Analysis of Resistance to Fusarium Head Blight (FHB) in Winter Wheat and Evaluation of Genetics and Cultural Practices for FHB Mitigation

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Abstract

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Fusarium head blight (FHB) caused by *Fusarium graminearum* is a fungal disease of wheat that can result in severe yield losses and contaminate grain with deoxynivalenol (DON). Wheat cultivars with different levels of FHB resistance were combined with fungicides application to control FHB. Results showed that foliar fungicide Prosaro™ combined with moderately resistant cultivars greatly reduced the risk of FHB. Integrating fungicide application with moderately resistant cultivars can be an effective strategy in controlling FHB. Quantitative trait loci (QTL) for resistance to FHB related traits were analyzed using a double haploid population. Four QTL associated with FHB resistance was detected on chromosomes 2B, 2D, 4D and 7A. The QTL on chromosome 2B and 4D were found to reduce multiple FHB-related traits and were more frequently detected than QTL on chromosome 2D and 7A. QTL on chromosome 2B and 4D could be valuable for improving FHB resistance in wheat.

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1.0 General introduction

Fusarium head blight (FHB), is one of the most destructive fungal diseases in wheat (*Triticum aestivum* L.) primarily caused by *Fusarium graminearum* Schwabe [telomorph:*Gibberella zeae* Schwein (Petch)], in North America (McMullen et al. 1997; Savard et al. 2000; Peiris et al. 2011). Wheat is most susceptible to FHB during the flowering stage (Bai and Shaner 1996). Infected spikes show premature bleaching and produce shriveled kernels, which are called Fusarium damaged kernels (FDK) and can lead to large yield loss and compromised end use quality (McMullen et al. 1997; Savard et al. 2000; Peiris et al. 2011). Production of trichothecene mycotoxins causes additional losses. Mycotoxins such as deoxynivalenol (DON) and its acetylated derivatives, 3-acteyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON) in FHB infected grain limit the use of the grain for food and feed purposes, thus lower the marketing value. *Fusarium* spp. can also cause seedling blight in wheat and severe infection can lead to poor establishment of crop stands (Jorgensen et al. 2012).

Several strategies can be used to control FHB and DON contamination in wheat, including cultural practices, fungicide application and use of resistant cultivars. Crop debris including maize stalks and grain, straw of barley, wheat and other cereals are important reservoirs of inoculum (Miller et al. 1998; Dill-Macky and Jones, 2000; Maiorano et al. 2008). FHB disease and DON contamination are more severe if the preceding crop was maize and are less severe following other crops such as soybean (Dill-Macky and Jones 2000). Removing or burying previous crop residues reduces

primary inoculum, thus the risk of FHB can be reduced.

Application of fungicides plays an important role in integrated FHB management to reduce FHB and mycotoxin contamination (Amarasinghe et al. 2013). However, the effectiveness of fungicides can be inconsistent. Fungicides with the active ingredients of cyproconazole, tebuconazole and azoxystrobin were found to significantly reduce FHB disease and DON contamination (Haidukowski et al. 2005). However, in Nakajima's (2010) field research, azoxystrobin was found to increase DON level significantly. In general, fungicides containing triazole (tebuconazole, metconazole and prothioconazole), imidazole or triazolinthione active ingredients, which inhibit the biosynthesis of ergosterol, were found to be most effective in controlling FHB disease and DON contamination (Haidukowski et al. 2005; Loss et al. 2005). Because of the complex and, in some cases, conflicting effects of fungicides on FHB management, resistant wheat cultivars should be included in the FHB management strategies to achieve more satisfactory results. Compared with other control strategies, breeding resistant wheat cultivars is considered to be the most effective, environmental friendly and economic measure to reduce the risk of FHB in wheat. Unfortunately, there are no highly resistant commercial wheat cultivars available (Amarasinghe et al. 2013).

Genetic resistance sources used for breeding FHB resistance are relatively limited. Only a few resistant genotypes have been used, including Wangshuibai, Sumai 3 and its derivates Ning 7480 and Ning 894037 from China (Bai and Shaner 2004), Frontana from Brazil and Nobeokabouzu from Japan (Badea et al. 2008; Rudd

et al. 2001)，Praa 8 and Novkrumka from Europe (Gilbert and Ketauz 2000), and Ernie and Freedom from the USA (Rudd et al. 2001). In wheat breeding programs, the extensive use of only a few sources of resistance may induce selection pressure on the pathogen and the effectiveness of the resistant genes involved may be reduced over time. Introduction of new sources of resistance could improve the level of wheat resistance to FHB and obtain more durable resistance. Therefore, it is important for wheat breeders to identify new sources of FHB resistance in the future in order to obtain satisfactory FHB resistance.

FHB resistance in wheat is a quantitative trait controlled by multiple genes on different chromosomes, making it difficult to breed resistant cultivars (Buerstmayr et al. 2009). Evaluation for FHB resistance is resource intensive and requires phenotyping of multiple plants within a large number of wheat breeding lines. DNA-based marker- assisted selection (MAS) is a recent tool that can be used to overcome these issues (McCartney et al. 2007; Buerstmayr et al. 2009). MAS is useful to enrich populations for FHB resistance in early generations by selecting markers or quantitative trait loci (QTL) linked to resistant alleles (McCartney et al. 2007). Therefore, to breed FHB resistant wheat cultivars, the first step is to identify the most promising breeding lines with resistant QTL. More than 100 QTL for FHB resistance in wheat have been reported on all wheat chromosomes except 7D, however, only 22 QTL were detected in more than one mapping population (Buerstmayr et al. 2009). Inconsistent detection of QTL may be due to their minor effects or they were falsely identified due to phenotypic bias caused by environmental effects (Chu et al. 2011).

There is no highly effective single control measure to manage either FHB or mycotoxin contamination in cereals (Yoshida et al. 2008). To effectively reduce the loss due to FHB, an integrated FHB management strategy including cultural practices, fungicide application and resistant cultivars should be considered. The objectives of the present study were to: (1) determine the influence of cultivar selection and seed- and foliar- applied fungicides on grain yield of spring and winter wheat, and investigate the effectiveness of integrating fungicide application and cultivar resistance in controlling FHB and DON accumulation in grain; (2) identify and map QTLs associated with resistance to FHB in a winter wheat double haploid (DH) population derived from a cross between Mironovskaja 808/AC Ron.

2.0 Literature review

2.1 Fusarium head blight (FHB) in wheat

Fusarium head blight (FHB), also called ear blight or scab, is an important fungal disease in wheat (*Triticum aestivum* L.) caused by several *Fusarium* spp. such as *Fusarium graminearum, F. culmorum, F. poae*, *F. avenaceum, F. sporotrichoides, F. verticillioides* and *F. equiseti* (Nicholson et al. 2003; Osborne and Stein 2007). This fungal disease has become one of the most important diseases in wheat production throughout much of the world where wheat is grown. Among the *Fusarium* spp. that cause FHB, *F. graminearum* Schwabe [telomorph:*Gibberella zeae* Schwein (Petch)] is considered to be the predominant pathogen worldwide (Windels 2000; Nicholson et al. 2003; Osborne and Stein 2007). In North America, *F. graminearum* is also the major causal agent of FHB in wheat (McMullen et al. 1997; Savard et al. 2000).

Infection of wheat spikes by *Fusarium* species negatively affects both the grain yield and quality due to reduced grain size, weight, protein content and baking quality of the flour (Parry et al. 1995). In addition to the yield and grain quality losses caused by this disease, the value of harvested grain may be further reduced due to mycotoxin contamination (Savard et al. 2000). FHB is of particular concern in wheat production because of the ability of these fungi to produce a range of mycotoxins in the grain that are harmful to the health of humans and animals. Trichothecenes are the predominant mycotoxins produced by *Fusarium* spp. in cereals (Nicholson et al. 2003). Mycotoxins produced by *Fusarium* spp. may include trithothecenes such as deoxynivalenol (DON) and nivalenol (NIV), as well as zearalenone, moniliformin,

enniatins, beauvericin and fumonisins (Nicholson et al. 2003; Osborne and Stein 2007). In addition to *Fusarium* species, *Microdochium nivale* may also serve as the causal agent of head blight (Nicholson et al. 2003; Osborne and Stein 2007), however, *Microdochium nivale* does not produce mycotoxins and is particularly prevalent in areas with cool, wet conditions (Nicholson et al. 2003). The multiple negative effects of FHB, conducive weather conditions such as high humidity and warm temperature during wheat anthesis, and inadequate FHB resistance in wheat has resulted in FHB becoming the most important cereal disease in Manitoba (Clear and Patrick 2000).

2.2 Economic importance of FHB in wheat

FHB is one of the most important diseases of wheat. In recent years, FHB has re-emerged as a disease of economic importance (Windels 2000; Paulitz 1999). FHB affects the developing wheat spikes and produces shriveled, light-weight kernels known as Fusarium damaged kernels (FDK). FHB may cause severe yield losses during epidemic years. It was estimated that wheat yield loss in North Dakota, Minnesota, South Dakota and Manitoba reached as much as 4.8 million metric tons and yield loss for barley was about 1.6 million metric tons during the FHB epidemic in 1993 (McMullen et al. 1997). In addition to yield losses, grain quality may be negatively affected by contamination of trichothecene mycotoxins which downgrades the marketable level of the grain. FHB outbreaks in Manitoba (1993-1994) and Ontario (1996) caused significant damage to wheat production (Savard et al. 2000). In 1996, 2000, and 2004 severe epidemics of FHB has caused more than $200 million

losses to Ontario's winter wheat industry (Tamburic-Ilincic et al. 2011). FHB epidemics in the US during the 1990s caused over three billion US dollars in losses in wheat and barley production. Therefore, FHB is considered to be the most costly disease of the last half of the 20th century (Paulitz 1999; Windels 2000). McMullen et al (1997), Paulitz (1999) and Windels (2000) summarized the factors that promote FHB disease epidemics. For instance, unseasonably wet weather during wheat anthesis, the prevalence of no-till and low-till agricultural practices that increase host crop residues on the soil surface which allow the pathogen to overwinter and produce more inoculum during wheat growing season, high percentages of farm lands planted to susceptible host crops, short crop rotation intervals between susceptible crops, and lack of resistance to FHB in wheat contribute to FHB epidemics.

2.3 FHB disease cycle

Fusarium spp. survive and over-winter as saprophytic mycelia (Goswami and Kistler, 2004) on, or within, host plant tissue residues such as wheat and maize that are left on the soil surface (Fernando et al. 1997; Paulitz 1999). In the following spring, perithecia and /or sporodochia are formed to produce ascospores (sexual spores) and macroconidia (asexual spores), respectively (Fernando et al. 1997). Ascospores are considered to be the primary inoculum of FHB in eastern North America (Paulitz 1999). Spores can be dispersed by wind, insects or water-splash to wheat spikes (Parry et al. 1995; McMullen et al. 1997). Given adequate moisture, spores start to germinate and infect the spikes. Wheat spikes are susceptible to

infection in the flowering to the soft dough stages (McMullen et al. 1997; Windels 2000), with corresponding Zadoks cereal growth stages range from GS 60 to GS 75. This may be due to the thin-walled flower parts, such as the surface of stigma, which may be penetrated more readily than vegetative organs such as leaves (Ngugi and Scherm 2006). One theory is that the spores germinate and can enter the floret via the filament of the stamen. Another critical factor that may make wheat more susceptible at the flowering stage may be the production of various types of nutrients, such as stigmatic exudates and pollen exudates that can be utilized by the pathogenic fungi (Ngugi and Scherm 2006). Lu et al. (2013) also noted a negative correlation between FHB severity and anther extrusion, which may indicate the important role of anthesis for Fusarium infection. Under warm, wet conditions, mycelium and sporodochia are often formed on the infected spikelets to give rise to macroconidia and may cause infection on secondary tillers that flower later (Paulitz 1999). However, Fusarium head blight is considered to be a monocyclic disease (Fernando et al. 1997; Paulitz 1999), because the wheat spike is most susceptible during anthesis and wheat spike susceptibility would be low when the secondary inoculum is formed. FHB spread between plants is rarely caused by the secondary inoculum (Paulitz 1999).

2.4 Symptoms of FHB

Wheat spikes are most susceptible to *Fusarium* spp. infection during anthesis (Bai and Shaner 1996). Sutton (1982) reported a wide range of temperature (10-30 ºC) for ascospore release and suggested that warm temperature together with a 92-94%

relative humidity during the flowering stage of wheat were ideal for FHB to establish and develop. Primary infection occurs when there are conducive temperature and humidity conditions and ascospores or macroconidia are released from infected host crop residues and deposited on, or inside, flowering florets (Pritsch et al 2000). Wheat florets infected by *Fusarium* spp. first show a tan, brown or bleached discoloration at the base. As disease progresses, the disease symptoms spread to neighboring florets within the same spikelet (Bushnell et al. 2003). Disease symptoms may be limited to one spikelet, or spread to other spikelets if the fungus invades the rachis of the susceptible host. The base of the infected spikelets and portions of the rachis often develop a dark brown color. When weather conditions are conducive for FHB development, the fungus may produce orange to pink colored sporodochia (Paulitz, 1999) or black perithecia on the surface of glumes (Parry et al. 1995). In wheat, the mycelium grows from an infected floret to neighboring florets within wheat spikelet by way of the vascular bundles of the rachilla. Likewise, spread from one spikelet to another occurs through the vascular bundles of rachis (Bushnell et al. 2003). Rachis infection usually causes premature senescence of spikelets above the infected spikelet due to vascular dysfunction in the rachis which blocks the upward delivery of water and nutrients (Savard et al. 2000; Bushnell et al. 2003).

When the fungus invades the developing kernels, it usually causes kernels that are light weighted, shriveled, white and chalky in appearance, known as Fusarium damaged kernels (FDK). In some cases, the infected kernels may develop a red or pink discoloration. FDK are contaminated with trichothecene mycotoxins such as

DON.

DON is a virulence factor that facilitates FHB spread within the spike. Jansen et al. (2005) demonstrated the role of DON in promoting the spread of *F. graminearum* in wheat from one infected floret to the next by hyphal growth through the vascular bundles of the rachis. The DON concentrations of kernels immediately above and below the point of infection were reported to be significantly higher than those farthest from the infection point (Peiris et al. 2011). In another study, the spikelet and rachis below the infection point had much higher DON concentrations compared with those above the infection point (Savard et al. 2000).

2.5 Fusarium mycotoxins and their roles in pathogenicity

2.5.1 Fusarium mycotoxins

Trichothecenes are a large group of sesquiterpenoid mycotoxins produced by *Fusarium* spp. and other fungi (McCormick 2003). Four types of trichothecenes have been identified from trichothecene-producing fungi with type A and type B being the most relevant to FHB (Nicholson et al. 2003; Foroud and Eudes 2009). Type A and type B trichothecenes can be distinguished based on the type of substitution at the C-8 position. Type A trichothecenes have hydrogen, hydroxyl or ester groups at the C-8 position while type B have a ketone function at C-8 (McCormick, 2003; Wagacha and Muthomi 2007). There are more than 60 naturally occurring trichothecenes (Desjardins et al. 1993). T-2 toxin and HT-2 toxin are the major type A trichothecenes produced by some *Fusarium* species (Foroud and Eudes 2009). *F. sporotrichioides*

produces mainly T-2 toxin (Poctor et al. 2006). Although *F. graminearum* and *F. culmorum* produce type B trichothecenes including nivalenol (NIV), deoxynivalenol (DON), DON acetylated derivatives 3-acetyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON), most isolates of *F. graminearum* and *F. culmorum* produce DON (Nicholson et al. 2003).

2.5.2 Toxicology of deoxynivalenol (DON)

Ingestion of trichothecenes produces toxic effects in some animals and humans. DON, also known as vomitoxin, is one of the trichothecenes most commonly detected in grains (Pestka 2007). Trichothecenes are inhibitors of peptidyl transferase and, therefore, inhibit protein synthesis. Trichothecenes are also phytotoxic to a number of plant tissues and can cause chlorosis, necrosis and wilting (McCormick 2003). Among animal species evaluated to date, pigs are the most sensitive to DON. Acute exposure to relatively low doses of DON (≥50 ug/kg body weight) can cause vomiting in pigs, while exposure to extremely high DON (≥27mg/kg body weight) doses can cause mortality or severe tissue injury (Pestka 2007). A wide range of cereals such as maize, wheat, rye, barley and rice are hosts of these destructive trichothecene-producing *Fusarium* species. (Desjardins et al. 1993). As a result of the harmful effects induced by Fusarium toxins, several countries have adopted advisory limits to ensure minimum levels of DON in unprocessed cereals and cereal products for human consumption (European Mycotoxins Awareness Network 2012). For example, the United States recommends that DON levels should not exceed 1000μg/kg in finished

wheat products. In Canada, the maximum limits for DON in un-cleaned soft wheat for use in non-staple foods and baby foods are 2000μg/kg and 1000μg/kg, respectively. In China, the recommended limit for DON in wheat flour and breakfast cereals is 1000μg/kg.

2.5.3 Trichothecenes biosynthesis in *Fusarium* spp.

The biosynthesis of trichothecenes by *Fusarium* species begins from the hydrocarbon trichodiene through a series of complex steps to trichothecenes (Desjardins and Hohn 1993). The *TRI*5 gene encodes the trichodiene synthase, which catalyzes the first step in the biosynthesis of trichothecenes (Wagacha and Muthomi 2007). This *TRI5* gene has been cloned to improve our further understanding of trichothecene biosynthesis and their roles in plant disease development (McCormick 2003). Genes *TRI3* and *TRI7* are involved in the production of NIV and 4-acetyl NIV in *Fusarium* spp., receptively (Wagacha and Muthomo, 2007). Three trichothecene biosynthetic loci have been identified in *Fusarium* species, including a single-gene locus, a 2-gene locus and an 11-gene locus (Proctor et al. 2006). Most of these genes are found in *F. sporotrichioides*. *F. graminearum* shares at least four identical biosynthetic genes with *F. sporotrichioides* (McCormick 2003). The profile of trichothecenes produced by a particular *Fusarium* isolate depends on the biosynthetic genes and their ability to function.

Trichothecene biosynthesis is also influenced by host resistant levels and environmental factors (Mesterhazy 2002; Ramirez et al. 2006). Mesterhazy (2002) showed that DON production by *F. culmorum* and *F. graminearum* was significantly

reduced to near zero in the most resistant genotypes, while very high toxin levels were detected in susceptible cultivars. Environmental factors that influence FHB development and mycotoxin production include temperature and water availability (Ramirez et al. 2006). Ramirez et al. (2006) reported 25℃ and 30℃ to be the optimum temperature for fungal growth and DON production for *F. graminearum*, respectively. Production of trichothecenes by *F. culmorum* and *F. graminearum* was not significantly influenced by moisture of cultures when water activity values were in the range of 0.960 – 0.980 (Llorens et al. 2004). Llorens et al. (2004) showed that the optimal temperature values for DON, NIV and 3-acetyl DON production were 28, 20 and 15℃, respectively, for *F. culmorum* and *F. graminearum.*

2.5.4 Role of trichothecene mycotoxins in pathogenesis

Field and greenhouse studies have shown that trichothecenes play an important role in the virulence of *F. graminearum* and disease development (Desjardins et al. 1996; Eudes et al. 2001; Langevin et al. 2004; Jansen et al. 2005). Disruption of *TRI5* gene was used to generate mutants of *F. graminearum* without the ability of producing trichothecenes, but with no difference from the parent strain in any other way (McCormick 2003). The trichothecene non-producing mutant GZT40 (*TRI5*-) and the trichothecene-producing strain GZ3639 (*TRI5*+) were used to study the roles of trichothecenes in *F. graminearum* aggressiveness and FHB development (Eudes et al. 2001; Langevin et al. 2004). Their results showed that the trichothecene-producing strain was generally more aggressive than the trichothecene non-producing

mutant. These two strains showed extreme differences in aggressiveness in most of the wheat cultivars tested (Eudes et al. 2001). *F. graminearum* GZ3639 (*TRI5*+) spread in the spikes, while the GZT40 (*TRI5*-) mutant caused disease symptoms only at the inoculated floret or spread was significantly reduced (Eudes et al. 2001; Langevin etal., 2004). Consistent results were demonstrated by Jansen et al. (2005), where in the absence of trithothecene producing gene *TRI*5 fungal growth was inhibited by thickened cell walls at the bottom of rachis node. This suggests that trichothecene is important in the aggressiveness of the *Fusarium* spp. and the spread of disease symptoms in the wheat spike. These findings also indicate that trithothecenes are not necessary for development of FHB symptoms, but they are required for fungal growth into the rachis and for disease spread (Eudes et al. 2001; Jansen et al. 2005). In the field, trichothecene non-producing strains colonized wheat spikes but showed significantly lower FHB incidence and severity (Desjardins et al. 1996). The greenhouse and field experiments with trichothecene non-producing mutants *of F. graminearum* indicate that trichothecene production is an important factor in FHB development caused by *F. graminearum*.

2.6 FHB resistance in wheat

2.6.1 Types of Resistance

Wheat resistance to FHB infection is a complex trait. Mesterhazy (1995) summarized components for two FHB resistance mechanisms in wheat which include passive and active mechanisms. Passive resistance mechanisms are related to

morphological traits such as plant height, absence or presence of awns, spikelet density, and flowering time. Under natural epidemic conditions in the field, genotypes with awns were found to be more susceptible to head blight than genotypes without awns, and FHB infection rate was higher in dwarf genotypes than in tall genotypes (Mesterhazy 1995). Buerstmayr et al. (2000) and Lu et al. (2013) also noted a negative correlation between plant height and FHB resistance in wheat. Anther extrusion was a morphological trait negatively correlated with FHB severity (Lu et al. 2013). Moreover, Klahr et al. (2007) showed that FHB resistance was significantly correlated with plant height and heading date. One explanation for the negative relation between FHB symptoms and plant height might be that it is easier for the *Fusarium* pathogen to land on the spikes of short wheat plants (Mesterhazy 1995). The other possibility might be that the spikes of taller plants are surrounded with less moisture than those of short plants, leading to a bias in the FHB resistance evaluation (Buerstmayr et al. 2000). However, Buerstmayr et al. (2000) didn't find negative effects of awns on FHB resistance but that awned progeny had slightly reduced FHB. Noticeable associations between FHB resistance and morphological traits such as plant height, spike length, number of spikelets and heading date were not observed by Jiang et al. (2006). Consistency of correlations between FHB resistance and morphological traits are generally inadequate. Buerstmayr et al. (2000) concluded that selection for FHB resistant cultivars should be largely independent of plant height, flowering date and awnedness.

Active mechanisms of FHB resistance have been classified as five types of resistance components. Type I: resistance to spike initial infection (Schroeder and Christensen 1963); Type II: resistance to spread of infection within the spike (Schroeder and Christensen, 1963); Type III: resistance to trichothecene mycotoxin accumulation (Miller et al. 1985); Type IV: resistance to kernel infection (Mesterhazy 1995; Mesterhazy et al. 1999); and Type V: tolerance (Mesterhazy 1995; Mesterhazy et al. 1999).

Inoculation methods employed for resistance evaluation are variable. Screening of genotypes for type I resistance is achieved by spray inoculation followed by disease incidence evaluation in field nurseries. In contrast, type II resistance is commonly evaluated under controlled conditions by inoculating a floret within the spikelets and assessing disease spread within the spike (Peiris et al. 2011). Under heavy disease pressure and favorable environmental conditions, a high proportion of spikelets may be infected in plants with type II resistance, but without type I resistance, thus causing severe disease symptoms (Bai and Shaner 2004). The Chinese spring wheat cultivar Sumai 3 and its derivatives have been widely used as genetic source of type II resistance in wheat breeding for FHB resistance (Bai and Shaner 2004).

Resistance types III, IV, and V are more difficult to assess and often are more expensive to screen (Gilbert and Tekauz 2000). Resistance to mycotoxin accumulation (type III) is an important component of FHB resistance in wheat. Resistance to toxin accumulation is the ability of the wheat host to maintain a low level of toxins in

infected grains. Low mycotoxin levels in the grain might be due to the capacity of host plant to detoxify or degrade the trichothecene mycotoxins, or inhibit their synthesis (Boutigny et al. 2008). Resistance to kernel infection (type IV) is assessed by measuring the percentage of diseased kernels. Tolerance (type V) is characterized as the ability of the host to maintain yield under disease pressure (Bai and Shaner 2004).

2.6.2 Molecular mechanisms of host defenses

Although QTL related to FHB resistance in wheat have been extensively studied, molecular events involved during pathogen infection are still poorly understood (Steiner et al. 2009). Defense response genes are activated rapidly in wheat spikes in response to FHB pathogen infection. A few defense response pathogenesis-related proteins are able to be detected as early as six hours after inoculation (Pritsch et al. 2000; Steiner et al. 2009). Accumulation of defense response gene transcripts was found to reach maximum level at 36 to 48 hours after inoculation (Pritsch et al. 2000). In contrast, Steiner et al. (2009) noted that 48-72 hours after inoculation was the time period with the strongest host-pathogen interaction since the majority of the pathogen-induced transcripts were detected during this period. Pritsch et al. (2000) demonstrated that defense response genes encoding pathogenesis-related proteins (PRs) such as peroxide, PR1 and PR2 (β-1, 3-glucanases), PR3 (chitinase), PR4 and PR5 (thaumatin-like protein) were activated in wheat spikes inoculated with *F. graminearum*. The expression patterns of defense-

related proteins were generally the same between resistant cultivar (Sumai 3) and susceptible cultivar (Wheaton), except that transcripts of PR4 and PR5 accumulated earlier and were greater in Sumai 3. Genes encoding these six proteins were also induced during infection of the wheat stem base with *F. pseudograminearum* that causes crown rot (Desmond et al. 2008). Mackintosh et al. (2007) developed seven transgenic lines carrying defense response genes encoding α-1-purothionin (one line), tlp-1 (two lines), and β-1,3-glucanase transgenes (four lines) by using the wheat cultivar Bobwhite. Six out of seven lines showed enhanced capacity to reduce at least one of the FHB symptoms (FHB severity, DON accumulation, and percentage of infected kernels) compared with the non-transgenic parent Bobwhite under field conditions. Results suggest that these defense response genes play important roles in the defense reaction to FHB pathogen infection. However, comparison of genes induced by *F. pseudograminearum* and the rust pathogen *Puccinia triticina* showed large overlap in most functional classes of induced genes, including genes encoding pathogenesis-related proteins (PRs) such as peroxide, PR1 and PR2 (β-1, 3-glucanases), PR3 (chitinase), PR4 and PR5 (Desmond et al. 2008). Results suggest that expression of these genes in wheat is not specific to *Fusarium* spp. infection. They may play some roles in general defense against infection by *Fusarium* spp., but it is unknown whether they are the key genes involved in FHB resistance.

DON is one of the major trichothecene mycotoxins produced by *Fusarium* spp. in infected grains. Glucosyltransferases are thought to be able to detoxify DON into the less toxic product DON-3-O-glucoside. Expression of glucosyltransferase

encoding genes enhances the ability to detoxify DON and improve FHB resistance in wheat. Desmond et al. (2008) noted that several glucosyltransferase genes were more highly induced in a crown rot resistant cultivar than a susceptible cultivar. Steiner et al. (2009) found that UDP-glucosyltransferase is one of the enzymes expressed by the up-regulated genes in FHB resistant wheat lines during infection by *F. graminearum*, other products include phenylalanine ammonia-lyase, DNA-J like protein, and pathogenesis-related proteins. There are many genes with unknown functions in the profile of genes that are up-regulated during FHB pathogen infection in wheat (Golkari et al. 2007; Kong et al. 2007). Golkari et al. (2007) found that 46.67% of 185 up-regulated expressed sequence tags did not show homology with sequences of known functions in GeneBank. Kong et al. (2007) used GeneCallingTM (an open-architecture mRNA-profiling technology) to identify differentially expressed genes induced or suppressed in spikes of FHB resistant wheat cultivar Ning 7840 after inoculation with *F. graminearum*. Forty-two out of the 125 identified differentially expressed cDNA fragments were found to lack homology to sequences in the available database. Studies of expression of defense-related genes may provide more insight into molecular events involved in defense against *Fusarium* spp, however, there are still large gaps in understanding the molecular mechanisms of FHB resistance in wheat.

2.7 FHB management strategies

2.7.1 Cultural control

Several cultural control techniques are available for reducing the risk of FHB epidemics, including crop residue management and crop rotation. Ascospores produced on host residues are the primary inoculum for FHB in the fields (Gilbert and Tekauz, 2011). Previous crop residues such as maize stalks and grain, and straw of barley, wheat, and other cereals serve as inoculum sources for *Fusarium spp.* (Miller et al. 1998; Dill-Macky and Jones 2000; Maiorano et al. 2008). Reducing inoculum of *Fusarium* spp in host debris and other reservoirs may be a key to prevent and manage FHB in wheat (Dill-Macky andJones 2000). Crop residues on the soil surface can be reduced by removing residues from the field or tilling fields to bury the residues(Pirgozliev et al. 2003).

Crop rotation is an important strategy in controlling crop diseases by breaking the disease cycle. It is generally understood that wheat rotated with maize should be avoided since they are both hosts of *Fusarium* spp. A three-year survey on the effects of crop residues and tillage practices on FHB of wheat showed a significant decrease in FHB severity and DON after planting wheat into soybean residues compared to wheat following maize or wheat (Dill-Macky and Jones 2000). Yield of wheat was approximately 15% higher when wheat followed soybean than in wheat following maize or wheat. In a later study, maize residues on the soil surface caused 38% higher DON contamination in wheat grain compared with plots without residues on the soil surface. FHB incidence and severity were significantly higher in no-till plots with

maize residues than tilled plots with maize residues (Maiorano et al. 2008). These results were consistent with those found by Dill-Macky and Jones study (2000), where lower FHB infection and higher yield were found in moldboard plowed plots than in no-till plots.

Success of tillage in controlling FHB may depend on its effectiveness in reducing crop residues that serve as potential FHB inoculum sources. Miller et al. (1998) found no effect of tillage or rotation on overall disease incidence or kernel infection. Similarly, a three-year study showed no effect of tillage on disease and DON in wheat following maize (Lori et al. 2009). Results demonstrated that severe FHB infection and high DON values were observed in the year with weather conditions that were conducive to disease development. No significant difference was observed between tilled and zero tillage plots, but differences were observed between cultivars with different levels of FHB resistance (Lori et al. 2009). These results suggested that favourable weather conditions were more important than tillage for FHB epidemics. Similar results were demonstrated in another study by Suproniene et al. (2012) which showed that tillage system had no clearly evident influence on FHB disease level. Although crop rotation and tillage can reduce inoculum sources, ascospores are air-borne and can be spread kilometers away from an inoculum source, which makes single cultural control methods insufficient to prevent occurrence of FHB (Gilbert and Tekauz 2011). In addition, reduced tillage and zero tillage are used to avoid soil erosion, reduce production costs, and increase organic matter in the soil. The contribution of tillage and crop rotation is limited in FHB epidemic seasons.

Other control strategies should be taken into account to gain improved management results.

2.7.2 Fungicide control

Fungicide application is an important strategy used to manage FHB and DON contamination in grain. In general, triazole fungicides were reported to be very effective in suppressing *Fusarium* spp. and reducing FHB and DON accumulation (Simpson et al. 2001; Mesterhazy, et al. 2003; Pirgozliev, et al. 2008; Wegulo, et al. 2011; Amarasinghe, et al. 2013). It was noted that azole fungicides were significantly more effective at reducing FHB symptoms and DON accumulation in grain than azoxystrobin (Pirgozliev et al. 2008; Blandino, et al. 2009)). In regions where weather conditions are favourable for FHB development, such as warm and wet conditions after wheat spike emergence, and the expected economic returns can overcome the cost of fungicide application, then a foliar fungicide application should be considered (Gilbert and Tekauz 2011). Several registered triazole based foliar fungicides are available for management of FHB and DON contamination, including Caramba ® (metconazole), Folicur® (tebuconazole), Proline ® (prothioconazole) and Prosaro ® (prothioconazole+tebuconazole) (Gilbert and Tekauz, 2011). The effects of fungicide applications on FHB control were found to be inconsistent. The inconsistency of fungicide effectiveness may be due to timing of the fungicide application, cultivar resistance level, virulence of the *Fusarium* species used for inoculation, and interaction between fungicide and *Fusarium* species (Mesterhazy et al. 2003; Pirgozliev, et al. 2003).

Timing of fungicide application is an important factor in obtaining optimal control of FHB and DON contamination. Wheat is most susceptible to *Fusarium* infection during anthesis (Yoshida and Nakajima 2010), and therefore anthesis is identified as the optimal growth stage for fungicide application to control FHB (Pirgozliev, et al. 2008; Yoshida and Nakajima 2010; Yoshida, et al. 2012). Yoshida and Nakjima (2010) inoculated wheat spikes at 0 days after anthesis (DAA), 10 DAA and 20DAA in a greenhouse experiment. They demonstrated that FHB severity declined with the delay in time of infection. Spike infection as late as 20 days after anthesis was found to cause grain contamination with DON and NIV without disease symptoms. Yoshida et al. (2012) also conducted a two-year field experiment to evaluate the effect of various timings (anthesis, 10, 20 and 30 DAA) of fungicide application on FHB and mycotoxin accumulation in wheat. Thiophanate-methyl fungicide was tested in this study and colonized maize kernels were used as the inoculum source. In both years, fungicide application at anthesis significantly reduced FHB incidence and severity compared with other application dates and the no-fungicide control. Results showed that fungicide applications at 10, 20 and 30 DAA were not significantly different from no-fungicide treatment in reducing FHB incidence and severity. Effect of fungicide application time on mycotoxin accumulation was inconsistent across the two experimental years. Fungicide application at anthesis was found to have the lowest mycotoxin level compared with other treatments in the first year's study. In contrast, the second year's results showed that fungicide application at anthesis had the highest mycotoxin content. Pirgozliev et

al. (2008) found that fungicides applied two days pre- or two days post-inoculation at anthesis were most effective in reducing FHB severity and DON accumulation in grain. They suggested that fungicide application as near to the pathogen infection as possible would help to obtain more consistent and reliable chemical control of FHB.

Conflicting results have been observed in field trials conducted to evaluate the efficacy of fungicides against FHB and DON accumulation. Application of particular fungicides may lead to an increase in DON content in grain (Simpson, et al. 2001; Pirgozliev, et al. 2008; Amarasinghe, et al. 2013). For example, azoxystrobin and fluquinconazole were found to significantly increase DON compared to the untreated control in field trials artificially inoculated with *Fusarium* spp mixture (Simpson, et al. 2001). Results from this study also showed that neither tebuconazole nor fluquinconazole or azoxystrobin significantly controlled F. avenaceum compared to the untreated control, although tebuconazole provide the most effective control of *F. culmorum*. Similar results were observed by Pirgozliev et al. (2008). They found that tebuconazole was more effective than azoxystrobin in reducing FHB severity and the colonization of grain by trichothecene-producing *Fusarium* species. Azoxystrobin application tended to increase the level of DON accumulation in grain compared to the unsprayed inoculated control. Field plots treated with triazole fungicides prothioconazole+tebuconazole and prothioconazo also showed inconsistent control effects on DON content in grain samples (Amarasinghe, et al. 2013). These findings may indicate that due to the various sensitivities of *Fusarium* spp to fungicides, fungicide selection is an important factor in order to obtain sufficient control of FHB

and DON contamination in grain.

Due to the inconsistent or conflicting effects of fungicide on FHB and DON accumulation, fungicide application integrated with genetic resistance to achieve more sufficient FHB control has been studied. Mesterhazy et al. (2003) observed that more FDK, FHB severity, and DON accumulation in grain were reduced when fungicides were applied to cultivars with higher levels of FHB resistance than when applied to susceptible cultivars. Similarly, Wegul et al. (2011) found that fungicide efficacy in reducing FHB index, FDK and DON was higher in FHB moderately resistant cultivars Harry than in susceptible cultivars Jagalene and 2137. Their results were confirmed by Amarasinghe et al. (2013), where they found triazole fungicides such as tebuconazole, metconazole and prothioconazole were more effective and consistent in reducing FHB index, FDK, and DON, and increasing yield in the FHB moderately resistant cultivar Glenn, than in the susceptible cultivar Roblin (Amarasinghe et al. 2013).

Studies on the effects of control strategies traditionally have been based on a single strategy or combined with two management strategies. Integrated management by combining multiple strategies showed advantage in improving FHB control results (Wegul et al. 2011; Amarasinghe et al. 2013). Results of FHB control strategies in wheat suggest that an integrated use of all the available strategies, including fungicide application, crop rotation, crop residue management, and resistant cultivars should be considered in order to reduce economical loss due to FHB epidemics and mycotoxin contamination in wheat.

2.7.3 **Biological control**

Results of many studies indicated the use of *Fusarium* spp. antagonists for biological control of FHB is an alternative, promising measure to increase the power of integrated management of FHB. Several microbial antagonists have been screened and identified as potential biocontrol agents for FHB, including bacteria, fungi and yeast. Biocontrol of FHB focuses on disrupting the pathogen life cycle at certain points, including inoculum production in infected crop debris, spikelet infection and FHB spread within wheat spikes (Luz et al. 2003). Antibiosis and competition are the mechanisms of biocontrol of FHB for many of the studied antagonists (Luz et al. 2003). Other mechanisms postulated are mycoparasitism, induced resistance, and metabolic inhibition of mycotoxin synthesis (Luz et al. 2003; Jochum et al. 2006; Matarese et al. 2007).

Khan et al. (2001) identified two promising *Bacillus* strains that significantly reduced FHB disease severity. Another bacterial antagonist *Lysobacter enzymogenes* strain C3 was evaluated for control of FHB under both greenhouse and field conditions (Jochum et al. 2006). They found that *L. enzymogenes* strain C3 was effective in reducing FHB severity under both greenhouse and field conditions. However, control effectiveness was inconsistent across all the cultivars tested.

Biocontrol isolates may be successfully combined with fungicides. In a field trial, it was demonstrated that *L. enzymogenes* strain C3 combined with the fungicide

tebuconazole was more consistently effective in reducing FHB severity or incidence than the biocontrol agent or fungicide application alone (Jochum et al. 2006). Xue et al. (2009) found that *Clonostachys rosea* strain ACM941 significantly reduced *Gibberella zeae* perithecial production in leaf disks, infected maize kernels, and spikelet debris in the field. They also found that the *C. rosea* strain ACM941 significantly reduced FHB index, FDK and DON content but was less effective than the fungicide tebuconazole. Use of *C. rosea* as a potential biocontrol agent for FHB was further studied by Palazzini et al (2013). Results demonstrated that *C. rosea* (strain 1457) was a promising antagonist to reduce *Fusarium spp.* on naturally infected wheat stalks in the fields. However, *C. rosea* strain 1457 showed inconsistent effectiveness in controlling *F. verticillioides*.

Matarese et al. (2012) studied the interactions between *Trichoderma* spp. and DON-producing *Fusarium* spp. Three *Trichoderma* strains, *T. gamsii* 6085, *T. gamssi* 6317 and *T. velutinum* 4837, significantly reduced *F. graminearum* and *F. culmorum* growth in at least one of potato dextrose agar and water agar substrates. These strainsalso greatly decreased DON production by *F. graminearum* when co-inoculated on a rice substrate. Fourteen days after inoculation, *T. gamsii* 6085 reduced DON production by 92% of that produced by *F. graminearum* when cultured alone. The other two *Trichoderma* strains, *T. gamssi* 6317 and *T. velutinum* 4837, reduced DON production by 60% and 67%, respectively. *Trichoderma* spp. were more effective in reducing FHB severity in greenhouse experiments than *Alternaria* spp. and *Epicoccum* spp. (Musyimi et al. 2012).

A couple of yeast strains have been identified as effective antagonists of *Fusarium* spp. (Khan et al. 2001; Khan et al. 2004). Several *Cryptococcus* spp. decreased FHB severity by as much as 50-60% in field tests and the lowest level of disease was observed when antagonists were applied to a moderately resistant cultivar (Khan et al. 2004).

There are some concerns about application of potential antagonists of *Fusarium* spp. to control FHB. Infected crop debris is a source of inoculum. Application of antagonists alone may not be sufficient to significantly reduce inoculum levels. Furthermore, the harsh environment could negatively affect the viability or growth of the antagonists applied to reduce inoculum produced from infected crop residues (Yuen and Schoneweis 2007). Several options can be taken into consideration in order to enhance FHB biocontrol efficacy. For example: application of biocontrol agent combinations; application of biocontrol agents with compounds that would stimulate the growth or activity of biocontrol agents; or integration of biocontrol agents with fungicide application (Luz et al. 2003).

2.7.4 Breeding for FHB resistant cultivars

Relatively few sources of resistance are available for FHB breeding programs. The spring wheat cultivar Sumai 3 and its derivatives such as Ning 7840 from China have been widely used as resistant sources in breeding programs worldwide (Rudd et al. 2001; Bai et al. 2003; Badea et al. 2008). Sumai 3 was developed by crossing a land race (Taiwanxiaomai) from China with an Italian cultivar (Funo), both of them

are moderately susceptible to FHB (Bai et al. 2003). Inheritance of FHB resistance from Sumai 3 was found to be more stable and consistent across environments than other sources of resistance. However, problems such as susceptibility to other diseases and shattering may occur when using Sumai 3 as a parent in a breeding program (Rudd et al. 2001). Ning 7480 has the same resistance as Sumai 3, but is more resistant to rust and power mildew and has better agronomic characteristics than Sumai 3 (Bai et al. 2003). Failure of resistance in the Sumai 3 source was not observed, and it has been considered as the best source of resistance to spread of FHB in the spike (Bai and Shaner 2004).

Other sources of resistance that have been widely used including Wangshuibai and Ning 894037 from China (Bai and Shaner 2004), Frontana from Brazil, Nobeokabouzu from Japan (Badea et al. 2008; Rudd et al. 2001)，and Praa 8 and Novkrumka from Europe (Gilbert and Ketauz 2000). Ernie and Freedom from the USA are also used as sources of resistance in some breeding programs (Rudd et al. 2001). The use of limited resistance sources may eventually cause erosion of the effectiveness of the genes involved. Deployment of various sources of resistance genes may maintain long-term effectiveness of these resistance genes and improve the level of resistance in wheat (Badea et al. 2008).

Among the strategies available for FHB management, growing of wheat cultivars resistant to FHB should be the most economic, environmentally friendly and effective method (Wagacha and Muthomi 2007). The breeding goal is to develop locally adapted superior genotypes, bringing together different types of disease

resistance with desired agronomic traits. However, Fusarium head blight resistance in wheat is a quantitatively inherited trait and controlled by a few QTLs with major effects and many minor genes (Buerstmayr et al. 2002; Sneller et al. 2010). Although there are a few sources of resistance available, it is difficult to make rapid progress in breeding for FHB resistance in wheat due to the complexity of resistance and screening for FHB resistance is labor intensive and time-consuming.

Application of QTL mapping is required to study the quantitative inheritance of FHB resistance in wheat. Molecular assisted selection (MAS) using FHB resistance QTLs allows breeders to screen breeding lines at the seedling stage and identify the most promising lines prior to disease and yield testing, thus shortening the breeding cycle and reducing breeding costs (McCartney et al. 2007). Identification of FHB resistance QTLs without pleiotropic effects or linkage drag on agronomic or quality traits is important to make MAS successful. The pleiotropic effects or linkage drag of identified resistance QTLs should be adequately examined in order to avoid undesirable consequences (Anderson and Liu 2007).

McCartney et al. (2007) evaluated the effects of FHB resistance QTL alleles from Nyuubai, Sumai 3, and Wuhan 1 on FHB resistance, and agronomic traits in elite Canadian spring wheat backgrounds. FHB resistance tended to be improved when more resistance QTLs were incorporated. However, they found that the Sumai 3 5AS resistance allele was negatively associated with grain protein content and the Wuhan 1 resistance allele was associated with increased plant height. Studies have not found any negative effects associated with the major FHB resistance gene *Fhb1* on

chromosome 3BS (Anderson and Liu 2007).

FHB resistance QTL in wheat has been extensively studied. Buerstmayr et al. (2009) summarized that FHB resistance QTL were found in all chromosomes except in chromosome 7D. The major resistance QTL on chromosome 3BS, designated *Fhb1*, is the most consistently identified QTL (Bonin and Kolb 2009). It is found in most Chinese resistance sources and serves as the best source for type II resistance (Bai and Shaner 2004). This QTL explained up to 60% of the phenotypic variation for type II FHB resistance (Buerstmayr et al. 2002). Two other major QTL that were repeatedly detected in different mapping populations are *Qfhs.ifa-5A* on chromosome 5AS which is associated with type I resistance and *Fhb2* on chromosome 6BS (Buerstmayr et al. 2009).

Most studies focus on QTLs associated with type I and type II resistance. QTLs associated with low DON and kernel damage are not as well-known as those associated with type I and type II resistance. The resistance QTL associated with low kernel damage have been identified across different populations on chromosome 4B (Abate et al. 2008; Bonin and Kolb, 2009). Bonin and Kolb (2009) identified three QTL that confer resistance to kernel damage on chromosomes 2B, 4B and 6B using recombinant inbred lines (RILs) derived from the cross IL94-1653/Patton. Phenotypic variation for kernel damage in the greenhouse and field trials explained by the 4B QTL were 7 and 12.3%, respectively. Three QTL for both low DON and FDK were consistently identified in RILs derived from the cross Erin/MO 94-317 in a two-year greenhouse evaluation (Abate et al. 2008). These three QTL located on chromosomes

3BSc, 4BL and 5AS together explained 31 and 42% of the total phenotypic variation in DON and FDK, respectively.

The introgression of type I and type II resistance QTL into locally adapted wheat cultivars has successfully improved their overall FHB resistance levels (Rudd et al. 2001; McCartney et al. 2007). It is important for wheat breeders to identify consistent QTL with large effects in reducing kernel damage and DON accumulation in order to breed a cultivar with multiple types of resistance (Bonin and Kolb, 2008).

3.0 Evaluation of the Role of Genetics and Fungicides on the Development of Fusarium Head Blight in Wheat

3.1 Abstract

The effects of genetics and different fungicide treatments on Fusarium head blight (FHB) in wheat were evaluated after artificial inoculation under field conditions with a mixture of four isolates of *Fusarium graminearum*. Field trials were conducted in 2012 and 2013 at two sites (Winnipeg and Carman) in Manitoba, Canada. Seed treatment fungicide thiamathoxam+difenoconazole+metal-axyl-M+S-isomer (Cruiser Maxx® Cereals) and the combination foliar fungicide tebuconazole+prothioconazole (Prosaro 250 EC™) were tested on four wheat cultivars to evaluate their effectiveness in FHB control. Two spring wheat (Carberry and Harvest) and two winter wheat (Emerson and CDC Falcon) cultivars with different levels of FHB resistance (moderately resistance and susceptible) were tested. Five treatments (inoculated-untreated control, seed treatment fungicides Cruiser Maxx® Cereals, foliar fungicide Prosaro 250 EC™, seed + foliar fungicides, uninoculated-untreated control) were applied to each cultivar. The moderately resistant cultivars Carberry and Emerson had lower FHB index, Fusarium-damaged kernels (FDK) and deoxynivalenol (DON), and higher yields than the more susceptible cultivars. Treatments that included the foliar fungicide significantly reduced FHB traits (FHB index, percent FDK, and DON levels) and increased thousand kernel weight and yield compared to the inoculated-untreated control. Seed treatment alone did not significantly reduce FHB traits or increase yield compared to the inoculated-untreated control. Yield was negatively

correlated with FHB traits, whereas FHB traits were positively correlated. The results of this study indicated that an integrated FHB management strategy including a moderately resistant cultivar and foliar fungicide application can be effective in reducing the risk of FHB and DON contamination.

3.2 Introduction

Fusarium head blight (FHB) is one of the most important fungal diseases in wheat because of its direct detrimental effects on yield and grain quality. Severe FHB outbreaks can cause up to 70% yield loss in epidemic years (Haidukowski et al. 2005). In addition to reduced yield and grain quality, the most common causal agent of this disease, *Fusarium graminearum* Schwabe [telomorph:*Gibberella zeae* Schwein (Petch)], produces deoxynivalenol (DON) and its acetylated derivatives, 3-acetyl DON (3-ADON) and 15-acetyl DON (15-ADON) (Nicholson et al. 2003; Osborne and Stein., 2007). The occurrence of mycotoxins in harvested grain is a big concern in wheat production. DON can threaten human and animal health because of its haematic and anorexic syndromes, and neurotoxic and immunotoxic effects in mammals (Haidukowski et al. 2005). Feeding farm animals such as swine with DON contaminated grain causes weight loss, food refusal, and vomiting when sufficient doses are ingested (Pestka 2007).

The effects of FHB management strategies such as cultural practice，fungicide application, biological control, planting resistant cultivars or modifying cropping system have been studied. However, a single control strategy usually fails to sufficiently control the disease. Commercial cultivars of wheat vary in their response to FHB. Fungicide application plays an important in controlling FHB (Simpson et al. 2001). However, results of FHB control with fungicide have been variable. There are a number of reports of the successful control of FHB using triazole-based fungicides (Mesterhazy et al. 2003; Paul et al. 2008; Wegulo et al. 2011; Amarasinghe, et al.

2013), but several studies have shown that azoxystrobin application led to increased DON contamination of grain in artificially inoculated field trials (Simpson et al. 2001; Mesterhazy et al. 2003; Pirgozliev, et al. 2008). Integration of triazole-based fungicides, alone or in combination, with moderately resistance cultivars tended to be more effective in reducing FHB index and DON accumulation in grain when compared with susceptible cultivars (Mesterhazy et al. 2003; Wegulo et al. 2011; Amarasinghe, et al. 2013). In addition, fungicide effectiveness was more stable in resistant cultivars. The variability of fungicide efficacy in controlling FHB might due to the timing of the fungicide application, fungicide selection and application technology, virulence of the *Fusarium* isolates, and level of resistance in cultivars planted (Mesterhazy et al. 2003).

Seed treatments using tebuconazole + imazalil, fludioxonil and difenoconazole resulted in significant reductions in the attack of soil-borne *Fusarium* spp. to roots and coleoptiles of seedlings in a trial carried out under greenhouse conditions (Jorgensen et al. 2012). However, information on the role of seed treatment in controlling FHB is very limited. It is recommended that an integrated approach including cultural control, cultivar resistance, crop rotation, and fungicide application be used to protect wheat from FHB.

The objectives of this study were to: (1) determine the influence of cultivar selection and seed- and foliar- applied fungicides on grain yield of spring and winter wheat; and (2) investigate the effectiveness of integrating fungicide application and cultivar resistance in controlling FHB and DON accumulation in grain.

3.3 Materials and methods

3.3.1 Plant materials

The following two commercial spring wheat cultivars and two commercial winter wheat cultivars with different levels of FHB resistance were used for evaluation:

Spring wheat cultivars: Carberry – moderately resistant (MR); Harvest – susceptible (S) (Seed Manitoba, 2015).

Winter wheat cultivars: Emerson - moderately resistant (R); CDC Falcon –susceptible (S) (Seed Manitoba, 2015).

3.3.2 Fungicides

Commercially available foliar fungicide - Prosaro EC™ - manufactured by Bayer Crop Science Inc. (Canada) and seed treatment fungicides and insecticide –- Cruiser Maxx® Cereals – manufactured by Syngenta Crop Protection Canada, Inc. were used in field experiments. Prosaro 250 EC™ combines two active ingredients (prothioconazole and tebuconazole) available for cereal disease control. Prosaro protects against diseases such as Fusarium head blight and leaf diseases in wheat and other cereal crops. Cruiser Maxx® Cereals seed treatment is a combination of the insecticide, thiamethoxam, and the fungicides, difenoconazole and metalaxyl-M (and S-isomer) that control or suppress wireworms and seed-borne and soil-borne diseases of cereal crops. It is registered for control of seedling blight, root rot, and damping-off caused by seed-and soil-borne *Fusarium* in wheat and other cereal crops. Prosaro is a Group 3 fungicide, while Cruiser Maxx® Cereals belong to Group 3 and Group 4.

Group 3 fungicides act against many different fungal pathogens by preventing the formation of sterols, which are needed in fungal cell walls. Group 4 fungicides suppress fungal pathogens by inhibiting nucleic acid synthesis. The recommended rate of application of Prosaro is 324 ml/acre, and for Cruiser Maxx® Cereals is 325 ml/100 kg of seed.

3.3.3 Fungal inoculum preparation

Four different *F. graminearum* isolates were selected for the experiment and were chosen to reflect the predominant chemotypes of the area. Two of the isolates were of the 3-ADON chemotype (M7-07-1 and M9-07-1), and two (M1-07-2 and M3-07-2) were of the 15-ADON chemotype. Isolates were first cultured in spezieller nahrstoffar agar (SNA) media plates for one week. After that the SNA cultures were transferred to aerated liquid carboxy methyl cellulose (CMC) media for another week to produce macroconidia under UV light. Afterward, the media were strained and decanted into sterile glass bottles. Macroconidia concentration was determined by using a hemocytometer cell counter. Equal concentrations of each isolate were combined and adjusted to 5×10^4 spores/ml for inoculation. Tween 20 was used as a surfactant and added at a volume of 4 ml per one liter of inoculum.

3.3.4 Field experimental design and treatments

Two spring wheat cultivars, Carberry and Harvest, and two winter wheat cultivars, Emerson and CDC Falcon, were grown in the field at Carman and Winnipeg, Manitoba, during the 2012 and 2013 growing seasons. Three fungicide treatments plus two controls were assigned to each cultivar. The experimental design

was a four replicate split-split-split-plot design. The first split was by growth habit (spring wheat vs winter wheat) and the second split was by cultivar (moderately resistant cultivar vs susceptible cultivar), and the third split was by treatment. The main plot effect was the wheat cultivar used for evaluation. The sub plot effect was the five experimental treatments for each wheat cultivar (Table 3-1).

Table 3-1. Experimental treatments for each cultivar in trials conducted in Winnipeg and Carman Manitoba in 2012 and 2013.

Cultivar	Treatment
spring wheat: Carberry & Harvest winter wheat: Emerson & CDC Falcon	Inoculated-untreated
	Inoculated+ seed fungicide
	Inoculated+foliar fungicide
	Inoculated+seed fungicide+foliar fungicide
	Uninoculated-untreated

Note: seed fungicide was Cruiser Maxx® Cereals and foliar fungicide was Prosaro 250EC.

There were four replicates at each site, to make a total of 80 plots (4 cultivars x 5 treatments x 4 replicates) at each site in each growing season. The plots were three meters in length and 1.5 meters in width. Each plot contained six rows spaced 17 cm apart and was sown at a seeding rate of 1200 viable seeds/plot.

Inoculated-untreated control plots were inoculated with *F. graminearium* and did not have seed treatment or foliar fungicide application, while uninoculated-untreated control plots were not inoculated and had no seed treatment or foliar

fungicide application. With the exception of the uninoculated-untreated plots, all plots were inoculated with a *F. graminearum* macroconidia suspension (5×10^4 spores/ml) at a rate of 1 L/plot when wheat reached 50% anthesis (Zadoks GS 65). The uninoculated plots were sprayed with 1L distilled water. In order to ensure later spikes were inoculated at the appropriate stage, a second inoculation was performed three days after the first inoculation. An overhead mist irrigation system was switched on after inoculation to maintain a humid environment conducive to the development of FHB symptoms. The irrigation system was programmed to irrigate field plots for 10 minutes every hour for 10 hours daily for five to seven days to promote FHB symptom development.

The seed treatment Cruiser Maxx® Cereals was applied to seeds prior seeding using the manufacturer's recommended rate of 325 ml/100 kg of seed. The amount of foliar fungicide used for one plot was 0.36 ml Prosaro 250 EC™ and mixed with one liter water. According to the manufacturer's instructions, foliar fungicide was sprayed to wheat spikes after most wheat plants had finished heading but were not yet at anthesis. Plots were inoculated two days after foliar fungicide application. Different CO_2 powered back-pack sprayers with a six-nozzle boom were used for foliar fungicide application and fungal inoculation. Air pressure for the sprayers was calibrated at 30psi during inoculation and fungicide application.

The average temperatures and total rainfall during June, July and August in the 2012 and 2013 growing seasons at Carman and Winnipeg are listed in Table 3-2. Data were from the Environment Canada weather station at Carman and the weather

station at the Point at University of Manitoba.

Table 3-2. Monthly mean temperature and total rainfall from June to August 2012 and 2013 in Carman and Winnipeg, Manitoba.

		2012		2013	
Site	Month	Mean temp (°C)	Total rain (mm)	Mean temp (°C)	Total rain (mm)
Carman	June	19.6	22.2	16.9	11.9
	July	21.9	29.4	18.7	52.8
	Aug	20.0	55.4	21.7	11.2
	Mean June-Aug	20.5	35.7	19.1	25.3
Winnipeg	June	18.7	63.2	18.6	64.5
	July	23.5	21.2	20.3	57.8
	Aug	20.9	50.8	20.8	24.0
	Mean June-Aug	21.0	40.1	19.9	48.8

3.3.5 Disease and agronomic traits evaluation

3.3.5.1 Seedling stand density determination

Seedling stand density for winter and spring wheat was assessed by counting two-one meter rows in each plot and converted to number of plants/ square meter. The assessment was conducted before seedlings started tillering. Seedling stand for winter wheat was counted in the fall of 2011, spring of 2012 and spring of 2013. In the fall of 2012, seedling stand for winter wheat was not counted due to late seeding and limited emergence prior to freeze-up.

3.3.5.2 FHB index evaluation

Disease incidence and severity were evaluated 18-21 days after the first inoculation by randomly collecting 50 spikes from the middle four rows of each plot. Samples were stored in a -20 ^{o}C freeze until they could be processed. Disease incidence was measured as the percentage of infected spike in the plot, while severity was measured as the percentage of diseased spikelets in the infected spike. FHB index for each plot was calculated using the following formula:

FHB index = (percent disease incidence × percent disease severity)/100.

3.3.5.3 Grain yield, percentage of Fusarium damage kernels and thousand kernel weight

After maturity, plots were harvested with a small plot combine. The wind speed of the combine was reduced from normal by 60% to retain as many Fusarium damaged kernels (FDK) as possible. Samples were placed on drying beds for three days after harvest to ensure that all samples were dry and at similar moisture content. After drying, samples were cleaned using a blower to remove straw and chaff, but not FDK. Grain yield of each plot was determined by weighing the clean, dried samples. One thousand seeds from each plot were used to determine the weight of 1000 thousand kernels (TKW). FDK were counted from a random 250 seed sample from each plot. Kernels with a shrunken, pinkish or whitish appearance, or mycelial growth, were considered as FDK and expressed as a percentage of the total sample.

3.3.5.4 Analysis of DON concentration and protein content in grain

A sample of 50g grain from each plot was ground into flour to pass through a 0.85 mm sieve and well mixed. Deoxynivalenol (DON) was extracted by adding 50 ml of deionized water into a subsample of 10g flour, and then quantified using EZ-Quant® Vomitoxin Enzyme-linked Immunosorbent Assay (ELISA) DON identification kit from Diagnostix. The minimum concentration that can be quantified by this method is 0.5 ppm.

The nitrogen content of samples from each plot was determined by using the FP-528 Nitrogen/Protein Determinator. A sample of 50g grain from each plot was ground into flour to pass a 0.5 mm sieve and well mixed. A subsample of 0.5g flour was used for nitrogen content determination on a 0% moisture basis. Protein content was obtained by multiplying nitrogen content by a typical protein factor for milling wheat (5.7) as recommended in the FP-528 Nitrogen/Protein Determinator operation manual.

3.3.6 Statistical analysis

Statistical analyses were performed using the SAS program (SAS version 9.2, SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) test was performed using PROC GLM statement. ANOVA for all response variables for each site/year (i.e 2012 Carman), site/year/growth habit (i.e. 2012 Carman spring wheat) and a combined analysis for site years were performed. A Levenes homogeneity test was conducted to determine whether all site years could be combined. Results showed that experimental treatments provided similar information in each site/year and site/year/growth habit

(data not shown), and the data for all variables from these two years and locations could be combined. Spring wheat and winter wheat data were analyzed separately since there were seeded and inoculated at different times. The least significant difference (LSD, p=0.05) was used to compare significant differences between cultivar and treatment means for each response variable. Pearson's correlation coefficients between the response variables were generated using the PROC CORR procedure of the SAS version 9.2. The effects of fungicide treatment, cultivar, and their interactions on FHB variables were determined.

3.4 Results

3.4.1 Weather conditions

Winter and spring wheat were inoculated and rated for FHB index during the period from June to August. Temperature during this period was lower in 2013 compared with 2012 at both experimental sites (Table 3-2). More rainfall was recorded during these three months in 2012 than in 2013 at Carman while Winnipeg had more rainfall in 2013. Although the same misting regime was used at both sites over the two years, in general, higher FHB index, FDK and DON concentration were observed in 2013 based on the data analysis results for site/year and site/year/growth habit (data not shown).

3.4.2 Analysis of variance for response variables

F values for cultivar were significant for all measured variables in both growth habits (spring wheat VS winter wheat) except for protein content in spring wheat (Table 3-3 and Table 3-4). The effect of site/year (SY) was significant for all measured variables in both spring and winter wheat and contributed a large portion to the total variation, which indicates the importance of environment in disease development (Table 3-3 and Table 3-4). The treatment effect was not significant for seedling stand density in winter wheat, but significant for all other variables in both spring and winter wheat (Table 3-3 and Table 3-4). The effect of cultivar * treatment interaction was not significant for protein content in spring wheat and for yield, protein content and seedling stand in winter wheat, but significant for other variables. The significant interaction between cultivar and treatment mainly derived from changes in magnitude between treatments for the cultivars rather than changes in rank (Table 3-11 and 3-12). The interaction among SY * cultivar * treatment had a significant effect on FHB index and FDK in both spring and winter wheat, however, no significant effect was observed on other variables except for TKW in spring wheat. Although effects of interactions were statistically significant for most of the traits, in most cases their contributions to the total variation are quite small relative to the contribution of treatment effect.

3.4.3 Correlation between the measured variables

In spring wheat, all measured variables were highly correlated with each other

except seedling stand density (Table 3-5). The highest Pearson correlation coefficients were found between FHB index and DON, and FDK and TKW. Both have a correlation coefficient as high as 0.99. FHB index and FDK were negatively correlated with TKW and yield, while TKW and yield were negatively correlated with protein and DON content. Protein content was highly correlated with other traits except with seedling stand density. Similar results were not observed in winter wheat (Table 3-6).

In winter wheat, protein content was only significantly correlated with seedling stand density (Table 3-6). The highest correlation coefficient was observed between FDK level and DON concentration (r=0.99). Winter wheat was similar to spring wheat in that FHB index and FDK were negatively correlated with TKW and yield, while TKW and yield were negatively correlated with DON content. The correlation coefficients between TKW and yield (r= 0.74), and TKW and DON (r= -0.79) in winter wheat were not as high as those in spring wheat. The correlation coefficients between TKW and yield, and TKW and DON in spring wheat were 0.96 and -0.95, respectively (Table 3-5). DON concentration was also negatively correlated with yield (r= -0.92) in winter wheat (Table 3-6).

Table 3-3. Combined 2012 and 2013 analysis of variance for Fusarium head blight (FHB) index, Fusarium damaged-kernels (FDK), deoxynivalenol (DON) concentration, thousand kernel weight (TKW), yield, protein content and seedling stands in spring wheat.

Source of variance	DF	FHB index (%)		FDK (%)		DON (ppm)		TKW(g)		Yield(t/ha)		Protein (%)		Seedling stands (/m^2)	
		MS	P>F	MS	P>F	MS	P>F	MS	P>F	MS	P>F	MS	P>F	MS	P>F
SY	3	3857.35	<.0001	7137.22	<.0001	3588.10	<.0001	184.3	<.0001	5.02	<.0001	186.76	<.0001	20014.43	<.0001
Rep(SY)	12	65.85	<.0001	26.65	0.3917	22.51	0.0745	2.30	0.0009	0.40	<.0001	0.73	0.0038	390.79	<.0001
Cultivar	1	3007.62	<.0001	7653.52	<.0001	808.69	<.0001	234.93	<.0001	2.46	<.0001	0.96	0.0628	6375.63	<.0001
SY*Cultivar	3	590.92	<.0001	844.80	<.0001	97.40	0.0002	11.13	<.0001	0.43	0.003	4.65	<.0001	1555.49	<.0001
Rep(SY)*Cultivar	12	33.84	0.0046	35.49	0.1663	26.94	0.0272	0.86	0.3205	0.43	<.0001	0.25	0.5116	200.46	0.0119
Treatment	4	5042.74	<.0001	6671.42	<.0001	2455.75	<.0001	188.20	<.0001	7.20	<.0001	2.21	<.0001	150.44	0.1471
SY*Treatment	12	539.52	<.0001	482.20	<.0001	345.47	<.0001	24.97	<.0001	0.70	<.0001	0.43	0.1058	155.24	0.0592
Cultivar*Treatment	4	344.69	<.0001	423.61	<.0001	65.45	0.0011	17.58	<.0001	0.83	<.0001	0.16	0.6634	253.56	0.0245
SY*Cultivar* Treatment	12	76.76	<.0001	91.97	0.0001	15.95	0.2824	4.90	<.0001	0.08	0.5376	0.11	0.9564	56.43	0.7912
Error	96	12.91		24.84		13.1		0.74		0.09		0.27		86.38	

Note: SY=SiteYear (Environment); DF= degree of freedom; MS=mean square

Table 3-4. Combined 2012 and 2013 analysis of variance for Fusarium head blight (FHB) index, Fusarium damaged-kernels (FDK), deoxynivalenol (DON) concentration, thousand kernel weight weight (TKW), yield, protein content and seedling stands in winter wheat.

Source of variance	DF	FHB index (%)		FDK (%)		DON (ppm)		TKW(g)		Yield(t/ha)		Protein (%)		Seedling stands (/m^2)	
		MS	P>F	MS	P>F	MS	P>F	MS	P>F	MS	P>F	MS	P>F	MS	P>F
SY	3	3832.63	<.0001	11530.70	<.0001	4256.54	<.0001	75.05	<.0001	33.60	<.0001	280.69	<.0001	160739.63	<.0001
Rep(SY)	12	84.32	0.0008	36.92	0.0036	78.59	<.0001	6.42	<.0001	0.64	<.0001	5.32	<.0001	1351.79	<.0001
Cultivar	1	3161.44	<.0001	8656.71	<.0001	4535.97	<.0001	1.21	0.2654	14.22	<.0001	9.51	<.0001	38297.18	<.0001
SY*Cultivar	3	745.96	<.0001	1546.50	<.0001	530.60	<.0001	12.50	<.0001	10.89	<.0001	0.88	0.0003	13416.53	<.0001
Rep(SY)*Cultivar	12	89.00	0.0005	31.92	0.0115	36.59	0.0176	3.37	0.0003	0.57	<.0001	2.08	<.0001	780.64	0.0039
Treatment	4	3068.80	<.0001	3316.82	<.0001	1612.34	<.0001	82.26	<.0001	4.60	<.0001	2.24	<.0001	1646.34	0.0004
SY*Treatment	12	294.24	<.0001	382.84	<.0001	204.41	<.0001	7.87	<.0001	0.54	<.0001	0.52	<.0001	239.92	0.6283
Cultivar*Treatment	4	190.53	<.0001	247.57	<.0001	230.93	<.0001	3.56	0.0079	0.19	0.2014	0.18	0.2273	142.11	0.7457
SY*Cultivar* Treatment	12	67.05	0.0066	41.57	0.0012	29.11	0.0685	0.86	0.5632	0.29	0.0139	0.07	0.8722	373.60	0.2442
Error	94	26.71		13.66		16.64		0.97		0.13		0.13		292.12	

Note: SY=SiteYear (Environment); DF= degree of freedom; MS=mean square

Table 3-5. Combined 2012 and 2013 Pearson correlation coefficients for seedling stand, FHB index, Fusarium damaged kernel (FDK), thousand kernel weight (TKW), yield, protein content and deoxynivalenol (DON) concentration in spring wheat

	Seedling stand (/m^2)	FHB Index (%)	FDK (%)	TKW (g)	Yield (t/ha)	Protein (%)	DON (ppm)
Seedling stand (/m^2)	1.000	0.26ns	0.43ns	-0.43ns	-0.24ns	0.25ns	0.20ns
FHB Index (%)		1.000	0.95****	-0.94****	-0.97****	0.97****	0.99****
FDK (%)			1.000	-0.99****	-0.97****	0.95****	0.96****
TKW (g)				1.000	0.96****	-0.92****	-0.95****
Yield (t/ha)					1.000	-0.95****	-0.98****
Protein (%)						1.000	0.97****
DON (ppm)							1.000

Note: site year/cultivar/treatment means were used for correlation analysis; ns, no significant; ****significant at p<0.0001. N=10

Table 3-6. Combined 2012 and 2013 Pearson correlation coefficients for seedling stand, FHB index, Fusarium damaged kernel (FDK), thousand kernel weight (TKW), yield, protein content and deoxynivalenol (DON) concentration in winter wheat

	Seedling stand (/m^2)	FHB Index (%)	FDK (%)	TKW (g)	Yield (t/ha)	Protein (%)	DON (ppm)
Seedling stand (/m^2)	1.000	-0.24ns	-0.41ns	-0.15ns	0.50ns	0.80**	0.41ns
FHB Index (%)		1.000	0.96 ****	-0.90 ***	-0.91***	0.24 ns	0.95****
FDK (%)			1.000	-0.80**	-0.94****	0.09ns	0.99****
TKW (g)				1.000	0.74*	-0.59ns	-0.79**
Yield (t/ha)					1.000	0.06ns	-0.92 ***
Protein (%)						1.000	0.09ns
DON (ppm)							1.000

Note: site year/cultivar/treatment means were used for correlation analysis; ns, no significant; *, **, ***, and ****significant at P<0.05, P< 0.01, P<0.001, and P<0.0001, respectively. N=10

Table 3-7. Combined treatment means across years (2012 and 2013) and locations (Carman and Winnipeg) for measured variables across the two spring wheat cultivars Carberry and Harvest

Treatment	FHB index (%)	FDK (%)	DON (ppm)	TKW(g)	Yield(t/ha)	Protein (%)	Seedling stands (/m^2)
Inoculated-untreated	28.08a	42.86a	21.91a	26.74a	2.54a	17.16a	214.06a
Seed Fungicide	28.92a	41.82a	21.45a	26.58a	2.61a	17.16a	214.63ab
Foliar Fungicide	10.83b	27.93b	10.49b	29.04b	3.18b	16.88b	216.88ab
Seed+Foliar fungicide	8.48c	26.48b	9.59b	29.11b	3.14b	16.76bc	218.94b
Uninoculated-untreated	0.63d	7.31c	1.28c	32.56c	3.70c	16.55c	213.94a
LSD(0.05)	1.78	2.47	1.80	0.43	0.15	0.26	4.61

Means followed by the same letter within a column are not significantly different at P =0.05

Table 3-8. Combined treatment means across years (2012 and 2013) and locations (Carman and Winnipeg) for measured variables across the two winter wheat cultivars Emerson and CDC Falcon

Treatment	FHB index (%)	FDK (%)	DON (ppm)	TKW(g)	Yield(t/ha)	Protein (%)	Seedling stands (/m^2)
Inoculated-untreated	25.48a	30.31a	19.29a	25.53a	3.58a	13.89a	143.55a
Seed Fungicide	25.04a	29.36a	18.98a	25.62a	3.75ab	13.92a	158.45c
Foliar Fungicide	16.11b	25.28b	15.21b	26.58b	3.92bc	14.03a	147.5ab
Seed+Foliar fungicide	15.90b	24.83b	16.17b	26.78b	3.99c	13.99a	155.75bc
Uninoculated-untreated	0.55c	4.76c	1.67c	29.57c	4.60d	13.39b	143.06a
LSD(0.05)	2.58	1.85	2.04	0.49	0.18	0.18	8.54

Means followed by the same letter within a column are not significantly different at P =0.05

Table 3-9. Combined cultivar means across years (2012 and 2013) and locations (Carman and Winnipeg) for spring wheat across treatments

Cultivar	Susceptibility	FHB index (%)	FDK (%)	DON (ppm)	TKW(g)	Yield(t/ha)	Protein (%)	Seedling stands (/m^2)
Carberry	Moderately resistant	11.05a	22.37a	10.74a	30.02a	3.16a	16.98a	209.38a
Harvest	Susceptible	19.72b	36.20b	15.16b	27.60b	2.91b	16.82a	222.00b
LSD(0.05)		1.13	1.56	1.14	0.27	0.09	0.163	2.92

Means followed by the same letter within a column are not significantly different at P =0.05

Table 3-10. Combined cultivar means across years (2012 and 2013) and locations (Carman and Winnipeg) for winter wheat across treatments

Cultivar	Susceptibility	FHB index (%)	FDK (%)	DON (ppm)	TKW(g)	Yield(t/ha)	Protein (%)	Seedling stands (/m^2)
Emerson	Moderately resistant	12.01a	15.37a	8.77a	26.91a	4.28a	14.08a	164.97a
CDC Falcon	Susceptible	20.89b	30.09b	19.49b	26.75a	3.68b	13.62b	134.70b
LSD(0.05)		1.63	1.17	1.29	0.31	0.11	0.11	5.4

Means followed by the same letter within a column are not significantly different at P =0.05

Table 3-11. Combined Fungicide treatment means across years (2012 and 2013) and locations (Carman and Winnipeg) by cultivar and treatment for FHB index, Fusarium damaged kernel (FDK), deoxynivalenol (DON), thousand kernel weight (TKW), yield, protein content and seedling stand in spring wheat

Cultivar	Treatment	FHB index (%)	FDK (%)	DON (ppm)	TKW(g)	Yield(t/ha)	Protein (%)	Seedling stands (/m^2)
Carberry	Inoculated-untreated	20.68a	31.81a	18.53a	28.47a	2.81a	17.01a	204.50a
Carberry	Seed Fungicide	21.01a	32.54a	17.46a	28.24a	2.87a	17.01a	208.38ab
Carberry	Foliar Fungicide	7.08b	20.58b	8.40b	30.62b	3.35b	16.88ab	210bd
Carberry	Seed+Foliar fungicide	6.09b	21.32b	7.97b	30.25b	3.16c	16.73bc	217.13c
Carberry	Uninoculated-untreated	0.39c	5.59c	0.84c	32.52c	3.60d	16.5c	206.88ad
Harvest	Inoculated-untreated	35.48d	53.92d	25.30d	25.01d	2.26e	17.32d	223.63e
Harvest	Seed Fungicide	36.82d	51.10e	25.45d	24.91d	2.35e	17.31d	220.88e
Harvest	Foliar Fungicide	14.58e	35.29f	12.11e	27.47e	3.01c	16.88ab	223.75e
Harvest	Seed+Foliar fungicide	10.88f	31.64a	11.21e	27.97e	3.12c	16.78be	220.75e
Harvest	Uninoculated-untreated	0.86c	9.04h	1.72c	32.61c	3.80f	16.61ce	221.00e
LSD(0.05)		1.80	2.49	1.82	0.43	0.15	0.26	4.65

Means followed by the same letter within a column are not significantly different at P =0.05.

Table 3-12. Combined Fungicide treatment means across years (2012 and 2013) and locations (Carman and Winnipeg) by cultivar and treatment for FHB index, Fusarium damaged kernel (FDK), deoxynivalenol (DON), thousand kernel weight (TKW), yield, protein content and seedling stand in winter wheat

Cultivar	Treatment	FHB index (%)	FDK (%)	DON (ppm)	TKW(g)	Yield(t/ha)	Protein (%)	Seedling stands (/m^2)
Emerson	Inoculated-untreated	19.48ae	20.53a	11.96a	26.15ac	3.90a	14.18a	159.60ad
Emerson	Seed Fungicide	17.79a	19.08ab	11.00ab	25.99a	4.15b	14.19a	177.33b
Emerson	Foliar Fungicide	11.05b	17.73bc	9.68bc	26.73bcf	4.18b	14.23a	165.75ac
Emerson	Seed+Foliar fungicide	10.99b	16.75c	10.16abc	26.50cf	4.18b	14.18a	168.75c
Emerson	Uninoculated-untreated	0.26c	2.11d	0.72d	29.27d	4.97c	13.76b	158.25d
CDC_Falcon	Inoculated-untreated	30.35d	38.89e	25.89e	25.07e	3.29d	13.65b	130.75e
CDC_Falcon	Seed Fungicide	31.28d	38.4e	26.22e	25.32e	3.39d	13.77b	142.88f
CDC_Falcon	Foliar Fungicide	21.17e	32.84f	20.74f	26.83f	3.65e	13.83b	129.25e
CDC_Falcon	Seed+Foliar fungicide	20.81e	32.92f	22.17f	26.67f	3.81e	13.81b	142.75f
CDC_Falcon	Uninoculated-untreated	0.84c	7.41g	2.44d	29.86g	4.24f	13.03c	127.88e
LSD(0.05)		2.61	1.87	2.08	0.50	0.18	0.18	8.64

Means followed by the same letter are not significantly different at P =0.05.

3.4.4 FHB traits (FHB index, FDK and DON) assessment

In both spring and winter wheat, the disease traits FHB index, FDK and DON were significantly reduced by the application of foliar fungicide compared with the seed fungicide treatment and the inoculated-untreated control (Table 3-7 and 3-8). No significant difference was observed between seed treatment and the inoculated-untreated control. Although seed+foliar fungicide had significant lower FHB index across spring wheat cultivars, there was no significant difference between foliar fungicide treatment and seed + foliar fungicide treatment in other measured variables across spring and winter wheat cultivars (Table 3-7 and 3-8). The numerical differences between foliar fungicide treatment and seed+foliar fungicide treatment in reducing FHB index, FDK and DON were very small and in most cases were not statistically significant. This fact indicates that seed + foliar fungicide was not superior to foliar fungicide alone in controlling FHB disease.

The effects of genetics were significant in controlling FHB disease (Table 3-3 and 3-4). The moderately resistant cultivars Carberry and Emerson had significantly lower FHB index, FDK and DON than the more susceptible cultivars (Table 3-9 and 3-10). The inoculated-untreated control in moderately resistant cultivars had significantly lower FHB index, FDK and DON than in the susceptible cultivars (Table 3-11 and 3-12).These results suggest that by growing FHB resistant cultivars, the cost of wheat production can be reduced due to less fungicide use. Seed treatment alone was not effective in controlling FHB in either moderately resistant or susceptible cultivars as it was not significantly different from inoculated-untreated control for FHB traits. In contrast, foliar fungicide application significantly reduced FHB traits in both moderately resistant and susceptible cultivars (Table

3-11 and 3-12). In most cases, there was no significant difference between foliar fungicide treatment and seed + foliar fungicide treatment in reducing FHB index, FDK and DON in these four cultivars (Table 3-11 and 3-12). The only significant difference between foliar fungicide and seed + foliar fungicide treatments was observed for FHB index and FDK in Harvest (Table 3-11). Comparison of the foliar fungicide treatments (foliar fungicide and seed + foliar fungicide treatments) between moderately resistant and susceptible cultivars, showed that FHB index, FDK and DON were approximately 40%-50% lower in moderately resistant cultivars than in susceptible cultivars (Table 3-11 and 3-12). Although uninoculated-untreated plots were also infected by FHB either due to wind drifting effects during artificial inoculation or natural inoculum from fields, FHB index, FDK and DON concentrations were low in both spring and winter wheat for this treatment (Table 3-11 and 3-12). It is worth mentioning that in the uninoculated-untreated controls, DON concentrations in moderately resistant cultivars were under the maximum allowable level (1 ppm) in processed wheat products. These results suggest that under low FHB disease pressure, genetic resistance alone is enough to minimize economic loss.

3.4.5 Thousand kernel weight (TKW) and grain yield

Application of foliar fungicide significantly increased TKW and grain yield compared with the inoculated-untreated control, but no significant differences were found between foliar fungicide and seed + foliar fungicide treatments (Table 3-7 and 3-8). No significant differences for TKW and grain yield were observed between the seed fungicide

treatment and the inoculated-untreated control. In both spring and winter wheat, grain yield in the inoculated-untreated control was more than 1 t/ha lower than that in uninoculated-untreated control.

The moderately resistant cultivars Carberry and Emerson had significantly higher yield compared with the more susceptible cultivars across years, locations, and treatments (Table 3-9 and 3-10). In spring wheat, Carberry had significantly higher TKW than Harvest (Table 3-9). In winter wheat, there was no significant difference between Emerson and CDC Falcon for TKW (Table 3-10).

Within each cultivar, under inoculation, the highest TKW and grain yield were found in foliar fungicide or seed + foliar fungicide treatment (Table 3-11 and 3-12). In addition, foliar fungicide or seed + foliar fungicide treatments always had significant higher TKW and grain yield than the inoculated-untreated control (Table 3-11 and 3-12). In most cases, there was no significant difference between seed treatment and the inoculated-untreated control for TKW and yield in each cultivar. High FHB disease pressure in plots without fungicide application (inoculated-untreated plots) led to severe grain yield loss. Grain yield for the inoculated-untreated control was 0.79 t, 1.54 t, 1.07 t and 0.95 t less than that for uninoculated-untreated control in Carberry, Harvest, Emerson and CDC Falcon, respectively (Table 3-11 and 3-12).

3.4.6 Protein content and seedling stand density

In spring wheat, the inoculated-untreated control and seed fungicide treatment plots had significant higher protein content than plots sprayed with foliar fungicide (Table 3-7). However, the same results were not observed in winter wheat (Table 3-8). Significant

differences in protein content between moderately resistant and susceptible cultivars were not observed in spring but were in winter wheat (Table 3-9 and 3-10). The different cultivar response for winter wheat vs spring wheat may be due to relatively large inherent differences in the genetic potential for protein content between Emerson and CDC Falcon. Emerson is a Canada Western Red Winter (CWRW) wheat and CDC Falcon has been moved to the general purpose class because its protein content is too low for the CWRW class. In both winter wheat cultivars, all three fungicide treatments and the inoculated-untreated control had significantly higher protein content than the uninoculated-untreated control (Table 3-12). In Carberry and Harvest, the inoculated-untreated control, seed fungicide treatment and foliar fungicide treatment had significantly higher protein content than uninoculated-untreated control (Table 3-11).

There was no significant effect of treatment on seedling stand for spring wheat (Table 3-3), however, a significant effect was found in winter wheat (Table 3-4). No significant difference for winter wheat seedling stands was observed between cultivars and among treatments in the fall of 2011 (data not shown) while significant differences were observed in spring seedling stands (Table 3-8 and 3-10). There was a significant difference in spring seedling stand density between the winter cultivars, where Emerson had significant higher spring seedling stand than CDC Falcon. This supports the field observation that Emerson has better winter hardiness than CDC Falcon (Seed Manitoba, 2015). Seed treatments (seed fungicide and seed + foliar fungicide) improved spring seedling stands in winter wheat (Table 3-8). In winter wheat cultivars Emerson and CDC Falcon, the two highest seedling stand densities were found in the treatments of seed fungicide and seed + foliar fungicide (Table 3-

12). The FHB susceptibility level of the cultivar did not affect its seedling stand density, since the susceptible cultivar might have higher or lower seedling stand density than moderately resistant cultivar (Table 3-9 and 3-10).

3.5 Discussion

During the two experimental seasons 2012-2013, the field plots were mist-irrigated after inoculation to provide an environment that was favorable to the development of FHB disease in wheat. All inoculated plots successfully developed FHB symptoms. Some of the wheat plants in un-inoculated plots were also infected at low levels by FHB due to possible drifting effects during inoculation or natural inoculum in the environment.

In the present study, FHB index and DON, FHB index and FDK, and FDK and DON were positively and highly correlated in both spring and winter wheat ($r \geq 0.95$). This result is consistent with some published results (Haidukowski et al. 2004; Wegul et al. 2011). The strongest two correlations were found between FHB index and DON in spring wheat ($r=0.99$) and FDK and DON in winter wheat ($r=0.99$). Test of DON content in grain samples is expensive, these strong correlations suggest that FHB Index and FDK can be used as good indicators of DON content in severe FHB epidemics. Paul et al. (2005) analyzed 163 published and unpublished studies to determine the overall mean correlation coefficients between FHB traits. They found that FDK had the strongest positive correlation with DON. However, Wegul et al. (2011) observed that the strongest correlation was consistently between FHB index and DON in their study. In this study, although the highest correlation between FHB traits differed between wheat growth habits, correlations between FDK and

DON, and FHB index and DON were all very strong in each wheat growth habit. High correlations between FHB index, FDK and DON are not always observed in other studies. Amarasinghe et al. (2013) reported weak correlations between FHB index and DON (r=0.367), and FDK and DON (r=0.339) in field trials in 2010. In another study, weak correlations between FHB index and DON, FHB index and FDK were observed though they were significantly correlated (Tamburic-Ilincic, 2012). FHB index can be assessed in two ways, either by visually estimating FHB incidence and severity in the field plots, or by counting FHB incidence and severity in collected wheat spikes to calculate FHB index. The accuracy of visual rating depends on personal experience and rating skills. FDK percentage can be expressed either by weight or number. The differences in the results of correlations between FHB traits in studies may be due to differences in the methods used for assessing FHB index and FDK. In addition, variation in weather conditions in experimental fields can be another factor that leads to different results among studies.

Yield and TKW were negatively correlated with FHB index, FDK and DON in both spring wheat and winter wheat. These correlations were stronger in spring wheat than in winter wheat. In contrast, protein content was positively correlated with FHB index and FDK in spring wheat though their correlations were not significant in winter wheat. This would be expected since FHB infection is associated with smaller seed size, leading to a higher protein concentration in the seed. It was interesting to find that protein content significantly correlated with all the measured traits except seedling stand density in spring wheat, while protein content was only significantly correlated with seedling stand density in winter wheat. In spring wheat, significant differences in protein content among some treatments were

observed (Table 3-11). In winter wheat, there was no significant difference among fungicide treatments for protein content in each cultivar (Table 3-12). These results might be a good explanation for protein correlation differences in spring and winter wheat.

In this study, cultivar, fungicide treatment, and their interactions had significant effects on most of the measured variables. Application of triazole-based foliar fungicide Prosaro (tebuconazole + prothioconazole) significantly reduced FHB index, FDK and DON content and yield loss compared with the inoculated-untreated control. These results confirm the effectiveness of triazole-based fungicide application in managing FHB and DON contamination in harvested grain. Mesterhazy et al. (2003) reported that fungicides containing tebuconazole tended to be more effective in reducing FHB than those without tebuconazole. Paul et al. (2007) analyzed 139 studies for the effect of tebuconazole on FHB index and 101 studies for the effect of tebuconazole on DON contamination of harvested grain in susceptible cultivars and found that the overall mean percent control of FHB index and DON was 40.3 and 21.6%, respectively. However, the efficacy of tebuconazole was variable. Variation in the efficacy of triazole-based fungicides in managing DON contamination had also been noted in another recent study (Amarasinghe et al. 2013). Amarasinghe et al. (2013) found that DON content in some grain samples treated with prothioconazole and prothioconazole + tebuconazole increased when compared with the controls without fungicide application, although these fungicides successfully reduced DON in most of the treatments. They explained that fungicide application reduced FHB symptoms and increased seed size sufficiently that diseased seeds were not lost in harvest and thus could contribute to a higher percentage of FDK and DON in the samples. Variability of fungicide

treatment effects may be due to differences in weather conditions in the target experimental fields. For example rainy weather during fungicide application may result in low efficacy (Sip et al. 2010). Other sources of variability including fungal virulence, level of cultivar resistance, and timing and coverage of the fungicide application (Mesterhazy et al. 2003).

The role of fungicide in increasing the level of DON content in infected grains under field conditions is not well understood. Results from an *in vitro* study showed that the amount of DON produced by *F. graminearum* was influenced by complex interactions between water activity, temperature, and fungicide concentrations (Ramirez et al. 2004). They assumed that fungal strains responded to the presence of sub-lethal concentration of fungicide by increasing the production of secondary metabolites including DON. Audenaert et al. (2010) also reported that sub-lethal doses of triazole fungicide prothioconazole stimulated DON production. More research is needed to better understand the relationship between fungicide application and increased DON accumulation in infected grains under field conditions.

The effect of seed treatment on FHB management is not well documented. In this study, seeds treated with Cruiser Maxx® Cereals (thiamethoxam, difenoconazole, metalaxyl-M and S-isomer) didn't result in significant reduction of FHB index, FDK, DON and yield loss compared with inoculated-untreated control (Table 3-7 and 3-8). However, seed treatment significantly increased seedling stand density across the two winter wheat cultivars (Table 3-8) and yield in Emerson compared with the inoculated-untreated treatment (Table 3-12). In most cases, seed treatment was not significantly different from inoculated-untreated control for FHB index, FDK and DON in each cultivar (Table3-11 and Table 3-12). Results from this study suggest that seed treatment is not effective in

controlling FHB under high disease pressure. Seed treatment is more commonly used to prevent disease rather than controlling disease. In order to prevent Fusarium seedling blight, seed treatment is suggested when growing wheat in fields with high levels of *Fusarium* inoculum. Fusarium-infested seed results in poor seedling emergence and number of tillers, therefore, yield can be significantly reduced if the proportion of infested seed is high (Gibert et al. 2003). Seed treatments using bitertanol, difenconazole, triticonazole, maneb, fludioxonil or guazatine significantly improved germination and reduced Fusarium seedling blight in three field trials with 5-45% infested seeds, however, no significant improvements in yield were observed (Jorgensen et al. 2012). In another two field trials with more than 90% infested seeds, fludioxonil significantly improved germination rate and yield was increased by 1.2-1.5 t/ha compared with the control. However, seed treatments with fludioxonil failed to reduce FHB symptoms and DON contamination in the harvested grain (Jorgensen et al. 2012). Different findings were observed in another study, where fludioxonil was found to minimize the attack and spread of mycotoxins to wheat spikes (Klix et al. 2009). Seeds used in this study were healthy seeds and no Fusarium seedling blight was observed. Therefore, lack of significant effects of seed treatment in this study may be a function of the use of healthy seed and an environment that was not conducive to pathogens that affect young seedlings. Seed treatment may not be necessary in fields without the history of FHB, as yield returns may not be able to offset the cost of the application of seed treated fungicides.

In this study, the level of FHB resistance in wheat cultivars had a significant effect on all measured variables except protein content in spring wheat. Moderately resistant

cultivars had significantly lower FHB index, FDK and DON and higher yield compared with susceptible cultivars (Table 3-9 and Table 3-10). Similarly, McMullen et al. (2008) reported that using moderately resistant cultivars alone resulted in an 86% reduction in FHB field severity and a 64.7% reduction in DON compared to susceptible cultivars. Lower mycotoxin levels in resistant cultivars may be due to an inhibition of the spread of the fungus within the spike as well as a detoxification of the DON produce by the fungus (Peiris et al. 2011). Application of prothioconazole + tebuconazole at flowering of moderately resistant cultivars resulted in lower FHB and DON and higher yield (McMullen et al. 2008). In the current study application of Prosaro or Prosaro+Cruiser combined with moderately resistant cultivars resulted in lower FHB index, FDK and DON than when these treatments were applied to susceptible cultivars. This is consistent with the results of previous studies (Mesterhazy et al. 2003; Wegul et al. 2011) where fungicide efficacy in reducing FHB index, FDK and DON was higher in the moderately resistant cultivars compared to susceptible cultivars. These findings suggest that a combination of cultivar resistance and fungicide application in an integrated management strategy can result in a better control of FHB and reduce DON contamination than either individual disease control measure on its own. For the uninoculated-untreated plots, a low percentage of spikes were infected due to inoculation drift or natural inoculum, but the DON contents in the moderately resistant cultivars were less than 1ppm (Table 3-11 and 3-12). This fact indicates that in years with low FHB disease pressure, farmers can benefit from growing moderately resistant cultivars, reducing the need for fungicide application, as genetic resistance alone may be adequate in preventing economic loss due to FHB occurrence.

Results from this study provide useful information to help understand the impact of cultivar susceptibility and triazole fungicide application to control FHB in wheat. In this study, application of foliar fungicide and seed + foliar fungicide significantly reduced FHB index, FDK and DON and increased TKW and yield compared with inoculated-untreated controls in all tested cultivars. In addition FHB index, FDK and DON were lower and higher yield was observed in the more resistant cultivars. These results suggest that integrating cultivar resistance and fungicide application can be an effective strategy for managing FHB. However, we should notice that there is an urgency to breed cultivars highly resistance to FHB, as integrating moderately resistant cultivars, seed treatment and triazole foliar fungicide application under high FHB pressure failed to reduce DON content to a level below the maximum limit (1 ppm) allowed for some uses, which makes these infected grains difficult to market. In the future, other methods which have proven to have a possible effect on FHB control in wheat, such as previous crop residue management, crop rotation and biological control should be introduced into this integrated strategy and tested to evaluate their impact on FHB management.

4.0 QTL Analysis of Resistance to Fusarium Head Blight (FHB) in Winter Wheat

4.1Abstract

Breeding for Fusarium head blight (FHB) resistance in wheat is a promising strategy for FHB control. Breeders have relied heavily on Asian sources of FHB resistance. In this study, a European winter wheat was used as source of resistance. A population of 89 double haploid (DH) lines from the cross Mironovskaja 808 (moderately resistant)/AC Ron (susceptible) was used to identify quantitative trait loci (QTL) for resistance to FHB-related traits. This population was evaluated for Type II resistance (resistance to FHB spread within spike) under greenhouse conditions over a period of two years using dual-floret inoculation, and other types of resistance were evaluated in spray-inoculated field trials for one year at three different locations (Winnipeg and Carman MB, and Ridgetown ON). Four QTL were associated with FHB resistance under greenhouse and field conditions on chromosomes 2B, 4D, 2D and 7A. One QTL associated with type II resistance was consistently detected on chromosome 2B under greenhouse conditions in both 2012 and 2013. This QTL was also associated with resistance to field FHB incidence (type I resistance) in Ridgetown, field FHB severity (type II resistance) in Winnipeg and FHB index in Carman and Ridgetown. The QTL on chromosome 4D was responsible for resistance to Fusarium damage kernels (FDK) at the Carman and Ridgetown locations. It was also associated with resistance to field FHB incidence, FHB index and deoxynivalenol (DON) at Carman.

4.2 Introduction

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum* Schwabe [telomorph:*Gibberella zeae* Schwein (Petch)], is an economically important fungal disease that occurs in most wheat growing regions of the world (McMullen et al. 1997). FHB infects the wheat spike during flowering and may cause serious losses in yield and end-use quality in epidemic years. Furthermore, FHB infected grains, also called Fusarium damaged kernels (FDK), are contaminated with mycotoxins such as deoxynivalenol (DON) that are toxic to humans and animals. Thus, DON contamination leads to further economic loss. FHB management strategies such as fungicide application, crop rotation, crop residue management and tillage have been studied, producing in-consistent results. Breeding for FHB resistant cultivars is considered to be a key component in an effective and economical strategy to reduce the risk of FHB.

FHB resistance in wheat is a quantitative trait and controlled by a few quantitative trait loci (QTL) with major effects and many QTL with minor effects (Buerstmayr et al. 2002; Sneller et al. 2010). More than 100 QTL have been implicated in FHB resistance of which the most repeatedly detected QTL are *Fhb1* on chromosome 3BS, *Fhb2* on chromosome 6BS and *Qfhs.ifa-5A* on chromosome 5AS (Buerstmayr et al. 2009). Breeding for FHB resistance in wheat is time-consuming and labor intensive due to the need to screen adult plants and harvest seed to measure the different types of FHB resistance. Type I resistance (resistance to spike initial infection) and type II resistance (resistance to spread of infection within the spike) are the two types of resistance that are well characterized. Screening of genotypes for type I resistance is achieved by spray inoculation followed by disease incidence evaluation in

field nurseries. Type II resistance is commonly evaluated under controlled conditions by inoculating a floret within the spikelet and assessing disease severity of the inoculated spike. Evaluation of resistance to kernel damage and DON accumulation are performed by counting percentage of FDK and determining DON content in each genotype. Marker assisted selection for resistance can accelerate breeding progress, but requires reliable QTL.

Genetic variation for resistance to FHB and resistant genotypes has been identified in wheat, but no complete resistance or immunity to FHB has been detected (Gervais et al. 2003). Three origins of resistant wheat germplasm sources have been recognized, including Asian, Brazilian and European sources (Gervais et al. 2003). The Chinese spring wheat Sumai 3, and its derivatives, are the most commonly used Asian resistant sources in FHB breeding programs worldwide (Rudd et al. 2001; Bai et al. 2003; Badea et al. 2008). Other sources of genetic resistance include Nobeokabouzu from Japan, and Frontana from Brazil， Praa 8 and Novkrumka from Europe (Gilbert and Tekauz, 2000; Rudd et al. 2001; Badea et al. 2008). Ernie and Freedom from the USA are also used as sources of resistance in some breeding programs (Rudd et al. 2001). Over use of a limited source of resistance may give rise to selection pressure on the FHB pathogens and promote the development of pathogenesis against resistance genes (Gervais et al. 2003). In recent years, European winter wheat has attracted more attention from wheat breeders for their potential as an alternative source of FHB resistance (Draeger et al. 2007).

Mironovskaja 808 is a European winter wheat cultivar that was reported to possess resistance to the infection of some *Fusarium* species (Shpokauskene1977). AC Ron is a Canadian winter wheat cultivar that is susceptible to FHB. FHB severity and DON

accumulation was high in AC Ron after artificial spray inoculation (Tamburic-Ilincic and Schaafsma 2007). A cross between AC Ron and Mironovskaja 808 was made and a double haploid (DH) population with 89 lines was developed. The objectives of this study were to characterize FHB resistance in this DH population and to identify QTLs for FHB resistance.

4.3 Materials and Methods

4.3.1 Mapping population development

A double haploid (DH) population of 89 lines of winter wheat was developed from a cross between AC Ron and Mironovskaja 808 (cross designation ARM8). Wheat-maize hybridization followed by embryo rescue and cholchicine treatment were used to develop the double haploid population (Thomas et al. 1997).

4.3.2 Fusarium head blight phenotyping (Type II resistance) in the greenhouse

The 89 DH lines and parents of the mapping population，along with six checks (32C*17, FHB148, Freedom, Hanover, 43I*18, Caledonia) with different FHB resistant levels were grown in the greenhouse for FHB resistance evaluation. A three replicate randomized complete block design was used and repeated over two years in 2012 and 2013. One plant was planted per pot. Two plants from each DH line were included in each replicate. Supplemental incandescent light was set for16h daylight and the temperature ranged from 18 to 25 °C. Three spikes from each plant were inoculated when they reached 50% anthesis (Zadoks GS65). The primary and secondary floret in a spikelet that was two-thirds from the base or one-third from the top of the spike, were inoculated by injecting 10ul macroconidia suspension (50,000 spores/ml) between the lemma and palea. The inoculum solution was

prepared by mixing equal quantities of macroconidia of four isolates of *F. graminearum* (M7-07-1, M9-07-1, M1-07-2 and M3-07-2) in distilled water to make a concentration of 50,000 spores/ml (inoculum was produced as described in previous chapter). Tween 20 (polysorbate surfactant) was added at a rate of 5 ml/L. After dual floret inoculation, the inoculated spike was covered with a glassine crossing bag for 24 hours to maintain moisture around the inoculated spike and facilitate infection. In order to make sure that the inoculated spikes reached maximum disease symptoms before disease symptoms were indistinguishable from natural senescence color, disease severity was monitored 7, 14, 21 and 25 days after inoculation. Disease severity in the greenhouse was rated as the percentage of infected spikelets per spike at 25 days after inoculation (Type II resistance). FHB infection blocks the transport of water and nutrients to distal spikelets and causes early senescence that might be mistaken as FHB symptom. Therefore, only the number of infected spikelets and total number of spikelets below the inoculated florets (excluding the inoculated florets) were counted to determine disease severity (disease severity = infected spikelets / total spikelets x 100%) for each inoculated spike. Disease severity of the three inoculated spikes was averaged to calculate disease severity for each plant.

4.3.3 Fusarium head blight phenotyping in the fields

The materials used for field trials were 89 DH lines, the two parents of the mapping population and the same six checks as used in the greenhouse study (97 entries in total). The field trials were conducted at two locations in Manitoba (Winnipeg and Carman) and one location in Ontario (Ridgetown) in the 2013 field season. The field experimental design was a

randomized complete block design with four replicates. Field plots consisted of a single one meter row with 30cm row spacing. The seeding rate was 70 seeds per row. Dates of 50% heading and 50% anthesis were recorded for each plot. When the wheat in the plot reached 50% anthesis (Zadoks GS65), the plot was spray-inoculated with a mixed macroconidial suspension of four different isolates of *F. graminearum* (M7-07-1, M9-07-1, M1-07-2 and M3-07-2) with a concentration of 50,000 spores/ml. The inoculum was applied at a rate of 50 ml per plot, using a backpack-mounted sprayer pressurized to 30 PSI with CO_2 gas. A second inoculation was performed two or three days after the first inoculation. One hour after each inoculation, plots were mist-irrigated for five minutes every hour for 12 hours using an overhead misting system to maintain a humid environment conducive to disease development. All plots were mist-irrigated for a period of seven to ten days.

Plots were monitored for FHB symptom development. FHB incidence and severity of each plot were visually rated 18- 21 days after the first inoculation before disease symptoms became indistinguishable from natural senescence. FHB incidence for each plot was expressed as the percentage of spikes showing FHB symptoms (0-100%), and severity was recorded as a percentage of diseased spikelets on individual infected spikes (0-100%). FHB index for each plot was calculated by multiplying the disease incidence by the severity (disease incidence × disease severity/100). In order to verify the accuracy of visual rating, 50 spikes from each plot were randomly harvested from one of the three replicates at Winnipeg and Carman on the day of visual rating in the field and stored at -20°C until manual counts of FHB incidence and severity were conducted.

Plots were harvested at maturity when there was no green coloration remaining in the

peduncle. Harvested spikes were placed in cloth bags and dried for three days at 36°C using a forced air system. Wheat spikes were threshed and cleaned using a belt thresher. Wind speed was reduced to retain as many Fusarium damaged kernels (FDKs) as possible. A subsample of 250 seeds from each plot was used to determine the percentage of FDK (diseased seeds/total seeds*100). Any seed that was shriveled with mycelia growth on the surface, or chalky white, or pink discoloration was considered as FDK. After percentage of FDK was determined for each plot, seeds from the three replicates of each DH line at each location (two replicates at Ridgetwon) were mixed to generate a composite sample. A subsample of 50 g grain from the composite sample was ground into flour to pass through a 0.85 mm sieve for DON quantification. DON concentration in each sample was analyzed by using a certified procedure of Neogen Enzyme-linked immunosorbent assay (ELISA) veratox 5/5 DON kit. The minimum and maximum DON concentrations that can be quantified by this method are 0.5 ppm and 5ppm, respectively. When DON concentration exceeded 5 ppm within a sample, a standard dilution was used to ensure the accuracy of the measurement.

4.3.4 Genetic mapping and QTL analysis

Two to three pieces of young leaves (~4cm in length) from one plant of each DH line were collected and immersed in liquid nitrogen at two weeks after seeding. Leaf samples were freeze dried for 24 hours and stored at -20 °C until time was available for DNA extraction. DNA was extracted by using the modified CTAB method (Huang et al. 2000) (Appendix 7-1). PicoGreen dsDNA quantification reagent was used to quantify double-stranded DNA samples. The working DNA samples were adjusted to a concentration of 50

ng/ul. A high-density 90K SNP Infinium iSelect assay for wheat was used to assess SNP variation in the 89 DH lines and the two parental lines. Infinium iSelect genotyping data was analyzed using Genome Studio software (Illumina, USA). Markers with more than 10% missing data were culled. A framework linkage group was constructed using MSTMap software which was further refined using CarthaGene (version 1.3.beta). When multiple markers were in the same position of a linkage group, only the one with the least missing data was selected. All other markers were not included in the map. In MapDisto (version 1.7.7), for each segregating marker, a Chi square test (α= 0.01) was performed to test for deviation from the expected segregation ratio (1:1) to remove highly skew markers ($p<0.01$). Marker data were checked and error candidates in markers were replaced by missing data. After that, a total number of 980 SNP markers were used to generate linkage groups. Different logarithms of odds (LOD) scores and r values were tried to generate linkage groups. The LOD score of 4 and r value of 0.3 were chosen due to the improved LOD curve. The marker order in each linkage group was refined and recombination fractions were converted into map distances in centiMorgans (cM) using the Kosambi mapping function. Linkage groups were assigned chromosome names by comparing markers on previously published polyploid wheat high density SNP maps (Wang et al. 2014).

Phenotypic data from the greenhouse and field and the marker data were used for QTL analysis by using QGene software (version 4.3.10). The QTL analysis was performed for greenhouse disease severity in 2012, 2013 and the combined 2012 and 2013 data, and for field FHB traits at each experimental location and across three locations in 2013. The QTL analyses were performed using simple interval mapping (SIM) with QGene 4.3.10. A

permutation test of 1000 times for each variable in each year (greenhouse data) or at each location (field data) was performed to determine the appropriate LOD score for significant QTL. QTL scan interval was 1cM. QTL position, additive effect and the percentage of phenotypic variation explained were estimated. Charts of genetic linkage maps and QTL position were drawn by using Mapchart 2.2.

4.3.5 Phenotypic data analysis

Analysis of variance (ANOVA) for greenhouse disease severity, field disease incidence, field disease severity, field FHB index, FDK and DON were performed using the PROC GLM procedure of SAS version 9.2 (SAS Institute Inc., Cary, NC). Means for each experimental year in the greenhouse and each field site were generated and used for QTL analysis. Normality of residual distribution was tested using the PROC UNIVARIATE procedure, and homogeneity of phenotypic variance for years and locations was verified using Levene's test. A combined analysis was used for homogeneous data. The model statement used in the combined analysis for greenhouse disease severity was variable = year rep(year) genotype genotype*year. The model statement used in the combined analysis for field FHB response variables (FHB index, incidence, severity and FDK) was variables =location genotype rep(location) genotype*location. The model statement for DON was variable = genotype location.

4.4 Results

4.4.1 Trait analysis

In this study, 50 spikes from each plot were collected from one of the three replicates at Carman and Winnipeg to verify the accuracy of visual rating. The strong correlation (r = 0.93) between visually rated FHB index and FHB index determined on counted samples indicate that the visual evaluations in the field were reliable across the full range of values (Figure 4-1).

Analysis of variance (ANOVA) for greenhouse FHB severity showed significant effects of year, rep(year), genotype and genotype*year interaction (Table 4-1-1). Genotype, location, rep(location) and genotype*location interaction effects were significant for all FHB traits evaluated in the fields at three locations (Table 4-1-2).

Figure 4-1. Scatter plot between visually rated FHB index and FHB index by counting fifty collected spikes from one of three replicates at Carman and Winnipeg.

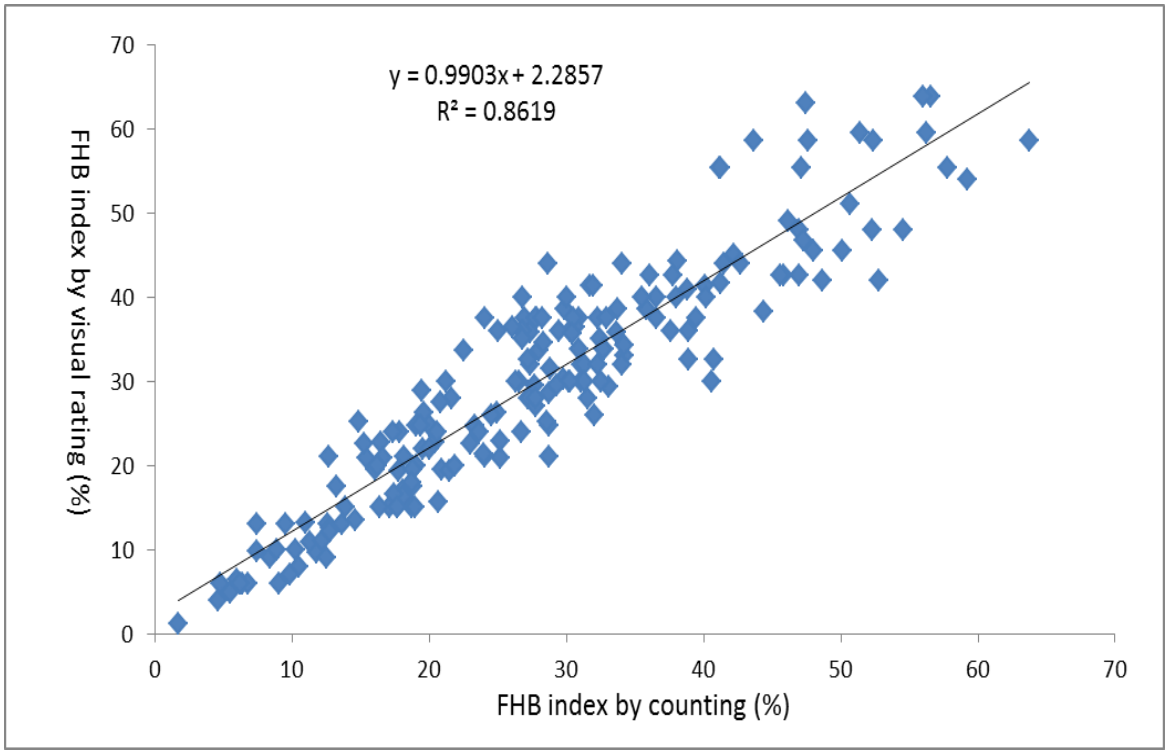


Table 4-1-1. Analysis of variance for greenhouse disease severity over two years (2012 and 2013) and field phenotypic variables from three locations (Carman, Winnipeg and Ridgetown).

Source	DF	Mean square	P-value
Year	1	2917.9	0.0023
Rep(Year)	4	6050.1	<.0001
Genotype	96	3710.6	<.0001
Genotype*Year	94	601.7	<.0001
Error	896	312.3	

Table 4-1-2. Analysis of variance for field FHB traits at three locations (Carman, Winnipeg and Ridgetown) in 2013.

Source	DF	Mean square	P-value
Field FHB Incidence			
Location	2	46409.1	<.0001
Genotype	96	1100.8	<.0001
Rep(Location)	6	285.3	0.0135
Location*Genotype	191	179.3	<.0001
Error	570	105.5	
Field FHB Severity			
Location	2	12745.5	<.0001
Genotype	96	797.9	<.0001
Rep(Location)	6	200.5	0.0068
Location*Genotype	191	105.0	<.0001
Error	570	66.8	
Field FHB Index			
Location	2	45601.4	<.0001
Genotype	96	1253.6	<.0001
Rep(Location)	6	244.5	0.0123
Location*Genotype	191	167.9	<.0001
Error	570	89.1	
Field FDK			
Location	2	42877.5	<.0001
Genotype	96	1504.0	<.0001
Rep(Location)	6	211.0	0.0316
Location*Genotype	191	156.23	<.0001
Error	474	85.3	
DON			
Location	2	69054.8	<.0001
Genotype	96	805.4	<.0001
Error	191	317.6	

The DH population developed from “Mironovskaja 808/AC Ron” displayed a wide and continuous distribution for greenhouse FHB severity (GH SEV) and all field evaluated FHB related traits (Figure 4-2). Transgressive segregation was observed for all the traits evaluated, where some DH lines showed higher or lower disease symptoms than their parents (Figure 4-2 and Table 4-2). The greenhouse disease severity of the DH population ranged from 12% to 95% and FHB severity of Mironovskaja 808 was 32% lower than that of AC Ron (83%) (Table 4-2). In the field studies, the two parents were very similar for FHB traits, while Mironovskaja 808 had an even higher percentage of Fusarium damaged kernel (FDK) than the susceptible parent AC Ron (Table 4-2).

Correlations between evaluated FHB traits were all positive and statistically significant (Table 4-3). However, correlations between the greenhouse disease severity and FHB related traits evaluated in the fields were not very strong, correlation coefficients ranged from 0.27 to 0.45 (Table 4-3). Poor correlation between greenhouse FHB severity and field evaluated traits may be due to differences in the environments where they were evaluated. Field evaluated FHB traits were all strongly correlated with each other. In the field, the two highest correlations were found in disease incidence (Field INC) and severity (Field SEV) with FHB index (Field IND), at $r = 0.98$ and $r = 0.93$, respectively. Deoxynivalenol (DON) concentration had relatively weaker correlations with other measured FHB traits in the field trials compared with correlations among other field measured FHB traits.

Figure 4-2. Frequency distribution of 89 double haploid lines developed from Mironovskaja 808/ AC Ron for Fusarium head blight (FHB) responses in greenhouse and field studies. Greenhouse disease severity (GH-SEV) was averaged over two years. Field visually rated Field FHB incidence (Field-INC), Field FHB severity (Field-SEV), Field FHB index (Field-IND), Fusarium damaged kernel (FDK) and deoxynivanol (DON) were averaged over three locations (Carman, Winnipeg, Ridgetown).

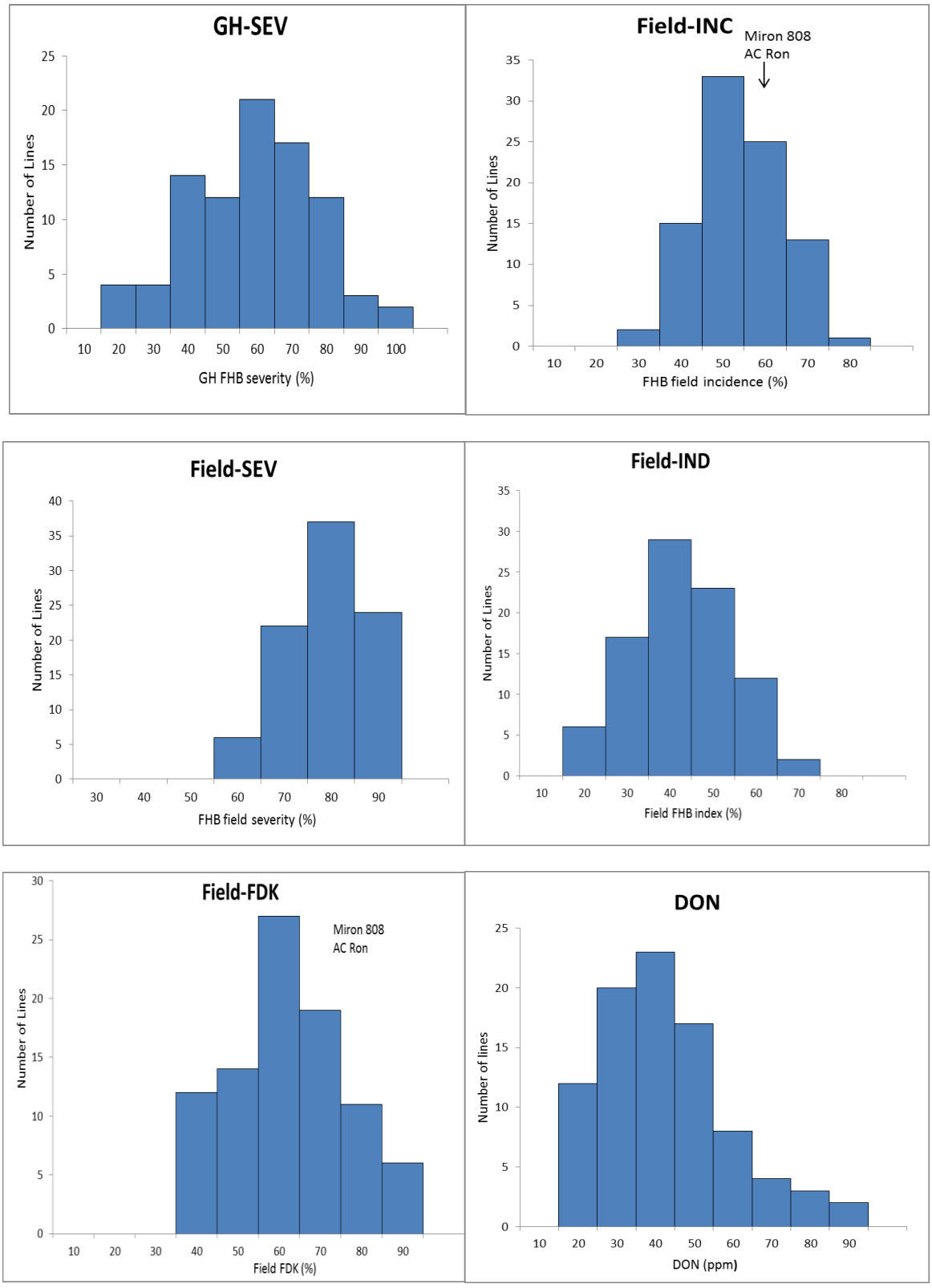


Table 4-2. Means and ranges for the evaluated traits of the population "Mironovskaja/AC Ron" (greenhouse mean value across two years 2012 and 2013, field mean value across three locations). Greenhouse disease severity (GH SEV), Field FHB incidence (INC), Field FHB severity (SEV), Field FHB index (IND), Fusarium damaged kernel (FDK) and deoxynivanol (DON)

Trait	Population mean	Population range	Mironovskaja 808	AC Ron
GH SEV(%)	54	12-95	51	83
Field INC(%)	49	30-74	54	59
Field SEV(%)	73	52-87	79	78
Field IND(%)	38	16-64	44	46
FDK(%)	57	30-85	65	60
DON(ppm)	38	13-84	56	64

Table 4-3. Pearson correlation coefficients between greenhouse disease severity (GH SEV), Field FHB incidence (INC), Field FHB severity (SEV), Field FHB index (IND), Field Fusarium damaged kernel (FDK) and deoxynivanol (DON) in the field evaluation using means across two years (GH) and three locations (field).

	GH SEV	Field INC	Field SEV	Field IND	FDK	DON
GH SEV	1					
Field INC	0.45****	1				
Field SEV	0.44****	0.89****	1			
Field IND	0.47****	0.98****	0.93****	1		
FDK	0.37***	0.88****	0.87****	0.89****	1	
DON	0.27**	0.70****	0.66****	0.70****	0.77****	1

Note: **, ***, **** significant at $p<0.01$, $p<0.001$ and $p<0.0001$, respectively. N=97

4.4.2 QTL analysis

QTL associated with FHB resistance localized on chromosomes 2B, 2D, 4D and 7A. QTL and their corresponding traits are listed in Table 4-4 and Table 4-5. QTL for 2D and 7A were from the susceptible parent, all other QTL were from the resistant parent. In the greenhouse evaluation for FHB type II resistance, one significant FHB resistance QTL on

chromosome 2B from Mironovskaja 808 was consistently detected in 2012 and 2013, explaining 19.6% and 26% of the phenotypic variation, respectively. This QTL explained 24.8% of the phenotypic variation for greenhouse severity over two years (Table 4-4). Altogether, four different QTL were identified on chromosome 2B, 2D, 4D and 7A for FHB related traits in the field traits (Table 4-5). The chromosome 2B QTL identified in the field was identical with the 2B QTL detected in greenhouse for type II resistance (Figure 4-3.) The QTL on chromosome 2B was also associated with field FHB incidence at Ridgetown, FHB severity at Winnipeg, and FHB index at Carman and Ridgetown. However, it was not consistently detected across locations for each trait. The susceptible parent AC Ron contributed two QTL that were assigned to chromosome 2D and 7A for reducing field disease severity at Ridgetown and Winnipeg, respectively (Table 4-5). They explained about 17% of the phenotypic variation for field FHB severity. The QTL on chromosome 4D for reducing FDK was detected at Carman and Ridgetown locations, explaining 37.7% and 18.4% of the phenotypic variation, respectively. In addition, FHB incidence, FHB index and DON at Carman were also associated with the chromosome 4D QTL from Mironovskaja 808 (Table 4-5). Although two QTLs from each parent were detected, resistant QTLs conferred by Mironovskaja 808 were more consistently detected than those conferred by AC Ron. It is also interesting to note that 2B and 4D FHB QTL were also detected for most of their corresponding traits in the combined QTL analysis across all locations (Table 4-5). Similar results were not observed for the 2D and 7A QTL.

Table 4-4. Summary of QTLs for FHB greenhouse severity (GH SEV) or type II resistance detected in the Mironovskaja 808/AC Ron population from the greenhouse in 2012, 2013, and the mean over two years

			Source of	2012			2013			Mean over two years		
Trait	QTL on chromosome	Map interval	resistance allele	LOD[a]	R^2(%)[b]	Additive effect	LOD	R^2(%)	Additive effect	LOD	R^2(%)	Additive effect
GH SEV	2B	wsnp_Ex_rep_c68386_67199155-	Mironovskaja 808	4.2	19.6	8.9	5.8	26.0	9.3	5.5	24.8	8.8
		RAC875_c8286_574										

[a]LOD is the highest LOD value detected within QTL interval.

[b]R^2(%) is the percentage of phenotypic variation explained by the QTL .

Table 4-5. Summary of QTLs for FHB resistance detected in the Mironovskaja 808/AC Ron population from the field evaluation at Carman, Winnipeg, and Ridgetown in 2013

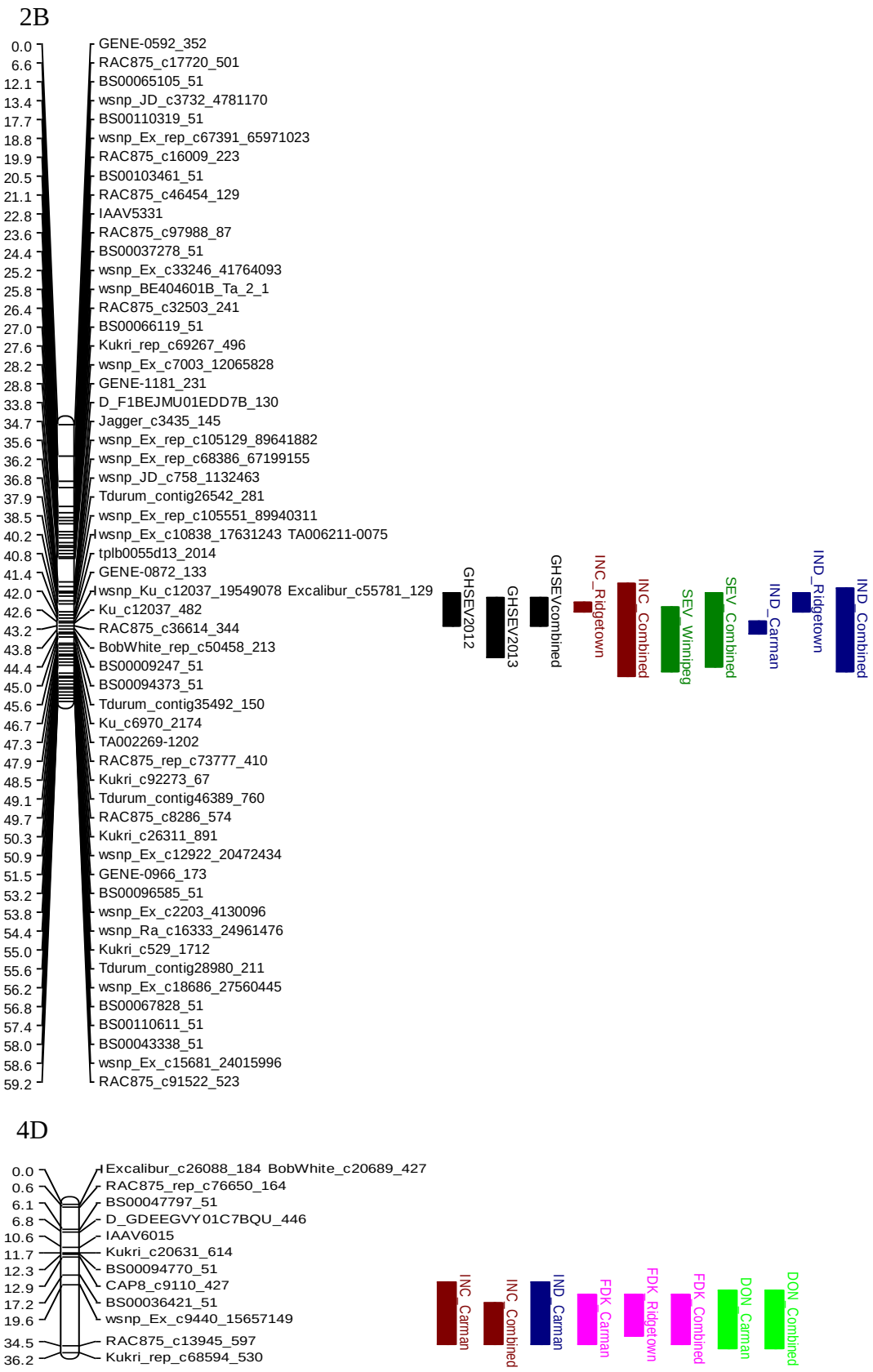
			Source of	Carman			Winnipeg			Ridgetown			Mean over three locations		
Trait	QTL on chromosome	Map interval	resistance allele	LOD[a]	R^2(%)[b]	Additive effect	LOD	R^2(%)	Additive effect	LOD	R^2(%)	Additive effect	LOD	R^2(%)	Additive effect
Field INC	2B	D_F1BEJMU01EDD7B_130-	Mironovskaja 808	ns	ns	ns	ns	ns	ns	3.6	17.0	5.4	3.6	17.0	4.2
		wsnp_Ex_c2203_4130096													
	4D	BS00036421_51-	Mironovskaja 808	4.2	19.5	5.2	ns	ns	ns	ns	ns	ns	3.4	16.3	5.1
		RAC875_c13945_597													
Field SEV	2B	wsnp_Ex_rep_c68386_67199155-	Mironovskaja 808	ns	ns	ns	5.3	24.1	5.4	ns	ns	ns	4.9	22.6	3.9
		GENE-0966_173													
	2D	Kukri_c51309_198-	AC Ron	ns	ns	ns	ns	ns	ns	3.6	17.2	-3.7	ns	ns	ns
		RAC875_rep_c70010_692													
	7A	RAC875_c27696_718-	AC Ron	ns	ns	ns	3.6	17.1	-4.6	ns	ns	ns	ns	ns	ns
		wsnp_Ku_c42539_50247426													
Field IND	2B	D_F1BEJMU01EDD7B_130-	Mironovskaja 808	3.8	17.8	4.3	ns	ns	ns	3.9	18.3	6.3	4.4	20.4	5.0
		BS00094373_51													
	4D	BS00036421_51-	Mironovskaja 808	4.0	18.9	5.3	ns	ns	ns	ns	ns	ns	ns	ns	ns
		RAC875_c13945_597													
FDK	4D	wsnp_Ex_c9440_15657149-	Mironovskaja 808	9.1	37.7	11.8	ns	ns	ns	3.9	18.4	7.4	6.9	30.1	8.5
		RAC875_c13945_597													
DON	4D	RAC875_c13945_597	Mironovskaja 808	6.4	28.2	20.9	ns	ns	ns	ns	ns	ns	5.2	23.7	9.1

[a]LOD is the highest LOD value detected within QTL interval.

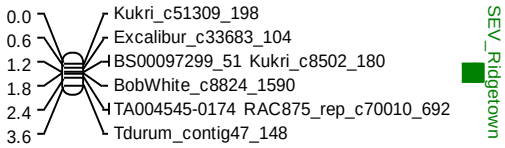
[b]*R^2(%)* is the percentage of phenotypic variation explained by the QTL.

INC, FHB incidence;SEV, FHB severity; IND, FHB index; FDK, Fusarium damaged kernel; DON, deoxynivalenol; ns, not significant.

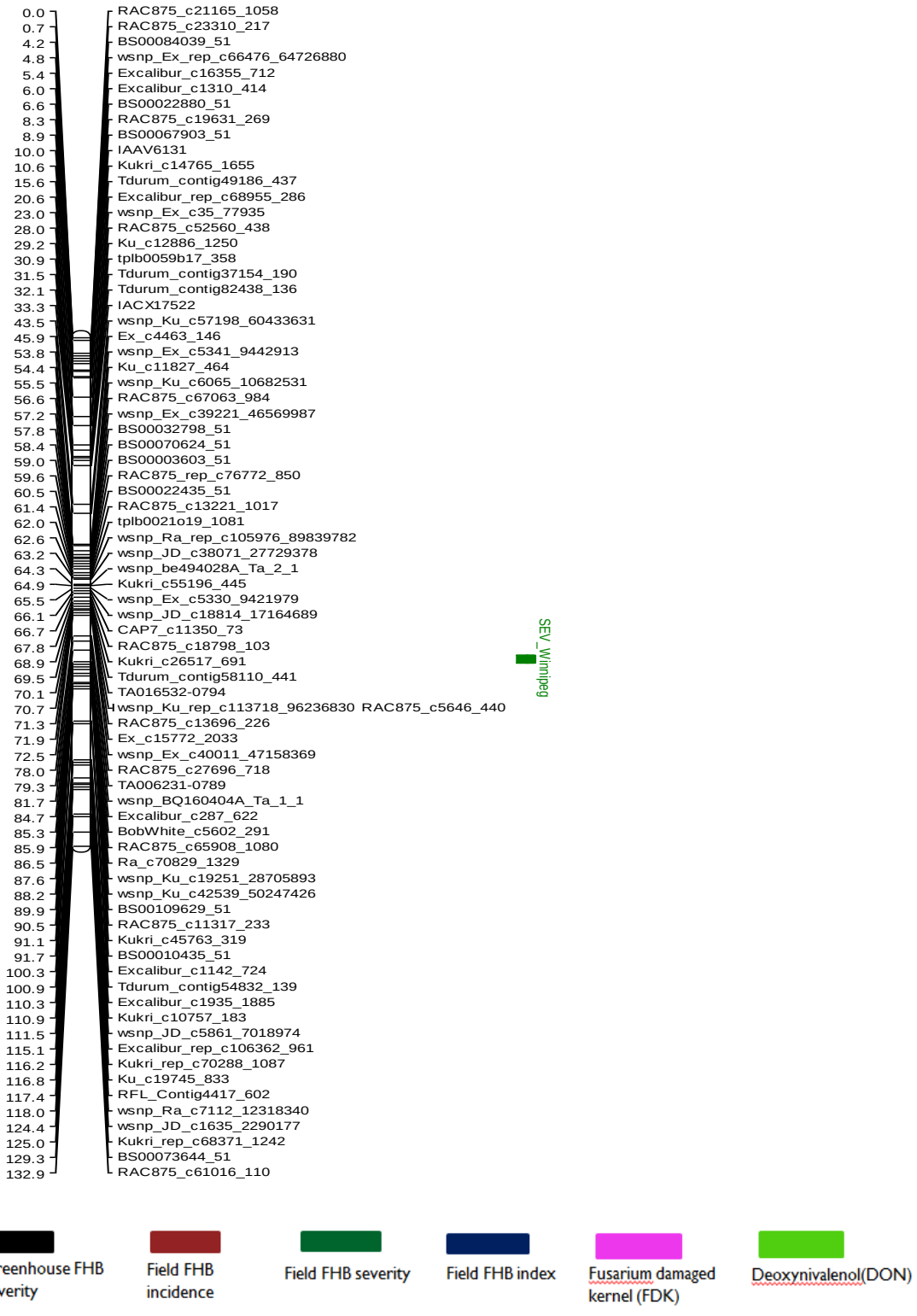
Figure 4-3. Genetic linkage map for QTL associated with Fusarium head blight traits evaluated in the greenhouse and field in the Mironovskaja 808/AC Ron population. Values on the left of chromosome bars are accumulative genetic distance (cM) of markers.



2D


0.0
0.6
1.2
1.8
2.4
3.6
Kukri_c51309_198
Excalibur_c33683_104
BS00097299_51 Kukri_c8502_180
BobWhite_c8824_1590
TA004545-0174 RAC875_rep_c70010_692
Tdurum_contig47_148
SEV_Ridgetown

7A


0.0 RAC875_c21165_1058
0.7 RAC875_c23310_217
4.2 BS00084039_51
4.8 wsnp_Ex_rep_c66476_64726880
5.4 Excalibur_c16355_712
6.0 Excalibur_c1310_414
6.6 BS00022880_51
8.3 RAC875_c19631_269
8.9 BS00067903_51
10.0 IAAV6131
10.6 Kukri_c14765_1655
15.6 Tdurum_contig49186_437
20.6 Excalibur_rep_c68955_286
23.0 wsnp_Ex_c35_77935
28.0 RAC875_c52560_438
29.2 Ku_c12886_1250
30.9 tplb0059b17_358
31.5 Tdurum_contig37154_190
32.1 Tdurum_contig82438_136
33.3 IACX17522
43.5 wsnp_Ku_c57198_60433631
45.9 Ex_c4463_146
53.8 wsnp_Ex_c5341_9442913
54.4 Ku_c11827_464
55.5 wsnp_Ku_c6065_10682531
56.6 RAC875_c67063_984
57.2 wsnp_Ex_c39221_46569987
57.8 BS00032798_51
58.4 BS00070624_51
59.0 BS00003603_51
59.6 RAC875_rep_c76772_850
60.5 BS00022435_51
61.4 RAC875_c13221_1017
62.0 tplb0021o19_1081
62.6 wsnp_Ra_rep_c105976_89839782
63.2 wsnp_JD_c38071_27729378
64.3 wsnp_be494028A_Ta_2_1
64.9 Kukri_c55196_445
65.5 wsnp_Ex_c5330_9421979
66.1 wsnp_JD_c18814_17164689
66.7 CAP7_c11350_73
67.8 RAC875_c18798_103
68.9 Kukri_c26517_691
69.5 Tdurum_contig58110_441
70.1 TA016532-0794
70.7 wsnp_Ku_rep_c113718_96236830 RAC875_c5646_440
71.3 RAC875_c13696_226
71.9 Ex_c15772_2033
72.5 wsnp_Ex_c40011_47158369
78.0 RAC875_c27696_718
79.3 TA006231-0789
81.7 wsnp_BQ160404A_Ta_1_1
84.7 Excalibur_c287_622
85.3 BobWhite_c5602_291
85.9 RAC875_c65908_1080
86.5 Ra_c70829_1329
87.6 wsnp_Ku_c19251_28705893
88.2 wsnp_Ku_c42539_50247426
89.9 BS00109629_51
90.5 RAC875_c11317_233
91.1 Kukri_c45763_319
91.7 BS00010435_51
100.3 Excalibur_c1142_724
100.9 Tdurum_contig54832_139
110.3 Excalibur_c1935_1885
110.9 Kukri_c10757_183
111.5 wsnp_JD_c5861_7018974
115.1 Excalibur_rep_c106362_961
116.2 Kukri_rep_c70288_1087
116.8 Ku_c19745_833
117.4 RFL_Contig4417_602
118.0 wsnp_Ra_c7112_12318340
124.4 wsnp_JD_c1635_2290177
125.0 Kukri_rep_c68371_1242
129.3 BS00073644_51
132.9 RAC875_c61016_110
SEV_Winnipeg

Greenhouse FHB severity
Field FHB incidence
Field FHB severity
Field FHB index
Fusarium damaged kernel (FDK)
Deoxynivalenol(DON)

4.6 Discussion

Breeding for FHB resistance in wheat is difficult due to the complexity of the genetics of resistance and different types of resistance that have not been well characterized. No wheat lines are completely immune to FHB although FHB resistance QTL have been identified in many breeding programs. A survey of the literature has shown that many FHB QTL have been identified in different experiments and a few major FHB resistance QTLs were repeatedly identified on chromosome 3BS, 5AS and 6BS (Buerstmayr et al. 2009). QTL for Type II resistance, resistance to spread of infection, derived from Asian resistance sources have been well studied. However, in breeding programs, QTL for multiple components of resistance need to be identified from various resistance sources to pyramid resistance genes into elite wheat lines. In this study, a double haploid winter wheat population with 89 lines was evaluated for multiple types of FHB resistance, including Type I resistance, Type II resistance, resistance to kernel damage, and resistance to DON accumulation.

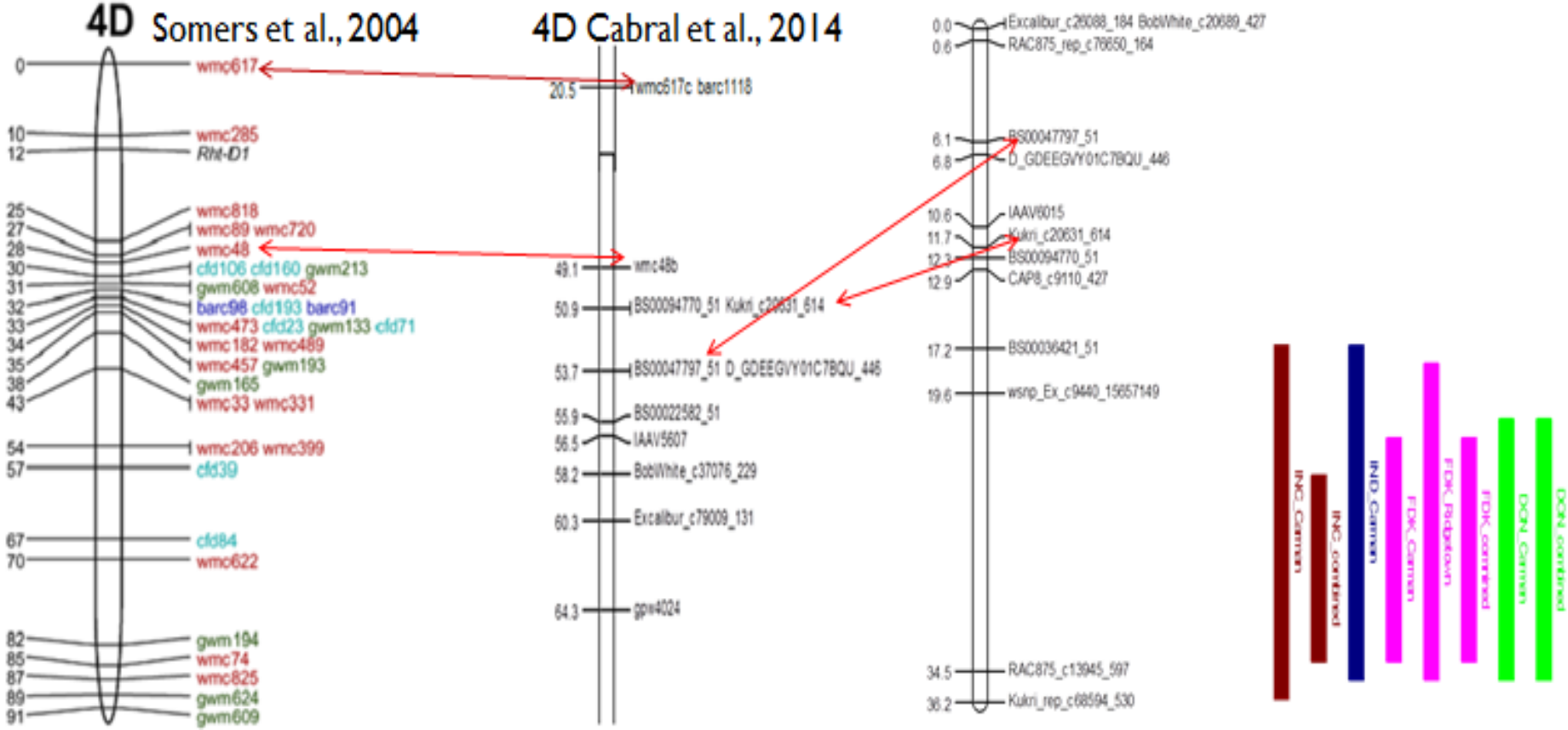
Development of FHB disease symptoms is greatly affected by environmental conditions. To optimize estimates of QTL number and their effect and interaction, evaluations under optimum conditions for disease development are an important key point (Vales et al. 2005). In this study, we created field conditions at all locations that were favorable for FHB development by using a standard inoculation protocol and mist irrigating plots after each artificial spray inoculation to minimize environmental effects. Despite these efforts, differences in FHB resistance QTL detection were observed across locations. In this study, four QTL were identified in the field trials at three locations. QTL on chromosomes 2B and 4D were both associated with reduced field FHB incidence and index, which might be a good explanation for the strong correlation among FHB-related traits evaluated in the fields. Three QTL for lower

field FHB severity (type II resistance) were identified on chromosomes 2B, 2D and 7A. Resistance to kernel damage and DON accumulation was associated to one QTL on chromosome 4D. Among the four QTL identified in the field trials, QTL on chromosomes 2B and 4D were more frequently detected than QTL on chromosomes 2D and 7A. None of the QTL detected in this study was consistently expressed over three locations. The inconsistent expression of resistance QTL across locations may be partially due to the effects of other uncontrollable environmental factors, including temperate and rainfall at each experimental location. The inconsistent expression of FHB resistance QTL in different environments was also observed in other studies in wheat (Bonin and Kobl 2009; Ruan et al. 2012). Environment may affect the expression of important FHB resistance QTL and the effectiveness of minor resistance QTL varies by environment (Bonin and Kolb 2009). If a QTL is consistently expressed in multiple environments, it may indicate that this is a QTL with significant effect on FHB control, and can be useful in marker-assisted breeding programs.

In wheat, FHB type II resistance (resistance to spread of infection) has been well studied. Significant and stable resistance QTLs were found on chromosomes 3BS and 6BS (Buerstmayr et al. 2009). Other types of resistance, including resistance to kernel damage and DON accumulation are not well documented compared with type II resistance. Resistance QTL for kernel damage and DON accumulation have been previously reported (Bonin and Kobl 2009; Somers et al. 2003). Kernel damage resistance QTL were identified on three chromosomes 2B, 4B and 6B in a RIL population derived from a cross between IL94-1653 x Patton (Bonin and Kobl 2009). However, QTL on chromosomes 2B and 6B were minor QTL explaining only 3 to 7% of the phenotypic variation, while QTL on chromosome 4B explained 12.3% of phenotypic variation for kernel damage in the field evaluation (Bonin and Kobl 2009).

Somers et al. (2003) identified two QTL on chromosome 3BS and 5AS together explaining 25% of the phenotypic variation in DON levels, with resistance contributed by 'Maringa'. In the present study, the QTL on chromosome 4D for kernel damage resistance was detected from trials conducted at Carman and Ridgetown, explaining 37.7% and 18.4% of phenotypic variation, respectively. Moreover, this QTL was found to reduce FHB incidence, index and DON accumulation at Carman. These results fall in line with the findings that the QTL on chromosome 4D was stable and significant (Draeger et al. 2007). Draeger et al. (2007) identified a QTL on the short arm of chromosome 4D which was associated with reduced FDK, DON accumulation, disease symptoms and other FHB-related traits. They suggested that the coincidence of QTL for multiple FHB-related traits might be due to the presence of linked genes separately affecting each trait, or it was a resistance capable of reducing multiple FHB-related traits (Draeger et al. 2007). Although this 4D QTL was significant and consistently observed in four trials, it was co-localised with dwarfing allele at Rht-D1 (Draeger et al. 2007). By comparing chromosome 4D linkage maps from previous studies, we found that Rht-D1 and the chromosome 4D QTL detected in the present study were both located between molecular markers wmc 617 and wmc 48 (Figure 4-4). It is possible that the chromosome 4D QTL identified in this study is identical with the 4D QTL identified by Draeger et al.(2007) since they are in the same chromosomal region and control some common FHB traits. In order to clearly reveal the relationship between the dwarfing allele and the chromosome 4D QTL identified in the present study, plant height data would need to be collected in future work.

Figure 4-4. Comparison of the positions of dwarfing allele Rht-D1 and chromosome 4D FHB QTL identified in this study.



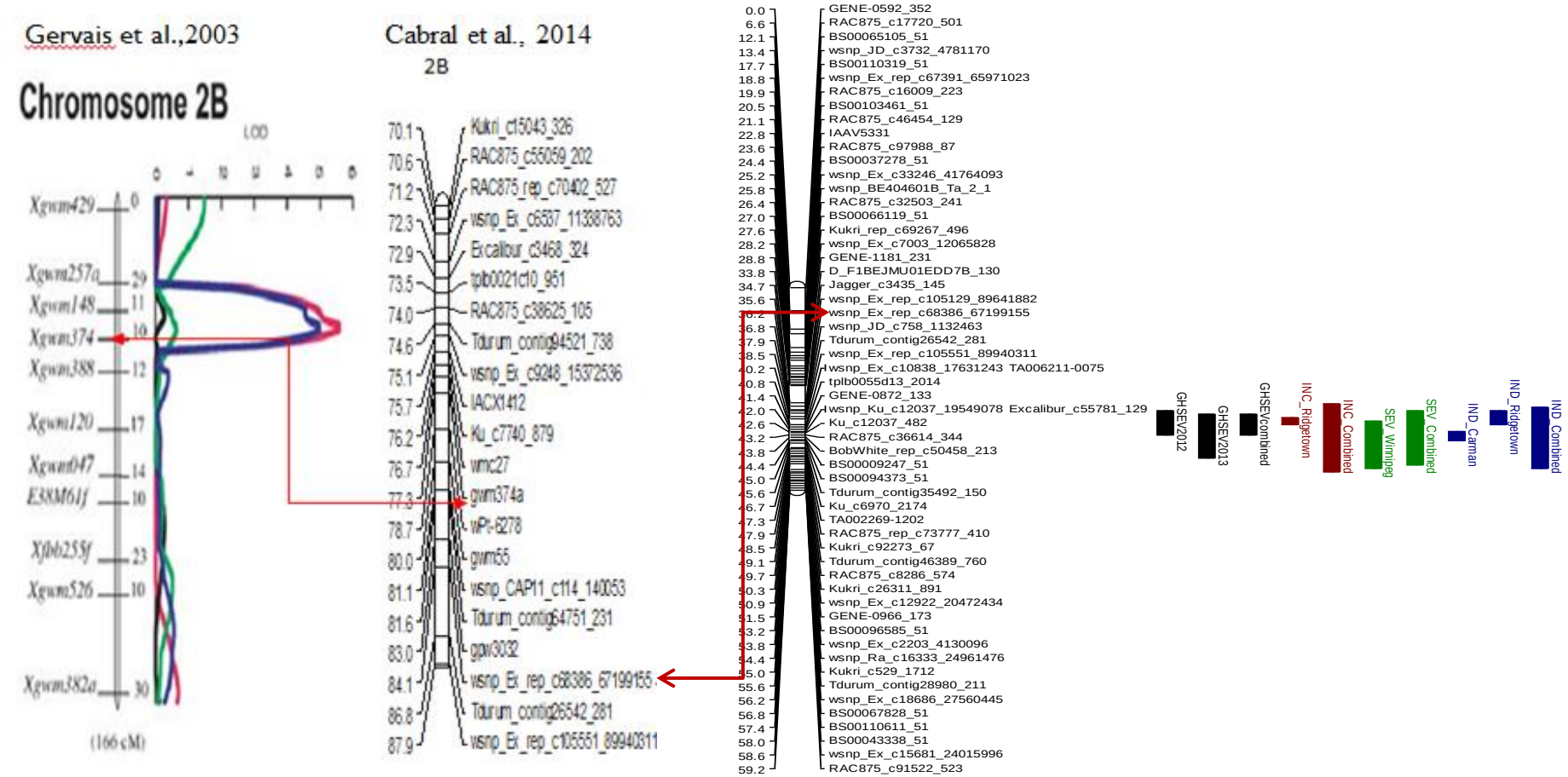
Note: Cabral et al's chromosome 4D linkage map was constructed using only part of the markers reported by Cabral et al. 2014. The arrows show the common markers and indicate the direction of chromosomes from different studies to compare QTL locations.

Most of the chromosome 2B regions are covered by QTL intervals contributed by Chinese, US and European winter wheat resistance sources (Buerstmayr et al. 2009). The QTL on chromosome 2B detected in this study influence more than one FHB-related trait and explained 17-24.8% of phenotypic variation (Table 4-4 and Table 4-5). This QTL was associated with reduced FHB severity (type II resistance) in the greenhouse (GH SEV), field FHB incidence (field INC), severity (field SEV) or FHB index (field IND) in at least one of the three field locations. Coincident QTLs suggest that the multiple types of resistance may be controlled by a single gene or by closely linked genes at certain QTL (Bonin and Kolb, 2009).

Although the QTL on chromosome 2B was not consistently detected across three field locations, it was consistently identified over two years for type II resistance evaluated in greenhouse. QTL consistently expressed in different environments are the most useful ones that can be used in marker-assisted selection to accelerate breeding progress (Ruan et al. 2012). Resistance QTL on chromosome 2B for FHB severity has been reported in a FHB resistant European winter wheat cultivar 'Renan' (Gervais et al. 2003). Renan is derived from the cross Mironovskaja 808/MarisHuntsman//VPM Moisson 4/Courtot. Nine QTLs for reduced field FHB severity were identified in RILs developed from Renan (resistant)/Recital (susceptible), and they explained 30-45% of total phenotypic variation (Gervais et al. 2003). They found a QTL on chromosome 2B was stably expressed over three years, which explained 12% of the phenotypic variance for FHB symptoms based on the adjusted means averaged over three years and was considered to be essential for resistance expression in 'Renan'. There is a high possibility that the QTL identified on chromosome 2B in the present study is identical with the one detected in Gervais et al.'s study (2003), since the mapping

populations used have a common ancestor ‘Mironovskaja 808’ and the marker closest to the chromosome 2B QTL identified by Gervais et al (2003) is only 6.7 cM apart from the 2B QTL detected in this study (Figure 4-5). FHB resistance QTL on chromosome 2B has also been reported in other studies (Abate et al. 2008; Bonin and Kolb, 2009). However, they explain only a small proportion of phenotypic variation for type II resistance and/or kernel damage.

Figure 4-5. Comparison of the positions of chromosome 2B FHB QTL identified by Gervais et al. 2003 and this study.



Note: Cabral et al.'s chromosome 2B linkage map was constructed using only part of the markers reported by Cabral et al. 2014. The arrows show the common markers and indicate the direction of chromosomes from different studies to compare QTL locations.

In the current study, both parents contributed resistance alleles. The QTL on chromosome 2D and 7A were from the more susceptible parent 'AC Ron'. However, they were only occasionally detected. Chromosome 2D QTL explained 17.2% of the phenotypic variation for field FHB severity at Ridgetown and 7A QTL explained 17.1% of the phenotypic variation for field FHB severity at Winnipeg. Resistance QTL contributed by Wangshuibai on chromosome 2D and 7A explained 10.6% and 12.6% phenotypic variance for FHB severity in natural infection field conditions (Jia et al. 2005). Resistance QTL on chromosome 2D from Wangshuibai for field FHB severity or incidence have also been reported in other studies (Mardi et al. 2005; Lin et al. 2006). Resistance QTL on chromosome 7AL from NK93604 and Ritmo explained 14.8% and 9.9% of phenotypic variance for field FHB severity, respectively (Semagn et al. 2007; Klahr et al. 2007). The results of this study showed that QTL from the more resistant parent 'Mironovskaja 808' control more FHB-related traits than those from 'AC Ron' and they were more stable across different environments. Compared with QTL that control only a single trait, the resistance QTL that affect multiple traits are usually preferentially used in breeding programs because selecting for one FHB resistance type will indirectly select for other types of resistance.

In the present study, transgressive segregation was detected in all the evaluated traits both in greenhouse and fields where some double haploid lines were more susceptible or resistant than the parents. These results and the fact that both 'Mironovskaja 808' and 'AC Ron' contribute resistance alleles indicate the feasibility of pyramiding resistance genes from different sources of resistance to improve FHB resistance. According to the results from two years' greenhouse experiment and one year's field trials, it appears that the FHB resistance in 'Mironovskaja 808' is due to two QTL on chromosomes 2B and 4D. It is notable that few QTL were detected in

this study and they explained a relatively high proportion of phenotypic variation. This may due to use of a small population size (89 DH lines). It was previously demonstrated that QTL number can be underestimated by using a limited population size. Other problems associated with limited population sizes include overestimation of QTL effects and failure to quantify QTL interactions (Vales et al. 2005). It is worth noting that the percentage of FDK for Mironovskaja 808 was higher than that of AC Ron, but QTL analysis results showed that resistance to kernel damaged was from Mironovskaja 808 (table 2-2). This result suggests that not all QTL segregating for FDK were detected in this mapping population. AC Ron might have QTL contributing to its lower FDK score. Not all QTL can be detected due to a small mapping population and there are major QTL on 4D and 2B segregating. Many linkage groups mapped in this study are short, and there may be additional QTL present in the unmapped portions of these chromosomes. Although we detected resistance QTL in both parents, additional experiments will be necessary to define QTL stability across various environmental conditions in multiple years and diverse genetic backgrounds.

5.0 General discussion and conclusion

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a devastating fungal disease of wheat that has become of increasing importance in wheat production regions. This disease can result in both grain yield loss and contamination of mycotoxins in the grain. Management of FHB in wheat production is challenging wheat growers and breeders as there are no wheat cultivars that are immune to FHB. Currently, only moderately resistant cultivars are available in commercial wheat production and fungicides are applied to prevent and control FHB. Without the availability of highly resistant cultivars, it is recommended that an integrated management strategy including using moderately resistant cultivars, fungicide application, and other control methods should be employed. In FHB epidemic years, partial genetic resistance and fungicide application may be inadequate to control this disease, making breeding for cultivars highly resistance to FHB a high priority.

The first study examined the effects of genetics and fungicide application on the development of FHB in wheat. Application of foliar fungicides tebuconazole and prothioconazole (Prosaro 250 EC™) significantly reduced the FHB index, FDK and DON content and increased the thousand kernel weight and yield in both moderately resistance and susceptible cultivars compared to the inoculated-untreated control. In most cases, there was no significant difference between seed treatment fungicides thiamathoxam+difenoconazole+metal-axyl-M+S-isomer (Cruiser Maxx® Cereals) and the inoculated-untreated control for the measured variables. Seed fungicides combined with foliar fungicides were generally not superior to foliar fungicides alone for FHB control. In the present study, the effectiveness of foliar fungicides in reducing FHB index, FDK and DON was consistent across locations and years. Inconsistent

results have been reported in other studies, where application of fungicides increased DON accumulation in grain (Simpson et al. 2001; Mesterhazy et al. 2003; Pirgozliev, et al. 2008; Amarasinghe et al. 2013). The variability of fungicide efficacy in controlling FHB might due to the timing of the fungicide application, fungicide selection and application technology, virulence of the *Fusarium* isolates and level of resistance in cultivars planted (Mesterhazy et al. 2003). Furthermore, climatic factors such as temperature and rainfall in target experimental fields can also contribute to the variability.

Results from this study showed that foliar fungicide application in moderately resistant cultivars was more effective in controlling FHB than when applied to susceptible cultivars. These results agreed with the finding that fungicide efficacy in reducing FHB traits was greater in moderately resistant cultivars than in susceptible cultivars (Wegulo et al. 2011; Amarasinghe et al. 2013). Results from this study also demonstrated that even foliar fungicide combined with moderately resistant cultivars was incapable of obtaining complete control of FHB under high disease pressure. However, integrating fungicide application and moderately resistant cultivar would minimize the economic loss due to FHB epidemics.

The second study identified QTL associated with FHB resistance. One of the key points to increase the accuracy of QTL analysis is to evaluate the trait of interest accurately. In this study, the accuracy of visual scoring for FHB incidence and severity in the fields was high, which made the results of QTL analysis reliable. FHB resistance in wheat was previously reported as a quantitative trait. The frequency distribution of FHB-related traits evaluated in the greenhouse and fields showed continuous variation, which confirmed that FHB resistance in wheat is quantitatively inherited. Among the four QTL detected, none of them was consistently expressed

across three experimental locations. Although standard inoculation protocols and mist irrigation after inoculation were utilized to minimize variability, climatic factors such as temperature and rainfall varied between locations. These partially explain the inconsistent expression of QTL across locations.

Field FHB incidence, severity and index were highly correlated to each other. The QTL analysis results showed that QTL on chromosome 2B and 4D were both associated with field FHB incidence and index, furthermore, the 2B QTL was also associated with field FHB severity. These results may explain the strong correlation among these three traits.

In this study, a relatively small DH population (89 lines) was used for QTL analysis. The problem associated with use of a small population is that not all QTL for the trait of interest will be identified, which might lead to underestimation of QTL number and overestimation of QTL effects. In this study, the DH population was evaluated in three locations in 2013 and four QTL were detected. Although the QTL on chromosome 2B and 4D were stably detected, the QTL on chromosome 2D and 7A were detected only once. Additional evaluation will be required in years and locations to determine the stability of QTL and whether the same, or distinct QTLs influence a trait under different field conditions.

In conclusion, integrating moderately resistant cultivars with foliar fungicide application is an effective strategy for reducing the risk of FHB and DON contamination. Seed treatment alone is not effective in protecting wheat from being attacked by FHB. An integrated management strategy would be necessary to minimize the economic loss due the FHB infection. Mironovskaja 808 is a useful source of FHB resistance. The stable QTL from Mironovskaja 808 can be used in wheat breeding programs to breed for cultivars with improved FHB resistance.

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7.0 Appendices

Appendix 7-1. Plant total DNA isolation with CTAB

1. CTAB extraction buffer (100 ml)
   - 1.5 g CTAB (Hexadecyl trimethyl-ammonium bromide)
   - 10.0 ml 1M Tris-HCl pH 8.0
   - 4.0 ml 0.5M EDTA pH 8.0 (Ethylenediaminetetra Acetic acid Di-sodium salt)
   - 21.0 ml 5M NaCl
   - 63.5 ml dd H_2O sterile
   - 15.0 ml 0.5% 2-mercaptoethanol (added to CTAB buffer immediately before use).

2. DNA extraction procedure

   - Grind 2-3pieces (~4 cm each piece) of frozen leaf tissue to a powder in liquid nitrogen in a chilled 2 ml microfuge tube.
   - In the fume hood, add 1ml of CTAB buffer to the tube and then immediately add 2 µl of greshly made proteinase K stock solution (20mg/ml).
   - Incubate the CTAB/plant extract mixture for 30 min at 60 ℃ in a recirculating water bath with occasional gentle swirling.
   - After incubation, remove tubes from the water bath and let equilibrate on the bench at room temperature for 5 min.
   - In the fume hood, add equal volumes of Chloroform : Iso Amyl Alcohol (24:1) to each tube and mix the solution by inversion
   - Mix the samples using the Rotator SB2 for 30 min.
   - Centrifuge at 40000 rpm at room temperature for 30 min.
   - In the fume hood, transfer the upper aqueous phase only (contains the DNA) to a new 2 ml microfuge tube using transfer pipet.
   - Add 2 µl RNaseA (10 mg/ml) to each tube and incubate for 30 min at room temperature.
   - In the fume hood, add 1 ml of cold 100% Isopropanol from a -20 ℃ freezer to the tube, mix gently and incubate for 30 min at 4 ℃.
   - Scoop out the precipitated DNA with a pipet tip and put the DNA into a 1.5 ml tube containing 1 ml of 70% ethanol. If the precipitated DNA cannot be scooped out, centrifuge at 3200 rpm at room temperature for 8 min, discard the supernatant and transfer the pellet to a 1.5 ml tube using a transfer pipet.
   - Wash the pellet with 1 ml of 70% ethanol twice until it is completely white. Let the ethanol drain and gently ‘dab’ the last droplet off with a clean Kimwipe.
   - Dry the DNA pellet in the fume hood.
   - Resuspend the DNA pellet in 300-500 µl TE buffer (Tri-EDTA, pH 8.0) and store at a -20 ℃ freezer.