies with North Dakota
State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Andrew Green

Chair

Dr. Andrew Friskop

Dr. Blaine Johnson

Dr. Jack Rasmussen

Approved:

October 28, 2019

Date

Dr. Rich Horsley

Department Chair

ABSTRACT

Due to scientific advancement, hybrid wheat may be the future of wheat breeding, but many questions about the hybrid system remain. How hybrids will perform in terms of Fusarium head blight (FHB) resistance is one of the most important. It is unknown if a hybrid produced from a resistant by susceptible cross will express acceptable levels. This experiment tested hybrids produced from four resistant parents crossed with a common susceptible parent. Genotype was a significant effect for all traits measured. Hybrids were usually different than one or both parents. Resistance levels showed numerical increase with additional resistance genes. All hybrids were more similar to their respective resistant parent with percent similar ranging from 57-110% depending on parameter. This suggests that additive effects may play a role in hybrid wheat breeding and that incomplete dominance allows for production of hybrids using one FHB resistant parent.

ACKNOWLEDGEMENTS

I would first like to thank my advisor Dr. Andrew Green for his mentoring, guidance, and patience over the past two years. He gave a North Dakota farm boy the chance to learn more about plant genetics and gain valuable experience and knowledge about wheat breeding. I would also like to thank my graduate committee; Dr. Andrew Friskop, Dr. Blaine Johnson, and Dr. Jack Rasmussen.

I would like to thank everyone who made this experiment possible. Jesse Underdahl, Matt Abdallah, Andre Miranda, Adam Walz and Thor Selland were an invaluable source of guidance and help. I would also like to thank the NDSU Ag experiment station, Langdon NDSU Extension Center, and BASF for providing land for me to conduct my field trials on. I also need to thank the NDSU durum breeding crew for planting and helping maintain my plots at the Langdon Research Center. Thank you also to Corteva Agriscience™, Agriculture Division of DowDuPont for aid in funding this project and my education.

I am especially grateful to my friends and family for their support and encouragement during my time in graduate school. Special thanks to my parents, Mitch and Doris, for their love and support while I chased a dream rather than coming home to the farm. Finally I wish to thank my fiancé, Emily Trzpuc. Your love and encouragement drives me to become the best version of myself. Thank you.

TABLE OF CONTENTS

ABSTRACT	iii
ACKNOWLEDGEMENTS	iv
LIST OF TABLES	vi
LIST OF FIGURES	vii
INTRODUCTION	1
MATERIALS AND METHODS.....	3
Plant Material	3
Field Design & Agronomic Practices.....	5
Field Evaluations of FHB.....	8
Post-harvest Evaluations of FHB	8
Statistical Analysis	9
RESULTS	11
Genotype Verification	11
Genotype Effect on Fusarium Head Blight Resistance	11
Comparing All Genotypes within Each Parameter	15
DISCUSSION.....	19
LITERATURE CITED	24

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Presence and absence of resistance genes based on marker analysis for the five parents used in this experiment.....	4
2. Least Square Means and standard error for all parameters and genotypes across environments.....	18

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. Genotypic response of mean FHB index values for all genotypes in four separate environments.....	12
2. Genotypic response of mean FDK values for all genotypes in four separate environments.....	13
3. Genotypic response of mean test weight values for all genotypes in four separate environments.....	13
4. Genotypic response of mean DON accumulation for all genotypes in four separate environments.....	14
5. Genotypic response of mean DISK values for all genotypes in four separate environments.....	14

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important crops in the world and certainly in North Dakota. In 2018, North Dakota planted spring wheat hectares totaled 2.65 million and valued over \$1.6 billion [USDA/NASS, 2019]. One of the most limiting factors in wheat production is the spike disease Fusarium head blight, known as scab.

Several *Fusarium* species can cause scab and the most important species in North Dakota (ND) is *F. graminearum* (Friskop et al., 2018). This disease can cause yield reduction up to 70% under conditions conducive to the disease, temperatures of about 25°C accompanied by moist, humid air (>80%) extending for 36 to 72 hours during anthesis and seed development (Bai & Shaner., 1994; Osborne & Stein., 2007; McMullen et al., 2012). The fungus also secretes mycotoxins, such as deoxynivalenol (DON), which can cause health concerns in humans and livestock.

Management of Fusarium head blight (FHB) relies on an integrated strategy with varietal resistance being the most important tool. One of the first FHB resistance quantitative trait loci (QTL) located was Qfhs.ndsu-3 BS (*Fhb1*), derived from ‘Sumai 3’, which was introduced by Waldron et al., (1999). Breeders have produced resistant cultivars containing *Fhb1* such as NDSU cultivars ‘Alsen’ and ‘Faller’, and Syngenta’s cultivar ‘Soren’ (Browne, 2007). ‘Sumai 3’ and *Fhb1* are not the only resistance source. US cultivars such as ‘Freedom’ and ‘Ernie’ express resistance that is unrelated to resistance that is derived from ‘Sumai 3’. Complete resistance has not been found in any cultivar worldwide. Due to the massive economic impact and the health concerns that are caused by this disease, resistance-breeding efforts have been ongoing.

Wheat varieties are typically bred and released as inbred lines because wheat is a self-pollinating crop. However, advances in chemical hybridizing agents (CHAs) and cytoplasmic

male sterility (CMS) systems have opened the possibility of hybrid wheat becoming a viable release or commercialized product target. Small plot research studies have shown yield increases of 5-15% over the better parent using parents from the same class; however, drill-sown trials show yield increases at or over 20% (Singh et al., 2010; Whitford et al., 2013). When using parents from differing classes of wheat, such as soft and hard red, heterosis levels reach nearly 30% (Singh et al., 2010). An efficient hybrid system could help generate a yield boost in wheat that will help feed the world's increasing population.

Questions remain about the effectiveness of the hybrid production system. One question is how disease resistance will be affected by the hybrid system. Miedaner et al. (2017) found that hybrids, on average, expressed FHB resistance higher than the mean of their parents. FHB resistance is quantitatively inherited showing an abundance of additive effects, although in some hybrids, dominance and epistatic gene action has been observed (Miedaner et al., 2017). Knowledge of the type of resistance gene action could help formulate crossing strategies. Complete dominance of all FHB resistance genes would be desirable to hybrid breeders because the F₁ progeny obtained by pairing a resistant parent with a susceptible parent would have acceptable levels of resistance. Hybrids produced with two parents carrying resistance genes express levels of resistance similar to that of the better parent (Miedaner et al., 2017). However, it is currently unknown if a hybrid produced from only one resistant parent will express sufficient resistance to be commercially acceptable. Therefore, the objectives of this experiment were to evaluate FHB resistance of F₁ hybrids and their parents by measuring visual symptoms, Fusarium damaged kernels, grain volume weight and DON accumulation when the hybrids and parents were grown in the field across multiple environments using artificial inoculation.

MATERIALS AND METHODS

Plant Material

F₁ hybrids were created by crossing four cultivars resistant to *Fusarium* head blight with a common susceptible cultivar. The resistant parents were ‘Faller’ (PI 648350), ‘Glenn’ (PI 639273), ‘Alsen’ (PI 615543) and ‘SY Soren.’ The susceptible parent is ‘2398.’ ‘Alsen’ is a hard red spring wheat (HRSW) cultivar bred at North Dakota State University (NDSU) and released by the North Dakota Agricultural Experiment Station (NDAES) in 2000 (Frohberg et al., 2006). ‘Alsen’ originated from a three-way cross; ND674//ND2710/ ND688, and includes ‘Sumai3’ in its background. This variety warranted release by combining high levels of FHB resistance with excellent yield and end-use quality. ‘Faller’ is a cultivar of HRSW bred at NDSU and released by the NDAES in 2009 with a pedigree of ND2857/‘Dapps’ (Mergoum et al., 2008). ND2857 includes ‘Sumai3’ in its background. ‘Faller’ expresses FHB resistance similar to both ‘Alsen’ and ‘Glenn’ in nursery screenings. NDSU variety trials report the FHB resistance level of ‘Faller’ is a five on a 1-9 scale (Ransom et al., 2017). ‘Glenn’ is a HRSW cultivar bred at NDSU and released by the NDAES in 2005 (Mergoum et al., 2006). ‘Glenn’ is the result of a cross between experimental line ND2831 that has ‘Sumai3’ as a parent, and another NDSU experimental. ‘Glenn’ expresses high levels of FHB resistance at 19% incidence in screening nurseries and NDSU variety trials report the FHB resistance level is a three on a 1-9 scale (Ransom et al., 2017). ‘SY Soren’ is a Syngenta cultivar with moderate FHB resistance. According to NDSU variety trials the FHB resistance level of ‘SY Soren’ is a five which is the same rating score as ‘Faller’ (Ransom et al., 2017). ‘2398’ is a variety released by the NDAES and developed by the Pioneer Hi-Bred HRSW program in the 1980’s. It has been historically used as a very susceptible check in the NDSU scab nursery as referenced to in registrations for

‘Faller’, ‘Glenn’, and ‘Alsen’ (Frohberg et al. 2006; Mergoum et al. 2006; Mergoum et al. 2008). All initial seed was sourced from within the wheat breeding program at NDSU. Subsequent plantings were sourced from the first round of planting and purified using molecular markers and phenotypic observations.

Parents were used both as male and as female donors in order to generate reciprocal crosses. Resistant parents were selected on basis of the combination of resistance genes known to be present in their genomes. ‘Alsen’ contains *Fhb1*, *Fhb5*, and a QTL on chromosome 3A derived from ‘Frontana’ (Browne, 2007). ‘Faller’ expresses *Fhb1* and *Fhb5*. ‘SY Soren’ only contains resistance gene *Fhb1*. Based on molecular marker screening, ‘Glenn’ contains none of the known large effect resistance genes mentioned above, but expresses high resistance levels (EIDoliefy, 2015). Table 1 summarizes the varying resistance genes expressed in the resistant parents. ‘2398’ contains none of the genes mentioned and is rated as highly susceptible.

Table 1. Presence and absence of resistance genes based on marker analysis for the five parents used in this experiment.

Gene (chromosome)	Parent cultivar				
	Alsen	Faller	SY Soren	Glenn	2398
<i>Fhb1</i> (3B)	+ ^a	+	+	-	-
<i>Fhb5</i> (5A)	+	+	-	-	-
Frontana QTL (3A)	+	-	-	-	-

^a (+) indicates cultivar has the gene, (-) indicates the cultivar does not have the gene

Hybrid Production

All hybrid seed was produced by hand emasculations and pollinations in the NDAES greenhouse in Fargo, ND. In 2018 the parents were planted individually to maximize the number of tillers, allowing crossing of the same male parent with a large number of heads on one female parent, thereby reducing potential variability from seed source. Hybrid seed was bulk harvested by specific parent by parent cross in Spring 2018 to allow for marker verification of genotypes.

Seed production of parents and of hybrids was repeated again in Fall 2018 and Spring 2019. The genotypes had been verified, explanation given below, so two plants per pot were used to maximize hybrid production.

Genotype Verification

The identity of the parent plant material was verified using known molecular markers in the marker assisted selection program at NDSU during the summer of 2018. Three seeds from a self-pollinated head were grown in the greenhouse and tissue samples were harvested at the three leaf stage. The USDA Genotyping Lab, Fargo, ND, performed the marker analysis. Markers associated with loci in Table 1 (*Fhb3A*, *Fhb3B*, *Fhb5A*) were used along with Lr34, a leaf rust resistance gene, to verify the alleles present in the hybrids. All four loci were used to verify the parents' identity. Three hybrid seeds were also saved from each plant x plant combination and grown in the greenhouse to compare the identity of the hybrids to the parents. Visual verifications of hybridity were also made during the field trials. Plant height and anthesis date were measured to distinguish between F₁ hybrids and inbred lines. Both traits were expected to be intermediate to the parents. Plant height was recorded at physiological maturity. Any hybrid which exhibited incorrect or inconsistent genetic marker data or was phenotypically observed to be inconsistent with parental types was discarded.

Field Design & Agronomic Practices

In 2018, the F₁ hybrids, five parental lines, and two checks were planted in Fusarium head blight nurseries at Prosper and Langdon, ND on May 14 and 16, respectively. In 2019, Langdon and Prosper were used and an additional location was added; Sabin, MN. The 2019 nurseries in Sabin and Langdon were planted on May 16, and Prosper was planted on May 31.

The experimental design was a randomized complete block design, augmented with repeated checks in a row column design to allow for spatial adjustment. The design utilized six replications in 2018 and three replications per location in 2019. The repeated checks, included three times in each replication, were ‘ND2710’ (PI 633976) as the resistant check and ‘WB Mayville’ as the susceptible check. Plots consisted of three-seed hill plots. The experiment was planted using a four-row planter with 30cm spacing between rows and 58cm between plots. Trial plots were surrounded by a border of ‘WB Mayville’.

Planting date was dictated by weather but targeted to represent the typical planting time practiced in the region. Tillage was performed in accordance with regional practices and fertilizers was applied according to NDSU Extension recommendations (Franzen., 2018). Herbicide control program consisted of Axial XL (Pinoxaden), Bronate Advanced (Octanoic acid ester of bromoxynil, Heptanoic acid ester of bromoxynil, & 2-ethylhexyl ester of MCPA), and Starane Ultra (fluroxypyr 1-methylheptyl ester) in accordance with product labels and repeated as necessary. Hand weeding was utilized to remove weeds that herbicides failed to control. No fungicide was applied to the experimental plots or surrounding border plots.

At physiological maturity (Feekes 11.4) all heads from each plot were harvested. In 2018, the Prosper location was harvested on August 21 and dried for four days at 16°C. The Langdon location was not rated or harvested in 2018 due to lack of Fusarium infection. Harvest dates in 2019 were August 14 for Sabin, Aug. 22 for Langdon, and Aug. 29 for Prosper. Harvested spikes were dried for four days at 16°C. Harvested heads were threshed on a whole plot basis using a stationary thresher (Sparc Machine Design, Swift Current, Sask.). Air flow of the thresher was minimized and small chaff removed by hand in order to retain as many tombstone seeds as possible.

Inoculation

Fusarium graminearum colonized corn grain was used as inoculum to ensure a reoccurring source of spores. Inoculum was obtained from the North Dakota State University Plant Pathology Department, which creates the inoculum used in the wheat breeding scab nursery. Inoculum was composed of twenty isolates collected throughout North Dakota. Ten of the isolates were 3A DON producing isolates and ten 15A DON producing isolates. *Fusarium* inoculum preparation followed similar methods described by Balut et al., (2013).

Inoculum was spread by hand at multiple growth stages to ensure a high infection rate. In 2018 the Prosper nursery was inoculated at early jointing (Feekes 7) and early boot stage (Feekes 10) on June 18 and 25, respectively. The Langdon location was inoculated at the same stages on June 26 and July 3, 2018. In 2019, the Langdon location was inoculated on June 25, July 2, and July 9 which coincided with tillering (Feekes 3), jointing (Feekes 7), and early heading (Feekes 10.3). The Prosper location was inoculated on July 2, 8, and 12 which corresponded with the tillering (Feekes 3), jointing (Feekes 7), and boot growth stage (Feekes 10). In each location the total combined rate of corn grain inoculum was 61 g/m². Experimental plots were irrigated using an overhead mist irrigation system, with the first irrigation coinciding with the day of first inoculation in 2018. In 2019 the Langdon irrigation began at Feekes 3 and irrigation at Prosper began at Feekes 7. Irrigation water was supplied at one to two minutes durations per hour, and four days per week. In Langdon, the irrigation operated twelve hours during daytime. The Prosper irrigation system operated on a twenty-four hour basis. During periods of excessively dry or wet conditions, the irrigation system was adjusted accordingly.

Inoculum and misting methods used at Sabin included an application of water for 30 minutes late in the afternoon every other day starting on the first day of corn inoculum (spawn)

spread. For Sabin only, the colonized corn grain inoculum was prepared by BASF and contained one 3A DON producing isolate and one 15A DON producing isolate. The combined rate across the three separate applications was 61 g/m². The corn spawn was spread on June 19, June 26, and July 3, which corresponded to late tillering (Feekes 3), jointing (Feekes 7), and boot (Feekes 10) growth stages.

Field Evaluations of FHB

Both disease incidence and severity were assessed for all hill plots and those values were used to create a FHB index. Evaluations were conducted at around growth stages Feekes 11.1-11.2. Anthesis was determined as 50% of the heads in a plot with visible anther extrusion. The disease incidence was calculated by counting the number of diseased heads out of ten randomly selected heads and expressed as a percent (Khorzoght et al., 2010). Ten infected heads were also evaluated for disease severity which is expressed as the percentage of diseased spikelets per head (Khorzoght et al., 2010; Balut et al., 2013). The disease index was calculated as below according to Khorzoght et al., (2010).

$$\text{Incidence} = \frac{\text{\# of diseased heads}}{10} \times 100$$

$$\text{Severity} = \frac{\text{\# of diseased spikelets}}{\text{Total spikelets}} \times 100$$

$$\text{FHB Index} = \frac{(\text{Incidence} \times \text{Severity})}{100}$$

Post-harvest Evaluations of FHB

Seed lots were visually evaluated for Fusarium damaged kernels (FDK), using the methods described by Jones & Mirocha., (1999), with the FDK value being expressed as

percentage of damaged kernels in the sample. Micro test weight was also measured and converted using a standard conversion table. A sample from each plot was evaluated for deoxynivalenol (DON) accumulation at a DON testing lab. Samples were processed at the University of Minnesota in 2018 and at NDSU in 2019. Samples were ground using a UDY Cyclone Sample Mill model 3010-014 (UDY Corporation; Fort Collins, CO). The mill was cleaned between each sample using a brush, forced air, and vacuum to ensure no cross-contamination between samples. Samples were also grouped by genotype in order to minimize contamination and the mill cleaned extra thoroughly when changing to a new genotype. The mill was also allowed to cool after every ten samples to avoid any sample damage through contact with hot rotor blades.

To observe the cumulative effect of multiple traits across the genotypes, a DISK rating was also calculated. This rating is a summation of four individual ratings and generates a single numeric rating that includes DON, incidence, severity, and kernel damage (FDK) (Bissonette et al., 2018). The DISK values were calculated as shown below according to Bissonette et al., (2018).

$$(0.2 \times \text{incidence}) + (0.2 \times \text{severity}) + (0.3 \times \text{FDK}) + (0.3 \times \text{DON}) = \text{DISK}$$

Statistical Analysis

Statistical analysis across all environments was conducted using SAS 9.4 (SAS Institute, Cary, NC). Calculations were made using PROC GLIMMIX to estimate and compare the magnitude of the genotypic effects. The experiment was analyzed as a randomized complete block (RCBD). Significant differences of least square means at the $P \leq 0.05$ were separated using *t*-tests. Genotype was considered a fixed effect, while replicate, nested within environment,

was considered a random effect. Minimal spatial variability was detected in the data analysis, and as such were not included in the final analysis.

RESULTS

Genotype Verification

Molecular markers were utilized to verify the identity of each cultivar used and ensure that the resistance genes were consistent with the published literature. All markers that ‘Alsen’, ‘Faller’, and ‘SY Soren’ are known to contain were consistent with our screening (Table 1). ‘Glenn’ and ‘2398’ had a null allele for all markers in the screening. The hybrids expressed both presence and absence for all the FHB resistance alleles as expected per the respective pedigrees. For instance, the 2398/Alsen hybrids appeared heterozygous for all three markers. There also were no anomalies in the Lr34 data, plant height, or anthesis data that caused discarding of any plots (data not included).

Genotype Effect on Fusarium Head Blight Resistance

Genotype was a significant effect ($p < 0.05$) for all traits measured and hybrids usually differed from either one or both parents. Reciprocal crosses were treated as separate genotypes in the field. After analyzing the data and observing no significant difference between any reciprocal pairs, they were combined for the analysis used in the experiment (data not shown). Since the main focus is on the effect of genotype, data are presented by hybrid.

Environmental Effects

Over the course of the two year experiment, the genotypes were tested in four environments that greatly varied. Four of the five environments produced adequate disease pressure. Two of those four locations received excessive rain in 2019 contributing to extremely high disease pressure and missing plots, which were accounted for by using a program that handled them as well as including at least three plots of each genotype per replicate.

Despite the magnitude differences of environments, genotypic response was consistent with environments producing similar responses (Figures 1-5). Susceptible genotypes, ‘2398’ and ‘WB Mayville’, show higher levels of FDK and FHB index as well as lower test weight than the resistant parents in all environments. For DON, the susceptible genotypes had levels higher than levels of all resistant parents in two of the four environments. In the other two environment, ‘WB Mayville’ had lower DON than one or more resistant parents. With DISK (Figure 5) the resistant parents have lower values than ‘2398’ and ‘WB Mayville’ in all four environments. The hybrids were also generally more affected by FHB than their resistant parents. Figure 3 shows an inverse of the trend we have seen in Figures 1 and 2. A resistant cultivar produces more healthy seeds, resulting in increased test weight. There are genetic differences for test weight between genotypes in the absence of FHB, but generally, lower test weight was observed under heavy disease pressure. This relationship explains why ‘2398’ and ‘WB-Mayville’ both have lower test weights than the resistant parents in most cases. In general, all genotypes followed a similar pattern across all four environments.

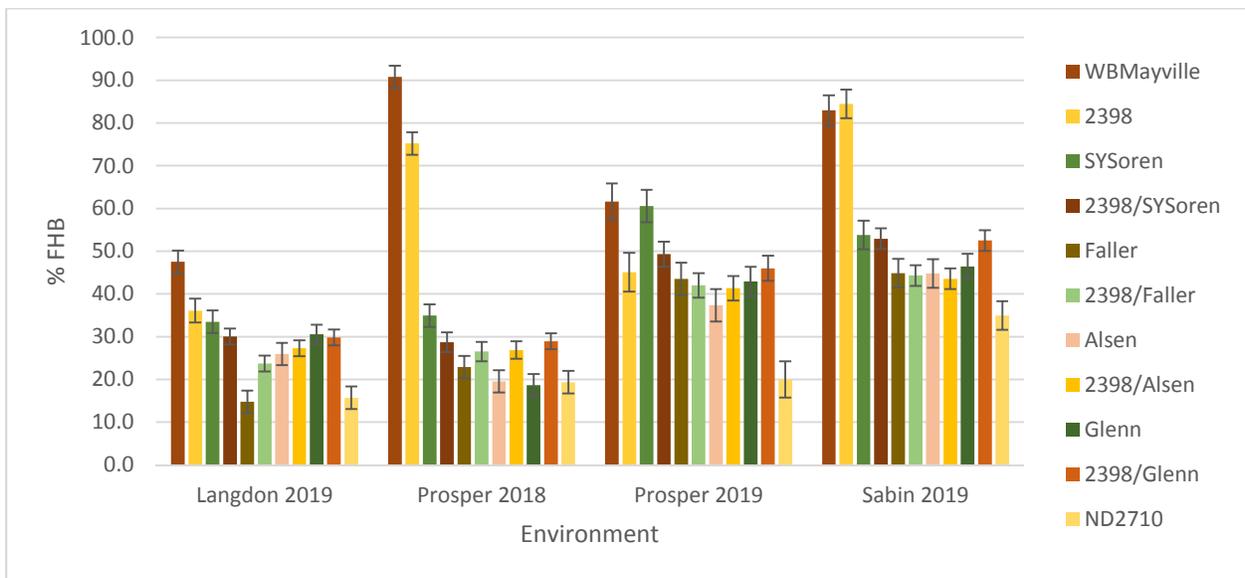


Figure 1. Genotypic response of mean FHB index values for all genotypes in four separate environments.

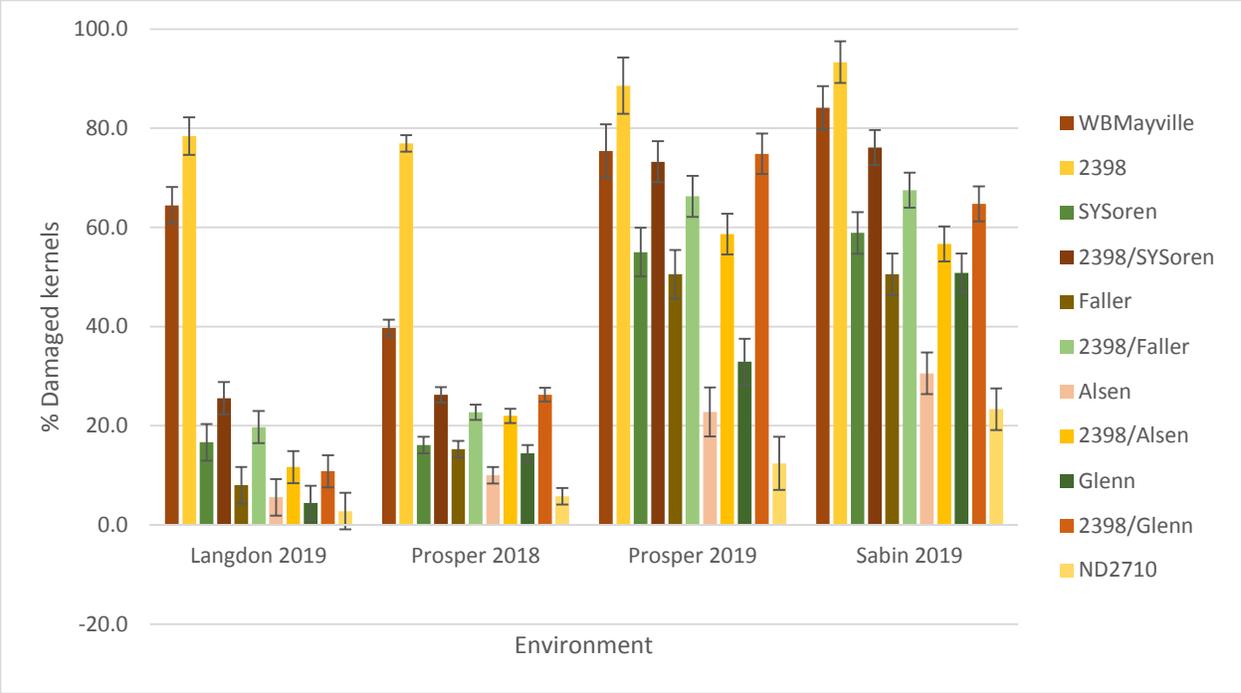


Figure 2. Genotypic response of mean FDK values for all genotypes in four separate environments.

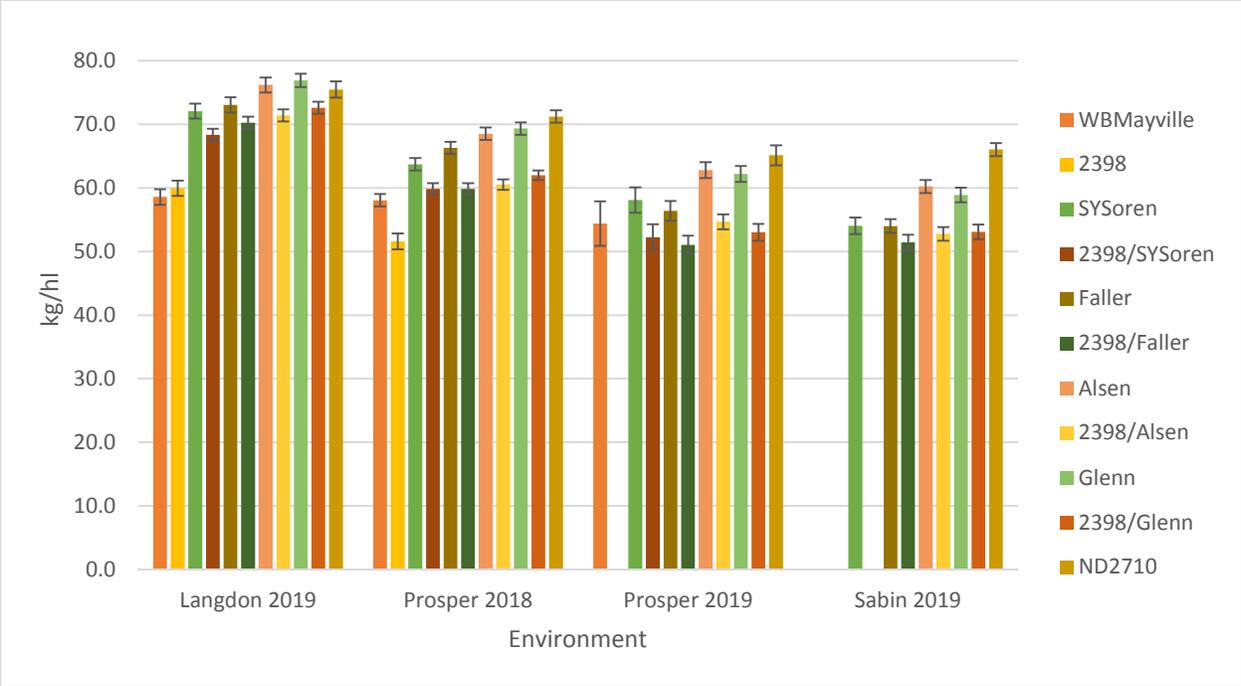


Figure 3. Genotypic response of mean test weight values for all genotypes in four separate environments.

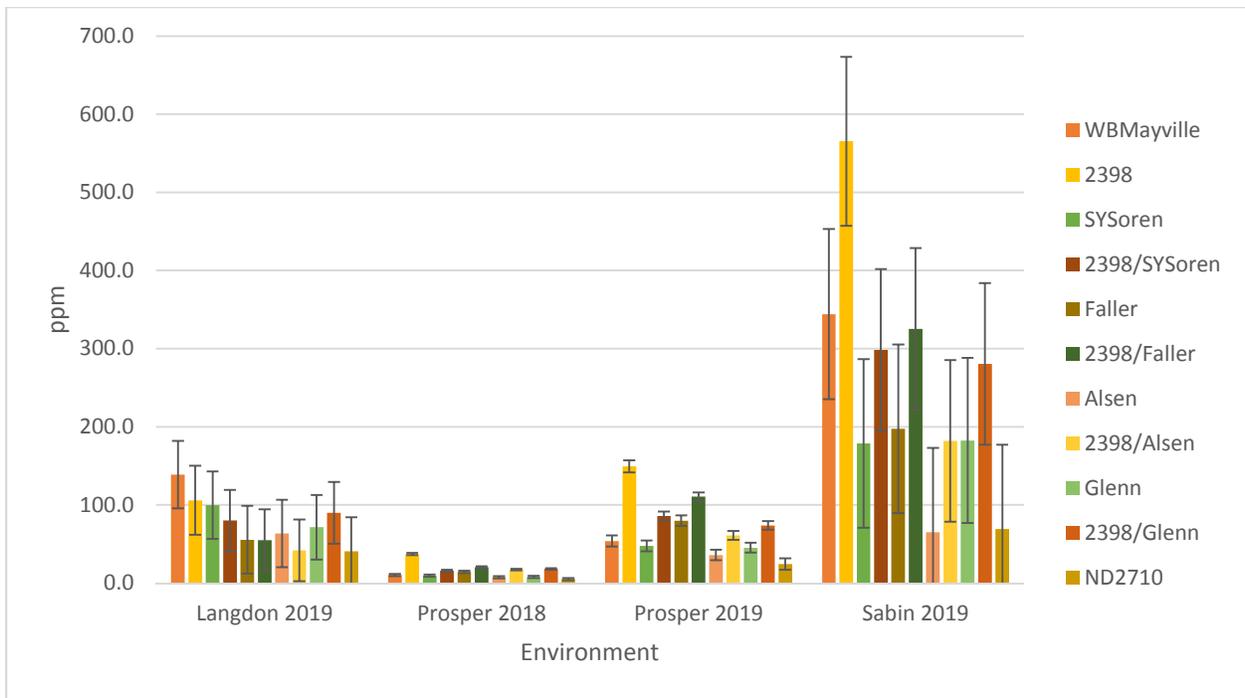


Figure 4. Genotypic response of mean DON accumulation for all genotypes in four separate environments.

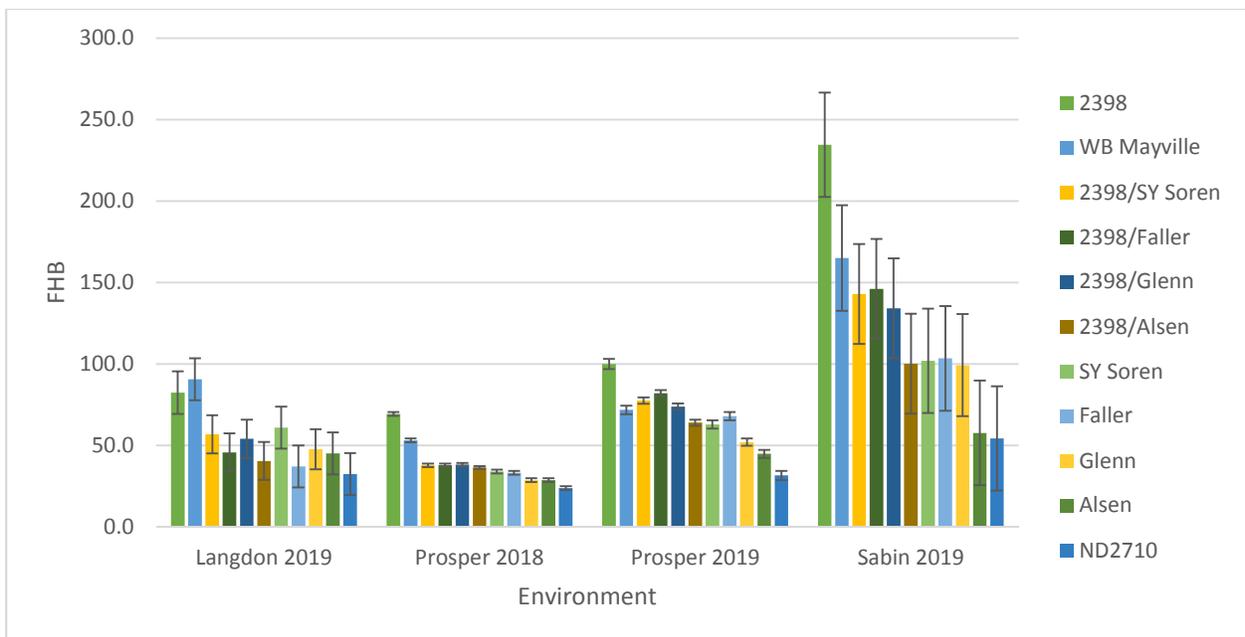


Figure 5. Genotypic response of mean DISK values for all genotypes in four separate environments.

Comparing All Genotypes within Each Parameter

FHB index appeared to be relatively unaffected by the number of resistance genes. The repeated checks were consistent, with 'ND2710' having the lowest score and 'WB Mayville' having the highest score. Rank of resistant parents and hybrids for the index deviated from rankings of other traits. The most resistant parent was 'Faller', followed closely by 'Alsen' which was then followed by 2398/Faller, then 'Glenn' (Table 2). The 2398/Alsen and 2398/Glenn hybrids followed after 'Glenn'. 'SY Soren' had the third highest index which was one rank higher than 2398/SY Soren. Despite this unusual ranking of resistant parents, 'Faller' is only different from 'SY Soren' ($p < 0.05$). We did observe hybrids outperforming the resistant parents as 2398/Faller was lower than 'Glenn', and all hybrids were lower than 'SY Soren'.

When evaluating FDK, the genotypes ranked in order based on number of resistance genes. Susceptible genotypes had the highest mean FDK scores. The rank order of the parents from lowest FDK to highest was 'Alsen', 'Glenn', 'Faller', and then 'SY Soren'. Rank of genotypes based on test weight were in order of resistance genes with 'Glenn' having the highest, followed by 'Alsen', 'Faller', and 'SY Soren'. 'ND2710' had the highest mean test weight. There exists inherent differences in test weight for the parents due to genetic differences. However, some of these differences can be explained by higher resistance resulting in more non-infected kernels which, as stated earlier, increases the test weight.

DISK and DON values show very similar results since DON is a large component of the DISK formula. For both, 'Alsen' and 'Glenn' were the two top ranked parents. The 2398/Alsen hybrid ranks in the top four for both parameters, while the other three hybrids fall below all resistant parents but still ranked higher than both the susceptible check and parent. The hybrids were numerically different but statistically similar for all measured traits.

Alsen Hybrid

Across environments, 2398/Alsen had an FHB index of 34.8% which was significantly lower than '2398' (60.8%) and not significantly different from 'Alsen' (31.8%) (Table 2). 2398/Alsen also expressed an FDK value of 37.2%, which is less than '2398' (84.5%), but higher than 'Alsen' (17.3%). Similar to FDK, the test weight data shows 2398/Alsen to be greater than '2398' and less than 'Alsen'. For both DON and DISK, 2398/Alsen (73.4 ppm and 59.7) was less than '2398' but not 'Alsen'. The 2398/Alsen hybrid was significantly different from 'WB Mayville' for FHB index, FDK, test weight and DISK, but not different for DON. 2398/Alsen was also different than 'ND2710' for FDK, test weight, and DON, but was not different for FHB index or DISK.

Faller Hybrid

The FHB index for 2398/Faller is 34.1% which is not significantly different from 'Faller' or 'ND2710' (Table 2). The hybrid's FHB index is also less than the susceptible check and parent. 2398/Faller has a lower FDK score than the susceptible parent and check. The hybrid's FDK score (44.0%) is not significantly different from its resistant parent but is different from 'ND2710'. Test weight for 2398/Faller was significantly greater than test weight of the two susceptible genotypes and less than 'Faller' and 'ND2710'. For DON, 2398/Faller had 125.3 ppm which was not different from '2398' (211.4 ppm), 'WB Mayville' (133.7 ppm), or 'Faller' (84.6 ppm). 'ND2710' was higher than 2398/Faller for DON. The 2398/Faller hybrid was lower than '2398' and higher than 'ND2710' for DISK, but was not different from 'WB Mayville' or 'Faller'.

Glenn Hybrid

The FHB index value for 2398/Glenn was 39.3%, not significantly different from ‘Glenn’ (Table 2). 2398/Glenn had a lower FHB index than did either the susceptible parent or the susceptible check. For FDK, 2398/Glenn performed intermediate and differed significantly from both parents. Test weight showed results similar to the results for FDK. Test weight for 2398/Glenn was different from both parents but still intermediate. The DON value for 2398/Glenn was lower than ‘2398’ and not different from ‘Glenn’ or either check. For DISK, 2398/Glenn had a value of 74.4 which was less than ‘2398’ (121.0) and higher than ‘ND2710’ (35.3), but not different from ‘WB Mayville’ (94.4) or ‘Glenn’ (56.4).

SY Soren Hybrid

The 2398/SY Soren hybrid was consistent with the other hybrids in having an FHB index value of 40.2% which was significantly lower than both ‘2398’ and ‘WB Mayville’ (Table 2). 2398/SY Soren also did not significantly differ from ‘SY Soren’ that had an FHB index of 45.6%. In regards to Fusarium damaged kernels, 2398/SY Soren was similar to the other hybrids, being lower than both susceptible genotypes. The 2398/SY Soren hybrid had an FDK value of 50.2%, which was less than ‘SY Soren’ at 36.6% damaged kernels. For test weight, this hybrid, at 58.0kg/hl⁻¹, performed intermediate to both parents and significantly higher than ‘2398’. For DON rating, 2398/SY Soren was different from ‘2398’ but not from the resistant parent or either check. The 2398/SY Soren hybrid was different from the susceptible parent and resistant check, but not from the susceptible check or resistant parent.

Table 2. Least Square Means and standard error for all parameters and genotypes across environments.

Cultivar	FHB Index		FDK		Test wt.		DON		DISK	
	-----%-----				-----kg/hl ⁻¹ -----		-----ppm-----			
WB Mayville	71.2 a ^a	(7.1) ^b	65.8 b	(10.9)	53.5 d	(4.0)	133.7 ab	(58.3)	94.4 a	(20.5)
2398	60.8 a	(7.1)	84.5 a	(10.9)	49.8 d	(4.1)	211.4 a	(58.4)	121.0 ab	(20.5)
SY Soren	45.6 b	(7.0)	36.6 de	(10.9)	61.9 b	(4.0)	81.6 bcd	(58.2)	64.4 cd	(20.5)
2398/SYSoren	40.2 bc	(7.0)	50.2 c	(10.8)	58.0 c	(4.0)	117.6 bcd	(57.8)	78.2 bc	(20.3)
Faller	31.4 cd	(7.0)	31.1 de	(10.9)	62.6 b	(4.0)	84.6 bcd	(58.2)	59.9 cde	(20.5)
2398/Faller	34.1 bcd	(7.0)	44.0 cd	(10.8)	58.3 c	(3.9)	125.3 abc	(57.8)	77.3 bc	(20.3)
Alsen	31.8 bcd	(7.0)	17.3 gf	(10.9)	66.9 a	(3.9)	41.6 cd	(58.2)	43.8 de	(20.5)
2398/Alsen	34.8 bcd	(7.0)	37.2 cde	(10.8)	59.8 bc	(3.9)	73.4 bcd	(57.7)	59.7 cde	(20.3)
Glenn	34.6 bcd	(7.0)	25.7 fe	(10.8)	66.9 a	(3.9)	74.6 bcd	(58.0)	56.4 cde	(20.4)
2398/Glenn	39.3 bc	(7.0)	44.1 cd	(10.8)	60.4 bc	(3.9)	113.2 bcd	(57.7)	74.4 bc	(20.3)
ND2710	22.6 d	(7.1)	11.2 g	(10.9)	69.5 a	(4.0)	33.4 d	(58.3)	35.3 e	(20.5)
<i>F</i> -values	8.35***		19.49***		25.06***		2.41*		5.87***	

^a Values in the same column followed by the same letter are not significantly different by the t-test at the 5% level of confidence.

^b Values in parenthesis are the Standard Error for each LS mean.

DISCUSSION

As stated previously, the main objective to this experiment was to compare resistance of hybrids to their respective parents. However, since the resistant parents used in this trial differ for resistance genes (Table 1), it is also appropriate and important to observe how their genetic compositions may have affected the separation of the means. FHB resistance is quantitatively inherited with a high frequency of additive effects and less frequently showing some dominance and epistatic action (Miedaner et al., 2017). Assuming only additive effects, the accumulation of additional resistance genes should confer increasing levels of FHB resistance. Recall that the parents used in this experiment vary in number of resistance genes (Table 1) and could be expected to vary for resistance according to their genetic composition. A resistance ranking based on genetic composition would result in the order of – ‘Alsen’, ‘Faller’, ‘SY Soren’, ‘Glenn.’ However, Glenn has historically been rated highly resistant despite not containing a marker for any of the known resistance genes (ElDoliefy, 2015). Taking this into account, a ranking based on resistance composition would result in the order of – ‘Glenn’, ‘Alsen’, ‘Faller’, ‘SY Soren’. The parents rank similar to both of these rankings for FDK, test weight, DON, and DISK but not FHB index. The hybrids follow the expected ranking based on resistance for DISK, and very similarly for three other parameters. In line with the parental results, the hybrids do not conform to the predicted resistance ranking for FHB index, although statistically, there were no significant differences among the means. The difference in ranking between FHB index and the other parameters is because visual field ratings showed less consistency across locations than kernel ratings. These inconsistencies in visual ratings suggests the importance of post-harvest ratings.

The results indicate that a hybrid could successfully be developed using only one resistant parent. This conclusion is based upon the levels of resistance exhibited by the hybrids, compared to the resistant parents, for the measures of resistance used in this experiment. Across environments, the resistance levels varied for each trait when compared to the resistant parent. The FHB index for the hybrid 2398/Alsen was 96% of the resistant parent 'Alsen'. Similarly, 2398/Faller and 2398/Glenn had FHB index scores that were 96% and 93% of their resistant parents, respectively. 'SY Soren' had a lower FHB index than 2398/SY Soren causing the hybrid to express 110% of the parental resistance. This is the only instance of a hybrid outperforming the resistant parent in this experiment when results are combined across all environments. This result could be attributed to error, due to small sample size and/or the inconsistency of visual ratings. Further experimentation is needed to determine the actual cause. The FDK scores for the 2398/Alsen hybrid was 76% as resistant as 'Alsen'. 2398/Faller had the greatest resistance in terms of FDK when compared to its resistant parent at 82% of 'Faller'. The last two hybrids, 2398/Glenn and 2398/SY Soren had FDK scores similar to their resistant parents at levels of 75% and 79% respectively. The test weight parameter revealed percent similar to resistant parents of 90, 93, 91, and 97% corresponding to 2398/Alsen, 2398/Faller, 2398/Glenn, and 2398/SY Soren respectively. Resistance in terms of DON accumulation showed the most consistency of similarity between hybrids and parents. The 2398/Alsen hybrid was 97% as resistant as 'Alsen', while all other hybrids were 96% as resistant as their respective resistant parents. The DISK rating, which is a cumulative rating including DON, incidence, severity, and FDK, showed the lowest levels of similarity between parents and hybrids. The lowest similarities were 2398/Faller and 2398/Glenn at 57% and 59% as resistant respectively. 2398/SY Soren was

61% as resistant as ‘SY Soren’ and 2398/Alsen had the highest value expressing 71% the resistance level of ‘Alsen’ when comparing DISK values.

We observed the FHB index of the hybrids to range from 93 to 110% compared to their respective resistant parents. We observed similar results for DON, where the hybrids expressed resistance of 96-97% of that of the respective resistance parents. Similarity ranges for the other parameters were 90-97% for test weight, 76-82% for FDK, and 57-71% for DISK. In general, hybrids in this experiment did not have the same resistance level as the resistant parents but they did express resistance levels more similar to that of the resistant parents, as compared to that of the susceptible parent. The high percentage of similarity to resistant parents suggests that these hybrids have resistance levels comparable to commercial cultivars.

‘SY Soren’ carries only one resistance gene, *Fhb1*, and had a worse rating for all but one parameter than the other resistant parents which contain more resistance genes (with the exception of ‘Glenn’). This trend noted in the parents was also seen in the hybrids, where 2398/SY Soren performed numerically worse than the other hybrids for all measures of resistance with the exception of DON. ‘Alsen’ expresses the most resistant alleles of all parents in this experiment (three) and maintained a rank as a top two parent for all resistance parameters measured. Though ‘Alsen’ and 2398/Alsen did not differ statistically from other lines carrying fewer resistance genes, means for ‘Alsen’ and 2398/Alsen were numerically higher in many instances. When evaluating FDK and test weight, ‘Alsen’ was significantly better than ‘SY Soren’ whereas 2398/Alsen was only numerically better than 2398/SY Soren for both parameters. This trend of ‘Alsen’ and 2398/Alsen generally performing better than lines with less resistance genes suggests that additive gene action plays a significant role in the level of FHB

resistance in hybrids. More research is needed to fully determine the importance of these additive effects.

A conclusion that could be derived from this experiment is that *Fhb1*, or any single resistance gene alone, does not provide adequate resistance. However, ‘SY Soren’ is the only parent in this study that carried one FHB resistance gene. To accurately determine this outcome, another line expressing only a single resistance gene would need to be included.

Finally, we are able to make inferences about the inheritance of the FHB resistance genes expressed in this experiment. Through the years, ‘2398’ has consistently been found to be highly susceptible to FHB. ‘2398’ has never tested positive for a resistance marker, thus we assume that it contains the susceptible alleles at all known resistance loci. If the resistance genes observed in this study, *Fhb1*, *Fhb5*, and Frontana QTL exhibit dominance gene action, the hybrid progeny would have the resistance level equal to that of their respective resistant parent. Equal resistance was not the case in this experiment. For three of the resistance parameters, levels were generally between 90 and 98% that of the resistant parent, while in two of the parameters the resistance never reached 85% of the resistant parents. These results are in line with those reported by Miedaner et al. (2017), who found hybrids to usually express FHB resistance most similar to the resistant parent. Our results suggest that genetic resistance to FHB is not completely dominant, but rather expressed by positive partial dominance. This is unfortunate in hybrid breeding as the resistance level is not a given simply based on the genes of one parent. However, the high percentage of similarity which suggests partial to nearly complete dominance, is promising. Knowing this, a breeder can develop lines with high resistance and lines with low resistance for use as hybrid parents, but still retain adequate resistance in any hybrid progeny. This allows focused breeding efforts for certain traits into separate lines knowing the other parent will

contain genes to offset deficiencies. The resulting hybrid produced by crossing such lines will show resistance similar to the resistant parent. These data suggest that commercially acceptable FHB resistance levels may be achieved through susceptible/resistant crosses.

LITERATURE CITED

- Bai, G. H., & G. Shaner. 1994. Scab of wheat: Prospects of Control. *Plant Disease* 78(8): 760-764
- Balut, A. L., A. J. Clark, G. Brown-Guedira, E. Souza, and D. A. Sanford. 2013. Validation of Fhb1 and QFhs.nau-2DL in Several Soft Red Winter Wheat Populations. *Crop Sci.* 53:934-945
- Bissonnette, K. M., F. L. Kolb, K. A. Ames. 2018. Effect of wheat cultivar on concentration of *Fusarium* mycotoxins in wheat stems. *Plant Disease* 102:2539-2544
- Browne, R. A. 2007. Components of Resistance to Fusarium Head Blight (FHB) in Wheat Detected in Seed-Germination Assay with *Microdochium majus* and the relationship to FHB Disease Development and Mycotoxin Accumulation from *Fusarium graminearum* Infection. *Plant Pathology* 56:65-72
- EIDoliefy, A. E. A. 2015. Molecular mapping of fusarium head blight resistance in two adapted spring wheat cultivars (Doctoral Dissertation). Retrieved from ProQuest Dissertations & Theses (Order No. 3685801). @ North Dakota State University; ProQuest Dissertations & Theses Global. (1664843621).
- Franzen, D. W. 2018. Fertilizing Hard Red Spring Wheat and Durum. NDSU Extension Service Bulletin
- Frohberg, R. C., R. W. Stack, T. Olson, J. D Miller, and M. Mergoum. 2006. Registration of 'Alsen' wheat. *Crop Sci.* 46:2311-2312
- Friskop, A., S. Zhong, R. Breuggeman. 2018. Fusarium Head Blight (Scab) of Small Grains. NDSU Extension Service Bulletin. PP804 (Revised).

- Jones, R. K., and C. J. Mirocha. 1999. Quality Parameters in Small Grains from Minnesota Affected by Fusarium Head Blight. *Plant Dis.* 83: 506-511
- Khorzoght, E. G., H. Soltanloo, S. S. Ramezani, and M. K. Arabi. 2010. Combining Ability Analysis and Estimation of Heterosis for Resistance to Head Blight Caused by Fusarium Graminearum in Spring Wheat. *AJCS* 4(8):626-632
- McMullen, M., G. Bergstrom, E. D. Wolf, R. Dill-Macky, D. Hershman, G. Shaner, and D. Van Sanford. 2012. A Unified Effort to Fight an Enemy of Wheat and Barley: Fusarium Head Blight. *Plant Disease* 96(12): 1712-1728
- Mergoum, M., R. C. Froberg, R. W. Stack, J. B. Rasmussen, and T. L. Friesen. 2008. Registration of 'Faller' wheat. *Journal of Plant Registrations* 2:224-229
- Mergoum, M., R. C. Froberg, R. W. Stack, T. Olson, T. L. Friesen, and J. B. Rasmussen. 2006. Registration of 'Glenn' wheat. *Crop Sci.* 46:473-474
- Miedaner, T., A. W. Schulthess, M. Gowda, J. C. Reif, C. F. H. Longin. 2017. High Accuracy of Predicting Hybrid Performance of Fusarium Head Blight Resistance by Mid-parent Values in Wheat. *Theor Appl Genet* 130:461-470
- Osborne, L. E., J. M. Stein. 2007. Epidemiology of Fusarium Head Blight on Small-grain Cereals. *International Journal of Food Microbiology* 119: 103-108
- Ransom J., A. Green, S. Simsek, A. Friskop, M. Breiland, T. Friesen, Z. Liu and S. Zhong, J. Rickertsen, E. Eriksmoen, B. Hanson, G. Martin, G. Pradhan, M. Ostlie. 2018. North Dakota Hard Red Spring Wheat Variety Trial Results for 2017 and Selection Guide. NDSU Extension Service & NDAES
- Singh, S. K., R. Chatrath, and B. Mishra. 2010. Perspective of Hybrid Wheat Research: A Review. *Indian Journal of Agricultural Sciences* 80(12): 1013-1027

- United States Department of Agriculture National Agriculture Statistics Service [USDA/NASS].
2019. 2018 State Agriculture Overview – North Dakota. Retrieved from
[https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=NORTH
%20DAKOTA](https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=NORTH%20DAKOTA)
- Waldron, B. L., B. Moreno-Sevilla, J. A. Anderson, R. W. Stack, and R. C. Frohberg. 1999.
RFLP Mapping of QTL for Fusarium Head Blight Resistance in Wheat. *Crop Science* 39:
805-811
- Whitford, R., D. Fleury, J. C. Reif, M. Garcia, T. Okada, V. Korzun, and P. Langridge. 2013.
Hybrid Breeding In Wheat: Technologies to Improve Hybrid Wheat Seed Production.
Journal of Experimental Botany 64(18): 5411-5428
- Verges, V. L., D. V. Sanford, and G. Brown-Guedira. 2006. Heritability Estimates and Response
to Selection for Fusarium Head Blight Resistance in Soft Red Winter Wheat. *Crop Sci.*
46:1587-1594