CONTROL OF FUSARIUM HEAD BLIGHT IN WHEAT: I. EVALUATION OF HOST PLANT RESISTANCE AND FUNGICIDES II. MOLECULAR MARKERS ASSOCIATED WITH QTL FOR RESISTANCE

BY

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THESIS

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ABSTRACT

Fusarium head blight (FHB) of wheat has become an increasingly important disease over the past 25 years. Significant grain and quality reductions due to FHB can be observed when there is a favorable environment for disease development. *Fusarium graminearum*, the primary fungal pathogen that causes FHB in the U.S. produces deoxynivalenol, a mycotoxin that can cause serious health problems for both humans and livestock when consumed in FHB infected grain. While cultural practices and fungicide treatments can suppress FHB, the use of resistant cultivars is also an essential tool for control of FHB. Breeding for resistance to FHB has become a very large part of wheat and barley breeding programs in temperate climates. Various sources of resistance have been used to develop new cultivars that have high levels of resistance. The primary objective of this study was to combine multiple sources of resistance using a recombinant inbred line (RIL) population derived from three FHB-resistant University of Illinois breeding lines (IL96-6472, IL97-6755 and IL97-1828) to obtain transgressive segregants that are significantly better than the three parents. The RIL population, consisting of 266 lines, was evaluated for FHB resistance in the greenhouse and in a mist irrigated, inoculated disease nursery. Forty-three simple sequence repeat (SSR) and 250 Diversity Arrays Technology (DArT) polymorphic markers were used to create a linkage map using Joinmap 3.0. PlabQTL was used for composite interval mapping and detection of significant QTL. QTL were found for all measured traits except for mean severity in the 2009 greenhouse evaluation. QTL on the short arm of chromosome 3B were identified for all measured traits and accounted for 4.2% to 18.8% of the phenotypic variation, depending on the trait. We believe that these markers are associated with *Fhb1* or QTL tightly linked to *Fhb1*. Minor QTL were also found on chromosomes 7B, 1A, 5D, 6B and 6A and explained a smaller amount of phenotypic variation

(between 2.5% and 8.7%). A total of 13 transgressive segregants were found that were significantly better than the mean of the three FHB-resistant parents for more than one trait. These thirteen lines were found to carry many of the resistance alleles associated with the QTL found in the study. Although the population was derived from three FHB-resistant parents, and there were likely QTL that were not detected due to a lack of polymorphism, we believe that multiple genes for resistance were combined in the transgressive segregants observed in the RIL.

The second study examined the performance of FHB-resistant and susceptible cultivars with three fungicide treatments. Until recently, there were few fungicides labeled for suppression of FHB. Numerous studies have shown that fungicides containing the active ingredient tebuconazole are very effective in reducing losses caused by FHB. While fungicides can be a useful tool for FHB suppression, they do not provide complete control, and their efficacy is greatly affected by timing. Planting cultivars that are resistant to FHB infection provides farmers with continual protection against the disease. The experiment was grown as a split plot with fungicide treatment (No Fungicide, Prosaro® (tebuconazole+prothioconazole) and Folicur® (tebuconazole) as the main plot and cultivar (6 susceptible and 6 resistant) as the sub-plots. Based on the results of this experiment, it is apparent that resistant cultivars are a necessity to provide the best control of FHB. Under the extremely heavy disease pressure of our FHB nursery, fungicides did not provide sufficient control of FHB on susceptible cultivars. Not surprisingly, we found the best method for controlling FHB is to plant a resistant cultivar in addition to applying a fungicide; however, we were interested to see how resistant cultivars alone would perform when compared to susceptible cultivars treated with a fungicide. Resistant cultivars performed impressively, and it was apparent that resistant cultivars are an essential first step of an effective program for controlling FHB. Resistant cultivars without fungicides were

able to yield well and provide excellent net economic returns that were not significantly different than resistant cultivars that were treated with a fungicide. This would suggest that under low to moderate disease pressure there no need for fungicide application for FHB control. This experiment illustrated that resistant cultivars provide sufficient protection from FHB; however, to achieve high quality grain with low levels of FDK and DON, fungicide application may be needed in years when there is a high risk of severe disease pressure.

ACKNOWLEDGEMENTS

I would like to extend my gratitude to all of the individuals who helped make these research projects successful. Thank you to my adviser, Professor Frederic L. Kolb, who was always there to offer excellent advice and support. I would also like to thank my committee members, Professor Brian W. Diers, Professor Jerald K. Pataky and Assistant Professor Carl A. Bradley, who gave guidance in my graduate studies and my projects. I also must thank the technicians who helped make these projects possible, Eric Brucker, Norman Smith and Keith Ames. Thank you also to my friends and family who have supported me throughout life and my academic career. The University of Illinois at Urbana-Champaign must also be thanked for providing the facilities that made these projects possible. Thank you also to the U.S. Wheat and Barley Scab Initiative for funding of these projects. Finally, my sincerest thanks to Pioneer Hi-Bred Int. Inc. for awarding me with the Jack Cavanah plant breeding fellowship.

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CHAPTER 1

LITERATURE REVIEW

INTRODUCTION

Fusarium head blight (FHB) caused primarily by *Fusarium graminearum* in the U.S. is a destructive disease of wheat that is of major importance for several reasons. FHB occurs most often in humid and semi-humid areas of the world (Schroeder and Christensen, 1963). FHB can significantly reduce grain yields, in some cases up to 100% yield loss on susceptible cultivars when conditions are favorable for disease (Bai and Shaner, 1994). Often yield loss is a result of shriveled or "tombstone" kernels being removed by the combine. When tombstone kernels are not removed, they can significantly reduce test weight (Bai and Shaner, 1994).

Another serious concern associated with FHB is the production of mycotoxins (Bai and Shaner, 2004). Deoxynivalenol (DON) is a mycotoxin that can cause serious health problems for humans and livestock when consumed in FHB infected grain (Parry et al., 1995). DON is considered the most important mycotoxin produced by *F. graminearum* and has been shown to cause vomiting in both swine and humans, often resulting in feed refusal in livestock. DON can also reduce starch and protein concentration in grain (Bai and Shaner, 2004). DON is regulated by both national and international grain markets, therefore infected grain is often rejected by elevators (Culler et al., 2007).

EPIDEMIOLOGY

F. graminearum can survive on many living hosts such as wheat, corn, and soybean (Bai and Shaner, 2004). It can also survive on crop residue left in the field which serves as a major source of primary inoculum (Sutton, 1982). Several fungal structures can act as the primary inoculum including ascospores, macroconidia, chlamydospores, and hyphal fragments (Bai and Shaner, 2004). Ascospores and conidia produced on debris serve as the principal primary

inoculum in the field (Bai and Shaner, 1994). Wheat heads are most susceptible to infection at anthesis when the anthers have formed (Sutton, 1982). Choline and betaine found in anthers have been found to greatly stimulate the growth of *F. graminearum* hyphae once the spores have germinated (Strange et al., 1974). The wheat flower tends to be susceptible to infection by *F. graminearum* from flowering to soft dough, and is very resistant to infection after the soft dough stage (Schroeder and Christensen, 1963).

Temperature and moisture play key roles in the development of FHB in the wheat head. Warm temperatures (20-30°C) and long periods (48-60 hours) of moisture are necessary for infection of wheat spikes (Sutton, 1982). *F. graminearum* cannot directly penetrate the thick epidermal cells of the glume, lemma, or palea (Bushnell et al., 2003). The spores germinate and hyphae quickly grow on the glumes. The network of mycelium that is quickly formed penetrates stomates, anthers, and crevices between the lemma and palea (Bushnell et al., 2003). After penetration, the fungus colonizes the spikes, glumes, rachis, and grain in the wheat flower (Sutton, 1982). The spread to these flower parts is the result of the fungus growing on the rachis both internally and externally and infecting other spikelets (Bai and Shaner, 1994). Infected spikelets will often have dark brown and water soaking symptoms on the glumes that extend to the rachis. Infected spikelets will also show bleaching symptoms when infected. When the rachis is infected with mycelia, uninfected spikelets will often have shriveled grain as a result of premature senescence (Bai and Shaner, 1994). Under heavy infections, pink mycelia can often be observed growing from infected spikelets (Bushnell et al., 2003). Perithecia and macroconidia will develop on spikelets in wet conditions (Sutton, 1982); however, these potential sources of secondary inoculum are not considered important in the disease cycle due to their inability to infect after the soft dough stage (Bai and Shaner, 1994).

Trichothecene mycotoxins such as deoxynivalenol have been found to have a significant effect on the virulence of *F. graminearum* (McCormick, 2003). These trichothecenes are peptidyl transferase inhibitors, therefore inhibiting the production of proteins. By transforming *F. graminearum* to disrupt the TRI5 gene with a disrupter plasmid one can stop production of trichothecenes such as DON (McCormick, 2003). This information allows for determining the role of DON in the virulence of FHB. When compared to wild type *F. graminearum* that produces DON, mutants produce significantly less disease than the wild type fungus (McCormick, 2003). This implicates DON in a key role in the disease cycle of FHB. Concentrating on resistance to DON accumulation could provide higher levels of resistance. The FDA requires DON levels to be below 10 ppm in grains used for ruminating beef and feedlot cattle older than 4 months and chickens, while swine and all other animals require DON levels below 5 ppm for feed use. Wheat products that are used for human consumption must be below 1 ppm (FDA). Millers and end-users must clean and mill grain to obtain levels lower than 1 ppm in products. This is an expensive process and end-users prefer to purchase grain below the 1 ppm threshold.

Fusarium head blight was first described by Arthur (1891) and has been recognized in North America for more than 100 years; however, it has only recently emerged as a major and chronic problem (Shaner, 2003). Fusarium head blight has become a very important disease in the last two decades as a result of changing farm practices (McMullen et al., 1997). A higher incidence of FHB is expected in wheat when it is grown continuously or if it is grown immediately after maize (Parry et al., 1995). This is a result of *Gibberella zeae* being the teleomorph stage of *F. graminearum* which causes ear rots in corn (Sutton, 1982). FHB became a severe problem in the early 1990s for a number of reasons. A major reason for the increase in

FHB was the wet weather that was experienced during this time. The high proportion of minimum tillage leaving significant amounts of corn and wheat residue on the ground to provide primary inoculum also contributed to the FHB epidemics in the early 1990s. Government programs encouraged farmers to plant continuous wheat in some states (McMullen et al., 1997). There has also been an increase in corn acreage and continuous corn practices that provide high levels of primary inoculum. When wheat is planted after corn there is significantly more blighting and higher DON concentrations (Teich and Hamilton, 1985). Perhaps most importantly, was the high percentage of acreage that was planted in susceptible cultivars (McMullen et al., 1997).

CONTROL

Fusarium graminearum is a ubiquitous fungus that is found on many hosts, making it very hard to control with a single strategy (Bai and Shaner, 1994). Therefore, combining multiple control strategies is essential in effective control of FHB. An important control practice is tillage to bury crop residue where *F. graminearum* can overwinter and provide a source of primary inoculum (Bai and Shaner, 1994). Crop rotation is another key cultural control practice. While *F. graminearum* does have a wide host range, and some infection is expected, rotations with wheat planted after corn or wheat show significantly higher levels of FHB (Bai and Shaner, 1994).

Fungicides are another possible means of controlling FHB. There are many variables that influence the effectiveness of fungicides such as cultivar resistance, fungicide coverage, and timing of application (Mesterhazy et al., 2003). There has been some disagreement on the effectiveness of fungicides to control FHB. New fungicide chemistries such as the triazoles are much more effective in controlling FHB than older fungicides; however, fungicides still do not

provide complete FHB control (Brucker et al., 2008). Most studies have shown that there is a significant reduction in FHB severity and an increase in yield when fungicides such as Folicur (tebuconazole) are used (Hollingsworth et al., 2008; Jones, 2000; Mesterhazy et al., 2003; Paul et al., 2008). Fungicides are an important tool in the control of FHB; however, they should be used in conjunction with other control strategies because of their inconsistent control of DON (Paul et al., 2007).

While fungicides are an important tool in the control of FHB, host plant resistance is by far the most consistent strategy for obtaining high yields and excellent grain quality under disease pressure (Hollingsworth et al., 2008). Schroeder and Christensen (1963) were the first to identify the two major types of resistance to FHB. Type 1 resistance is resistance to the initial *F. graminearum* infection. Type 2 resistance is resistance to the spread of the infection to other spikelets on the wheat head (Schroeder and Christensen, 1963). Other proposed types of resistance are resistance to kernel infection and resistance to DON accumulation (Mesterhazy, 1995).

SCREENING METHODS

There are various methods used to screen for resistance to FHB. Greenhouse screening using a needle inoculation method can be very effective in determining type 2 resistance (Argyris et al., 2005). This method involves injecting a conidial inoculum suspension into a single floret of a middle spikelet, then placing the plant in a mist irrigated chamber for 72 hours. The inoculated heads are then rated 21 days after inoculation to determine the number of spikelets exhibiting disease symptoms (Argyris et al., 2005). Field studies can also be used to evaluate both type 1 and type 2 resistance. Field studies are usually mist irrigated to promote infection. There are various methods for inoculating field studies. One method is to create a conidial

suspension and spray individual plots at anthesis (Culler et al., 2007). This method can be very time consuming if there are cultivars or experimental lines with varying maturities. To solve this problem a grain spawn method can be used.

The grain spawn method involves spreading infected seed throughout the disease nursery. In this method, wheat or corn kernels are colonized with several virulent isolates of *F. graminearum,* then spread on the soil surface at a rate of about 250 pounds per acre (Yang et al., 1999). Mist irrigation is then used to keep humidity high and promote the growth of *F. graminearum.* The grain spawn method provides inoculum over a prolonged period of time, allowing for infection of materials with variable maturities without the need for daily hand inoculations. Ratings are done in a similar fashion to the spray inoculated field trials.

There are several ways to evaluate FHB infection. Type 1 resistance can be estimated by visually determining the number of wheat heads in an inoculated plot that are affected by FHB. This estimation demonstrates a cultivar's resistance to initial FHB infection (Schroeder and Christensen, 1963). Type 2 resistance can also be determined by simply counting the number of infected spikelets on a wheat head, as well as the total number of spikelets on the wheat head. This provides an evaluation of a cultivar's ability to resist the spread of the FHB infection. Another type of resistance that can be evaluated is the percentage of *Fusarium* damaged kernels, which can be estimated by inspecting seeds threshed from a wheat head and evaluating the percentage of tombstone (shriveled) kernels. This evaluation shows a cultivar's ability to resist kernel infection (Bai and Shaner, 2004). A final method for evaluating FHB is determining DON levels from harvested samples. By measuring the amount of DON in harvested seed, a wheat cultivar's ability to resist DON accumulation can be determined (Bai and Shaner, 2004).

Using these evaluation methods in combination can effectively illustrate which cultivars have resistance to FHB.

BREEDING FOR RESISTANCE

Breeding for resistance is a very important part of controlling FHB worldwide. Resistance to FHB is a complicated quantitative trait which makes breeding for resistance fairly difficult (Bai and Shaner, 1994); however, resistance to spread within the spikelet seems to be controlled by a few genes, making breeding for type 2 resistance promising and popular (Bai and Shaner, 1994). There are various sources that can be used in a breeding program to provide FHB resistance (Bai and Shaner, 2004). Molecular markers are relatively new tools that can help in breeding for resistance to FHB.

Chinese land races of wheat have been found to have excellent resistance to FHB. These land races often have very poor agronomic traits such as small heads, tall stature, and late maturity. Sumai 3 and its derivatives (Ning series) have been very popular in breeding for resistance to FHB, but there are other sources of resistance that are also very promising. Sumai 3 sources of resistance provide excellent type 2 resistance; however, they often have poor agronomic traits (Bai and Shaner, 2004). There have also been reports of Brazilian sources of resistance in the cultivars Frontana and Encruzilhada. Frontanta shows excellent type 1 resistance but has relatively low type 2 resistance. European resistance sources have also been found such as Fundulea 201R from Romania (Bai and Shaner, 2004). Native sources of resistance have also been found in the United States including Bess, Truman, Goldfield, Ernie, Freedom and other breeding lines which exhibit low incidence and severity (Bai and Shaner, 2004). Breeders must use these sources of resistance to incorporate resistance into cultivars that are planted by farmers.

While there has not yet been a problem with *F. graminearum* overcoming the Sumai 3 source of resistance, other sources of resistance need to be included in breeding programs for various reasons (Bai and Shaner, 2004). First, if the Sumai 3 resistance were to "break down" and no longer be effective in controlling FHB, other sources of resistance would help prevent widespread epidemics. Second, type 1 resistance and resistance to DON accumulation are important resistance aspects that are often overlooked. Finally, by combining sources of resistance genes, transgressive segregants may provide higher levels of FHB resistance than is already available (Kolb et al., 2001).

Inheritance of resistance is mainly additive with evidence of other minor non-additive affects. There also is evidence of minor epistatic effects (Bai et al., 2000). Type 2 FHB resistance appears to be oligogenic. Bai et al. (2000) found that there are one to three major genes, as well as other genes with possible modifying affects controlling type 2 resistance. These genes have relatively high heritability. Some researchers have found different numbers of genes in the same cultivar (Bai and Shaner, 2004). Multiple reasons have been suggested for this disparity between studies including polygenic control of resistance, types of resistances evaluated, genotype by environment interaction and inoculation techniques (Kolb et al., 2001). These inheritance studies illustrate that selecting transgressive segregants from a cross to bring multiple resistance sources together is an effective method for developing highly FHB resistant cultivars (Bai et al., 2000).

Using molecular markers for Marker Assisted Selection (MAS) has become an important tool for breeders. High quality, validated quantitative trait loci (QTLs) are necessary to be able to effectively use MAS. QTLs that have been validated in multiple backgrounds and environments are ideal for MAS (Pumphrey et al., 2007). The most consistent QTL for type 2

FHB resistance comes from the Sumai 3 background and is located on the short arm of chromosome 3B. This 3BS region has been named *Fhb1* and has been validated using near-isogenic lines. (Pumphrey et al., 2007). Before a breeder can integrate MAS into their breeding program, QTL must be found using various techniques.

RAPD, RFLP, AFLP and SSR markers have all been found in association with FHB resistance (Kolb et al., 2001). Diversity array technology (DArt) markers have also recently been used for high throughput analysis (Akbari et al., 2006). FHB resistance QTL have been found on all of the wheat chromosomes except for 7D (Buerstmayr et al., 2009). While there have been many QTL for FHB resistance found, not all are stable across populations and environments. With plant height and heading date playing a major role in a plant's resistance to infection, it can be difficult to separate out true QTL effects for FHB resistance (Buerstmayr et al., 2009). Therefore a breeder must use validated QTLs when using MAS.

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CHAPTER 2
EVALUATION OF HOST PLANT RESISTANCE AND FUNGICIDE TREATMENT FOR SUPPRESSION OF FUSARIUM HEAD BLIGHT

INTRODUCTION

Fusarium head blight (FHB) caused primarily by *Fusarium graminearum* is a destructive disease of wheat that is of major importance worldwide. FHB occurs most often in humid and semi-humid areas of the world (Schroeder and Christensen, 1963). FHB can significantly reduce grain yields, in some cases up to 100% yield loss on susceptible cultivars when conditions are favorable for disease (Bai and Shaner, 1994). Substantial yield loss can result from shriveled or "tombstone" kernels being removed by the combine. When tombstone kernels are not removed they can significantly reduce test weight (Bai and Shaner, 1994).

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Fusarium graminearum is a ubiquitous fungus that is found on many hosts, making it very hard to control with a single effective control strategy (Bai and Shaner, 1994). Therefore, combining multiple control strategies is essential in controlling FHB. A historically important control practice in areas where *F. graminearum* can overwinter is tillage to bury crop residue (Bai and Shaner, 1994); however, recent trends in no-till and minimum tillage to reduce soil erosion have drastically reduced the use of this practice. Crop rotation is another key cultural control practice. Although *F. graminearum* has a wide host range, the amount of infection can be very high when rotations with wheat planted after corn or wheat are used (Bai and Shaner, 1994).

Fungicide application is another method used to reduce damage due to FHB. Many variables influence the effectiveness of fungicides such as cultivar resistance, fungicide coverage, and timing of application (Mesterhazy et al., 2003). The effectiveness of fungicides to reduce DON and the damage due to FHB has been the topic of extensive discussion. New fungicide chemistries such as the demethylation inhibitors (DMI) are much more effective in controlling FHB than other fungicides; however, they generally still do not provide complete FHB control (Brucker et al., 2008). Fungicides help reduce losses by reducing the amount of infection, DON concentration, yield losses, and by improving grain quality (Paul et al., 2010). Even with fungicide application, DON levels above 1 part per million are often observed when favorable conditions for disease development exist. Farmers have FHB prediction models available to them (http://www.wheatscab.psu.edu); however, it can still be very difficult to determine if there will be an economic benefit from application of a fungicide.

Most studies have shown that there is a significant reduction in FHB severity and an increase in yield when fungicides such as tebuconazole are used (Hollingsworth et al., 2008;

Jones, 2000; Mesterhazy et al., 2003; Paul et al., 2008). Fungicides that have been shown to be effective in reducing FHB severity include tebuconazole, prothioconazole, metconozole, and tebuconazole+prothioconazole (Paul et al., 2010).

While fungicides are an important tool in the control of FHB, they should be used in conjunction with other control strategies because of their inconsistent control of DON (Paul et al., 2007). Host plant resistance is by far the most consistent strategy for obtaining high yields and excellent grain quality under heavy disease pressure (Hollingsworth et al., 2008). Schroeder and Christensen (1963) were the first to identify the two major types of resistance to FHB. Type 1 resistance is resistance to the initial *F. graminearum* infection. Type 2 resistance is resistance to the spread of the infection to other spikelets on the wheat head (Schroeder and Christensen, 1963). Other proposed types of resistance are resistance to kernel infection, and resistance to DON accumulation (Mesterhazy, 1995).

Breeding for resistance is a very important part of controlling FHB worldwide. Resistance to FHB is a complicated quantitative trait which makes breeding for resistance fairly difficult (Bai and Shaner, 1994); however, resistance to spread within the spike seems to be controlled by a few genes, making breeding for type 2 resistance promising and popular (Bai and Shaner, 1994). Various sources of FHB resistance are available for use in breeding programs (Bai and Shaner, 2004). Some Chinese land races have been found to have excellent resistance to FHB. These land races often have very poor agronomic traits such as small heads, tall stature, and late maturity. Sumai 3 and its derivatives (Ning series) have been very popular in breeding for type 2 resistance to FHB, but there are other sources of resistance that are also very promising. Brazilian sources of resistance were identified in the cultivars Frontana and Encruzilhada. Frontana has excellent type 1 resistance but relatively low type 2 resistance.

European resistance sources have also been identified such as Fundulea 201R from Romania (Bai and Shaner, 2004). Native sources of resistance have also been described in the United States in soft red winter wheat germplasm. Bess, Truman, Goldfield, Ernie, Freedom and advanced breeding lines including some US cultivars also exhibit low incidence and severity (Bai and Shaner, 2004). It is important for breeders to use all of these sources of resistance. By combining resistance genes, transgressive segregants may provide higher levels of FHB resistance than is currently available (Kolb et al., 2001).

The objective of this study was to examine the effectiveness of two foliar applied fungicides on Fusarium head blight, and evaluate fungicide performance on cultivars with varying levels of resistance. A producer can save a considerable amount of money by simply planting a highly resistant cultivar, and not spraying a fungicide; however, resistance alone will not provide complete control when disease pressure is high. Likewise, fungicides alone on a susceptible cultivar will not provide complete control under severe disease pressure. I hypothesized that the combination of resistant cultivars with fungicide treatment reduces damage due to FHB to the lowest level, and results in the highest yield. I also hypothesized that the yield of highly resistant cultivars with no fungicide treatment will be similar to susceptible cultivars that have fungicides applied to them.

MATERIALS AND METHODS

The experiment was a split plot with 6 row plots 4.26 m in length with a row spacing of 17.8cm and a seeding rate of 1955 seeds per plot (4.3 million seeds/ha). The main plots consisted of an nontreated plot, a Folicur® (tebuconazole) treated plot, and a Prosaro® (tebuconazole+prothioconazole) treated plot. Buffer plots were planted to separate the main plots, and prevent overspray. The subplots consisted of twelve soft red winter wheat cultivars

ranging from FHB susceptible to FHB resistant. The resistant cultivars were IL00-8061, IL00-8530, IL01-11934, Excel 307, IL02-18228, and IL01-16170. The susceptible cultivars were Sisson, Kaskaskia, Branson, Pioneer 25R37, Pioneer 25R47, and Cooper. IL-02-18228 has Ning 7840 in the pedigree, but none of the other cultivars derive their FHB resistance from known and identifiable sources of resistance. The experiment was conducted for two years (2008 and 2009); each treatment was replicated four times each year. A new randomization was produced for the second year.

The experiment was grown in a mist irrigated, grain spawn inoculated FHB evaluation nursery at the Crop Sciences Research and Education Center at the University of Illinois at Urbana-Champaign, Illinois. The inoculum consisted of corn kernels infected with 10 different isolates of *F. graminearum* collected from various locations in Illinois. The grain spawn was broadcast at two, four, and six weeks prior to anthesis, providing a total rate of about 287 kg per hectare. Mist irrigation was applied three times for one hour every 24 hours during anthesis and continued for two weeks after the last genotype flowered. The mist irrigation system delivered approximately 3.05 mm of water per hour. Nitrogen fertilizer was applied at a rate of 44.8 kg/ha in the fall as a 28% liquid solution. A dry ammonium sulfate nitrogen application was also made in the spring with a rate of 61kg/ha. All plots were treated with Quadris® (azoxystrobin; Syngenta Crop Protection, Greensboro, NC) was applied at a rate of 7.55 mL per hectare (0.04 kg a.i./ha) to control foliar diseases at Feekes growth stage 8.0-9.0. Warrior® (lambda-cyhalothrin) was applied at a rate of 0.006 kg a.i./ha in the fall and spring to control aphids thereby preventing *Barley yellow dwarf virus* infection. Folicur® and Prosaro® were applied using a hand held CO_2 sprayer with TJ60-8002 nozzles and 0.25% NIS at 4.79 mL per hectare

(0.021 kg a.i./ha) and 7.79 mL per hectare (0.03 kg a.i./ha), respectively, at Feekes growth stage 10.51-10.54.

FHB incidence and severity were evaluated one month after the heading date of each cultivar. Incidence was recorded by randomly selecting three groups of approximately ten heads in the plot and visually estimating the percentage of infected heads. The mean of these three estimates was then used for the analysis. Severity was recorded by estimating the percent of infected spikelets on ten heads randomly selected throughout each plot. The average of these ten severity scores were used for the analysis. The plots were combine harvested with a low fan speed to prevent shriveled kernels from being removed. Yield and test weight were measured. Fusarium Damaged Kernel (FDK) percentage was visually estimated post-harvest using standards with a known ratio of scabby kernels/healthy kernels. FHB index (Inc*Sev) and incidence/severity/kernel quality (ISK) index ([.4(FDK)+.3(Inc)+.3(Sev)]) were calculated. The ISK index includes kernel FDK evaluations in the index calculation. It is an excellent broad indicator of resistance because it includes visual symptoms pre- and post-harvest. FDK evaluations are also highly correlated with DON concentration. DON concentration was also measured by gas chromatography-mass spectrometry by Dr. Yanhong Dong's lab at the University of Minnesota department of Plant Pathology. Net returns were calculated using wheat prices from the Chicago board of trade on the respective days of harvest. The yields multiplied by these prices and then the cost of respective fungicide application was subtracted from this overall net return.

Data were analyzed with SAS 9.2© using the Proc Mixed procedure to make contrasts and estimates (SAS Institute, 2007). Residuals were examined for normality, and transformations were used when necessary. Year variance was also examined to determine if

years could be combined. DON was the only measured variable that did not have homogeneous variance over years. Transformations did not provide homogeneous variance, so DON was analyzed separately for 2008 and 2009. Non-normal residuals were found for FDK, test weight, DON, and FHB index. Simple transformations provided normal errors for all measures except for DON in 2008. The following statistical model was used for data analysis:

$$y_{ijkl} = \mu + Y_i + \beta_{(i)j} + F_k + YF_{ik} + \varepsilon_1 + V_l + YV_{il} + FV_{kl} + YFV_{ikl} + \varepsilon_2$$

where 288 degrees of freedom were partitioned as follows:
$\mu = 1$
Y=1 – random effect of year.
β =6 – random effect of blocks.
F=2 – fixed fungicide treatment
YF=2 – interaction of fungicide by year
$\varepsilon_1 = 12$
V=11 – fixed cultivar treatment
YV=11 – interaction of year by cultivar
FV=22 – interaction of fungicide by cultivar
YFV=22 - 3-way interaction of year, fungicide, and cultivar
ε_2 =66+132=198

One of my main objectives in this experiment was to examine the effectiveness of resistant cultivars and determine how they perform in comparison to fungicide application on a susceptible cultivar as well as how resistant cultivars perform when combined with a fungicide application. As a result, specific contrasts were made using Best Linear Unbiased Predictions (BLUPs) instead of making all possible comparisons. This helped keep Type I experiment-wise error rate down and eliminated the need for using a Bonferoni adjustment that would have been necessary if all pair-wise comparisons had been made. BLUPs were also used to test for significant interactions that occurred between fungicide treatment and cultivar. Significant interactions were only found for yield and test weight. Cultivars were grouped as either being

resistant or susceptible for analysis. A review of the estimates I obtained from my analysis can be found in Table 1.

RESULTS

FHB Incidence and Severity. Host plant resistance and fungicide treatment were both effective methods of reducing both FHB incidence and severity and resulted in incidence and severity ratings significantly lower than nontreated susceptible plots (Table 1.1). Use of resistant cultivars lowered severity 17% and incidence by 26.5% when compared to susceptible cultivars when no fungicides were applied (Table 1.1). The average incidence for resistant cultivars with no fungicide treatment was 56.8% while susceptible cultivars had an average incidence of 83.3% with no fungicide treatment (Table 1.2). Similarly, when no fungicide was applied, resistant cultivars had a mean severity of 39.9% while susceptible cultivars had a mean severity of 57.0% (Table 1.2). Prosaro® and Folicur® both significantly reduced incidence and severity when compared to non-treated plots.

Prosaro® reduced severity by an average of 10.6% while Folicur® reduced severity by 8.8% when compared to nontreated plots (Table 1.1). Prosaro® reduced incidence by an average of 32.5% while Folicur® reduced incidence by an average of 20% when compared to nontreated plots (Table 1.1). Although Prosaro® provided lower values than Folicur® for both incidence and severity; it was not significantly different than Folicur®. Resistant cultivars that had no fungicide treatment were not significantly different for incidence than susceptible cultivars that had a fungicide treatment. Nontreated resistant cultivars were significantly better than Folicur® treated susceptible cultivars for average severity ($P=0.0039$). Nontreated resistant cultivars were not significantly different than Prosaro® treated susceptible cultivars for average severity. Fungicide combined with a resistant cultivar provided the lowest incidence and severity scores.

When treated with fungicide, resistant cultivars had significantly lower incidence and severity scores than susceptible cultivars treated with the same fungicide ($P<0.0001$). The combination of resistance and fungicide treatment proved to be the most effective method for reducing the incidence and severity of FHB, and susceptible cultivars had relatively high incidence and severity scores, even when treated with a fungicide (Figures 1.1 and 1.2).

Kernel Quality. Fungicides were important in reducing Fusarium damaged kernls (FDK). Both Folicur® and Prosaro® significantly reduced FDK when compared to nontreated plots (Table 1.1). Prosaro® was significantly better than Folicur® in reducing FDK ($P<0.0192$). Resistant cultivar was also an important factor in lowering FDK levels. Resistant cultivars reduced FDK by an average of 24.8% when nontreated susceptible and resistant cultivars were compared (Table 1.1). The average overall FDK level for resistant cultivars was 4.5% while the average overall FDK for susceptible cultivars was significantly higher ($P<0.0001$) at 22.1% (Table 1.2). When the resistant cultivars that received no fungicide treatment were compared to the susceptible cultivars that were treated with either Folicur® or Prosaro®, the resistant cultivars had significantly lower FDK ($P<0.0001$). Even when fungicides were applied to susceptible cultivars, relatively high FDK values were present with 21.3% for Folicur® and 13.2% for Prosaro® (Table 1.2). These FDK values represent substantial improvement over nontreated susceptible cultivars; however, considering that resistant cultivars with no fungicide applied had an average FDK value of 6.9%, it is obvious that resistant cultivars provided the best control for FDK (Table 1.2). Fungicides combined with resistant cultivars provided the lowest FDK values with Prosaro® providing 2.5% and Folicur® providing 4.06% averaged over the six resistant cultivars (Table 1.2). Figure 1.3 shows the drastic difference in FDK levels between

susceptible and resistant cultivars and illustrates the importance that resistant cultivars play in reducing FDK.

FHB and ISK Indices. *FHB* index is a value that is popular for assigning a general resistance value for visual FHB ratings. FHB index was another measure that illustrated the importance of host plant resistance. Resistant cultivars significantly ($P<0.0001$) reduced FHB index by 24.8% when compared to susceptible cultivars with no fungicide application (Table 1.1). With no fungicide treatment resistant cultivars had a FHB index of 22.5% compared to a FHB index of 47.3% for susceptible cultivars when no fungicides were applied (Table 1.2). Folicur® and Prosaro® also significantly reduced FHB index ($P=0.0018$ and $P=0.0039$, respectively). Prosaro® reduced the FHB index by 19.7%, and Folicur® reduced FHB index by 13.5% when compared to nontreated plots (Table 1.1). Resistant cultivars with no fungicide treatment had significantly lower FHB index values than Folicur® treated susceptible cultivars ($P=0.0020$); however, nontreated resistant cultivars were not significantly different than Prosaro® treated susceptible cultivars. When resistant and susceptible cultivars were both treated with either Folicur® or Prosaro®, resistant cultivars had significantly ($P<0.0001$) lower FHB index scores. Similar to other variables, resistant cultivars combined with fungicide treatment provided the lowest FHB index values (Figure 1.4). When resistant cultivars were treated with Prosaro® and Folicur® the FHB indices were 7.5% and 10.1%, respectively (Table 1.2).

Resistant cultivars significantly reduced ISK index when compared to susceptible cultivars when no fungicide was applied ($P<0.0001$). The resistant cultivars provided an ISK index reduction of 23% when compared to susceptible cultivars with no fungicide treatment (Table 1.1). When fungicides were applied, resistant cultivars provided a significant reduction in

ISK index, 17.2% for Prosaro® and 23.1% for Folicur® (P<0.0001) (Table 1.1). When resistant cultivars did not have a fungicide treatment the ISK index average was 31.8% which is much lower than the nontreated susceptible average of 54.8% (Table 1.2).

Both Folicur® and Prosaro® significantly reduced ISK index (P=0.0048 and P=0.0020, *respectively*). Prosaro® performed significantly better than Folicur® (P=0.0156) with an average ISK index 6.2% lower than Folicur® (Table 1.1). Resistant cultivars with no fungicide applied were not significantly different than Prosaro® treated susceptible cultivars; however, resistant cultivars with no fungicide treatment significantly outperformed susceptible cultivars treated with Folicur® (P<0.0001). Resistant cultivars that received the same fungicide treatment as susceptible cultivars had significantly better ISK index scores (P<0.0001). Similar to other measured triats, fungicide treatment combined with a resistant cultivar provided the best FHB control (Figure 1.5)

DON Concentration. Deoxynivalenaol is the tricothecene mycotoxin that is of primary concern to end users of wheat. Unfortunately the two years of data could not be combined due to non-homogeneous error variances. Therefore, DON concentration for 2008 and 2009 had to be analyzed separately. The raw DON values were much lower and less variable in 2008 than in 2009 which is the probable cause of the non-homogeneous error variance (Figures 1.6 and 1.7).

In both 2008 and 2009 resistant cultivars significantly lowered DON compared to susceptible cultivars when no fungicide was applied (P<0.0001). When fungicides were applied, resistant cultivars had significantly lower DON concentration than susceptible cultivars (P<0.0001) in both 2008 and 2009. Resistant cultivars alone reduced DON concentration by 3.9 parts per million (ppm) in 2009 and 2.9 ppm in 2008 when compared to susceptible cultivars (Table 1.1). The average DON concentration for susceptible cultivars with no fungicide

treatment was 4.16 ppm in 2008 and 6.5 ppm in 2009 (Table 1.2). The average DON concentration of resistant cultivars with no fungicide was 1.3 ppm in 2008 and 2.6 ppm in 2009 (Table 1.2). Neither of these values provides the required DON levels below 1 ppm for human food use; however, they do provide levels well below the 5 ppm required for animal feed, and end-users will typically accept grain that has 3-4 ppm and clean the tombstone kernels out and reduce DON during milling to get below the 1 ppm FDA guideline. It is also important to consider that the FHB disease pressure was very high in the inoculated and irrigated nursery.

To obtain DON levels below the FDA required 1 ppm for human products both resistant cultivars and fungicide application was required; however, fungicide applied to susceptible cultivars did not provide DON levels below 1 ppm. When Folicur® was applied to susceptible cultivars high DON levels were still observed with 4.1 ppm in 2009 and 2.5 ppm in 2008 (Table 1.2). When Prosaro® was applied to susceptible cultivars, lower DON levels were obtained, but with DON concentration at 1.5ppm in 2008 and 2.8 ppm in 2009, DON levels would still require cleaning to get below the 1 ppm level (Table 1.2). In 2008 and 2009 both Folicur® and Prosaro® significantly reduced DON levels (2009: Folicur® $P=0.0477$, Prosaro® $P=0.0014$; 2008: Folicur® $P=0.0148$, Prosaro® $P=0.0022$). Prosaro® provided significantly lower DON concentration than Folicur® in 2009 ($P<0.0213$) but was not significantly different than Folicur® in 2008 (Table 1.1). Although fungicide treatment did significantly lower DON concentration, the DON levels observed were not at a safe level for immediate human consumption.

Resistant cultivars that received no fungicide treatment had significantly lower DON concentration than susceptible cultivars that were treated with Folicur® in both 2008 and 2009 ($P=0.0115$ for both years). Resistant cultivars that received no fungicide treatment were not

significantly different than susceptible cultivars that had a Prosaro® application in either 2008 or 2009. In both 2008 and 2009, resistant cultivars with no fungicide treatment had significantly lower DON concentration than susceptible cultivars treated with Folicur® ($P=0.03$ and $P<0.0001$, respectively). When resistance was combined with fungicide treatment, DON values under 1 ppm were obtained. In 2009 Folicur® treated resistant cultivars had an average DON concentration of 2.2 ppm while Prosaro® treated resistant cultivars had an average DON concentration of 0.53 ppm (Table 1.2). In 2008 Folicur® treated resistant cultivars had an average DON concentration of 0.56 ppm while Prosaro® treated resistant cultivars had an average DON concentration of 0.31 ppm (Table 1.2). Although combining resistant cultivars and fungicide treatment provided DON levels below the 1ppm threshold, resistant cultivars with no fungicide application were not significantly different than resistant cultivars treated with either fungicide.

Test Weight. Fusarium head blight can drastically reduce test weight due to shriveled seed. Resistant cultivars significantly increased test weight compared to susceptible cultivars ($P<0.0001$). Resistant cultivars increased test weight by 56 kg/m^3 when compared to susceptible cultivars with no fungicide treatment (Table 1.1). When fungicides (both Prosaro® and Folicur®) were applied, resistant cultivars provided a significant increase in test weight ($P<0.0001$) with resistant cultivars increasing test weight by 28 kg/m^3 when treated with Prosaro® and 41 kg/m^3 for Folicur® (Table 1.1). Resistant cultivars with no fungicide applied had an average test weight of 733 kg/m^3 (Table 1.2).

Both Folicur® and Prosaro® significantly increased test weight ($P=0.0043$ and $P=0.0017$ respectively). Folicur® increased test weight by 21 kg/m^3 and Prosaro® increased test weight by 34 kg/m^3 (Table 1.1). Resistant cultivars with no fungicide application provided test weights

significantly higher than susceptible cultivars sprayed with either Folicur® or Prosaro® ($P<0.0001$ and $P=0.0059$, respectively). Folicur® treated susceptible cultivars provided an average test weight of 697 kg/m^3 while Prosaro® treated susceptible cultivars had an average test weight of 726 kg/m^3 (Table 1.2). When resistant cultivars were combined with fungicide treatment test weight was an average of 754 kg/m^3 for Prosaro® and 747 kg/m^3 for Folicur® (Table 1.2). Resistant cultivars combined with fungicides had the highest test weights (Figure 1.8).

Yield. Resistant cultivars significantly out-yielded susceptible cultivars when no fungicide treatment was applied ($P<0.0001$). When no fungicides were applied, the six resistant cultivars yielded 854 kilograms/hectare (kg/ha) more than the six susceptible cultivars (Table 1.1). Resistant cultivars significantly ($P=0.0012$) increased yield by 632 kg/ha when Folicur® was applied and, were numerically 312 kg/ha higher than when Prosaro® was applied although this difference was not significant (Table 1.1). It is important to note that the nursery was mist irrigated to ensure high levels of infection; however, this irrigation also helps boost yield, especially when disease symptoms of foliar diseases are well controlled.

Both Folicur® and Prosaro® significantly increased yield when compared to nontreated plots ($P=0.0153$ and $P=0.0278$ respectively). Folicur® increased yield by 603 kg/ha while Prosaro® increased yield by 832 kg/ha (Table 1.1). Although Prosaro® application resulted in yields slightly higher than Folicur®, Prosaro® was not significantly different than Folicur® for yield. Unlike many other traits, resistant cultivars with no fungicide treatment were not significantly different than susceptible cultivars that received a fungicide treatment. This implies that applying a fungicide to a susceptible cultivar will provide a yield equivalent to a resistant cultivar with no fungicide. When both resistant and susceptible cultivars were treated with

Folicur®, resistant cultivars significantly outperformed susceptible cultivars (*P<0.0001*). While yield is a very important factor, it is imperative to plant a resistant cultivar to protect grain quality as will be discussed in the discussion section.

Net Return. It is important to consider the added cost of application of a fungicide and if the added protection is worth the investment. I assumed that there was no difference between the cost of resistant and susceptible seed in the calculation of net return. Deductions for grain quality were also not factored into the calculation due to a lack of standardization of deductions for DON concentration. When no fungicides were applied resistant cultivars significantly (*P<0.0001*) increased the net return by $208/ha (Table 1.1). The cost of Folicur® application is much lower than the cost of Prosaro® application at $28.62/ha and $63.46/ha, respectively. Prosaro treated plots had a numerically higher net return than Folicur® treated plots, although this difference was not statistically significant. Susceptible cultivars treated with either Folicur® or Prosaro® were not significantly different than resistant cultivars that received no fungicide treatment. Even when both susceptible and resistant cultivars were treated with Folicur®, resistant cultivars significantly (*P<0.0001*) increased net return by $153/ha compared to susceptible cultivars (Table 1.1). Not surprisingly, Folicur treated resistant cultivars had the highest net return of $1603/ha (Figure 1.10). Although both Folicur® and Prosaro® treated resistant cultivars had numerically higher net returns ($86.5/ha and $73.5/ha, respectively), they were not significantly different than resistant cultivars that had no fungicides applied (Table 1.1). Resistant cultivars with no fungicide application had an average net return of $1517/ha with the lowest net return being susceptible cultivars with no fungicide treatment at $1308/ha (Table 1.2).

DISCUSSION

This experiment was conducted in a mist irrigated and inoculated nursery, providing extremely high disease pressure. Although the high levels of disease pressure present in the nursery are less likely in production fields, this level of disease severity is possible in years that favor disease development. It is important to realize that *F. graminearum* is ubiquitous and in years with good weather for disease development, very high levels of inoculum can be found in typical production fields. The irrigation also helped boost yields when FHB was well controlled by both fungicide and resistant cultivars. While there has been yield drag associated with resistance to FHB in the past, newer FHB resistant cultivars have the ability to yield comparably to yield standards even when high disease pressure is not present. There are also inherent differences between how a cultivar yields that are not related to levels of FHB infection. No yield drag was observed with this set of cultivars.

Another consideration is the possibility of pathogen resistance to fungicides. *F. graminearum* tolerance to tebuconazole has been found in *in vitro* experiments that did not use any mutagens. *F. graminearum* exposure to tebuconazole resulted in adapted virulent isolates that were resistant to tebuconazole (Becher et al., 2010). Although it is unlikely for entire wheat growing regions to use fungicide applications every year, it is still a cause for concern and another reason that resistant cultivars should be relied on as much as possible to ensure long-term effectiveness of important fungicides.

Relying on fungicide application alone to control a disease that can be controlled with resistant cultivars is a risky agronomic practice. While our data indicate that the combination of resistant cultivars with fungicide treatment provides the best control for FHB, there are many other factors that must go into the decision to apply fungicides. A consideration in fungicide

application decisions is the fact that application and efficacy can be limited by timing as well as weather. Flowering time varies among plants in the same plot, among tillers of the same plant and spikelets on the same head (Paul et al., 2007). This can be an issue for determining when to apply a tebuconazole fungicide that should be applied at mid-anthesis. Applying too early or too late may result in lowered levels of control. It may also be difficult to spray fungicides during anthesis in a production setting where many large fields that need to be sprayed are not geographically close together (Paul et al., 2007). The efficacy of fungicides in a field production setting may therefore be less than the results found in this experiment where fungicides were applied at the optimal Feekes Growth stage of 10.5.1. Resistant cultivars are therefore imperative for season long protection from severe FHB infection pressure in years when fungicide application is not feasible or practical.

The cost of fungicide application is another consideration that must be examined in the evaluation of practicality of spraying a fungicide. While Folicur® is much cheaper to apply than Prosaro®, its reduced cost does not compensate for its slightly higher infection levels seen when compared to Prosaro® treated plots. Folicur® treated plots did provide the highest net return but were not significantly different than Prosaro® treated plots, or nontreated plots. With Folicur® not being significantly different than Prosaro® for yield, severity, incidence, DON in 2008, or net return, it would be logical for a producer to apply Folicur® due to the reduced cost associated with the lower price of Folicur®. Under extremely heavy infection pressure, resistant cultivars were able to yield and provide profit comparable to susceptible cultivars treated with a fungicide. If yield and net return were the only concerns when referring to FHB, recommendations may be to either plant a resistant cultivar or use a fungicide on a susceptible cultivar; however, seed quality is also an important factor when it comes to controlling FHB.

Fusarium head blight severity prediction models are another tool that can help determine the decision to apply a fungicide (www.wheatscab.psu.edu). Prediction models use weather data to determine the risk of FHB infection and provide advice on when a fungicide should be applied. The model cited above also includes the use of resistant cultivars into the determination of FHB infection risk. Although the prediction models do provide information about the risk of FHB infection, it may still be difficult to determine if a fungicide application is necessary. If a susceptible cultivar has been planted, it is imperative to treat it with a fungicide if the prediction model indicates there is a high risk of disease infection. If a resistant cultivar has been planted the decision to spray a fungicide is much more difficult. Although resistant cultivars treated with either fungicide were significantly better than resistant cultivars not treated with fungicide for yield, resistant cultivars treated with either fungicide did not significantly increase the net return. In other words, applying a fungicide does not significantly improve profit when DON and grain quality discounts are not included in net return analysis.

While yield is a very important consideration when examining FHB resistance, it is the grain quality that is of utmost importance to end users who plan to use the grain for either human products or animal feed. Therefore, a cultivar that is resistant to FHB should yield comparably to yield standards, but should also have low FDK as well as low DON concentration. Percentage of Fursarium damaged kernels (FDK) is an excellent measure for determining damage resulting from FHB. Resistant cultivars were important in the reduction of FDK (Figure 1.1). When susceptible cultivars were not treated with a fungicide they had an average FDK rating of 32%. Fungicides lowered this value to 21% and 13% for Folicur® and Prosaro®, respectively; however, resistant cultivars with no treatment had an average FDK rating of 6.9% (Table 1.2). This illustrates the importance of a resistant cultivar. Even with fungicides applied to the six

susceptible cultivars at an optimal time and rate, they still failed to produce FDK ratings that the six resistant cultivars obtained with no fungicide treatment (Figure 1.3).

The relationship between DON levels and the environment is confusing at best. There was clearly a large difference between DON concentration in 2008 and 2009, with DON concentrations being much higher in 2009 (Table 1.2). This is somewhat surprising, as the other traits were not drastically different between the two years. In fact, FDK was slightly lower in 2009 than in 2008 (data not shown). Other studies have shown that DON is a variable trait that can show different concentration levels in multiple environments that have similar disease severity ratings for other traits such as FDK (Bonin and Kolb, 2009; Mesterhazy, 2002). It is clear that DON is highly sensitive to microenvironments; however, it is unclear what causes the fluctuations in DON concentration from year to year and between environments. Mesterhazy, (2002) found that rainfall during specific times in spring were significantly correlated with DON concentration. The variability in DON concentration from year to year observed in this study as well as others can make breeding for resistance to DON difficult. While DON levels were much higher in 2009, the cultivars were fairly consistent in their ranking.

DON concentration is clearly a very important trait because it is of concern to both human and animal safety. Even though it can vary from year to year it is very imperative to breed for reduced DON concentration. As discussed in the introduction, DON concentration must be below 1 ppm for grain that is used for products for human consumption while DON concentration must be below 5 ppm or 10 ppm for animal feed depending on the type of livestock. In this study, susceptible cultivars had average DON concentration values well above the 1 ppm threshold as well as above the 5 ppm threshold in 2009, even when treated with fungicides (Figure 1.5). DON levels were lower in 2008; however, DON concentration was still

high on susceptible cultivars averaging 4.2 ppm when no fungicide was applied (Table 1.2). With fungicides applied to susceptible cultivars average DON concentration was still above 1 ppm. To obtain DON values below 1 ppm a combination of both resistant cultivars as well as fungicide treatment was required in both 2008 and 2009. Folicur® treated resistant cultivars had an average DON concentration of 2.2 ppm in 2009, well above the 1 ppm threshold for human consumption. Even though fungicides combined with resistant cultivars were required in both 2008 and 2009 to obtain DON levels below 1 ppm, these values were not significantly different than resistant cultivars that received no fungicide treatment. This information further complicates the decision to apply fungicides. Using a combination of resistant cultivars and applying fungicides when forecasting models predict an extremely high risk of FHB infection is the best recommendation for reducing yield loss and DON concentration to acceptable levels.

Based on the results of this experiment it is apparent that resistant cultivars are a necessity to provide the best control of FHB. Under the extremely heavy disease pressure of our FHB nursery, for almost all measured variables fungicide treatment of susceptible cultivars did not provide sufficient control of FHB. Not surprisingly, we found the best method for controlling FHB is to plant a resistant cultivar in addition to applying a fungicide; however, we were interested to see how resistant cultivars alone would perform when compared to susceptible cultivars treated with a fungicide. Resistant cultivars performed impressively, and it was apparent that resistant cultivars are an essential first step of an effective program for controlling FHB. Resistant cultivars were able to yield well and provide excellent net economic returns that were not significantly different than resistant cultivars that were treated with a fungicide. This would suggest that under low to moderate FHB pressure, there no need for fungicide application. It is only necessary to apply fungicides in years when there is a high risk of FHB infection. This

experiment illustrated that resistant cultivars provide sufficient protection from FHB; however, to achieve high quality grain with low levels of FDK and DON, fungicide application may be needed in years when there is a high risk of severe disease pressure.

TABLES AND FIGURES

Table 1.1 Summary of best linear unbiased predictions (BLUPs) from PROC MIXED analysis of twelve soft red winter wheat cultivars classified as being either resistant or susceptible treated with no fungicide, Folicur® (tebucnonazole) or Prosaro® (tebuconazole+prothioconazole).

	Estimate	Yield (kg/ha)	Test Weight (kg/m^3)	Severity (%)	Incidence (%)	FDK (%)	FHB Index (%)	ISK Index (%)	DON 2008 (ppm)	DON 2009 (ppm)	Net Return ($/ha)
BLUP-(Res. Vs. Susc.)											
Overall	Resistant vs. Susceptible	599.7**	41.8**	(-)17.1**	(-)29.8**	(-)17.6**	(-)20.9**	(-)21.1**	(-)2.0**	(-)3.0**	148.8**
No Treatment	Resistant vs. Susceptible	854.6**	56**	(-)17.15**	(-)26.5**	(-)24.8**	(-)24.8**	(-)23**	(-)2.9**	(-)3.9**	208.8**
Prosaro	Resistant vs. Susceptible	312^{NS}	28.4**	(-)12.5**	(-)30.3**	(-)10.7**	(-)15.4**	(-)17.2**	(-)1.2**	(-)2.3**	84.5^{NS}
Folicur	Resistant vs. Susceptible	632.5**	41**	(-)21.5**	(-)32.6**	(-)17.3**	(-)22.6**	(-)23.1**	(-)1.9**	(-)2.9**	153.1**
BLUP-(Fungicide/Resistance comparison)											
	Prosaro vs. No Fungicide	830.99*	33.8**	(-)10.6*	(-)32.5*	(-)11.4**	(-)19.7**	(-)17.5**	(-)1.8**	(-)2.9**	135.7*
	Folicur vs. No Fungicide	603.7*	20.9**	(-)8.8	(-)20*	(-)6.6*	(-)13.5**	(-)11.3**	(-)1.2*	(-).91*	114.4^{NS}
	Prosaro vs. Folicur	227.3^{NS}	12.8*	$(-)1.8^{NS}$	$(-)12.5^{NS}$	(-)4.8*	(-)6.2*	(-)6.2*	$(-).62^{NS}$	(-)1.9*	21.3^{NS}
Susc. Posaro vs. Res. No Fungicide		247.7^{NS}	(-)8.4**	4.25^{NS}	$(-)4.1^{NS}$	6.3**	$.42^{NS}$	2.6^{NS}	$.22^{NS}$	$.23^{NS}$	11^{NS}
Susc. Folicur vs. Res. No Fungicide		$(-)139.8^{NS}$	(-)27.6**	10.5**	9.5^{NS}	14.4**	10.2**	11.8**	1.2*	2.5*	66.6^{NS}
Res. Prosaro vs. Res No Fungicide		559*	20.6**	(-)8.3**	(-)34.4**	(-)4.42**	(-)14.9**	(-)14.6**	$(-).94^{NS}$	(-)2.06**	73.5^{NS}
Res. Folicur vs. Res. No fungicide		492.6*	13.3**	(-)11**	(-)23.1**	(-)2.85**	(-)12.4**	(-)11.4**	$(-).70^{NS}$	$(-).44^{NS}$	86.5^{NS}

*=Contrast significant at P≤0.01-0.05
**=Contrast significant at P≤0.0001
NS=Contrast difference not significant at P≤0.05
FDK- Percentage of Fusarium damaged kernels estimated by comparisons to standards with known proportions of damaged/undamaged kernels.
FHB Index-Severity*Incidence
ISK Index- Incidence/Severity/Kernel Quality index calculated via [(.4*FDK)+(.3*Incidence)+(.3*Severity)]
Net Return- Calculated using soft red winter wheat prices from the Chicago board of trade on the respective day of harvest. No grain quality reductions were factored into the calculation.

Table 1.2 Mean Estimates of twelve soft red winter wheat cultivars classified as being either resistant or susceptible treated with no fungicide, Folicur® (tebucnonazole) or Prosaro® (tebuconazole+prothioconazole).

Estimate	Yield	Test Weight	Severity	Incidence	FDK	FHB Index	ISK Index	Don 2008	Don 2009	Net Return
	(kg/ha)	(kg/m^3)	(%)	(%)	(%)	(%)	(%)	(ppm)	(ppm)	($/ha)
Ovarall										
Susceptible	6298.2	702.702	50.5	67.4	22.1	34.2	44.2	2.7	4.8	1422
Resistant	6897.7	745.173	33.5	37.6	4.5	13.4	23.1	0.71	1.8	1570
Prosaro	6950.8	740.025	37.9	37.5	7.9	15.2	25.8	0.89	1.7	1548
Folicur	6723.7	727.155	39.6	50	12.7	21.4	32	1.52	3.6	1527
No Fungicide	6119.8	705.276	48.5	70	19.3	34.3	43.2	2.7	4.5	1412
Fungicide/Resistance Combination										
No Fungicide-Resistant	6548.1	733.59	39.9	56.8	6.9	22.5	31.8	1.3	2.6	1517
No Fungicide-Susceptible	5692.5	678.249	57	83.3	31.7	47.3	54.8	4.2	6.5	1308
Prosaro-Resistant	7106.7	754.182	31.6	22.4	2.5	7.5	17.2	0.31	0.53	1590
Prosaro-Susceptible	6794.9	725.868	44.1	52.7	13.2	22.9	34.3	1.5	2.8	1506
Folicur-Resistant	7039.5	747.747	28.9	33.7	4.1	10.1	20.4	0.56	2.2	1603
Folicur-Susceptible	6407.3	706.563	50.4	66.3	21.3	32.7	43.5	2.5	5.1	1450

FDK- Percentage of Fusarium damaged kernels estimated by comparisons to standards with known proportions of damaged/undamaged kernels.

FHB Index-Severity*Incidence

ISK Index- Incidence/Severity/Kernel Quality index calculated via [(.4*FDK)+(.3*Incidence)+(.3*Severity)]

Net Return- Calculated using soft red winter wheat prices from the Chicago board of trade on the respective day of harvest. No grain quality reductions were factored into the calculation.

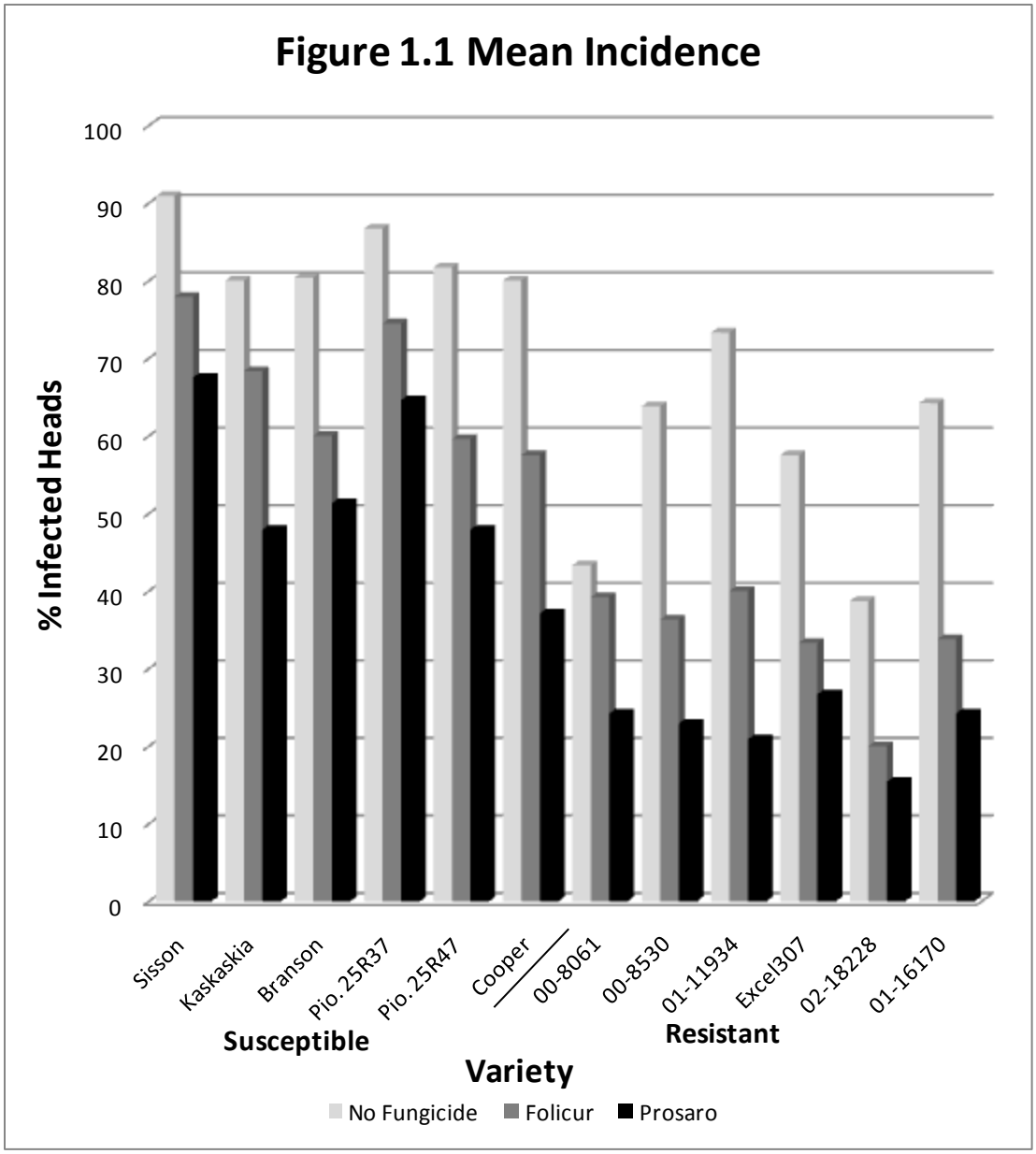

Figure 1.1 Mean incidence (% infected heads) for twelve soft red winter wheat cultivars. The six cultivars on the left are FHB susceptible while the six cultivars on the right are FHB resistant. Cultivars were grown in a mist irrigated, inoculated nursery at Urbana, Illinois in 2008 and 2009. For each cultivar three fungicide treatments were applied, Prosaro® sprayed, Folicur® sprayed, and unsprayed.

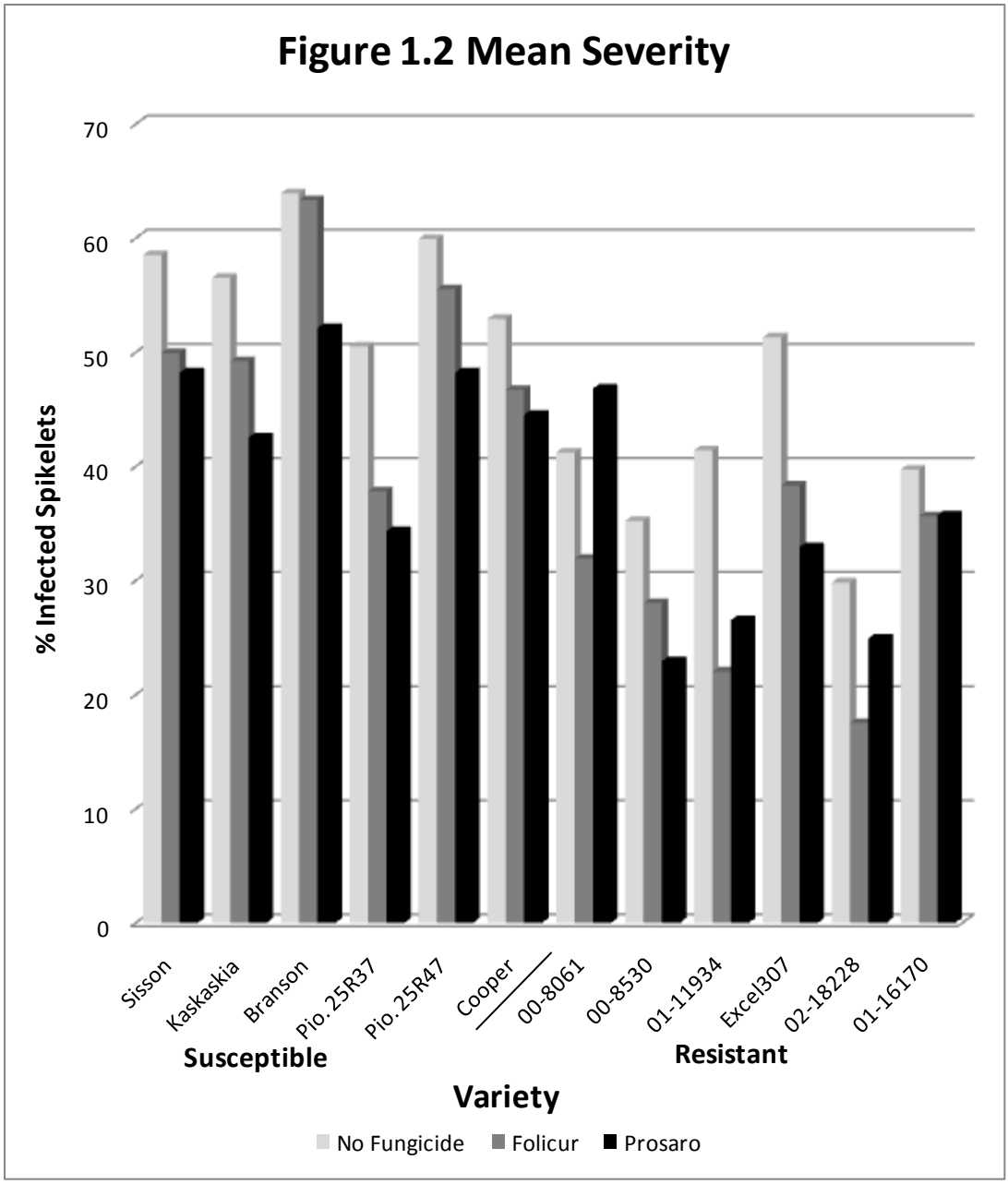

Figure 1.2 Mean severity (% infected spikelets) for twelve soft red winter wheat cultivars. The six cultivars on the left are FHB susceptible while the six cultivars on the right are FHB resistant. Cultivars were grown in a mist irrigated, inoculated nursery at Urbana, Illinois in 2008 and 2009. For each cultivar three fungicide treatments were applied, Prosaro® sprayed, Folicur® sprayed, and unsprayed.

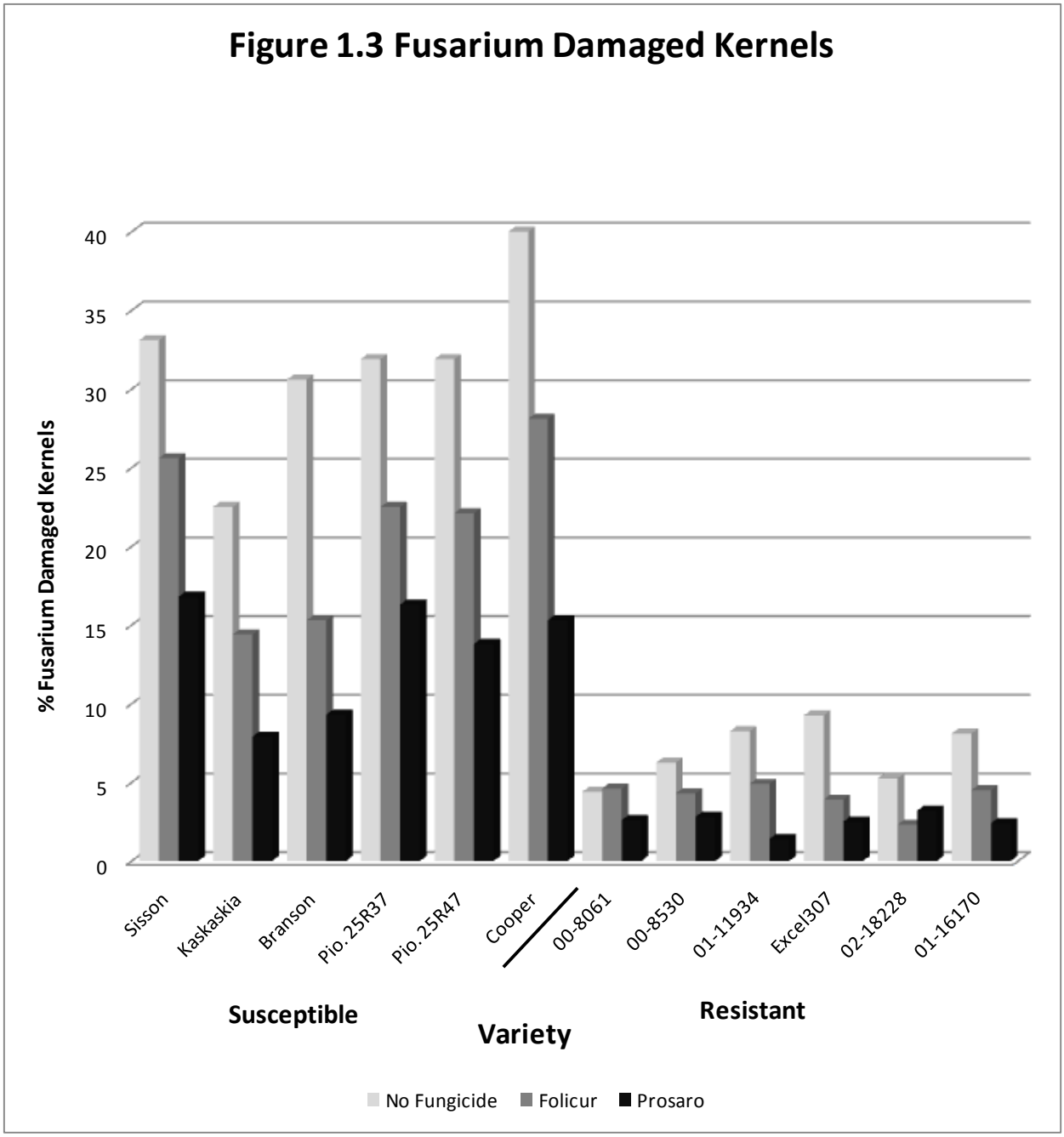

Figure 1.3 Percentages of Fusarium damaged kernels for twelve soft red winter wheat cultivars. The six cultivars on the left are FHB susceptible while the six cultivars on the right are FHB resistant. Cultivars were grown in a mist irrigated, inoculated nursery at Urbana, Illinois in 2008 and 2009. For each cultivar three fungicide treatments were applied, Prosaro® sprayed, Folicur® sprayed, and unsprayed.

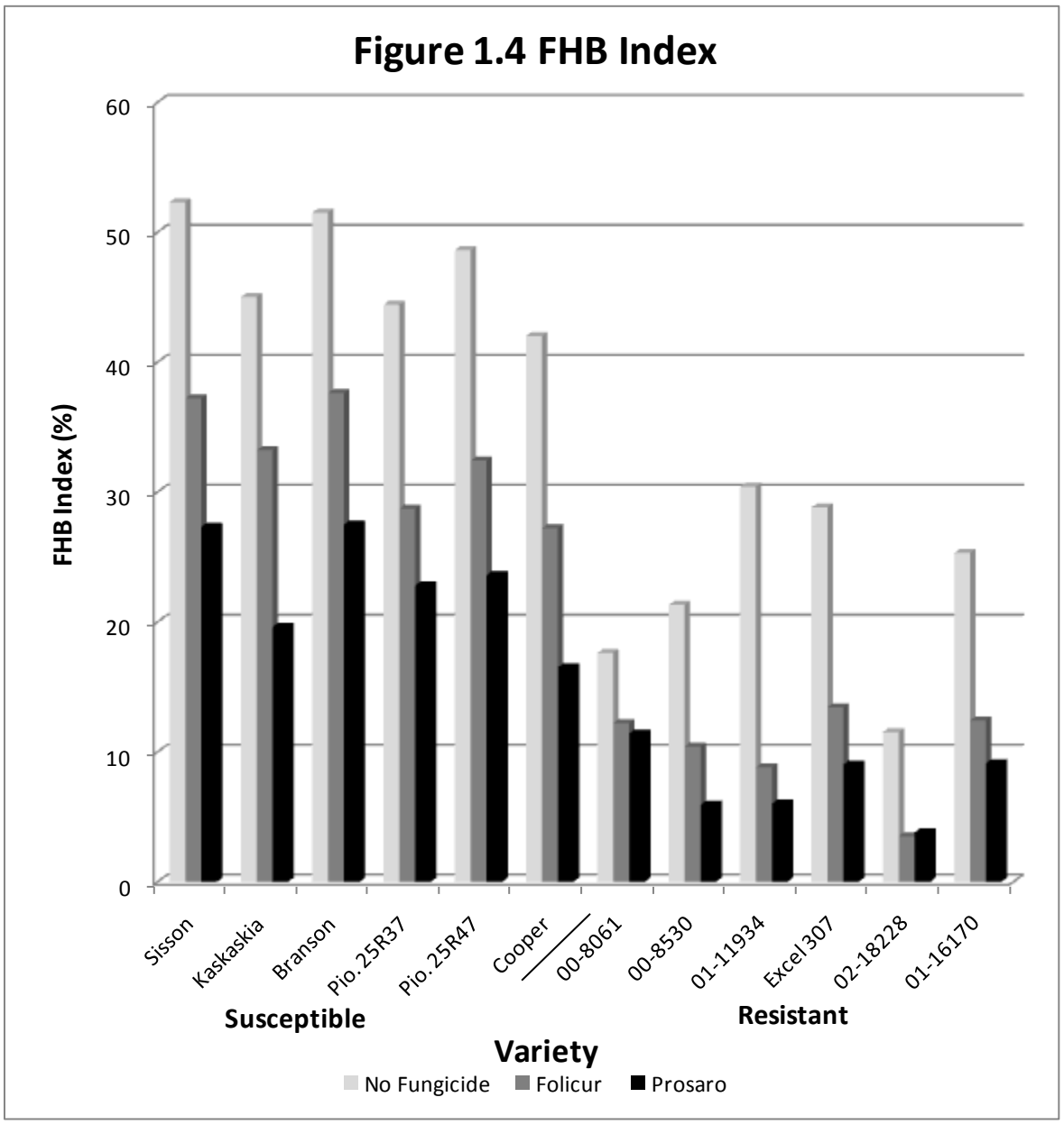

Figure 1.4 FHB index (%) for twelve soft red winter wheat cultivars (six FHB susceptible and six FHB resistant) grown in a mist irrigated, inoculated nursery at Urbana, IL in 2008 and 2009. For each cultivar three fungicide treatments were applied, Prosaro® sprayed, Folicur® sprayed, and unsprayed.

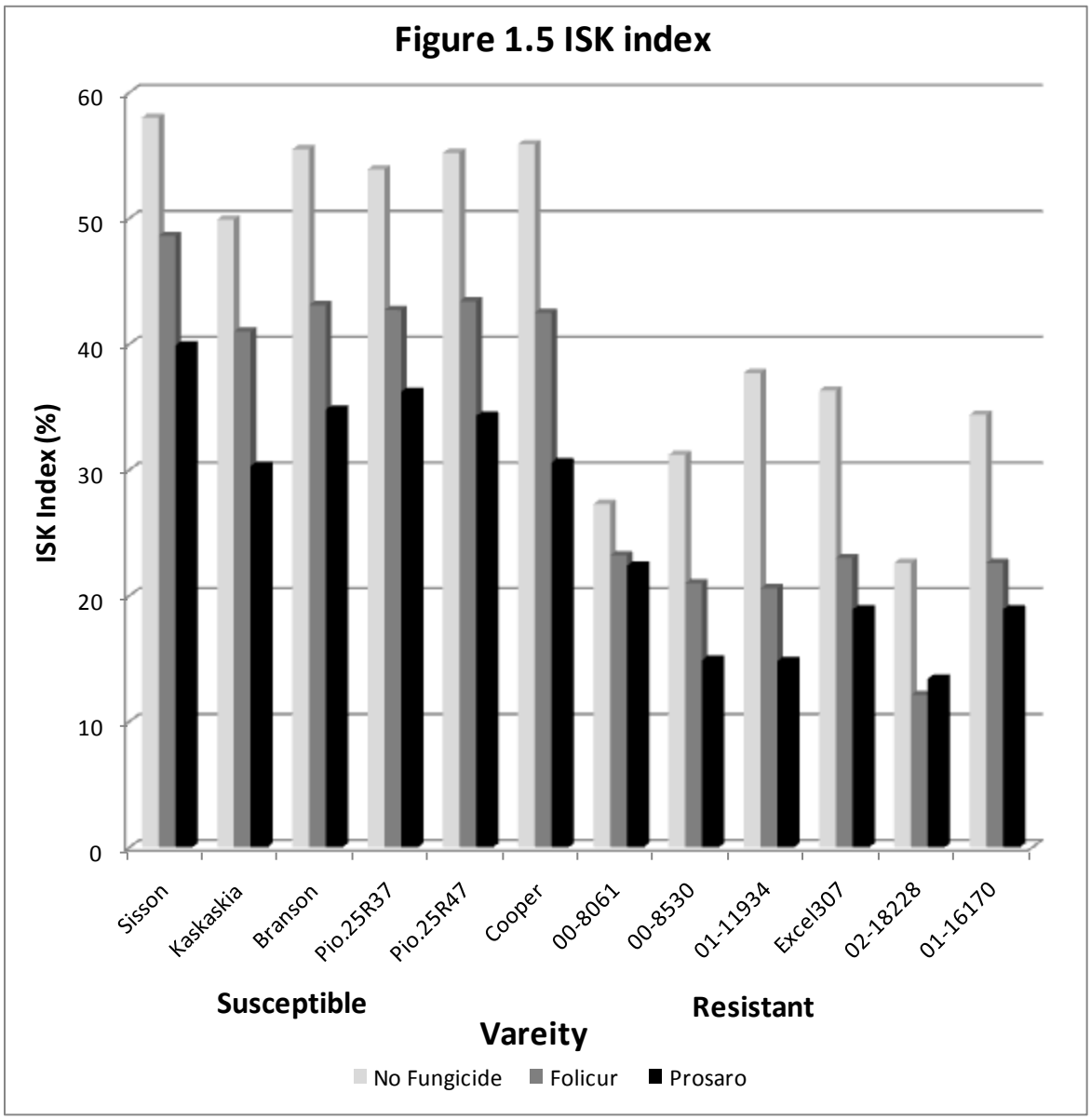

Figure 1.5 Incidence/Severity/Fusarium Damaged kernels (ISK) index for twelve soft red winter wheat cultivars (six FHB susceptible and six FHB resistant) grown in a mist irrigated, inoculated field nursery at Urbana, Illinois in 2008 and 2009. For each cultivar three fungicide treatments were applied, Prosaro® sprayed, Folicur® sprayed, and unsprayed.

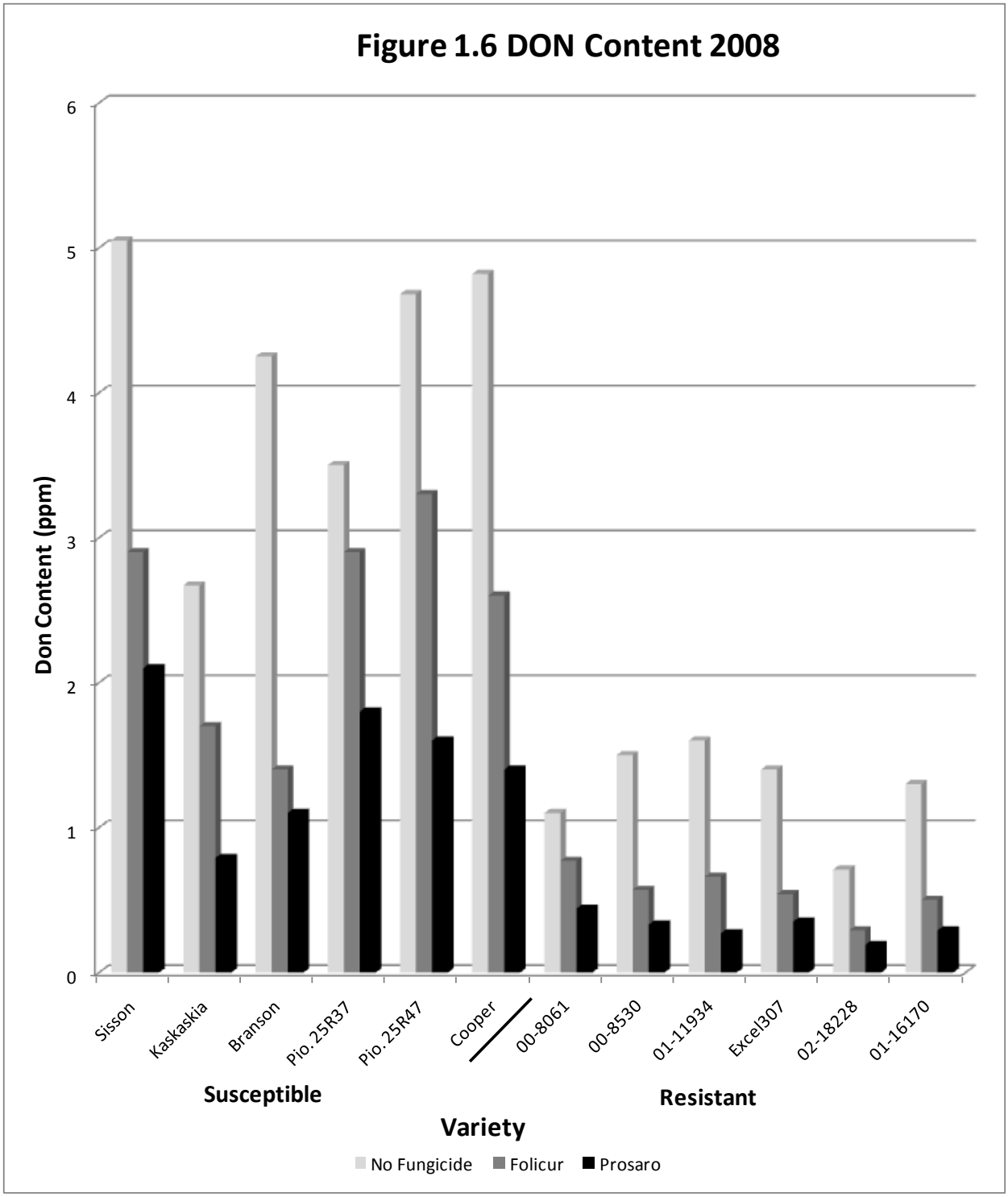

Figure 1.6 Deoxynivalenol concentration in parts per million for twelve soft red winter wheat cultivars (six FHB resistant and six FHB susceptible) grown in a mist irrigated, inoculated field nursery at Urbana, Illinois in 2008. For each cultivar three fungicide treatments were applied, Prosaro® sprayed, Folicur® sprayed, and unsprayed.

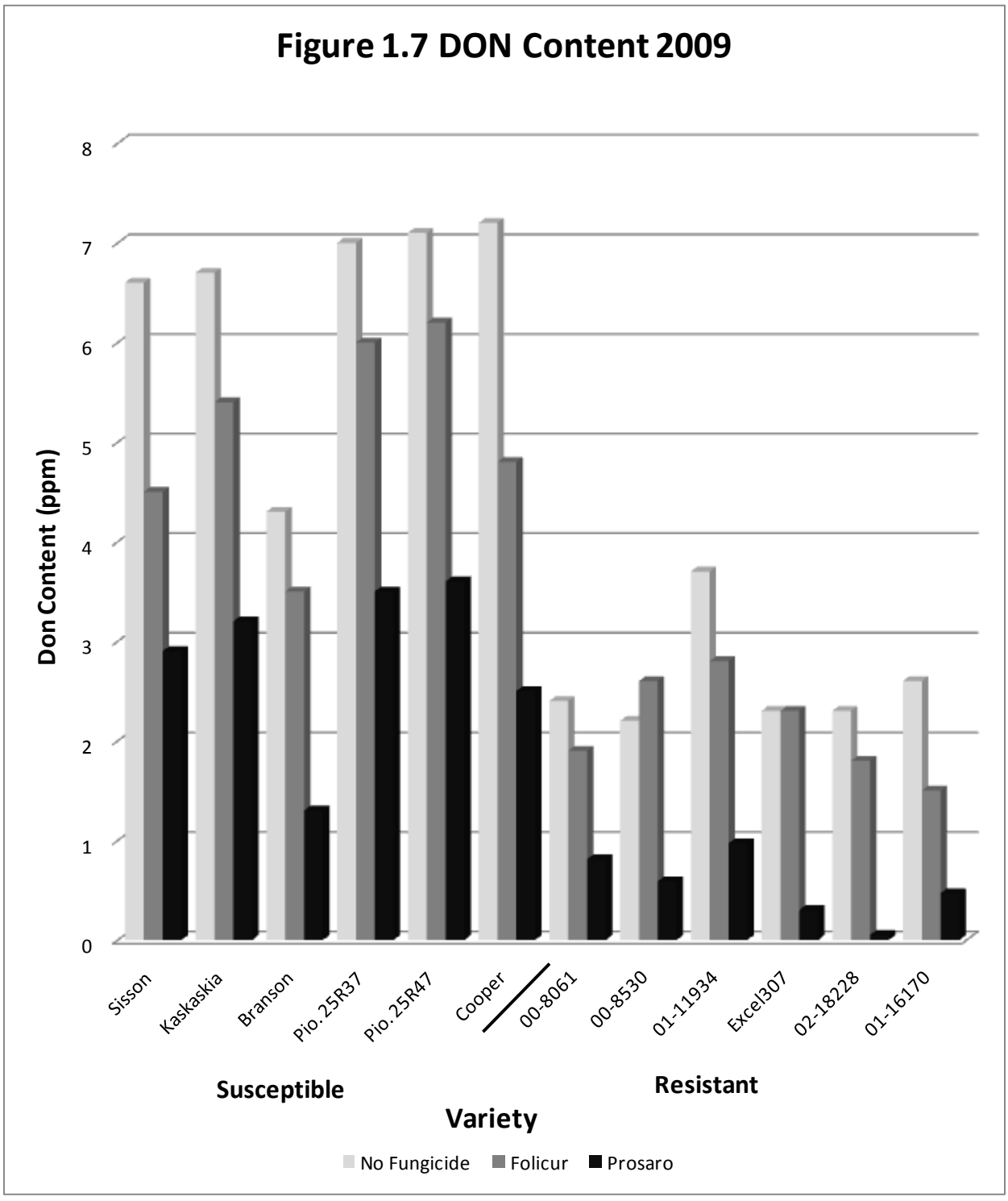

Figure 1.7 Deoxynivalenol concentration in parts per million for twelve soft red winter wheat cultivars (six FHB resistant and six FHB susceptible) grown in a mist irrigated, inoculated field nursery at Urbana, Illinois in 2009. For each cultivar three fungicide treatments were applied, Prosaro® sprayed, Folicur® sprayed, and unsprayed.

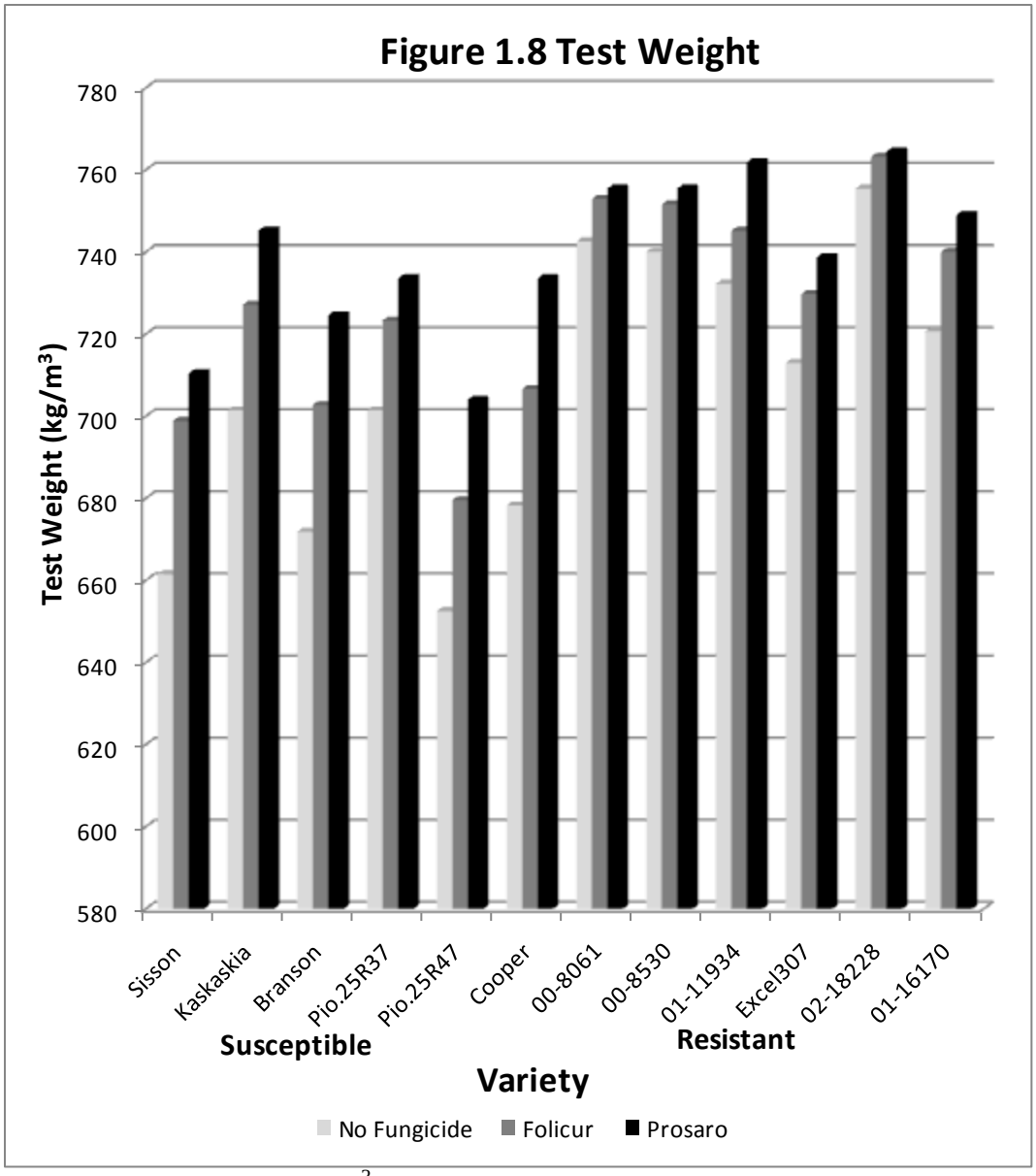

Figure 1.8 Test weight (kg/m^3) for twelve soft red winter wheat cultivars (six FHB susceptible and six FHB resistant) grown in a mist irrigated, inoculated nursery at Urbana, IL in 2008 and 2009. For each cultivar three fungicide treatments were applied, Prosaro® sprayed, Folicur® sprayed, and unsprayed.

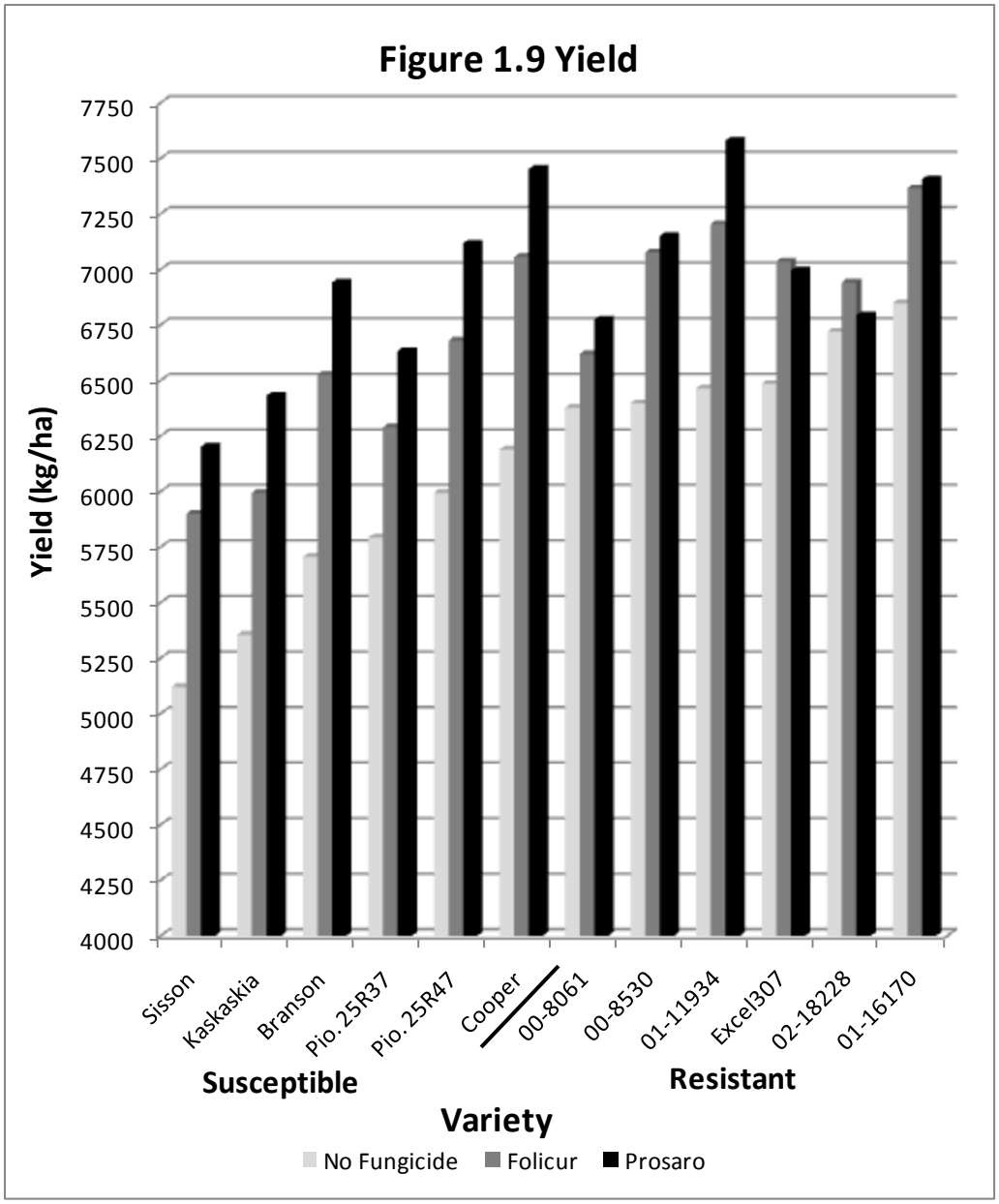

Figure 1.9 Yield (Kg/hectare) for twelve soft red winter wheat cultivars (six FHB susceptible and six FHB resistant) grown in a mist irrigated, inoculated nursery at Urbana, IL in 2008 and 2009. For each cultivar three fungicide treatments were applied, Prosaro® sprayed, Folicur® sprayed, and unsprayed.

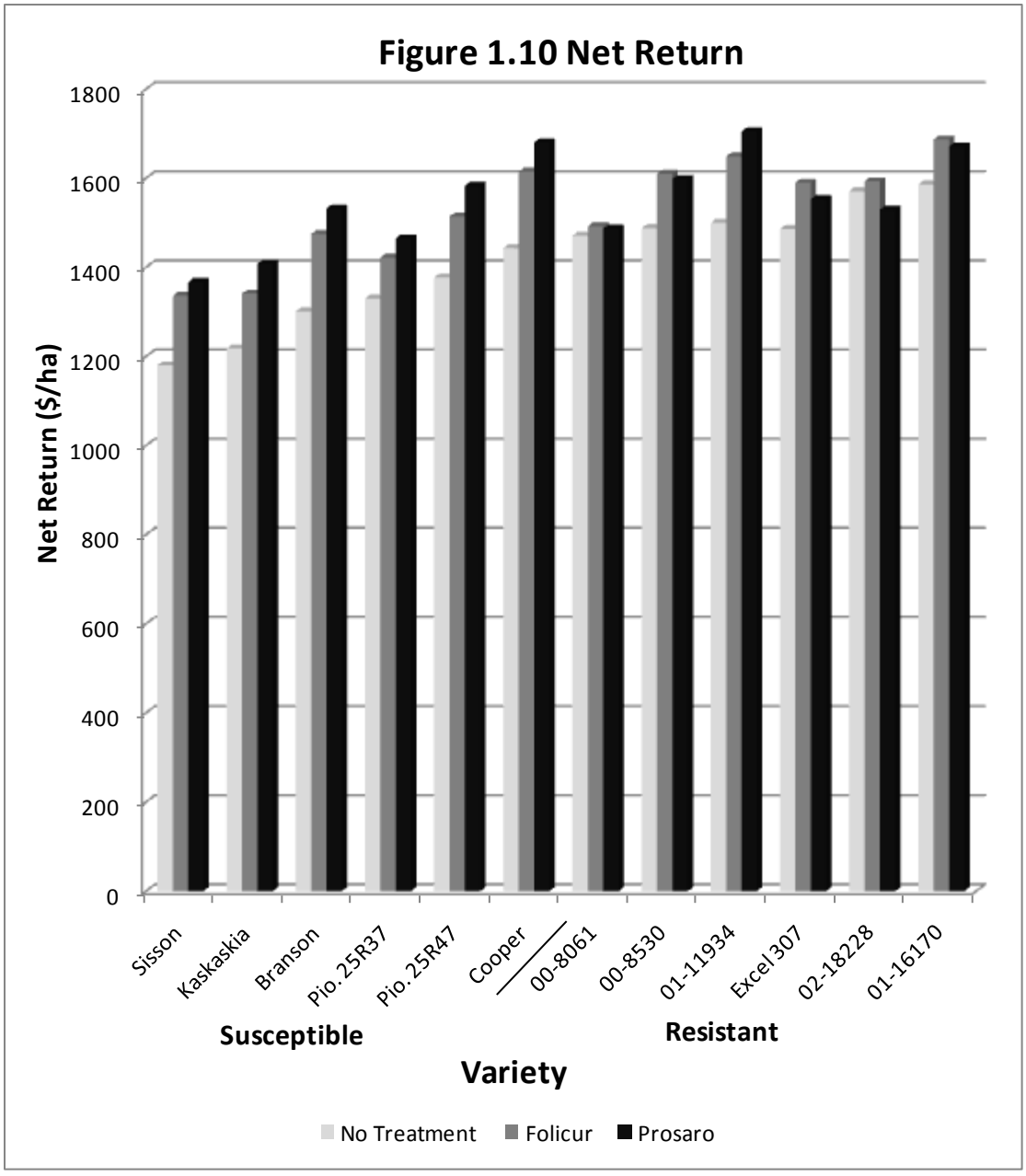

Figure 1.10 Net Return ($/hectare) for twelve soft red winter wheat cultivars (six FHB susceptible and six FHB resistant) grown in a mist irrigated, inoculated nursery at Urbana, IL in 2008 and 2009. For each cultivar three fungicide treatments were applied, Prosaro® sprayed, Folicur® sprayed, and unsprayed.

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CHAPTER 3
USING DART AND SSR MARKERS TO IDENTIFY QTL ASSOCIATED WITH FHB RESISTANCE.

INTRODUCTION

Molecular markers are useful tools for understanding plant processes as well as aiding in the plant breeding process. Using molecular markers, breeders can implement marker assisted selection to select lines with quantitative trait loci (QTL) for high levels of Fusarium head blight (FHB) resistance. There have been many QTL associated with FHB found throughout the wheat genome. An effective use of these markers is to select combinations of multiple genes for resistance (Anderson, 2007). Even though many QTL have been found to be associated with FHB resistance, many of these QTL have minor effects and are not good candidates for marker assisted selection. While there have been many types of molecular markers used for mapping and QTL detection (AFLP, RFLP, and RAPDs), simple sequence repeats (SSRs) have become dominant in mapping studies because they are highly polymorphic, exhibit co-dominant inheritance, are abundant and reproducible (Landjeva et al., 2007). SSRs are very useful but, they have a limited multiplexing ability (Akbari et al., 2006).

Diversity arrays technology (DArT) is an array based system that can be used for high throughput marker analysis. DArT technology uses solid state arrays to reveal polymorphism between samples (Jaccoud et al., 2001). Developing the DArT array is started using methylation sensitive restriction enzymes to digest genomic DNA, which reduces genomic complexity (Wenzl et al., 2004). The fragments of genomic DNA contain both constant fragments, as well as variable fragments. These variable fragments contain polymorphisms (DArT website, www.diversityarrays.com). The variable fragments are cloned to create a library (DArT website, www.diversityarrays.com). Clones from this library are amplified, purified and arrayed

onto a solid platform used for detecting QTL (Jaccoud et al., 2001). Individual samples are prepared in a similar way as the bulked samples used to create the array, using a complexity reduction. The clones on the array as well as the individual samples are then fluorescently labeled. The samples are then washed onto the array (DArT website, www.diversityarrays.com). Polymorphisms are detected through differences in hybridization signal as a result of samples being labeled with fluorescent dye. DArT is a dominant marker system and therefore relies on the presence or absence of a hybridization signal (Francki et al., 2009).

My objective in this study was to identify QTL for resistance to FHB in a recombinant inbred line (RIL) population derived from a three-way cross of resistant parents. FHB resistance was evaluated using several parameters including, type 1 and type 2 resistance, as well as Fusarium damaged kernels (FDK) and deoxynivalenol (DON) concentration. All three of the parents used to create the RIL population are relatively resistant to FHB infection. With three parents, it was impossible to determine which parent an allele originated from unless there was a different allele for each parent, or if the resistant allele donated from a specific parent is different than the alleles donated by the other two parents. If multiple QTL for FHB resistance can be detected in the RIL lines considered to be transgressive segregants, then the hypothesis that multiple genes for resistance can be identified from a three way cross will be confirmed and "gene stacking" was successful.

MATERIALS AND METHODS

Plant Materials. Three hundred wheat heads were selected from an F3 bulk population resulting from the three-way cross of Illinois lines IL96-6472/IL97-6755//IL97-1828. The heads were individually threshed and advanced to the F4 generation using single seed descent. F4:5 lines were increased in both the greenhouse and field. F4:6 was the generation used for

conducting both greenhouse evaluations as well as the field evaluation. The resulting RIL population consisted of 266 lines. Some lines were lost due to lack of vernalization and seedling death. While a typical mapping population would have a bi-parental cross with a single resistant and susceptible parent, the three resistant parents used in this experiment are all FHB resistant with resistances that are derived from different sources. IL97-6755 derives its resistance from Ning 7840 which contains the Sumai 3 source of resistance where the 3BS locus originates. Neither IL97-1828 nor IL96-6472 have a known source of resistance in their pedigrees.

Greenhouse phenotyping. Four to six seeds of each line were planted into 96 well flats filled with vermiculite. At seedling emergence, the flats were placed in a vernalizer for 8 weeks at 4^{o} C with a day length of 14 hours. Two seedlings of each line were transplanted into 12.7 cm clay pots and randomly organized on greenhouse benches. The pots were fertilized and treated with insecticide. The plants were watered daily, and day length was set at 16 hours for artificial lighting. All three parents, as well as a susceptible check (Sisson) were also transplanted for phenotypic evaluation.

The inoculum was prepared while the seedlings were vernalizing. Twenty previously collected isolates from various locations in Illinois were grown on potato dextrose agar (PDA) plates for two days at room temperature. Three 5mm^2 plugs were then removed from each culture and placed in flasks containing 70mL of carboxymethocellulose liquid media (Tuite, 1969). Flasks were swirled under fluorescent light for 4 days to produce macroconidia. The concentration of macroconidia in each flask was determined using a hemacytometer. A stock suspension of macroconidia was produced using 10 isolates with the highest spore production. The final concentration of macroconidia in the stock suspension was determined using a hemacytometer. An inoculation suspension was then prepared by diluting the stock suspension

to 50 macroconidia/µL in 2008 and 12 macroconidia/µL in 2009 depending on the inoculation technique. The inoculation suspension concentration was confirmed after dilution using a hemacytometer. The same isolates were used for each year of greenhouse screening.

In 2008, heads were inoculated using a needle inoculation technique. When two heads in a pot reached anthesis, they were inoculated. A central individual floret was inoculated with 20 µL of inoculum providing approximately 1,000 macroconidia per floret. The plants were then placed in a mist chamber for approximately 72 hours. Humidity was kept at 100% and temperature about 20º C. After 72 hours, the plants were removed from the mist chamber. Heads were rated for FHB severity 21 days after inoculation.

In 2009, heads were inoculated using a spray bottle technique. When at least two heads in a pot reached anthesis, heads were inoculated. The spray nozzle was calibrated to apply 2 mL of inoculum with each squirt. The heads were sprayed on each side with 1 squirt of the spray bottle. After accounting for overspray and runoff, an inoculation suspension of 12 macroconidia/µL was determined to deliver the desired 600 macroconidia per head. Plants were then evaluated for resistance 21 days after inoculation. In both years of greenhouse phenotyping the number of infected spikelets on each head was measured as well as the total number of spikelets per head to determine the percentage of infected spikelets on each head.

Field Phenotyping. Due to a limited supply of seed, only one year (2010) of phenotypic evaluation was done in a mist irrigated inoculated disease nursery at the Crop Sciences Research and Education Center at the University of Illinois at Urbana-Champaign. The 266 lines were grown along with 4 checks (Sisson, Ernie, IL00-8061 and IL02-18228) and the three parents used to create the recombinant inbred line population. The experiment consisted of three replications in a randomized complete block design, with each line being grown in a 1 meter

long row. The nursery was grain spawn infested using 10 different *F. graminearum* isolates. The grain spawn was spread two, four, and six weeks prior to anticipated anthesis giving a total rate of 116 kg per hectare. The mist irrigation ran three times per 24 hours during anthesis and continued for two weeks after the last genotype flowered delivering approximately 3.05 mm of water per hour. Quadris® (azoxystrobin; Syngenta Crop Protection, Greensboro, NC) was applied at a rate of 7.55 mL per hectare (0.04 kg a.i./ha) to control foliar diseases at Feekes growth stage 8.0-9.0. Warrior® (lambda-cyhalothrin) was applied at a rate of 0.006 kg a.i./ha in the fall and spring to control aphids thereby preventing *Barley yellow dwarf virus* infection.FHB incidence and severity were evaluated one month after the heading date of each genotype. Incidence was recorded by randomly selecting a group of heads from a plot and determining the number of heads that had been infected. Severity was recorded by estimating the percentage of infected spikelets on seven randomly selected heads. FDK was visually estimated post-harvest using standards with a known ratio of scabby kernels/healthy kernels. DON concentration was measured by Dr. Yanhong Dong's lab at the University of Minnesota department of Plant Pathology. SAS v9.2 was used to conduct analysis of variance using PROC MIXED and PROC UNIVARIATE to test for normality. When non-normal error variances were found, simple transformations provided normal error variances.

Genotyping. Tissue was collected from young F4:5 lines growing in the greenhouse. The tissue was lyophilized, and DNA was extracted using a slightly modified CTAB procedure found on the DArT website (http://www.diversityarrays.com/). The DNA concentration was quantified using a nanodrop quantifier. The DNA samples were then diluted to 60 ng/µL and were sent to the DArT facility in Yarralumla, Australia. The DArT group performed the marker analysis using the method described above and sent the data report back. A total of 2,500 DArT

markers were screened for polymorphism between the three parents and a total of 250 polymorphic markers were found.

SSR markers were also used to detect polymorphisms in the RILs because they show a lower level of clustering compared to DArT markers, making them a useful complement to the DArT markers (Francki et al., 2009). The SSRs also allow for more robust linkage map creation; however, with more than 2,000 DArT markers available for wheat, the coverage of the genome was relatively high. Another reason for using SSRs is that SSRs are not affected by DNA methylation as are DArT markers. DNA methylation plays a key role in gene regulation and may affect detection of QTL when using DArT markers (Francki et al., 2009).

The three parents from the cross used to create the population were screened for polymorphism using SSR markers with DNA at a concentration of 30ng/ µL. The products were amplified using a polymerase chain reaction (PCR) similar to Roder et al. (1998). The PCR products were then separated using 6% polyacrylamide gel electrophoresis. Gels were stained using ethidium bromide and visualized under ultraviolet (UV) light. A total of 616 SSR markers were screened on the three parents including Xbarc markers (Song et al., 2005), Xgwm markers (Roder et al., 1998), Xwmc markers (Gupta et al., 2002), and Xgdm markers (Pestsova et al., 2000). Of the 616 SSR markers, 43 markers were found to be polymorphic and provided clear banding patterns that were used to genotype the lines of the RIL population.

Map Construction. The linkage map was constructed using JoinMap 3.0 (Van Ooijen and Voorrips, 2001) with a logarithm of odds (LOD) score of 5.0 to group linked markers. Linkage groups from linkage map was placed on wheat chromosomes using the SSR consensus map created by Roder et al. (1998), the Triticarte wheat alignment map version 1.2

(www.triticarte.com.au), and marker information from GrainGenes 2.0 (http://wheat.pw.usda.gov). The map consisted of 47 linkage groups.

QTL Analysis. PlabQTL (Utz and Melchinger, 2003) was used for composite interval mapping and determination of threshold LOD scores using 1,000 permutations. Critical LOD values ranged from 2.76 to 2.89 at 10%. A threshold LOD score was set at 2.8 for all traits recorded in the field study. No QTL were found for the greenhouse evaluations with a LOD score of 2.4 with alpha=20%. A walking speed of 2.0 was used for detection of QTL. A QTL was considered major if it explained more than 10% of the phenotypic variation (Semagn et al., 2007). To confirm QTL location on their respective chromosomes, the Triticarte wheat alignment map version 1.2 (www.triticarte.com.au) was used to align the location of linkage groups on the consensus SSR chromosome map from Somers et al. (2004).

RESULTS

Phenotypic Evaluation. All traits measured in the field had a wide distribution of phenotypic values with most measurements approximately distributed normally. Severity, mean deoxynivalenol concentration, and Fusarium damaged kernels were all slightly positively skewed with a large number of individuals with lower values than the parents (Figures 2.1, 2.2 and 2.3). FHB index and ISK index had normal distributions with approximately equal numbers of RILs being higher and lower than the parents (Figures 2.4 and 2.5). Incidence was negatively skewed with a large number of individuals having a higher incidence than the parents (Figure 2.6). The severity for the 2008 greenhouse inoculation study was positively skewed with a very large number of RILs with very low severity scores (Figure 2.7). The severity for the 2009 greenhouse inoculation study was normally distributed and showed approximately equal number of transgressive segregants both above and below the parents (Figure 2.8). One of the main

objectives for this experiment was to combine resistance sources to produce transgressive segregants that would have a higher level of resistance than the three FHB-resistant parents used in creating the RIL population. There were observable transgressive segregants in all of the traits that were recorded (Figures 2.1-2.8), although the segregants may not be significantly better than the best parent in many cases.

There were lines that were significantly better than the mean of the parents for all measured variables except for FDK and both years of greenhouse severity evaluation (Table 2.1). Although there were no significantly different lines for these variables, there were a large number of lines that were numerically more resistant than the most resistant parent. For FDK there were 29 lines that had numerically better mean than the most resistant parent. For the 2009 greenhouse severity evaluation 51 lines had a numerically superior mean than the most resistant parent. It is likely that some of these RILs are transgressive segregants. For the greenhouse 2009 severity evaluation, lines 2 and 173 were numerically better than the most resistant parent while being significantly better than the mean of the resistant parents for other FHB resistance traits (Table 2.2). For FDK in the field evaluation, lines 2, 18, 168, 227, 266 and 284 were numerically better than the most resistant parent while being significantly better than the mean of the resistant parents for some of the other FHB resistance measures (Table 2.2).

There were a total of 13 lines that were significantly better than the mean of the parents for at least two of the measured FHB resistance traits. Line number 2 was significantly better than the mean of the resistant parents for all measured variables. Line numbers 18 and 168 were significantly better than the mean of the parents for incidence, severity, DON, FHB index and ISK index. Line 173 was significantly better than the mean of the parents for incidence, severity, DON, FHB Index and ISK index. Lines 42, 55, 259, 288 and 284 were all significantly better

than the mean of the parents for severity, DON, FHB index, and ISK index (Table 2.2). The lines that are significantly better than the mean of the parents cannot be considered transgressive segregants. Lines 2, 55 and 168 were considered transgressive segregants and were significantly better than the most resistant parent for at least two of the measured FHB resistance traits (Table 2.2).

As a whole the RIL population exhibited moderate levels of resistance with a handful of lines with very high levels of resistance. Overall, the mean of the recombinant inbred lines was never significantly different than any of the parents (Table 2.1). The mean resistance score for the RILs were significantly higher than the susceptible check, 'Sisson', for all measured traits except for severity measured in the greenhouse for 2009. In the greenhouse, the RIL population had mean severity values of 14.1% and 48.4% in 2008 and 2009, respectively (Table 2.1). The large difference in mean severity between the two years is believed to be due to the difference in inoculation techniques used. For the field phenotyping evaluation, the incidence and severity means for the RIL population were 84.4% and 42.4%, respectively (Table 2.1). The mean incidence and severity of the three parents were very close to the mean of the RIL population at 80.5% and 38.3%, respectively. The mean deoxynivalenol concentration for the RIL population was 4.8 ppm which was numerically lower than the mean of the parents at 5.3 ppm although the difference was not significant. The mean Fusarium damaged kernels score for the RIL population was 17.3%, higher than the mean of the parents at 11.7% but significantly lower than the susceptible check at 58.3%. The RIL population mean for both the FHB index and ISK index were very close to the mean of the parents. The RIL mean for FHB index and ISK index were 63.4 and 44.9, respectively, while the means for the parents were 59.4 and 40.3, respectively (Table 2.1).

All of the measured traits for the field phenotyping evaluation were significantly positively correlated (P<0.0001) (Table 2.3). Correlation between traits ranged from 0.33 to 0.89. There was a moderate correlation between FDK and DON concentration, at *r*=0.6. FDK was not highly correlated with FHB incidence or severity (*r*=0.39 and *r*=0.49, respectively), but was highly correlated with ISK index (*r*=0.85). FHB index and ISK index were also highly correlated, at *r*=0.89 (Table 2.3). Severity in both the 2008 and 2009 greenhouse evaluations were positively and significantly correlated to the severity in the 2010 field evaluation at *r*=0.19 and *r*=.33, respectively (Table 2.4), but these correlations are quite low. The 2008 and 2009 greenhouse evaluations were not significantly correlated. This lack of significant correlation is believed to be due to the use of a needle inoculation technique in 2008 and a spray inoculation technique in 2009.

Broad sense heritability ranged from 0.49 to 0.89 for phenotypic traits recorded (Table 2.1). The heritability of the 2009 greenhouse study was relatively low at 0.49, while the measures taken in the field evaluation had higher heritability, ranging from 0.71 to 0.89. These heritability values are likely slightly inflated due to a lack of genotype by environment interaction term in the calculation.

QTL Analysis. Significant QTL associated with FHB resistance measures are shown in Table 2.5. Two QTL were identified for severity measured in the field. The first QTL found for severity was located on chromosome 3Bs, linked to the DArT markers wPt-8446 and wPT-7225 and had a significant LOD value of 5.6 (Figure 2.9) explaining 9.6% of the phenotypic variation (Table 2.5). Although it was not possible to determine the parent that donated the allele for reduced severity, pedigree information of these two parents indicates that the allele was most likely donated by IL97-6755 as it has ‘Ning 7840’, a cultivar known to carry *Fhb1* in its pedigree

(Zhou et al., 2002). The second QTL associated with type 2 resistance in the field was located on chromosome 1A and was linked to DArT marker wPT-3904 with a significant LOD value of 3.77 (Figure 2.10). This QTL explained 6.6% of phenotypic variation in severity (Table 2.5). The allele for resistance was donated from parent IL97-1828 or IL97-6755.

Two QTL with minor effects were identified for incidence evaluated in the field. One of these was the same as the wpt-8446/wPt-7225 linked QTL found for severity on chromosome 3Bs but with a significant LOD value of 2.8 (Figure 2.9) explaining only 4.9% of the phenotypic variation in incidence (Table 2.5). The other QTL found for type 1 resistance was located on 1A and linked to a group of DArT markers (wPt-5934, wPt-5587, wPt-667473, wPt-6970, wPt-3459, wPt-6575 and wPt-6627) with a significant LOD value of 3.1 (Figure 2.11) explaining 5.3% of the phenotypic variation (Table 2.5). The allele that conferred reduced incidence was donated by either parent IL97-1828 or IL96-6472.

One QTL was found for FHB index evaluated in the field and was located in the same location as the QTL found for type 2 resistance on chromosome 3Bs. The QTL linked to wPt-8446/wPt-7225 had a significant LOD value of 5.36 (Figure 2.9) and explained 9.2% of phenotypic variation in FHB index (Table 2.5).

Three QTL were also identified for ISK index (Table 2.5). A QTL was located on chromosome 3Bs linked to DArT markers wPt-8446 and wPt-7225 with a significant LOD value of 5.68 (Figure 2.9) explaining 9.7% of phenotypic variation (Table 2.5). A minor effect QTL was also identified on chromosome 7B for ISK index linked to wPt-9215 with a significant LOD value of 2.97 (Figure 2.12) explaining 5.4% of the variation (Table 2.5). The allele associated with the QTL on chromosome 7B was also derived from parent IL96-6472. Another minor effect QTL was identified on chromosome 1A for ISK index linked to wPt-3904 with a

significant LOD value of 3.25 (Figure 2.10) explaining 5.7% of phenotypic variation (Table 2.5). The allele for reduced ISK index linked to this DArT marker was donated by parent IL96-6472.

Two significant QTL were found for FDK in the field evaluation. One QTL was identified on 3Bs and was linked to the same DArT markers as the other traits with QTL on 3Bs. The significant LOD value for this QTL was 3.91 (Figure 2.9), and explained 6.8% of phenotypic variation (Table 2.5). Another minor effect QTL was found for FDK on chromosome 7B linked to DArT marker wPt-9215 with a significant LOD value of 4.6 (Figure 2.12) accounting for 8.2% of phenotypic variation (Table 2.5). The allele for reduced FDK was donated by parent IL96-6472.

A single major QTL was identified for reduced DON concentration. This QTL was located on chromosome 3Bs linked to DArT markers wPt-8446/wPt-7225 with a significant LOD value of 5.92 (Figure 2.9) explaining 10.1% of phenotypic variation (Table 2.5). The allele for reduced DON concentration was donated by IL97-6755.

There were no significant QTL identified based on the 2008 or 2009 greenhouse evaluations with the significant LOD threshold at 2.8 for an alpha=0.10. With the LOD threshold at 2.4 with an alpha=.20, still no significant QTL were found for greenhouse severity evaluation.

DISCUSSION

By aligning the map created for this population with the consensus map created by Somers et al. (2004), the Triticarte wheat alignment map version 1.2 (www.triticarte.com.au) and the map created by Buerstmayr et al. (2009), that showed the location of previously discovered QTL for FHB resistance, I was able to determine if the QTL identified in this study had been previously discovered in other studies. Aligning the maps also allows for determining other

markers that map to a location similar to the QTLs identified in this study. These targeted markers could be used for further investigation of a locus and for fine mapping of QTL (Francki et al., 2009). It appears that all of the QTL found in this study have been identified in previous QTL studies; however, it cannot be determined if the alleles are the same or different.

The QTL found on chromosome 3B mapped to the short arm when the alignment was performed. The DArT markers align well to SSR markers that are associated with *Fhb1* leading me to believe that the QTL discovered on 3B is *Fhb1* or is closely linked to it (Pumphrey et al., 2007). The QTL linked to DArT markers wPt-8446 and wPt-7225 was found for all measured FHB resistance parameters.

There have been many studies that have identified QTL on the short arm of chromosome 3B (Anderson et al., 2001; Somers et al., 2003; Yang et al., 2005; Zhou et al., 2002). QTL have been identified in 'Ernie' derived populations close to the centromere of chromosome 3Bs that were not of Sumai 3 descent (Abate et al., 2008; Liu et al., 2007). I was curious if the QTL found on 3B was also in the centromeric region of 3B. After aligning the Triticarte wheat alignment map with our linkage map, the linkage map developed by Somers et al. (2004) and the maps developed by Abate et al. (2008) and Lui et al. (2007) I am confident that the QTL found on 3Bs is not in the centromeric region. The alignment suggests that the QTL found on 3Bs in this study is actually near the telomere. The QTL on 3Bs found in this population is likely derived from the Ning 7840 source of resistance. Therefore, it is not surprising that the QTL in this study did not align to the QTL found in Ernie which derives its resistance from a native source. The QTL that was found in this study on 3Bs did align well with the region of 3Bs where *Fhb1* is found.

The QTL on chromosome 7B for reduced FDK with a relatively minor effect, explained 8.2% of the phenotypic variation. Another QTL that mapped approximately 4 cM away from the QTL for reduced FDK was identified that conferred reduced ISK index. This QTL also had a relatively small effect, explaining only 5.4% of the phenotypic variation. Other studies have also found QTL in the same region of the 7B chromosome with slightly higher r^2 values (Gilsinger et al., 2005; Yang et al., 2005).

The minor effect QTL identified on chromosome 1A conferring reduced ISK index explained 5.7% of variation. A QTL was also found on chromosome 1A for reduced incidence and reduced severity explaining 5.3% and 6.6% of phenotypic variation, respectively. Jiang et al., (2007) also found a QTL in the same region of chromosome 1A for reduced FHB for multiple disease parameters.

A primary focus of this study was to combine resistance sources from three parents believed to have derived their resistances from different backgrounds. It would be ideal to be able to combine native sources of resistance with Chinese sources of resistance to develop highly resistant lines that could be used as parents in a breeding program. Pedigree information indicates that IL97-6755 is the only parent of the three parents in this population that carries the 'Sumai-3' derived Chinese source of resistance that has been characterized as *Fhb1* (Pumphrey et al., 2007). IL97-1828 and IL96-6472, however, do not appear to have Chinese sources of resistance and are believed to have a native source of resistance. Unfortunately, due to the nature of this population and the fact that DArTs are a dominant marker system, for some QTL it is impossible to determine the specific parent that donated a certain allele. Depending on the allele scores and effect of specific QTL, I was able to narrow the possible parent donating an allele to one of two of the parents. In some cases I was able to determine a specific parent that donated

an allele for increased resistance based on allele scores. It was my goal to combine the sources of resistance from the three resistant parents to obtain lines that exhibited higher levels of resistance than the three parental lines. The presence of transgressive segregants for all traits indicates that some of the lines in this experiment do have higher levels of resistance than the three resistant parents.

The thirteen lines that were significantly better for FHB resistance measurements than the mean of the three resistant parents are very promising for use in a breeding program to help introduce FHB resistance. These thirteen lines identified as being transgressive segregants have inherited many of the alleles associated with QTL that confer the increased level of resistance to the various FHB measures (Table 2.6). Ten of the twelve most resistant RILs carried markers associated with resistance QTL on 3Bs (assumed to be *Fhb1*)(Pumphrey et al., 2007). Also, 12 of the most FHB resistant RILs have the alleles associated with the QTLs found on either 1A or 7B that confer FHB resistance. Of the twelve most FHB resistant RILs, six carried markers for the resistance allele in all three regions, five carried markers associated with putative resistance QTL for two regions and one line carried only one putative resistance QTL.

The markers associated with FHB resistance on 3Bs are likely associated with *Fhb1,* and therefore are the best candidates for marker assisted selection in a breeding program. Markers that are used for marker assisted selection should be informative across populations (Van Sanford et al., 2001). Markers associated with 3Bs are therefore excellent candidates for marker assisted selection. Further investigation of the other small effect QTL in different populations would be necessary before using the small effect QTL found in this study for marker assisted selection. While this marker data on the individual lines is very useful for helping to develop the most resistant lines, it is also important to investigate the agronomic characteristics of these lines.

Although it is possible to combine multiple sources of resistance to obtain highly resistant transgressive segregants, some studies have shown it is very difficult to combine high levels of resistance with good agronomic traits (Jiang et al., 2006; Liu et al., 2007). FHB is a quantitatively inherited trait and therefore, selecting for a single molecular marker will not replace phenotypic evaluation; however, molecular markers can be used to assist in making selections prior to phenotypic evaluation to enrich populations for resistant genotypes (Van Sanford et al., 2001).

The RIL population as a whole was relatively resistant to all FHB resistance measurements. The population had a broad distribution for all measured traits except for the 2008 greenhouse evaluation which had a very large number of individuals with a low severity. For this reason, spray inoculation was used in the 2009 greenhouse evaluation instead of the needle inoculation method used in 2008. While this change in technique did provide a wide, normally distributed population, there were no QTL found for the 2009 greenhouse evaluation. The broad and continuous distributions were not surprising as resistance to FHB is a complex quantitative trait (Bai and Shaner, 1994).

The nature of this population may have caused fewer polymorphic markers to have been detected than would have been observed in a bi-parental resistant by susceptible derived population. Although a lower level of polymorphism may be expected in a cross between resistant adapted lines, 293 polymorphic markers is a sufficient number. The three-way cross may have provided the higher levels of polymorphism with three parents to provide different alleles. It would be beneficial to do bi-parental QTL analysis of each of the three parents used in this study using the same susceptible parent for each population. One population has already been developed and studied using IL97-1828. Examining a bi-parental mapping population of

IL96-6472 and IL97-6755 would provide additional polymorphic markers and would also aid in the determination of allele origin.

These bi-parental populations would also aid in QTL identification. It is likely that there were other QTL that could not be identified in this population due to the presence of three resistant parents. If all three parents carried an identical resistance allele, it would not be identified in QTL analysis due to the lack of polymorphism at linked markers. Additionally, if QTL inherited from the parents were tightly linked and in repulsion, it is possible for these QTL to "cancel each other out" preventing detection. While these issues suggest that more QTL may be present in the population, it is clear that both major and minor QTL that we identified are important for inheritance of FHB resistance.

Environment can significantly affect the level of FHB resistance for a line or cultivar (Ma et al., 2006). Ideally, additional years and environments would be beneficial for confirming QTL found in this study that involved only one year of field evaluation. Evaluation in the greenhouse only detected one QTL in 2008 and none in 2009. The 2008 greenhouse evaluation did confirm the QTL found on chromosome 3Bs linked to barc133 that was also found for multiple other field evaluation parameters. Although this experiment included limited environments, aligning our QTL to QTL found in other studies indicates that the QTL that we identified are not false positives. This study was successful in creating transgressive segregants with very high levels of resistance derived from multiple parents with different sources of resistance. Using these FHB resistant lines in a breeding program will introduce high levels of resistance from lines that are relatively well adapted with fair agronomic characteristics. Although further investigation for the minor effect QTL would be beneficial, the QTL in this study could also be used for screening breeding materials to aid in selection of individuals with high levels of resistance to FHB.

TABLES AND FIGURES

Table 2.1 Means, broad-sense heritability estimates (H^2), and 90% confidence intervals (CI) for heritability estimates for Fusarium head blight (FHB) measures in the greenhouse (GH) for mean severity (SVR) and field nursery mean incidence (INC), severity, Fusarium damaged kernels (FDK), deoxynivalenol concentration, FHB index and ISK index for the three parental wheat lines (IL96-6572, IL97-6755, IL97-1828), the derived recombinant inbred line (RIL) population and four check cultivars. 'Sisson' is a very susceptible cultivar while 'Ernie' and IL00-8061 are moderately resistant and IL02-18228 is very resistant. Transgressive segregants indicate the number of RILs with a mean significantly better than the mean of the three resistant parents for each respective FHB resistance measure. LSD signifies Fisher's LSD value at 0.05.

				Parent	RIL	RIL						Transgressive		90% CI
Trait	IL96-6472	IL97-6755	IL97-1828	Mean	Mean	Std. Dev.	Sisson	Ernie	IL00-8061	IL02-18228	LSD	Segregants	H^2	H^2
Field mINC	83.3	73.3	85	80.5	84.4	8.1	98.3	71.7	78.3	70	10.9	6	0.71	.64-.76
Field mSVR	34.5	38.1	42.3	38.3	42.4	13.9	63.8	43	34.6	30.6	15.8	15	0.79	.74-.83
Field mFDK	20	6.7	8.3	11.7	17.3	12	58.3	45	23.3	11.7	13.5	0	0.81	.76-.84
Field mDON	7	3.2	5.6	5.3	4.8	2.2	11.1	11	5.6	3.1	1.91	73	0.89	.87-.91
Field FHB Index	58.9	55.7	63.7	59.4	63.4	9.5	81.1	57.4	56.5	50.3	10.4	15	0.81	.76-.84
Field ISK Index	43.4	36.1	41.5	40.3	44.9	7.2	72	52.4	43.2	34.9	9.4	11	0.84	.80-.87
2008GH SVR	10.2	28.1	4.6	14.3	14.1	15.4	38.5	-	-	-	-	-	-	-
2009GH SVR	28.1	61.8	38.2	42.7	48.8	32.2	93.6	21.4	-	-	63.9	0	0.49	.38-.58

Table 2.2 Transgressive segregants found in a wheat recombinant inbred line (RIL) population derived from a three-way cross of Fusarium head blight (FHB) resistant parents where a transgressive segregant was defined as being significantly more resistant than the mean value of the three resistant parents for mean incidence, severity, deoxynivalenol (DON) concentration, FHB index, ISK index, greenhouse 2009 mean severity and Fusarium damaged kernels. An 'x' indicates that the RIL is significantly better than the mean of the parents for the respective FHB resistance measure, a '-' indicates that the RIL is not significantly different than the mean of the parents for the respective FHB resistance measure, and a '*' indicates that the RIL was numerically better than the best parent, but was not significantly different than the mean of the parents.

RIL	Incidence	Severity	DON	FHB Index	ISK Index	GH2009 SVR	FDK
2**	x	x	x	x	x	*	*
18	x	x	x	x	x	-	*
42	-	x	x	x	x	-	-
55**	-	x	x	x	x	-	-
77	x	-	-	x	-	-	-
168**	x	x	x	x	x	-	*
173	x	x	x	x	x	*	-
174	-	x	x	x	x	-	-
227	-	-	x	x	-	-	*
259	-	x	x	x	x	-	-
266	-	-	x	x	-	-	*
283	-	x	x	x	x	-	-
284	-	x	x	x	x	-	*

** Indicates line was significantly better than the most resistant parent for at least two FHB resistance measures.

Table 2.3 Correlations among Fusarium head blight (FHB) resistance measures taken on a wheat recombinant inbred line population (RIL) developed from a three-way cross involving three FHB resistant parents (IL96-6572, IL97-6755, IL97-1828) in the field phenotypic evaluation grown in Urbana, IL in 2010.

Parameter	INC	SVR	FDK	DON	FHB Index	ISK Index
INC	1					
SVR	0.45	1				
FDK	0.39	0.49	1			
DON	0.33	0.44	0.6	1		
FHB Index	0.76	0.92	0.52	0.46	1	
ISK Index	0.68	0.82	0.85	0.6	0.89	1

-All correlations significant at the $P<0.0001$ level.

Table 2.4 Correlations among three phenotypic evaluations of mean Fusarium head blight (FHB) severity(SVR) taken on a wheat recombinant inbred line population (RIL) developed from a three-way cross derived from three FHB resistant parents (IL96-6572, IL97-6755, IL97-1828) in two years of phenotypic greenhouse evaluations and a single year of phenotypic evaluation in a field inoculated, mist irrigated nursery. A needle inoculation technique was used in the 2008 greenhouse evaluation and a spray inoculation technique was used in the 2009 greenhouse evaluation.

Parameter	2008GH SVR	2009GH SVR	Field SVR
2008GH SVR	1		
2009GH SVR	0.11	1	
Field SVR	0.19**	0.3*	1

**correlation significant at P<0.0001
*correlation significant at p≤.05

Table 2.5 Overview of quantitative trait loci (QTL) found for Fusarium head blight (FHB) disease measures in the field evaluation study for severity (SVR), incidence (INC) FHB index, ISK index, Fusarium damaged kernels (FDK) and deoxynivalenol concentration (DON), in a wheat recombinant in bred line population derived from a three-way cross of FHB resistant parents (IL96-6572, IL97-6755, IL97-1828). Source indicates the parent from which the allele conferring resistance was derived.

Trait	Chr	Marker	LOD value	R^2
Field SVR	3B	wPt-8446/wPt-7225	5.6	9.6
	1A/5B	wPt-3904	3.77	6.6
Field INC	3B	wPt-8446/wPt-7225	2.8	4.9
	1A	wPt-5934*	3.1	5.3
FDK	3B	wPt-8446/wPt-7225	3.91	6.8
	7B	wPt-9215	4.6	8.2
FHB	3B	wPt-8446/wPt-7225	5.36	9.2
ISK	3B	wPt-8446/wPt-7225	5.68	9.7
	7B	wPt-9215	2.97	5.4
	1A/5B	wPt-3904	3.25	5.7
DON	3B	wPt-8446/wPt-7225	5.92	10.1

*Markers wPt-5587, wPt-667472, wPt-6870, wPt-3459, wPt-6575 and wPt-6627 also mapped to this position.

Table 2.6 Transgressive segregants found in a wheat recombinant inbred line (RIL) population derived from a three way cross of Fusarium head blight (FHB) resistant parents where a transgressive segregant was defined as being significantly more resistant than the mean value of the three resistant parents for various FHB resistance measures. Table shows resistance alleles associated with QTL that confer increased levels of FHB resistance where an '*' indicates the RIL carries the resistance allele for marker linked to the respective QTL found on chromosomes 3B, 7B and 1A, a '-' indicates resistant allele is not present and 'N/A' indicates there is no marker data for the RIL.

RIL	3Bs	7B	1A
2	*	N/A	*
18	*	*	*
42	-	*	*
55	*	*	-
77	-	*	-
168	*	-	*
173	*	*	*
174	*	*	*
227	*	*	*
259	*	*	*
266	*	*	-
283	*	*	*
284	N/A	N/A	N/A

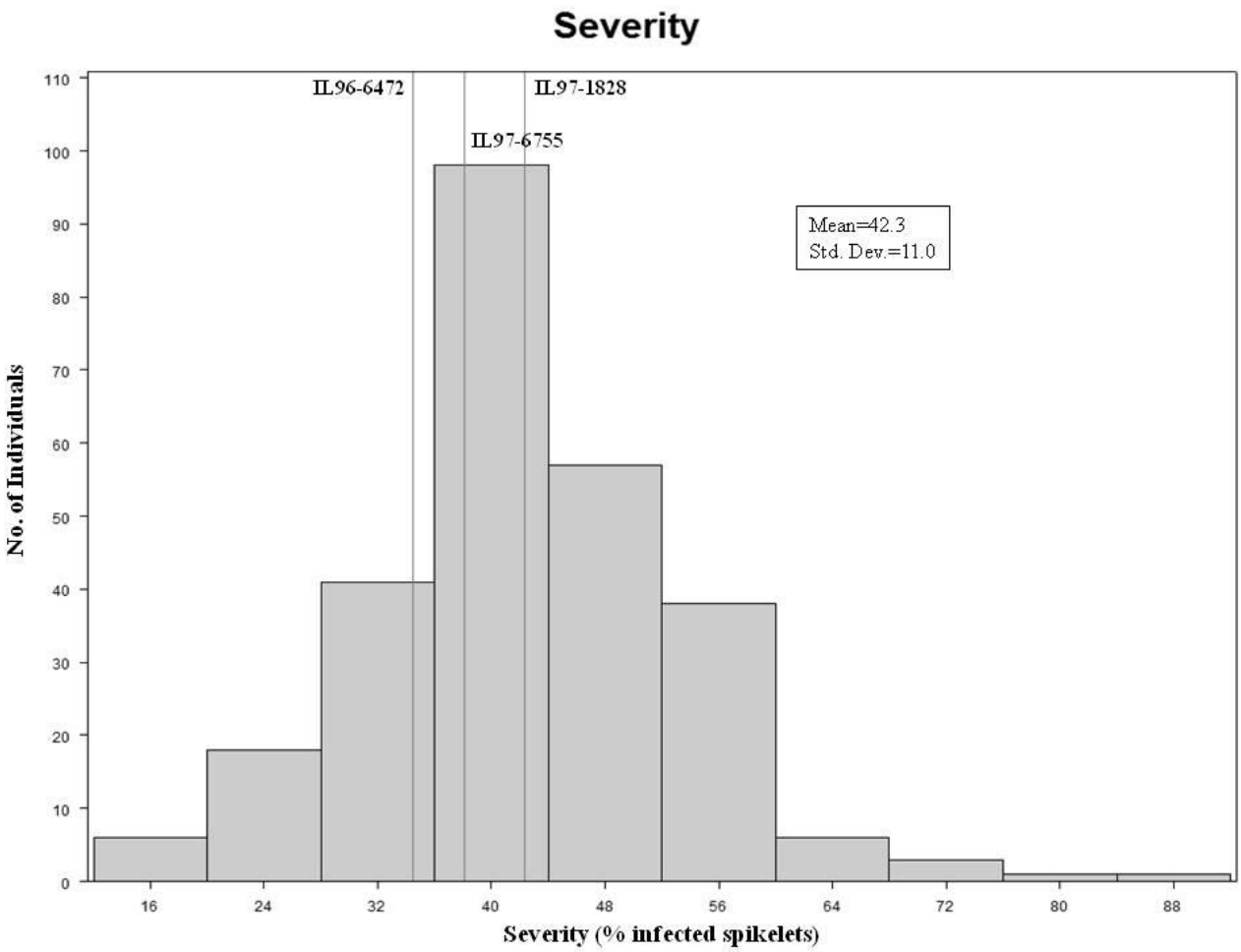

Figure 2.1 Frequency distribution of 266 soft red winter wheat recombinant inbred lines developed from the cross IL96-6472/IL97-6755//IL97-1828 for average severity in the field evaluation in 2010.

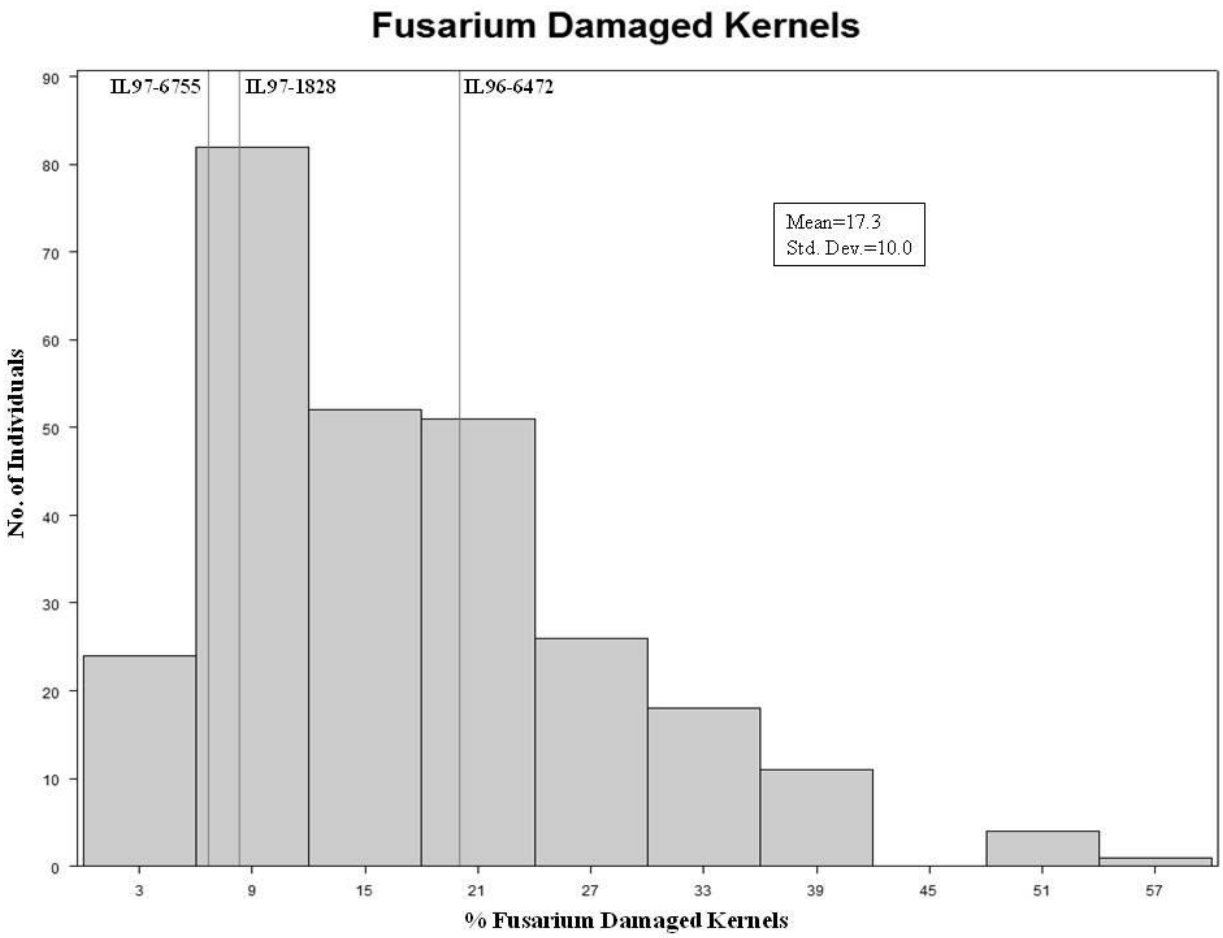

Figure 2.2 Frequency distribution of 266 soft red winter wheat recombinant inbred lines developed from the cross IL96-6472/IL97-6755//IL97-1828 for average percentage of Fusarium damaged kernels in the field evaluation in 2010.

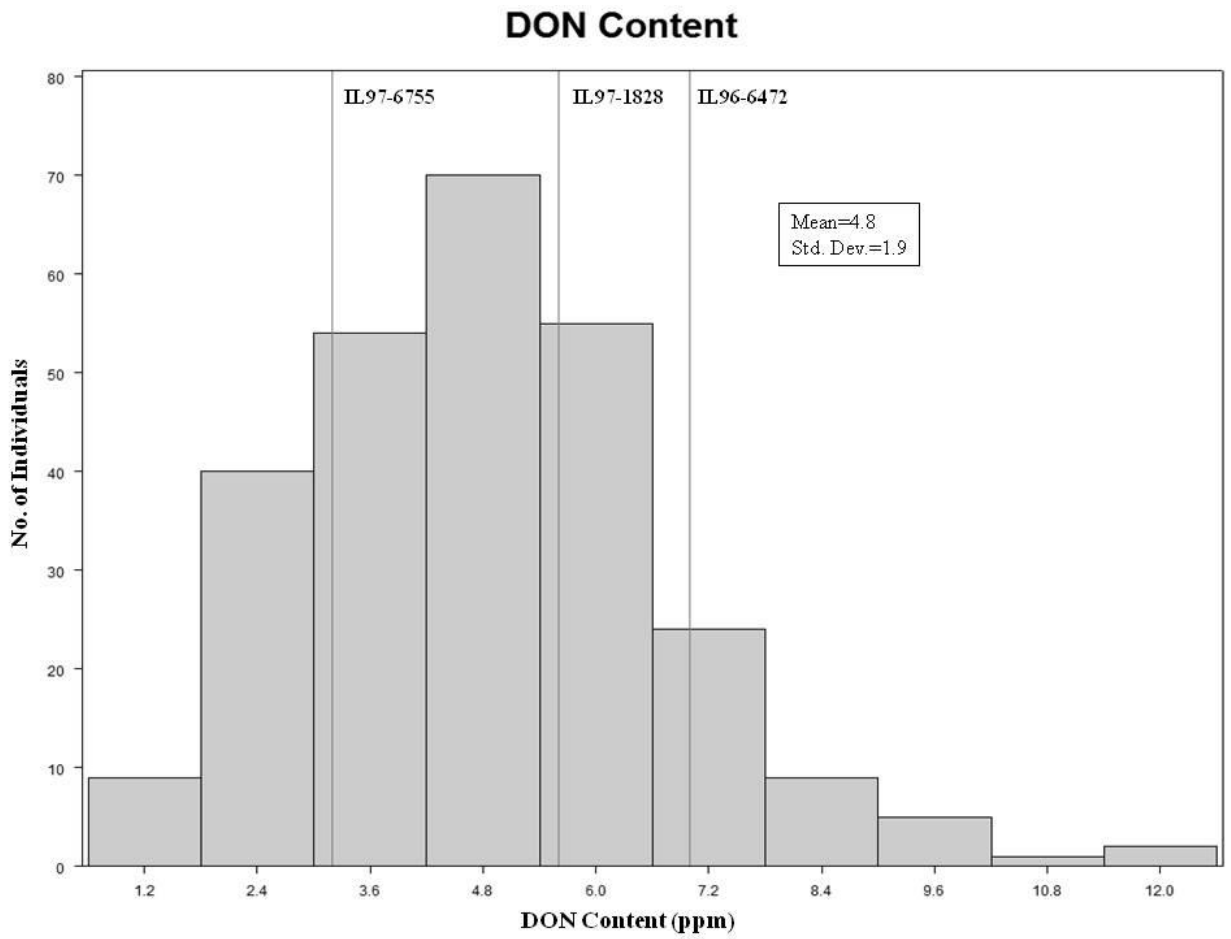

Figure 2.3 Frequency distribution of 266 soft red winter wheat recombinant inbred lines developed from the cross IL96-6472/IL97-6755//IL97-1828 for average deoxynivalenol concentration (ppm) in the field evaluation in 2010.

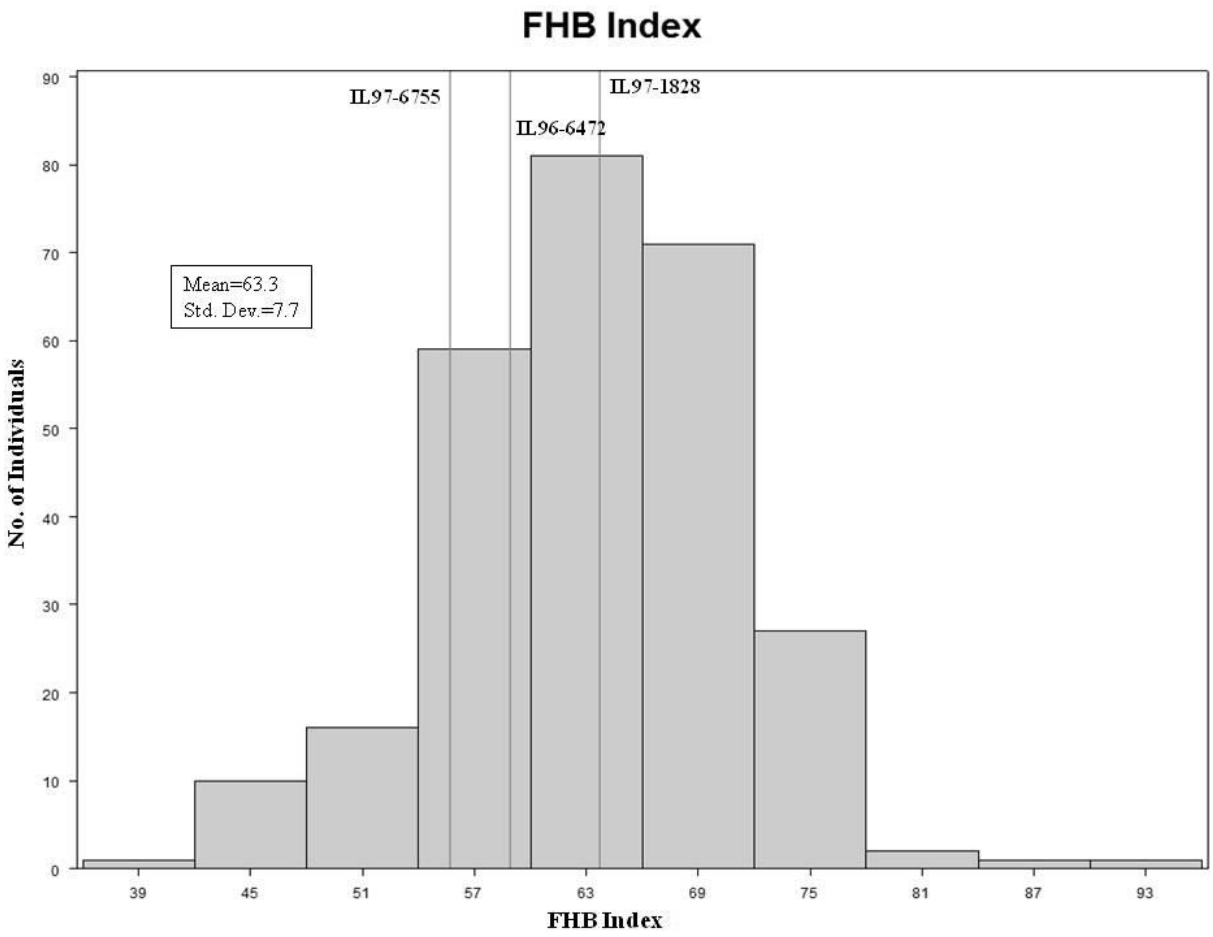

Figure 2.4 Frequency distribution of 266 soft red winter wheat recombinant inbred lines developed from the cross IL96-6472/IL97-6755//IL97-1828 for average FHB index value in the field evaluation in 2010.

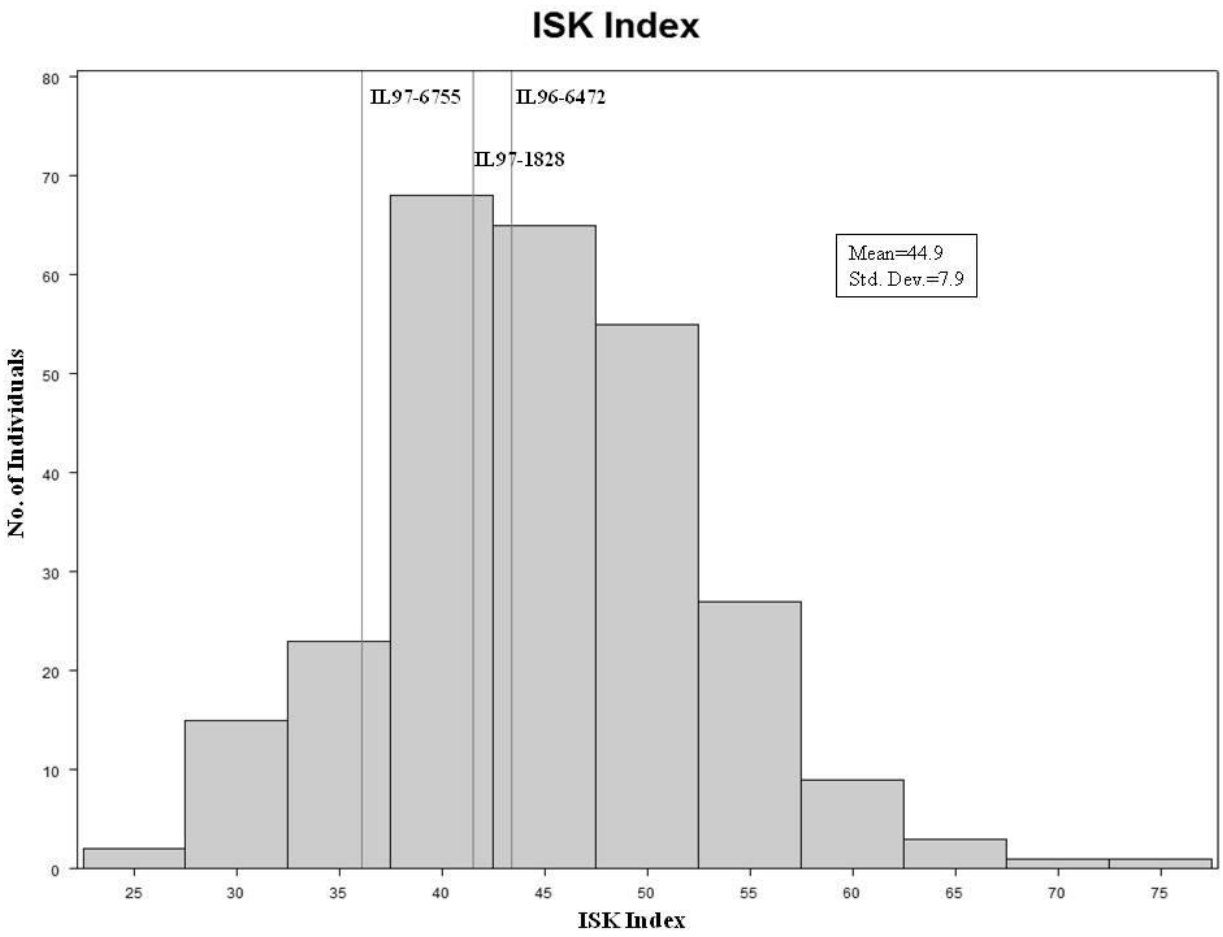

Figure 2.5 Frequency distribution of 266 soft red winter wheat recombinant inbred lines developed from the cross IL96-6472/IL97-6755//IL97-1828 for average Incidence, Severity, Kernel rating (ISK) index value in the field evaluation in 2010.

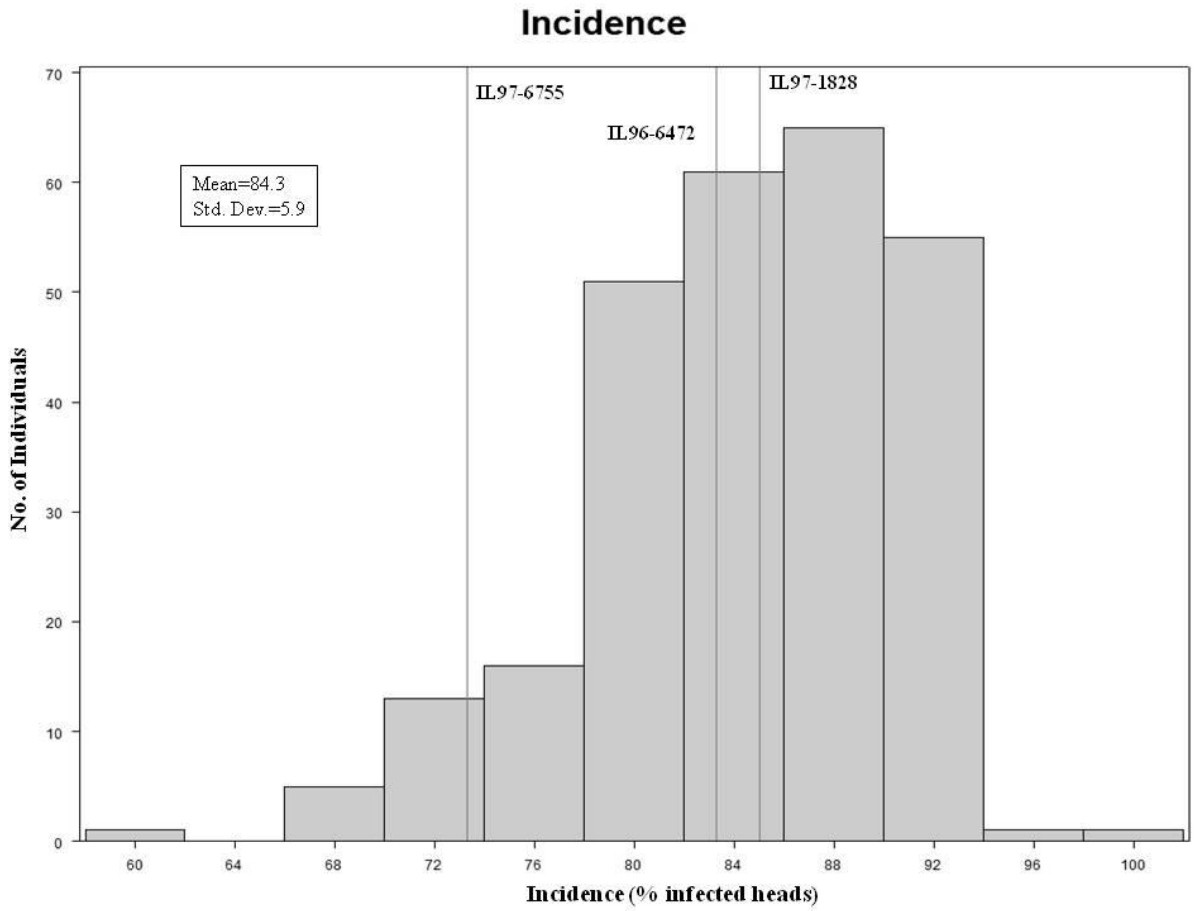

Figure 2.6 Frequency distribution of 266 soft red winter wheat recombinant inbred lines developed from the cross IL96-6472/IL97-6755//IL97-1828 for average incidence in the field evaluation in 2010.

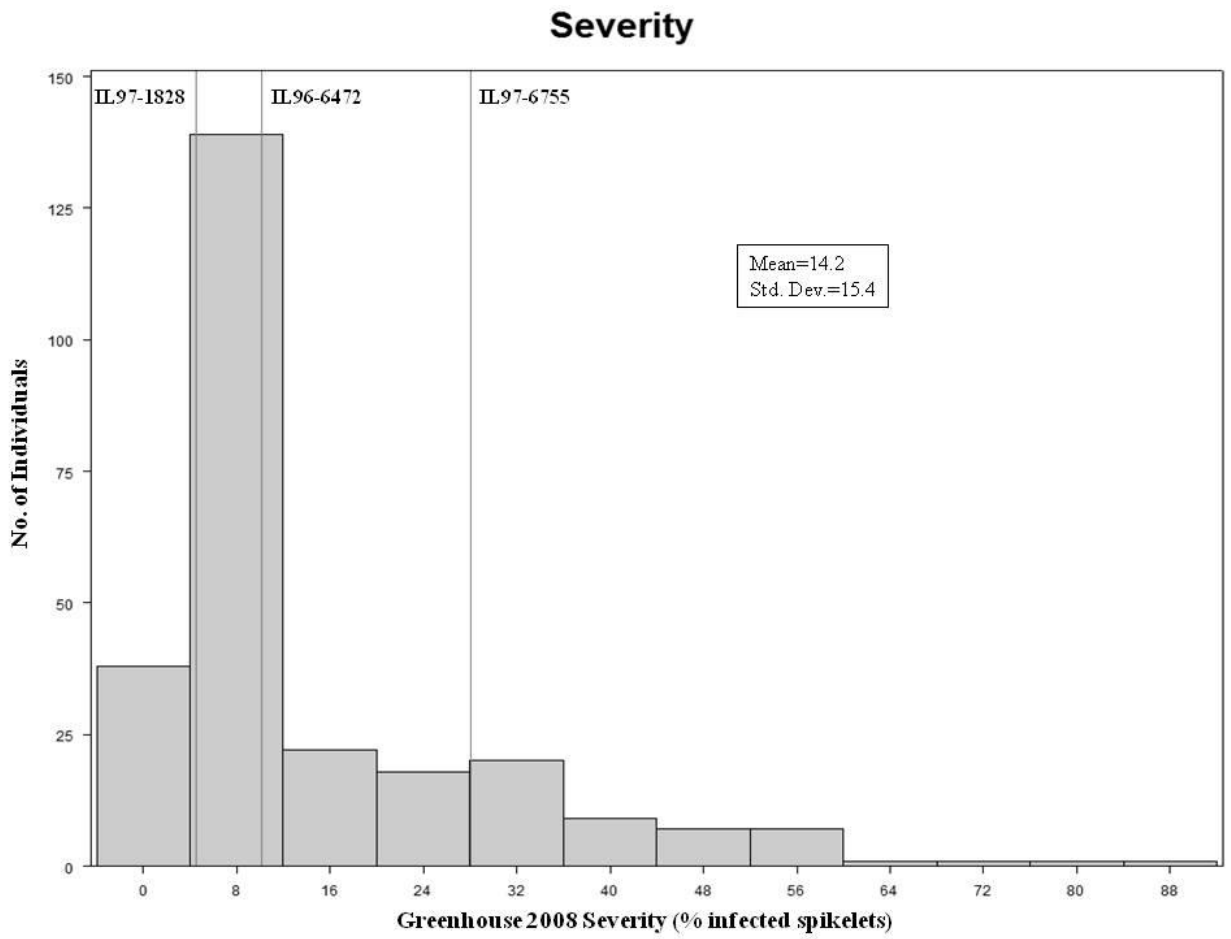

Figure 2.7 Frequency distribution of 266 soft red winter wheat recombinant inbred lines developed from the cross IL96-6472/IL97-6755//IL97-1828 for average severity in needle inoculated greenhouse evaluation in 2008.

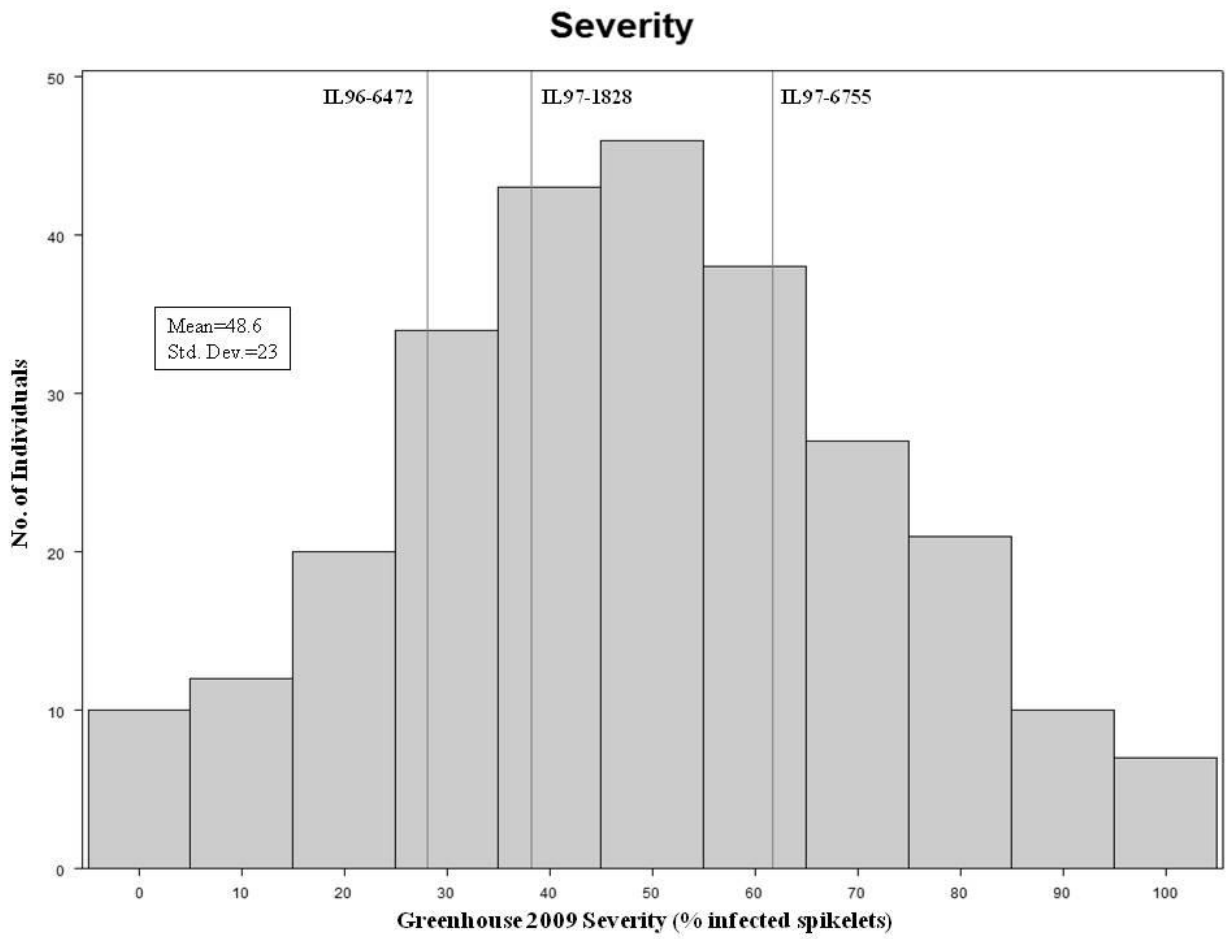

Figure 2.8 Frequency distribution of 266 soft red winter wheat recombinant inbred lines developed from the cross IL96-6472/IL97-6755//IL97-1828 for average severity in the spray inoculated greenhouse evaluation in 2009.

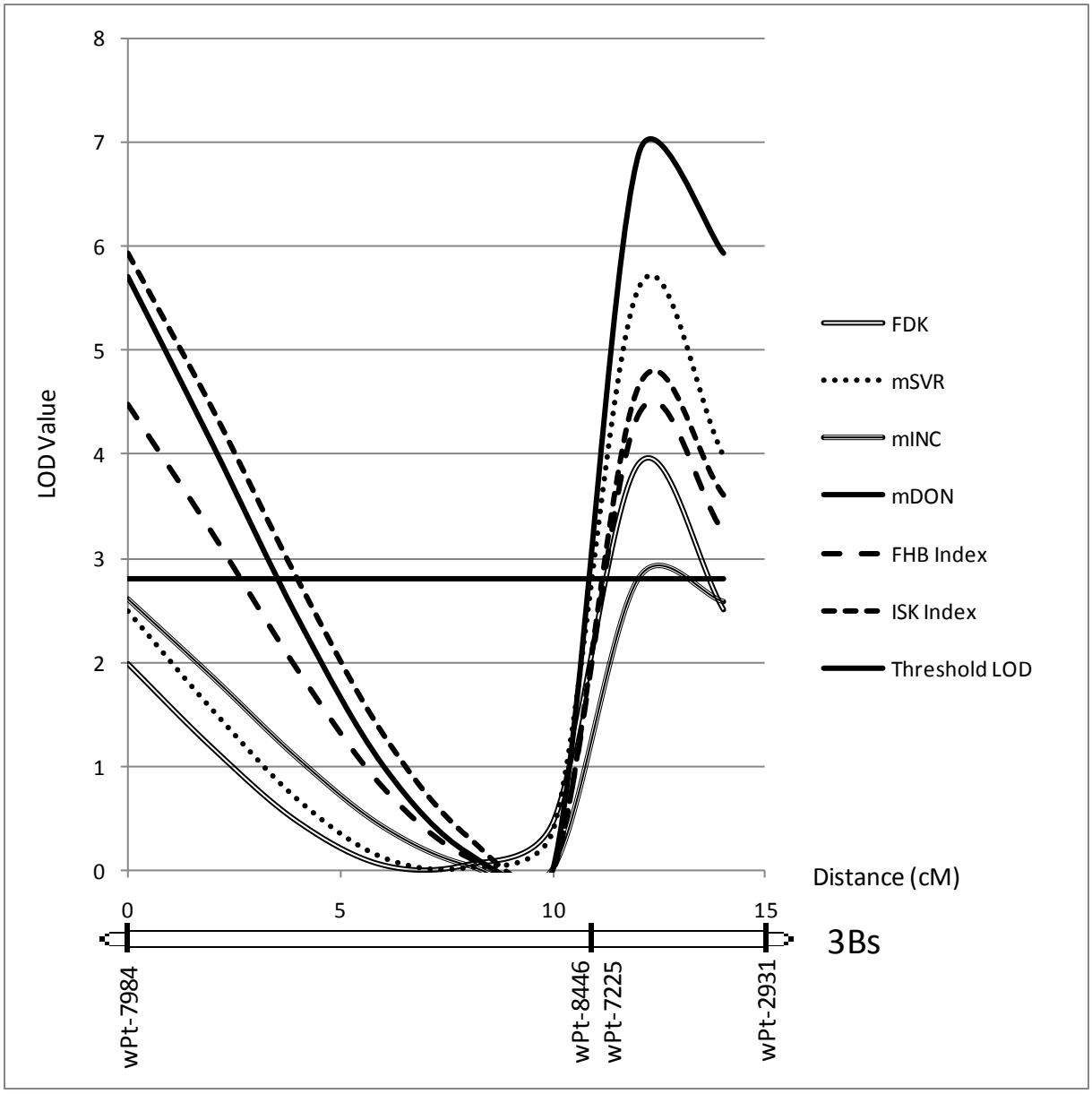

Figure 2.9 Linkage map of chromosome 3Bs derived from a recombinant inbred line population developed from a cross between three Fusarium head blight resistant parents. Quantitative trait loci LOD graphs for mean severity, mean incidence, mean DON concentration, Fusarium damaged kernels, FHB index and ISK index with a critical LOD threshold of 2.8 are also depicted above the linkage map.

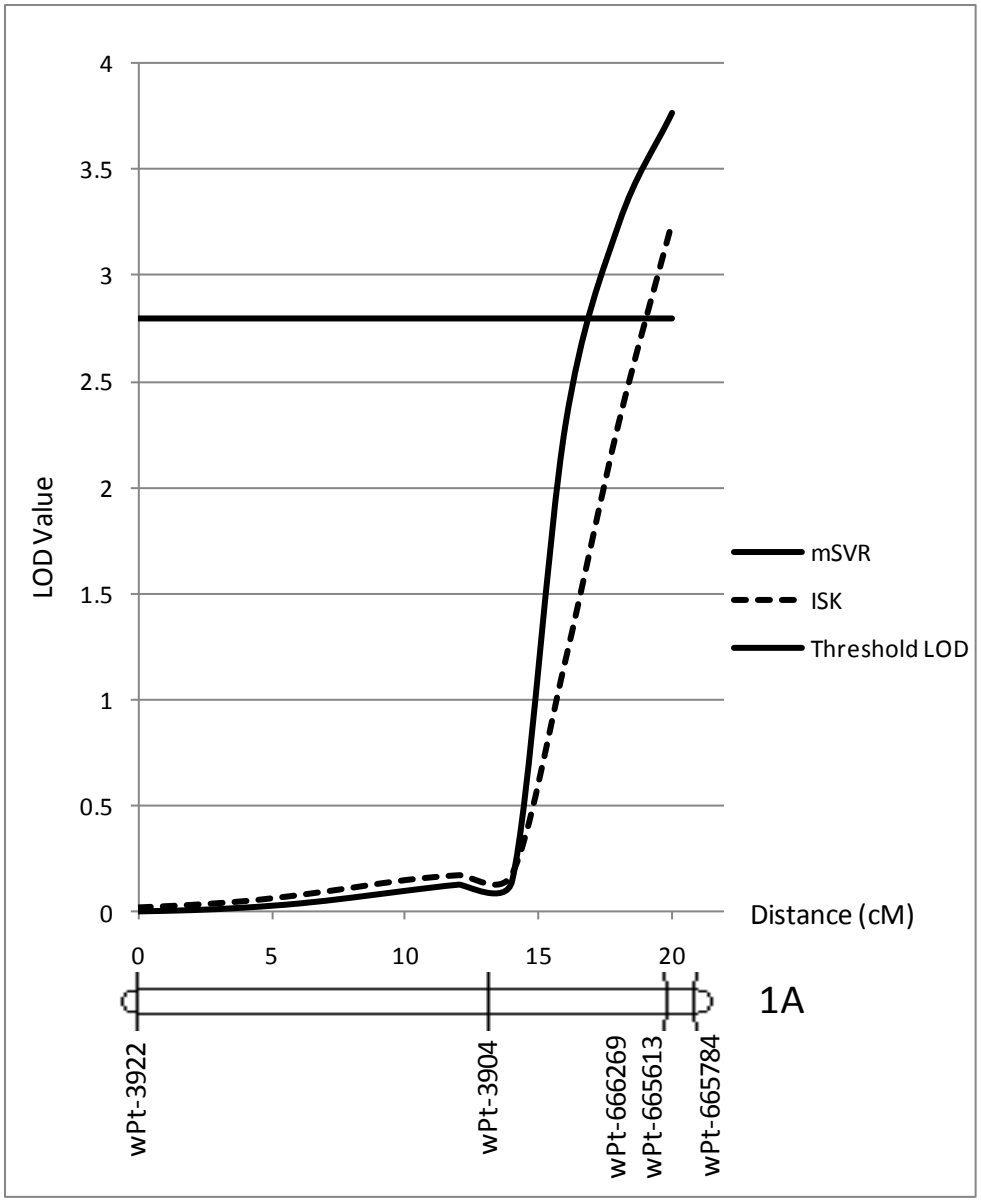

Figure 2.10 Linkage map of chromosome 1A derived from a recombinant inbred line population developed from a cross between three Fusarium head blight resistant parents. Quantitative trait loci LOD graphs for mean severity and ISK index with a critical LOD threshold of 2.8 are also depicted above the linkage map.

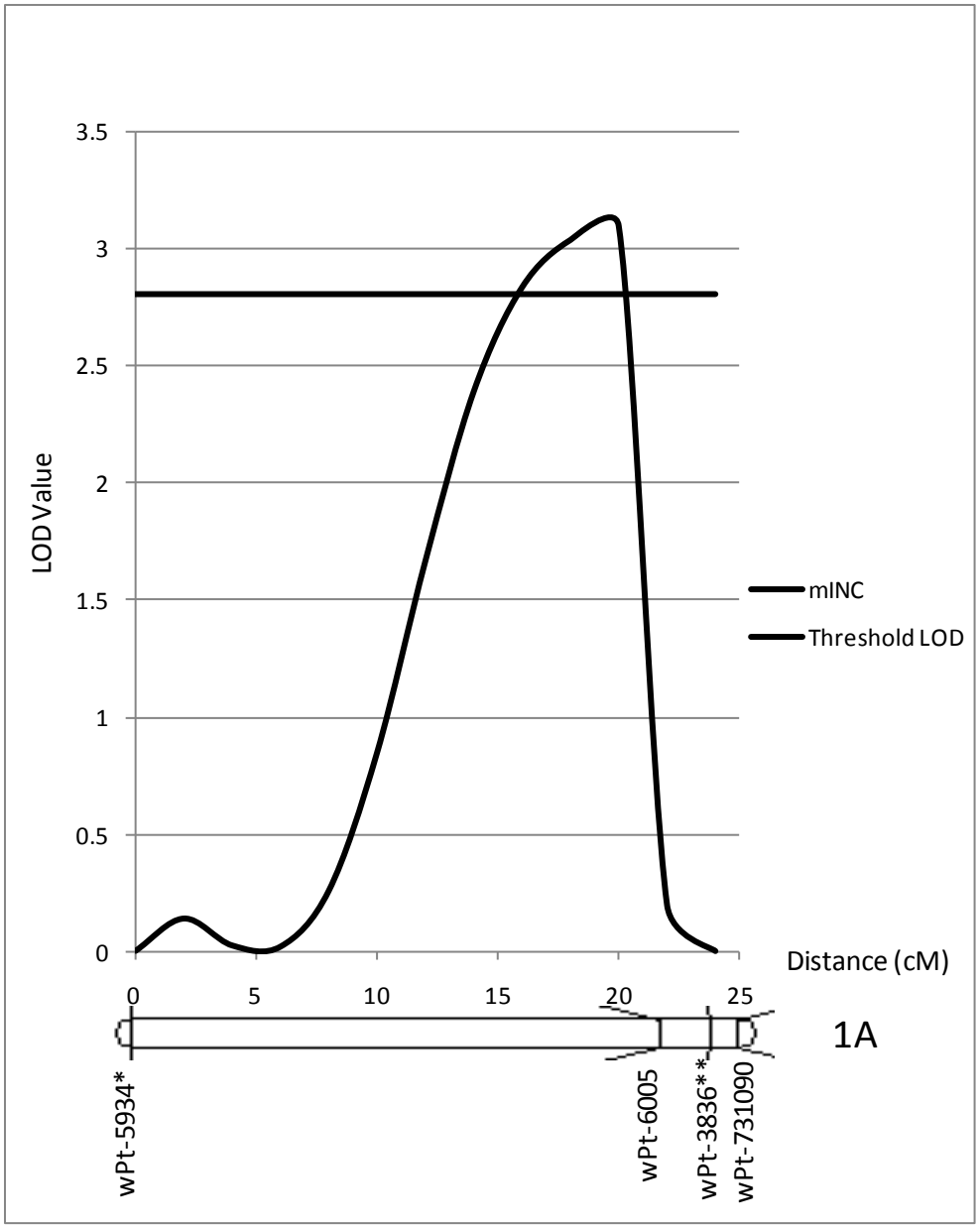

Figure 2.11 Linkage map of chromosome 1A derived from a recombinant inbred line population developed from a cross between three Fusarium head blight resistant parents. Quantitative trait loci LOD graph for mean incidence with a critical LOD threshold of 2.8 are also depicted above the linkage map.

*Markers wPt-5587, wPt-667472, wPt-6870, wPt-3459, wPt-6575 and wPt-6627 also mapped to this position.

**Markers wPt-8644, wPt-5274, wPt-669294, wPt-2406, wPt-669800, wPt-4709, wPt-6754, wPt-667288, wPt-730902, wPt-666087, wPt-0164, wPt-2976 also mapped to this position.

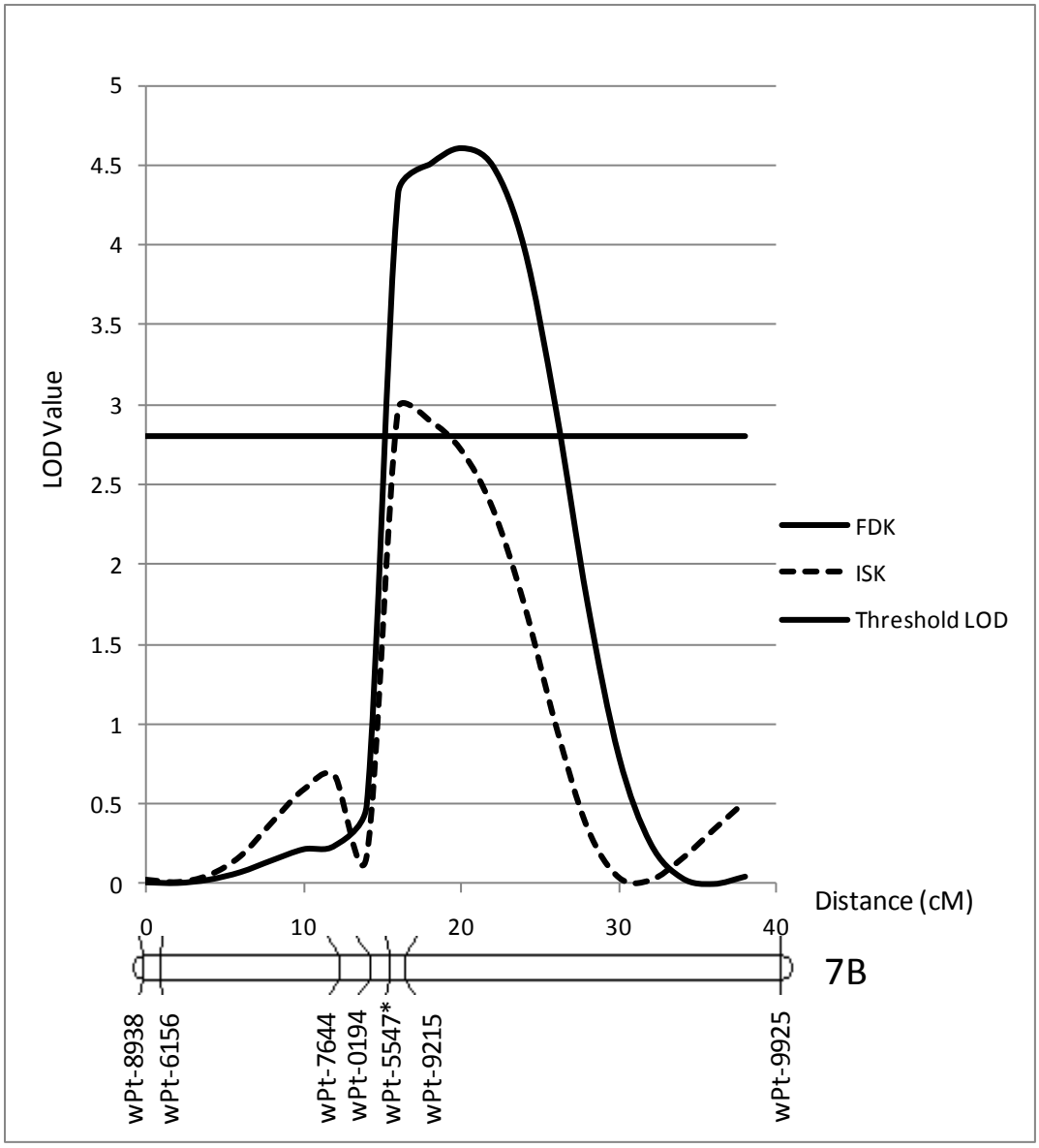

Figure 2.12 Linkage map of chromosome 7B derived from a recombinant inbred line population developed from a cross between three Fusarium head blight resistant parents. Quantitative trait loci LOD graphs for Fusarium damaged kernels and ISK index with a critical LOD threshold of 2.8 are also depicted above the linkage map.
*Markers wPt-664119 and wPt-5280 also mapped to this position.

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APPENDIX A

Figure A.1 The known pedigree information for University of Illinois soft red winter wheat breeding line IL97-6755.

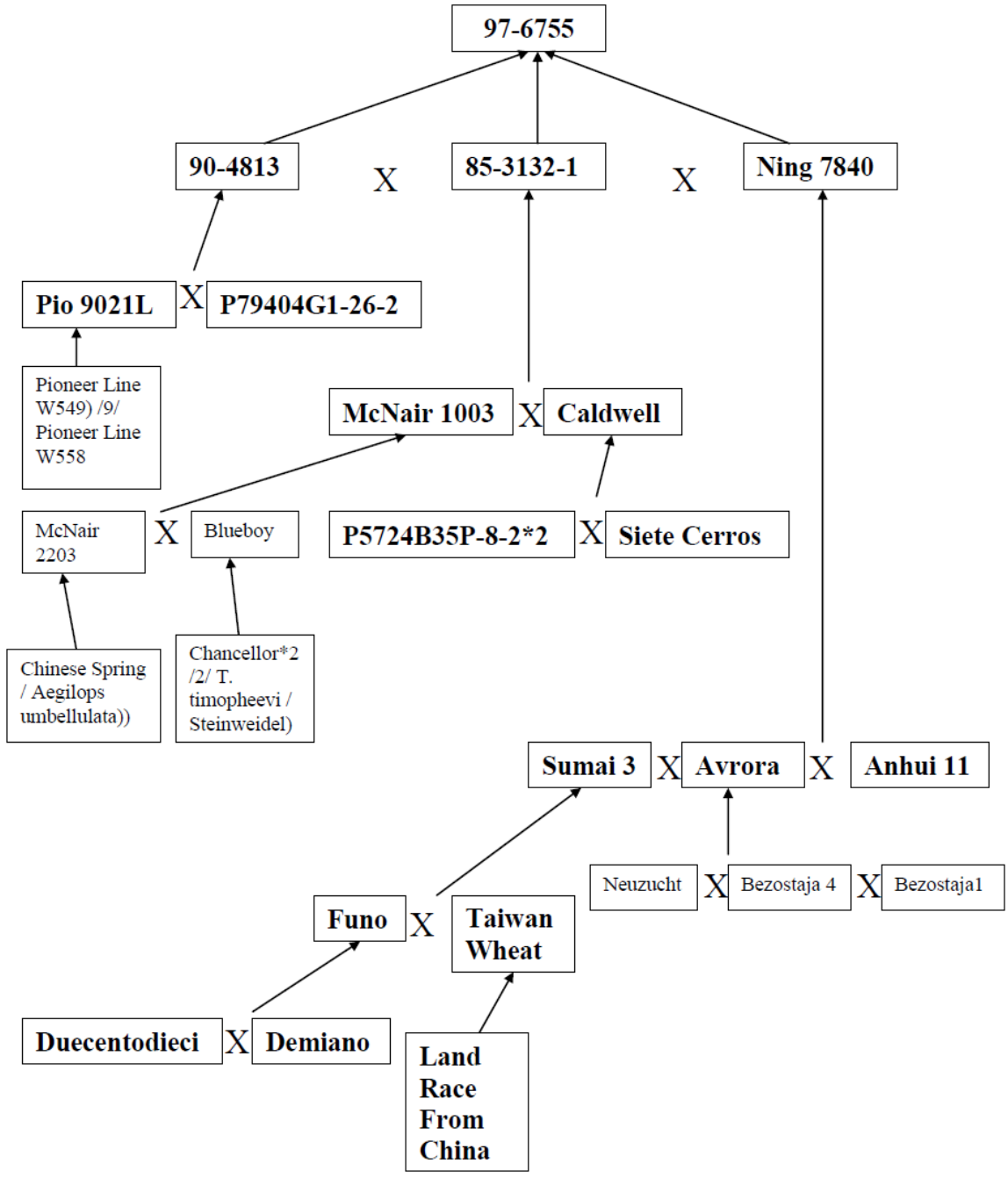

Figure A.2 The known pedigree information for University of Illinois soft red winter wheat breeding line IL97-1828.

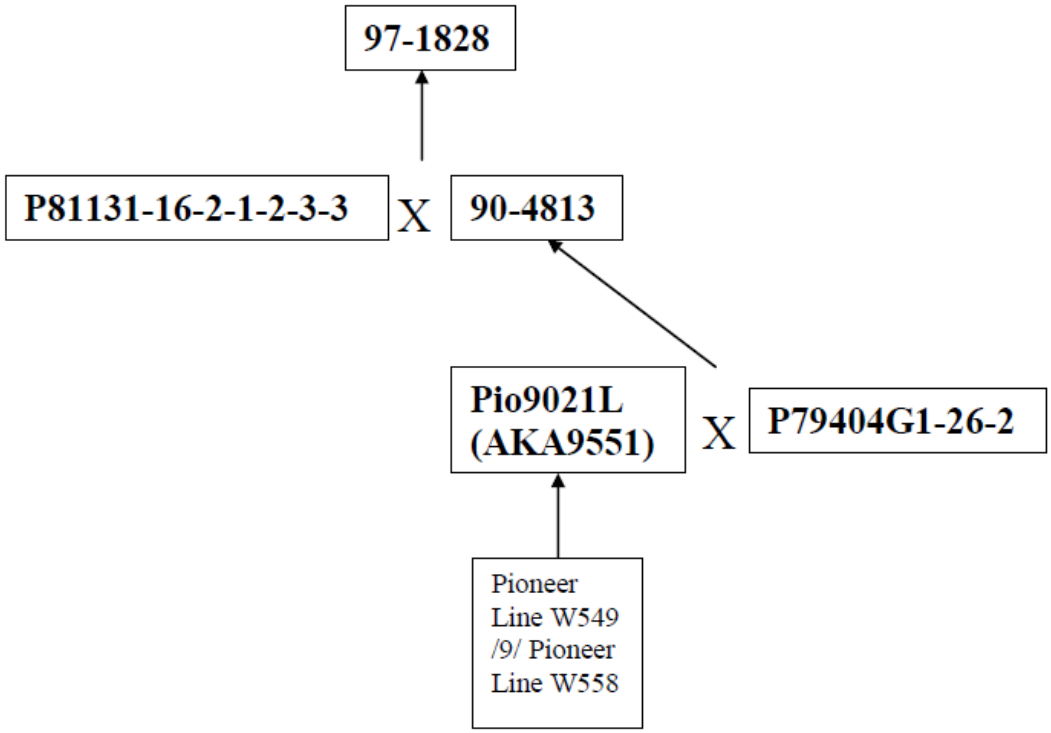

Figure A.3 The known pedigree information for University of Illinois soft red winter wheat breeding line IL96-6472.