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A comparative evaluation of the effects of 3-ADON and 15-ADON chemotypes of *Fusarium graminearum* on spring wheat and selected QTL lines

by

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ABSTRACT

Gauthier, Victoria Margot. M.Sc., The University of Manitoba, August 2010. An evaluation of the effects of 3-ADON and 15-ADON chemotypes of *Fusarium graminearum* on spring wheat and selected QTL lines. Major professor: Anita L. Brûlé-Babel.

Fusarium head blight (FHB) is a serious disease of wheat, primarily caused by the pathogen *Fusarium graminearum*. FHB results in yield losses and decreased grain quality due to the ability of the pathogen to produce the mycotoxin deoxynivalenol (DON) as well as acetylated derivatives of DON such as 3-acetyl DON (3-ADON) and 15-acetyl DON (15-ADON). Research shows that the 15-ADON chemotype is being replaced by the 3-ADON chemotype in eastern and central Canada. The first study investigated the potential for differences between the two chemotypes in terms of disease progression, effect on yield, Fusarium damaged kernels (FDK) and DON levels. Results showed that 3-ADON isolates were able to produce significantly more DON and FDK, and had significantly greater negative effects on yield than 15-ADON isolates, although there were no differences in symptom disease progression. The second study investigated if there were differences in resistance for the two chemotypes on 3BS and 4B quantitative trait loci (QTL) lines for disease severity and FDK levels. No differences were detected between chemotypes for disease progression but there were for FDK levels. One 3BS line was identified as partially resistant with significantly lower disease severity and FDK levels than the other QTL and null lines.

1.0 GENERAL INTRODUCTION

Fusarium head blight (FHB) is one of the most important diseases facing wheat production globally. The most common causal agent of FHB is *Fusarium graminearum* Schwabe [telomorph:*Gibberella zeae* Schwein (Petch)], representing over 95% of the *Fusarium* isolates found in Manitoba (Gilbert et al., 2009). FHB of wheat is such a devastating disease because it results in yield and quality losses (Kolb et al., 2001; Ludewig et al., 2005). Losses in Canada related to FHB in the last 30 years have been estimated to be upwards of one billion dollars (Clear and Nowicki 2009).

Fusarium graminearum has the ability to produce trichothecene toxins, specifically deoxynivalenol (DON) and its acetylated derivatives, 3-acetyl DON (3-ADON) and 15-acetyl DON (15-ADON) (Goswami and Kistler, 2004; 2005; Osborne et al., 2007; Ward et al., 2008). Until recently, the 15-ADON chemotype population was predominant in eastern Canada, however, in a study done by Ward et al. (2008) it was demonstrated that the 3-ADON chemotype, which is generally more common in Asia and Europe, is replacing the resident 15-ADON population (Miller et al., 1991). Ward et al. (2008) suggested that this shift in chemotype populations is due to differences in fitness (i.e. differences in fertility, and size of conidia) which have been conserved over multiple speciation events, thus giving the 3-ADON isolates a competitive advantage over the 15-ADON population.

Control methods such as crop rotation, tillage, fungicides, and biological control have been and continue to be used, but there is little known if any of these control methods will be effective against the new chemotype population. Resistant cultivars

remain an attractive solution for combating FHB in wheat, but more research needs to be done in this area to continue to develop FHB resistant wheat cultivars. The objectives of the present study were to evaluate:

1. the interaction between *F. graminearum* and spring wheat genotypes using isolates that differ in DON chemotype production and wheat genotypes that differ in reaction to *F. graminearum*

2. the reaction of 3BS and 4B FHB Quantitative Trait Loci (QTL) lines in reponse to inoculation with 3-ADON and 15-ADON isolates.

2.0 LITERATURE REVIEW

2.1 Wheat

2.1.1 Wheat origin and distribution

Triticum aestivum L. (common or bread wheat) is a major food crop that was domesticated over 10 000 years ago in south-western Asia (Sleper and Poehlman, 2006; Carver, 2009). Today, wheat accounts for 29-30% of the world's total cereal production (Carver, 2009). The majority of wheat crops are grown between 25^0- 60^0N and 25-45^0S (Sleper and Poehlman, 2006; Carver, 2009). The primary wheat-producing regions are in temperate and southern Russia, the central plains of the US, southern Canada, the Mediterranean Basin, northern China, India and Argentina and Australia (Carver, 2009).

2.1.2 Importance of wheat and production statistics

Canada produces around 5% of the world's wheat production, exporting 80% (18 MMT ± 3.23) of what is produced (Bonjean and Angus, 2001). Canadian wheat accounts for approximately 20% of internationally traded wheat (Bonjean and Angus, 2001). In the last five years, Canada has produced on average 17 million tonnes of spring wheat per year, almost 3 million tonnes of which, on average, were produced in Manitoba alone (Government of Canada, 2009).

Canada has a reputation of producing high-quality wheat (Bonjean and Angus, 2001) and its uses include, but are not limited to, bread, flour, confectionary products, unleavened bread, semolina, bulgar, and breakfast cereals (Sleper and Poehlman, 2006). Many studies have identified fusarium head blight (FHB) caused by *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* Schwein (Petch)] as one of the

most important diseases of wheat in Canada and other major wheat producing countries in the world.

2.1.3 Wheat Genome

The genome of common wheat consists of three homeologous chromosomes belonging to the A, B, and D genomes (Moolhuijzen et al., 2007). Hexiploid wheat (*Triticum aestivum*) has a chromosome number of 2*n* = 6x=42, AABBDD and has the largest genome at 16 000Mb (Moolhuijzen et al., 2007).

2.2 *Fusarium graminearum*

Fusarium graminearum is the most common causal agent of FHB in the world (Gilbert et al., 2001; Goswami and Kistler, 2004) and consists of at least nine phylogenetically distinct species (O'Donnell et al., 2004). In Canada and North America, FHB is an important disease of wheat because it directly reduces yield and quality (Gilbert et al., 2001; Kolb et al., 2001; Walker et al., 2001; Gilbert and Fernando, 2004; Ludewig et al., 2005). In Manitoba, *F. graminearum* consists of 97% of *Fusarium* isolates from *Fusarium* damaged kernels (FDK) ranking it the most common causal agent of FHB in Manitoba (Gilbert et al., 2009). More frequent occurrences of FHB epidemics have been reported in Asia, Canada, Europe and South America (Goswami and Kistler, 2004). Losses since the Canadian *Fusarium* epidemic of 1993 have been estimated to be over one billion dollars (Clear and Nowicki, 2009).

2.2.1 *Fusarium graminearum* taxonomy

Fusarium head blight was first described in England in 1884 by W.G. Smith (Stack, 2003). In 1935, Wollenweber and Reinking published *Die Fusarien* which described Fusarium species as mitosporic Ascomycetes (Liddell, 2003). Based on the sexual state *Gibberella zeae*, the pathogen belongs to Superkindgom Eukaryota, Kingdom Fungi, Phylum Ascomycota, Subphylum Pezizomycotina, Class Sordariomycetaidae, Subclass Hypocreomycetidae, Order Hypocreales, Family Nectriaceae and Genus Gibberella (Goswami and Kistler, 2004). *Gibberella zeae* is a homothallic ascomycete (Goswami and Kistler, 2004) meaning that it is able to sexually reproduce on its own by forming endogenous meiospores in asci and has a restricted dikaryon (Kendrick, 2000).

Fusarium head blight pathogens fall into four sections which share several characteristics, although each section is biologically distinct (Liddell, 2003). These sections are: Discolor, to which *F. graminearum* belongs, Roseum, Gibbosum and Sporotrichiella (Liddell, 2003). The most common causal agents of FHB belong to the *F. graminearum* species complex, consisting of at least nine phylogenetically distinct species as evaluated by genealogical concordance phylogenetic species recognition (O'Donnell et al., 2000; O'Donnell et al., 2004; Starkey et al., 2007). The study done by O'Donnell, et al. (2004) evaluated 13.6kb of DNA sequence from 11 nuclear genes including the mating-type locus. The distinct species identified in the study were shown to be descended from a single taxon and apomorphic origin of homothallism within the Fg clade. Ward et al. (2008) demonstrated that the population subdivision among North American *F. graminearum* isolates is widespread.

2.2.2 Symptoms and Life Cycle

To fully understand a disease, one must first understand its life cycle(s). *Fusarium graminearum* is a monocyclic disease (Bai and Shaner, 1994; Shaner, 2003), meaning that only one cycle of the disease is completed per season. Dill-Macky and Jones (2000) postulated that the reason for FHB being a monocyclic disease is due to the short period of time following anthesis, which is usually 10 to 20 days, when wheat spikes are most susceptible. The pathogen has both sexual and asexual lifecycles.

The disease cycle of FHB begins with the fungus overwintering on crop debris or FDK as saprophytic mycelia (McMullen et al., 1997; Dill-Macky and Jones, 2000; Fernando et al., 2000; Schaafsma et al., 2001; Bailey et al., 2003; Inch and Gilbert, 2003a; Inch and Gilbert, 2003b; Liddell, 2003; Markell and Francl, 2003; Shaner, 2003; Goswami and Kistler, 2004; Osborne and Stein, 2007). In the spring, when environmental conditions are favourable (i.e. warm weather and precipitation), the pathogen is able to produce sporodochia which give rise to conidia (asexual stage) and perithecia which give rise to ascospores (sexual stage) (McMullen et al., 1997; Clear and Patrick, 2000; Fernando et al., 2000; Schaafsma et al., 2001; Inch and Gilbert, 2003b; Markell and Francl, 2003; Osborne and Stein, 2007). Sutton (1982) identified ascospores, macroconidia, chlamydospores and hyphal fragments as inoculum components, although ascospores and macroconidia were identified as the most important types of inoculum in epidemics, which was also supported by Liddell (2003), Markell and Francl (2003), and Shaner (2003). Markell and Francl (2003) suggested that ascospores likely adhere better to the wheat spikes than conidia, due to the stickiness of ascospores (Parry et al., 1995; Trail et al., 2002). *Fusarium graminearum* readily forms

perithecia, giving rise to ascospores which are able to cause disease of aerial plant parts (Eudes et al., 2001; Bushnell et al., 2003). Rainfall has been shown to be important for perithecial formation and ascospore development (Fernando et al., 2000). Ascospore release is usually 1 to 3 days after a rainfall event (Fernando et al., 2000). The spores, regardless of type, are dispersed by wind, rain, or insects to host plants (Sutton, 1982; Parry et al., 1995; McMullen et al., 1997; Bailey et al., 2003; Gilbert and Fernando, 2004; Paul et al., 2004; Schmale III et al., 2005; Osborne and Stein, 2007). Wheat is most susceptible to head blight beginning at anthesis through to the soft dough stage (McMullen et al., 1997; Windels, 2000; Bailey et al., 2003; Osborne and Stein, 2007).

Symptoms are first seen on the first florets to flower, generally near the middle of the spike (Bushnell et al., 2003). Initial symptoms include water-soaked brown spots which spread up and down the rachis (Parry et al., 1995; Pirgozliev et al., 2003). Characteristic FHB symptoms are premature bleaching and senescence of the spikes (Parry et al., 1995; Bailey et al., 2003; Bushnell et al., 2003; Miedaner et al., 2003; Pirgozliev et al., 2003; Osborne and Stein, 2007). The premature bleaching of infected spikes is thought to be due to a vascular dysfunction in the rachis (Bushnell et al., 2003). This premature ripening results in seeds which have not been able to fill properly thus resulting in shrivelled, light-weight and chalky white or pink kernels, known as FDK (Bailey et al., 2003; Bushnell et al., 2003; Pirgozliev et al., 2003; Goswami and Kistler, 2004). Occasionally dark coloured perithecia (sexual fruiting bodies) or orange to pinkish coloured sporodochia (asexual fruiting bodies) may be seen on the infected wheat spikes, especially around the glumes (Bailey et al., 2003; Osborne and Stein, 2007). Goswami and Kistler (2005) found that the less aggressive strains of the pathogen caused

black lesions on infected spikelets as opposed to bleaching and deformed awns, as would be typically expected. Bushnell et al. (2003) and Shaner (2003) reported that symptoms spread both apically and basally from the point of infection. Symptoms usually appear approximately seven days after infection has occurred (Shaner, 2003) and progress until spike senescence.

2.2.3 Infection process in wheat

Fusarium graminearum is the most common causal agent of FHB likely because it is adapted to a wider range of environmental conditions than the other *Fusarium* species (Osborne and Stein, 2007). The spores of the pathogen, namely macroconidia and ascospores are able to survive saprophytically on residue of host crops such as wheat, barley and corn (McMullen et al., 1997; Osborne and Stein, 2007) for up to two years (Pereyra et al., 2004). In order for these spores to infect, warm, wet conditions are required at the time of anthesis (Osborne and Stein, 2007).

As identified in section 2.2.2, many studies have shown that wheat is most susceptible at anthesis. Flowering occurs when the lodicules swell and push apart the lemma and palea so that the anthers are exposed thus allowing spores to infect the floret via air current or water splash (Bushnell et al., 2003). Osborne and Stein (2007) suggested that anthers are able to provide nutrients and could promote fungal growth at the point where anthers are mature and beginning to senesce. It has been suggested by some studies that the presence of floral extracts such as betaine and choline aid in the germination of infectious spores, however, a study done by Engle, et al. (2002) showed that these compounds did not promote colonization by the pathogen neither in the sexual

nor asexual stage. Other factors are involved in the successful colonization of the host by the pathogen, including the amount of inoculum and the amount of time that the host and pathogen are exposed to periods of wetness at a temperature conducive for fungal infection, colonization and growth (Bushnell et al., 2003).

Ascospores are the sexual spores borne from perithecial ascomata during the pathogen's sexual stage (*Gibberella zeae*) (Kendrick, 2000). Macroconidia are the asexual spores borne from sporodochia during the pathogen's asexual stage (*Fusarium graminearum*) (Kendrick, 2000). Ascospores are transported by air currents to infect susceptible hosts (Osborne and Stein, 2007). The ascospores are forcibly discharged via turgor pressure from the perithecia; however ejection distances are at maximum a few millimetres (Trail et al., 2002; Osborne and Stein, 2007). In addition to wind dissemination, spores are also moved by rain splash, insects and other agents, but do not require a period of dormancy (Sutton, 1982; Parry et al., 1995; Bushnell et al., 2003; Paul et al., 2004). A few researchers have looked at the requirement of light for discharge and development of spores. Trail et al. (2002) found that light was not required for ascospore discharge, however, its presence resulted in a moderate increase in the ascospore release compared to complete darkness. A study done by Gunther, et al. (2005) found that light was a requirement for perithecia development in stomatal openings.

Visible symptoms can appear within three days of inoculation and it has been shown that macroconidia are able to germinate and grow on the anthers and filaments especially in the presence of pollen grains (Miller et al., 2007). In a study done by Pritsch et al. (2000), both resistant and susceptible lines were colonized within 48 to 72 hours after inoculation and a number of defence responses accumulated in wheat spike

tissue. Kang and Buchenauer (2000b) found that hyphal distribution differed between susceptible and resistant cultivars. In the susceptible cultivar, the pathogen's spread was not restricted and moved from the lemma and ovary to the rachilla and rachis more quickly than in the resistant cultivar which showed a restriction of fungal spread. Regardless of resistance or susceptibility of a cultivar, the mycelia appear more apt to spread toward the bottom of the spike than the top (Eudes et al., 2001). The thick-walled epidermal and or hypodermal cells of the outer surfaces of the glume, lemma and palea prevent direct penetration by the fungus (Kang and Buchenauer, 2000a; Bushnell et al., 2003). However, hyphae are able to directly penetrate ovaries, glumes and the inner walls of the palea and lemma (Pritsch et al., 2000; Bushnell et al., 2003). The most common point of entry is through the anthers and filaments (Miller et al., 2007). It is possible that the pathogen is able to enter the host through the stomates or through spaces between the palea and lemma (Pritsch et al., 2000; Guenther and Trail, 2002; Bushnell et al., 2003). Once the pathogen reaches the floret, the anthers, stigma and lodicules are highly susceptible to colonization (Goswami and Kistler, 2004). Pritsch et al. (2000) found that macroconidia were able to germinate within five to six hours after being placed on the glumes of a susceptible host. The pathogen is then able to move from one floret to another via vascular tissues in the rachilla and rachis (Schroeder and Christensen, 1963; Kang and Buchenauer, 2000a; Kang and Buchenauer, 2000b; Ribichich et al., 2000). Ribichich et al. (2000) identified a horizontal and a vertical infection path. In the horizontal path, the fungus colonized anthers and bracts of nearby florets in the inoculated spikelet, and then moved via the rachis and rachilla to the adjacent spikelet. In the vertical path, the fungus travelled through vascular bundles and

parenchyma tissues to invade spikelets above and below the point of infection. Pritsch et al. (2000) noticed subcuticular colonization of glumes 48 to 76 hours after inoculation.

Fusarium graminearum was found in most tissues of infected wheat spikes (Kang and Buchenauer, 2000a). Pritsch et al. (2000) suggested two patterns of colonization, the first as subcuticular hyphae on the inoculated epidermis and second, intracellular colonization of parenchyma tissues. Kang and Buchenauer (2000a) showed that the fungus was found in the xylem vessels, phloem and phloem parenchyma, and led to the distortion or collapse of these cells thereby causing premature death of the spikelets above the point of infection. Presence of the fungus led to thickening of cells walls, deposition of appositions, and occlusions of vessels of the host (Kang and Buchenauer, 2000a; Ribichich et al., 2000). A study done by Jansen et al. (2005) showed that antibodies reacting with cellulose, xylans and pectin were able to degrade host cells and thus gave proof of the release of enzymes by the pathogen for cell wall degradation at early stages of infection which is in agreement with studies done by Kang and Buchenauer (2000a) and Mesterhazy (2002).

Jansen et al. (2005) noted that cell death was initiated as soon as the pathogen entered the cytosol of the epicarp cells, and therefore concluded that a biotrophic phase of the pathogen is impossible. These findings conflict with results from a study done by Goswami and Kistler (2004) and a review by Bushnell et al. (2003), which identified a brief biotrophic stage before switching to a necrotrophic stage.

Goswami and Kistler (2005) concluded that the ability of isolates within the *F. graminearum* species complex to cause disease is isolate-specific rather than species

specific, and that it is the amount, rather than the type, of trichothecene produced that is a key factor in the level of aggressiveness on the host. However, the level of host resistance determines the incubation period and disease severity (Ribichich et al., 2000).

Trichothecenes produced by the pathogen are not considered virulence factors, although host defence is inhibited by trichothecenes (Bai et al., 2001b; Jansen et al., 2005). Deoxynivalenol appears to be important in the aggressiveness of the pathogen (i.e. disease causing ability), but is not a phytotoxin (Mesterhazy, 2002). Goswami and Kistler (2005) suggested that in the case of highly aggressive isolates, trichothecenes could be translocated in the plant and lead to further development of symptoms and progress of the fungus. Kang and Buchenauer (2000b) suggested that defence reactions may be retarded due to the effects of toxin production and extracellular enzymes. The wheat plant is able to sequester toxins in cytosolic ribosomes, chloroplasts, plasma-lemma, cell walls and vacuoles (Kang and Buchenauer, 2000b; Jansen et al., 2005). Kang et al. (2000b) found that the toxin densities differed between resistant and susceptible cultivars in that the toxin accumulation in host tissue at three to five days was much higher in the susceptible cultivar. It was shown in a study by Mesterhazy (2002) that DON concentration is significantly affected by amount of precipitation subsequent to infection.

Bushnell et al. (2003) found that kernel size and numbers were reduced when infections occurred early in the season. They postulated that the reason for reduced kernel size was a direct result of the premature ripening of the spike. Del Ponte et al. (2007) noticed a high incidence of fungal colonization of kernels between anthesis and dough stages of kernel development and a decline in DON concentration between kernel

watery ripe and hard dough stages. In a study done by Ludewig et al. (2005), DON content in the grain was lower than in the chaff and rachis and suggested that the kernels might be protected due to their position in the spike decreasing the passive transfer of DON through the xylem.

2.2.4 Mycotoxins and chemotypes

Trichothecenes are sesquiterpenoid alcohols which contain the trichothecene tricyclic ring system and are biosynthesized by trichodiene through the farnesyl pyrophosphate pathway (McCormick, 2003; Mirocha et al., 2003; Pestka and Smolinsky, 2005). Trichothecenes are considered antibiotics because they are able to inhibit protein synthesis (McCormick, 2003). Trichothecenes are divided in two groups based on the type of substitution at C-8; Type A trichothecenes have an ester group whereas type B trichothecenes have a keto group (McCormick, 2003). Type B trichothecenes include three strain specific profiles (chemotypes): nivalenol (NIV), 3-acetyl DON (3-ADON) and 15-acetyl DON (15-ADON) (McCormick, 2003; Pirgozliev et al., 2003; Goswami and Kistler, 2004; O'Donnell et al., 2004; Ward et al., 2008). DON is able to produce acetylated derivatives with an acetate group on carbon 3 (3-ADON) or carbon 15 (15-ADON) (Mirocha et al., 2003). The 15-ADON chemotype is more common in the USA whereas the 3-ADON chemotype is more prevalent in Asia (Miller et al., 1991). Two chemotypes of *F. graminearum* were first identified by Ichinoe et al. (1983) as DON (which produced deoxynivalenol and 3-ADON) and NIV (nivalenol and 4-acetyl nivalenol). Miller,et al. (1991) then identified two chemotypes of deoxynivalenol, 3-ADON (IA) and 15-ADON (IB). The chemotypes of *F. graminearum* were later characterized as NIV (nivalenol producers), 3-ADON (deoxynivalenol producers that

also make DON and 3-ADON) and 15-ADON (deoxynivalenol producers that also make DON and 15-ADON) (Goswami et al., 2004; Goswami and Kistler, 2005; Osborne and Stein, 2007; Ward et al., 2008).

Deoxynivalenol (DON), the main mycotoxin produced by *F. graminearum* is a type B trichothecene (McCormick, 2003; Mirocha et al., 2003). Chemotype differences are correlated with allelic polymorphisms within the trichothecene biosynthetic gene cluster (O'Donnell et al., 2000; Ward et al., 2002), but are not well related with evolutionary associations within the Fg clade (O'Donnell et al., 2000). The lack of relationship between chemotype differences and evolutionary origin within the Fg clade shows that the chemotypes have developed from more than one ancestral origin within the B-trichothecene lineage (Ward et al., 2002). Ward et al. (2002) further suggested that chemotype differences can have a significant impact on the pathogen's fitness due to the fact that the chemotype polymorphisms have been conserved in multiple speciation events.

Deoxynivalenol was first isolated from barley in Japan in 1972 (Yoshizawa and Morooka, 1973; Desjardins, 2006) and is also known as vomitoxin, or "the refusal factor" (Mirocha et al., 2003). Isolates that are 3-ADON producers are common in Japan and northern Europe, whereas 15-ADON producers are more common in North America (Mirocha, et al., 2003). 3-ADON producers were first isolated from Ontario in 1979 (Gilbert et al., 2001). This finding was supported by a study done by Ward et al. (2008) which found that 3-ADON populations have been in eastern Canada for thirty years. Ward et al. (2008) reported the rapid replacement of the 15-ADON chemotype by the 3-ADON chemotype in western Canada. The paper suggested that the chemotype shift is

likely because of a recent range expansion causing a composition shift at the population level and likely could be due to transcontinental introgression. The same study, as well as a study done by Goswami and Kistler (2004) suggested that the quick and considerable influx of 3-ADON producers in western Canada demonstrates the selective advantage over 15-ADON producers in areas such as reproductive capacity, *in vivo* growth rates and ability to use resources more efficiently. This selective advantage implies that the 3-ADON population is more toxigenic and vigorous than 15-ADON producers (Ward et al., 2008).

A study done by Gilbert et al. (2001) revealed that highly aggressive isolates were able to produce high levels of mycotoxins whereas the least aggressive isolates produced the lowest levels of mycotoxins. This agrees with studies done by Mesterhazy (2002) and Ludewig et al. (2005) which showed that DON is involved in aggressiveness. Results from a study done by McCormick (2003) revealed that DON production is a key virulence factor in head blight. Bai et al. (2001b) concluded that resistant cultivars have lower DON levels than susceptible cultivars. DON is not a reliable indicator of disease severity (Gilbert et al., 2001), although others found that FDK were a good predictor of DON levels (Mesterhazy et al., 2005; Zhang et al., 2008). Ludewig et al. (2005) concluded that yield is a reliable assessment of the pathogen's aggressiveness due to a strong correlation between disease incidence and percent developed kernels. Bai et al. (2001a) showed similar results. Eudes et al. (2001) concluded that trichothecenes are pathogenicity factors.

The field of molecular biology has increased knowledge about DON as well as its acetylated derivatives and has helped to identify chemotype differences on a molecular

level. The 'Tri cluster' contains the majority of the genes needed in the biosynthesis of the basic trichothecene structure (i.e. Tri4 and Tri5) (Goswami and Kistler, 2004). The flanking genes to the Tri cluster differentiate the chemotypes; 15-ADON isolates contain Tri 7 and Tri 13 as pseudogenes whereas 3-ADON isolates contain Tri 8 and Tri 13 as pseudogenes and Tri 7 is deleted (Goswami et al., 2004). Transformation-mediated gene disruption has been used to develop knock-out mutant strains to understand the biosynthetic pathway as well as to understand the importance of trichothecenes in plant disease which have revealed that DON production is not required for symptom development (McCormick, 2003). The study also suggested that by introducing genes for resistance to toxins, disease severity should decrease.

It is important to quantify DON toxin. Enzyme-linked immunosorbent assay (ELISA) is a quick and common method for DON quantification involving a simple extraction procedure (Mirocha et al., 2003). ELISA allows two antibodies or binding proteins that bind in synchrony to the analyte, in this case DON, which is then bound to a surface containing an enzyme which imparts colour to the reaction (Drolet et al., 1996; Mirocha et al., 2003). ELISA is simple, quick and extremely precise (Mirocha et al., 2003). Thin layer chromatography (TLC) was a commonly used method before the advent of ELISA. In a comparison between the two methods, ELISA was found to give consistently higher values and better extraction efficiency (Wolf-Hall and Bullerman, 1996). One draw back of ELISA is cross reactivity, which was suggested by Wolf-Hall and Bullerman (1996) and confirmed by Mirocha et al. (2003). Mirocha et al. (2003) found that other compounds could be detected by DON's acetylated derivatives.

2.2.5 Effects of mycotoxins

The trichothecenes have negative impacts on plants, end-use grain quality, animals, and humans. It has been documented that trichothecenes are phytotoxic resulting in chlorosis, necrosis and wilting (McCormick, 2003). In a study done by Wakulinski (1989), DON and 15-ADON inhibited germination, reduced seedling growth rates, inhibited root growth more than shoot growth, and showed phytotoxic activity at 1ug/ml to 10ug/ml. DON is concentrated in the bran layer of the grain, therefore, when the bran is removed during dry milling, there is a 40% reduction of DON level in the flour (Pestka and Smolinsky, 2005). Baking or heating of any kind has no affect on the toxin molecule (Pestka and Smolinsky, 2005).

Humans and animals are severely affected when grain contaminated with mycotoxins is ingested (Parry et al., 1995). Trichothecenes prevent eukaryotic protein synthesis, alter immune function, and can cause skin lesions (Mirocha et al., 2003; Pestka and Smolinsky, 2005). DON is biologically active and is able to disturb cell signalling, differentiation, growth and macromolecular synthesis as well as impact gastrointestinal homeostasis, growth, neuroendocrine function and immunity (Pestka and Smolinsky, 2005). Acute exposure results in diarreah, vomiting, leukocytosis and gastrointestinal haemorrhage (Pestka and Smolinsky, 2005). Higher doses can lead to circulatory shock, reduced cardiac output and in some cases, death, whereas chronic exposure results in reduced weight gain, altered nutritional efficiency and immunotoxicity (Pestka and Smolinsky, 2005). Monogastric animals such as humans and pigs are the most sensitive to the effects of DON, whereas ruminant animals are able to tolerate higher concentrations (Pestka and Smolinsky, 2005). Studies comparing the monoacetylated

forms of DON in mammals are limited, however the relative toxicity of the derivatives of DON are likely a minor issue because studies have shown accumulation of DON regardless of the pathogen's ability to produce DON derivatives (Ward et al., 2008).

2.3 Control

2.3.1 Genetic control

Although, genetic resistance alone is not the sole solution, it remains the most attractive management option for FHB (Gilbert and Tekauz, 2000) because regardless of epidemic potential, the host is able to tolerate invasion by *F. graminearum*. Overcoming FHB will require an integrative approach, combining cultural, chemical, and biological control as well as genetic control. Resistance is also affected by morphological or physiological characteristics such as spike anatomy or position, presence of awns, presence of a short peduncle or a compact spike (Rudd et al., 2001). McMullen et al. (1997) noted that breeders and pathologists have been able to identify and incorporate partial resistance genes, but the next challenge is to develop efficient standardized screening methods, and to understand inheritance of partial resistance.

Currently, resistance of Canadian wheat cultivars to FHB ranges from fair to very poor (Gilbert and Tekauz, 2000; Seed Manitoba, 2009); there is not a single registered Canadian wheat cultivar which is resistant to FHB, although there are some moderately resistant cultivars. Resistance sources have been identified, such as Sumai 3 and its derivatives from China, and Frontana from Brazil (Fedak et al., 2001; Kolb et al., 2001; Rudd et al., 2001; Xue et al., 2004; Ludewig et al., 2005; Yabawalo et al., 2009).

Disease screening remains a large expense of breeding programs for disease resistance. Jones (2000) suggested that the best measures of FHB, visual incidence and severity ratings, percentage of FDK, and DON quantification, provide consistent assessment of the disease and are able to show treatment differences.

There are two types of resistance against pathogen infections. Type I resistance is defined as resistance to initial infection, and type II resistance as resistance to spread of the fungus in the spike. A cultivar is said to have type I resistance if it is resistant to spray inoculation but susceptible to point inoculation and type II resistance if it is susceptible to spray inoculation but resistant to point inoculation (Schroeder and Christensen, 1963). Type I resistance can only be determined if there is some type II resistance present (Kolb et al., 2001). Since the acceptance of Type I and II resistance, three other types of resistance have been identified. Type III resistance is defined as resistance to kernel infection, Type IV resistance as tolerance to infection and Type V resistance as resistance to mycotoxin accumulation (Mesterhazy, 1995). Types III, IV and V resistance are more difficult to manage and/or more expensive to screen and are therefore not used in current breeding programs in Canada (Gilbert and Tekauz, 2000).

Type II resistance is most commonly evaluated in greenhouse studies (Kolb et al., 2001) due to the ease of the screening procedures and repeatability of results. Point inoculation can be used to evaluate Type II resistance and is frequently used in molecular marker screening and quantitative trait loci (QTL) mapping for FHB resistance in controlled environments (Bai et al., 1999; Waldron et al., 1999). Point inoculation, as described by Waldron et al. (1999), is inoculation by placing 10µL of a conidial suspension within the floret, specifically, between the palea and lemma, of a single

spikelet near the centre of the spike. Under greenhouse conditions, Type II resistance in wheat is a quantitative trait with high heritability (Bai and Shaner, 1994) and controlled by a few major genes with major effects (Van Ginkel et al., 1996). Cuthbert et al. (2006) found that *Fhb1*, located on chromosome 3B, is an additive gene and is essential for providing Type II resistance. Cuthbert et al. (2007) determined that the gene *Fhb2*, located on the 6BS QTL, controlled Type II field resistance to FHB. Haberle et al. (2009) also found that the 6BS QTL was necessary for FHB resistance.

The genetics of Sumai 3, an FHB resistance source from China, have been studied in linking molecular markers to QTL associated with Type II FHB resistance have been identified (Bai et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2002; Zhou et al., 2002b; Yang et al., 2003; Yang et al., 2005; Osborne and Stein, 2007). The FHB resistance QTL from Sumai 3 are the most widely used QTLs, especially the 3BS QTL close to the centromere region (Kolb et al., 2001; McCartney et al., 2004; Buerstmayr et al., 2009). Other sources of FHB resistance have been identified from spring wheats from Asia, Brazil and winter wheats from Europe (Snijders, 1994). Frontana has both type I and II resistance (Ludewig et al., 2005; Yabawalo et al., 2009). These two types of resistance were identified by Schroeder et al., (1963) and are widely accepted. Sumai 3 and Frontana are commonly used as resistance sources because their resistance is both heritable, stable and has low genotype-environment interaction (Rudd, et al., 2001). Sumai 3 in particular is a good source of resistance because it produces seed with low DON concentrations even under high disease pressure (Bai et al., 2001b). Use of a single resistance source is not desirable because of the potential for breakdown of resistance genes, thus, new sources of resistance need to be identified (Gilbert and Tekauz, 2000).

Unfortunately, the use of resistance from wild relatives has not been successful due to the lack of pairing of chromosomes between the wild relative and wheat in addition to the quantitative inheritance of resistance and undesirable agronomic traits of the hybrid (Chen et al., 1997).

One of the biggest challenges to incorporating disease resistance is that genetic resistance of FHB is complex because there are so many reported chromosomal locations for resistance genes and these components are quantitatively inherited (Buerstmayr et al., 1999; Kolb et al., 2001; Miedaner et al., 2001; Rudd et al., 2001; Miedaner et al., 2003). Various studies discuss FHB resistance involving anywhere from 2-6 genes (Kolb et al., 2001).

As mentioned before, FHB resistance is a quantitative trait, influenced by environmental effects such as temperature, humidity, plant development stage and abundance of inoculum (Bai and Shaner, 1994; Snijders, 1994; Parry et al., 1995; Zhou et al., 2002b; Shen et al., 2003; Yang et al., 2003; Steiner et al., 2004; Collard et al., 2005; Cuthbert et al., 2006; Osborne and Stein, 2007) and its inheritance involves several loci on several chromosomes (Bai and Shaner, 1994; Steiner et al., 2004). The quantitative nature of FHB resistance was postulated by (Osborne and Stein, 2007) to be due to many minor genes and a few major genes leading to resistance.

Quantitative trait loci (QTL) are regions within a genome which contain genes associated with a quantitative trait (Collard et al., 2005). QTL analysis is based on the ability to associate a phenotype to a genotype of the marker (Collard et al., 2005). QTLs are useful when they are closely linked to a marker (i.e. 20 centiMorgans or less) because

this lowers the chance of recombination between the marker and the QTL (Collard et al., 2005).

Several studies have identified the merits of using QTLs as a tool for marker assisted selection (MAS) in wheat breeding for resistance to FHB. Marker assisted selection uses the presence or absence of a marker for selection which in turn increases efficiency, effectiveness, reliability and reduces costs compared to conventional plant breeding (Collard et al., 2005). Kolb et al. (2001) identified four steps that are typically followed to develop molecular markers based on QTL for MAS: (1) identify and locate major QTL for FHB resistance, (2) confirm the magnitude and map position of the QTL, (3) identify markers that are closely linked and flanking the QTL, (4) develop "user-friendly" markers.

Many studies have tried to identify the number of genes involved in FHB resistance, with results ranging from two to six genes (Kolb et al., 2001). In a recent review, Buerstmayr et al. (2009), noted more than 100 QTLs for FHB resistance in wheat have been published, the majority of which are located in the B genome. FHB resistance genes are located throughout the genome and are cultivar dependent (Bai and Shaner, 1994). With the exception of chromosome 1A, all chromosomes associated with FHB resistance have been identified in multiple sources (Kolb et al., 2001; Yang et al., 2005). A summary of the identified and confirmed chromosome location for FHB resistance QTL are listed in table 2.1.

Table 2.1 Literature references for identified and confirmed FHB resistance QTL

Chromosome location	Reference
1A	Jiang et al. 2007; Semagn et al. 2007; Schmolke et al. 2008
1B	Ittu et al. 2000; Buerstmayr et al. 2002; Shen et al. 2003; Steiner et al. 2004; Zhang et al. 2004; Zhou et al. 2004; Schmolke et al. 2005; Yang et al. 2005; Mardi et al. 2006; Klahr et al. 2007; Semagn et al. 2007; Haberle et al. 2009; Zhang et al. 2010
1D	Ittu et al. 2000; Yang et al. 2005; Klahr et al. 2007
2A	Waldron et al. 1999; Anderson et al. 2001; Kolb et al. 2001; Zhou et al. 2002; Gervais et al. 2003; Paillard et al. 2004; Steiner et al. 2004; Yang et al. 2005; Ma et al. 2006; Semagn et al. 2007; Garvin et al. 2009
2B	Zhou et al. 2002; Gervais et al. 2003; Steiner et al. 2004; Gilsinger et al. 2005; Schmolke et al. 2005; Somers et al. 2006; Liu et al. 2007; Abate et al. 2008; Schmolke et al. 2008
2D	Shen et al. 2003; Somers et al. 2003; Jia et al. 2005; Mardi et al. 2005; Yang et al. 2005; Lin et al. 2006; Ma et al. 2006; Jiang et al. 2007; McCartney et al. 2007; Handa et al. 2008; Zhang et al. 2010
3A	Anderson et al. 2001; Otto et al. 2002; Bourdoncle et al. 2003; Shen et al. 2003; Paillard et al. 2004; Steiner et al. 2004; Yang et al. 2005; Mardi et al. 2006; Chen et al. 2007; Yu et al. 2008
3B	Bai et al. 1999; Waldron et al. 1999; Ban et al. 2000; Zhou et al. 2000; Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002; Bourdoncle et al. 2003; Buerstmayr et al. 2003; Guo et al. 2003; Liu and Anderson 2003; Shen et al. 2003; Somers et al. 2003; Yang et al. 2003; Zhou et al. 2003; Liu et al. 2004; Paillard et al. 2004; Zhang et al. 2004; Zhou et al. 2004; Jia et al. 2005; Lemmens et al. 2005; Mardi et al. 2005; Yang et al. 2005; Chen et al. 2006; Cuthbert et al. 2006; Liu et al. 2006; Ma et al. 2006; Miedaner et al. 2006; Jiang et al. 2007; Klahr et al. 2007; Liu et al. 2007; McCartney et al. 2007; Abate et al. 2008; Yu et al. 2008; Zhang et al. 2010; Zhou et al. 2010
4A	Paillard et al. 2004; Steed et al. 2005; Yang et al. 2005
4B	Buerstmayr et al. 1999; Waldron et al. 1999; Anderson et al. 2001; Somers et al. 2003; Steiner et al. 2004; Jia et al. 2005; Yang et al. 2005; Lin et al. 2006; Liu et al. 2007; McCartney et al. 2007; Abate et al. 2008
4D	Yang et al. 2005; Ma et al. 2006; Draeger et al. 2007; Srinivasachary et al. 2008
5A	Ban and Suenaga 1997, 1998; Anderson et al. 2001; Xu et al. 2001; Buerstmayr et al. 2002; Buerstmayr et al. 2003; Gervais et al. 2003; Shen et al. 2003; Somers et al. 2003; Paillard et al. 2004; Steiner et al. 2004; Yang et al. 2005; Chen et al. 2006; Lin et al. 2006; Ma et al. 2006; Miedaner et al. 2006; Jiang et al. 2007; Liu et al. 2007; McCartney et al. 2007; Abate et al. 2008
5B	Xu et al. 2001; Bourdoncle et al. 2003; Paillard et al. 2004; Jia et al. 2005; Yang et al. 2005; Klahr et al. 2007; Haberle et al. 2009
5D	Yang et al. 2005; Yu et al. 2008
6A	Anderson et al. 2001; Schmolke et al. 2005; Haberle et al. 2007
6B	Waldron et al. 1999; Anderson et al. 2001; Shen et al. 2003; Yang et al. 2003; Liu et al. 2004; Steiner et al. 2004; Yang et al. 2005; Somers et al. 2006; Cuthbert et al. 2007; Semagn et al. 2007; Draeger et al. 2008; Zhang et al. 2010
6D	Paillard et al. 2004
7A	Zhou et al. 2004; Jia et al. 2005; Mardi et al. 2006; Klahr et al. 2007; Kumar et al. 2007; Semagn et al. 2007; Zhang et al. 2010
7B	Gilsinger et al. 2005; Schmolke et al. 2005; Yang et al. 2005; Jiang et al. 2007; Haberle et al. 2007; Klahr et al. 2007

Chromosome 3BS carries a gene with a major effect for FHB resistance and has been identified in several studies (Waldron et al., 1999; Chen et al., 2000; Gupta et al., 2000; Anderson et al., 2001; Kolb et al., 2001; Zhou et al., 2002b; Shen et al., 2003; Somers et al., 2003; Yang et al., 2003). In a study done by Anderson et al. (2001), the 3BS QTL had more than twice the impact of any other QTL, based on a multiple regression model. The 3BS QTL was consistently detected across a range of environments and made the largest contribution to types I and II resistance, reducing the number of FDK and FHB symptoms (Bai et al., 1999; Waldron et al., 1999; Anderson et al., 2001; Buerstmayr et al., 2002; Steiner et al., 2004; Yang et al., 2005; McCartney et al., 2007). The 3BS QTL (*Fhb1*) has been consistently detected across a range of environments which leads researchers to believe that there is genetic control of the three types of FHB resistance i.e. Type I, Type II and Type III resistance, but it is unclear whether this is due to a single pleiotrophic locus or due to multiple linked loci (Yang et al., 2005).

Another important QTL for FHB resistance, although described by some as a minor QTL, is located on chromosome 4B (Waldron et al., 1999; Somers et al., 2003). In a study done by McCartney et al. (2007), the QTL on chromosome 4B was an even more effective FHB resistance QTL, than the QTLs on 3BS and 3BSc in relation to FHB disease parameters. The QTL on 4B has been found in Asian resistance sources and was shown to lower disease incidence (Buerstmayr et al., 2009). QTL on 4B are typically associated with plant height, which has been thought to be associated with pleiotropy or linkage effects with reduced height (*Rht*) genes, specifically *Rht-B1* (Buerstmayr et al., 2009). Yang et al. (2005) identified QTL with digenic epistasis between additive QTL on

chromosome 4B which supports results by Buerstmayr et al. (1999) and Somers et al. (2003).

Some alternative methods of genetic control of FHB have been proposed. Haber et al. (2009) are working to develop resistant wheat cultivars by controlling the expression of critical resistance genes which are already present in all wheat genotypes. Chen et al. (2009) made intercrosses between alien translocation lines with FHB resistance to pyramid different resistance genes and then used these lines as recurrent parents in wheat breeding programs.

Resistant cultivars still remain a practical and effective method for controlling FHB (Miedaner et al., 2003), however the hightest level of resistance of Canadian wheat cultivars is moderately resistant. Mesterhazy (2002) suggested that resistant cultivars combined with the use of fungicides would combat highly aggressive, DON producing isolates, but this remains to be achieved. Current thinking is that resistant cultivars should be developed by recombining resistance from different sources, different types of resistance, and desirable agronomic traits (Kolb et al., 2001; Goswami and Kistler, 2005).

2.3.2 Cultural control

There are many ways to control or help to reduce the amount of primary inoculum in fields with host crops that can be infected by FHB. In section 2.2.2 it was noted that primary inoculum is able to survive saprophytically on host crop debris. Inch and Gilbert (2003a) showed that the rate of decomposition of host tissues will have an effect on the survival of ascospores, and suggested that rotations of at least two years are required to avoid subsequent infection. These results are consistent with other studies (Dill-Macky

and Jones, 2000; Schaafsma et al., 2001; Pereyra et al., 2004). Dill-Macky and Jones (2000) found that FHB levels were higher when wheat was grown after corn than after wheat. Schaafsma et al. (2001) speculated that because the decomposition of corn residues is slower than other residues and that corn may act as a medium for increased inoculum production. By burying residues, decomposition occurs more rapidly due to increased surface area contact with microorganisms; therefore, tillage systems which keep more residue on the soil surface are more likely to provide substrate for inoculum production (Pereyra et al., 2004). Tillage is the most common way of incorporating crop residues and disease incidence and severity were decreased when stubble was plowed under Miller et al., 1998; Dill-Macky and Jones, 2000; Schaafsma et al., 2001). Dill-Macky and Jones (2000) stated that burying residue would prevent the development of the sexual stage of FHB, however, a review by Gilbert and Tekauz (2000) and a study by Pereyra et al. (2004) showed that Fusarium-infested residues resurfaced during tillage events and were still able to provide the necessary substrate for the pathogen to produce perithecia and ascospores. The results of the previously mentioned studies are in accordance with Miller, et al. (1998) who stated that inoculum persists for an unknown length of time, but more so on no-till land where there is a large amount of crop residue on the soil surface. Therefore, conservation and reduced tillage are contributing to FHB epidemics (McMullen et al., 1997; Dill-Macky and Jones, 2000). An integrative approach including tillage on conventional land seems to be a good option; however, tillage causes issues related to soil health, susceptibility to erosion and degradation (Lori et al., 2009).

Tillage and crop sequence affect the incidence and severity of FHB (Schaafsma et al., 2001). By introducing non-host crops for a period of at least two years, there would be no more substrate added to the land which would provide the time for the previous crop residues to decompose and thus help to break the cycle of inoculum production. Crop rotations are one of the more effective ways at reducing the risk of severe epidemics (McMullen, et al., 1997; Pereyra, et al., 2004).

A review by McMullen et al. (1997) stated that aside from favourable environmental conditions for pathogen development, a high occurrence of minimum tillage, large areas of susceptible crops and short rotation gaps will lead to FHB epidemics in the future. Other cultural control methods could include staggered planting dates or using winter wheat instead of spring wheat because winter wheat flowers earlier and frequently escapes mid-season infection (Krupinsky et al., 2002).

2.3.3 Chemical control

Chemical control is commonly used to control FHB, although it is not completely effective. Fungicides are management options for reducing DON accumulation and disease incidence (Gilbert and Tekauz, 2000; Mesterhazy, 2002). The most effective fungicide application is a narrow window after heading and before anthesis. This narrow application window is the reason for the inconsistent success, in addition to the spikes not being at the same stage on all plants (McMullen et al., 1997). Unfortunately, the entire crop does not flower at the same time due to elevation differences, availability of water, and tillering. Variability of results from fungicide studies can be attributed to timing of fungicide application and coverage, timing and severity of infection, virulence of isolates

and level of resistance of the cultivars planted (Gregoire, 2002; Mesterhazy et al., 2003; Veskrna et al., 2009). Although, when a fungicide application is used with accurate disease forecasting, fungicide treatments at heading can significantly reduce FHB and thus minimize losses related to yield and quality reductions (Jones, 2000; Yuen and Jochum, 2002; Mesterhazy et al., 2003; Mullenborn et al., 2008; Veskrna et al., 2009). Fungicide cost may also be a limiting factor for fungicide use to control FHB (McMullen et al., 1997).

According to the Guide to Crop Protection (MAFRI, 2010) there are three fungicides registered in Manitoba that are capable of suppressing FHB: Bravo 500, Folicur 432F and Proline 480 SC. The recommended fungicide application time is early flowering i.e. when at least 75% of the wheat main stem spikes are fully emerged to when 50% of the main stem spikes are in flower (MAFRI, 2010). These fungicides are all foliar sprays. The active ingredient of Bravo 500 is chlorothalonil which is a chloronitrile fungicide with multi-site contact activity (MAFRI, 2010). Folicur 432F and Proline 480SC are both triazole fungicides which provide broad-spectrum activity. The active ingredient in Folicur 432F is tebuconazole, and prothioconazole is the active ingredient in Proline 480SC (MAFRI, 2010).

Triazole fungicides including prothioconazole and tebuconazole had better control against *Fusarium* species than other fungicides tested by Mullenborn et al. (2008). These results were consistent with reports by Mesterhazy et al. (2003) and Karplus et al. (2009). In a study done by Matthies et al. (1999), tebuconazole was shown to inhibit fungal growth, although it increased 3-ADON production relative to the control. With some fungicides, there are inconsistent relationships between mycelia growth and toxin

production, which lead Mesterhazy (2002) to note that partially effective fungicides may cause lower disease levels but higher toxin contamination. Therefore fungicides that decrease mycelial growth and increase toxin production are only partially effective fungicides and therefore would not be desirable (Matthies et al., 1999).

Jones (2000) found that triazole fungicides including tebuconazole, most consistently reduced FHB in the field, in addition to FDK and DON concentration. These experiments also showed that propiconazole was not different from tebuconazole in reducing disease incidence and severity. A study by Matthies, et al. (1999) showed that tebuconazole and prochloraz did not inhibit toxin synthesis which could explain why DON concentrations are not reduced as much as disease symptoms with fungicides. Yuen and Jochum (2002) found that disease severity was reduced by a tebuconazole fungicide.

Gilbert and Tekauz (2000) noted that the more specific a fungicide, the more likely the pathogen will develop resistance to that fungicide. Fungicides can effectively reduce disease symptoms and toxin accumulation if accurate disease forecasting is employed. Producers need to remember that just as crop rotation is important for disease control, fungicide rotation is important to reduce the chance of a pathogen's resistance to a fungicide.

2.3.4 Biological control

Biological control is an attractive method for control of FHB because some consider it a more "natural" form of pest control than chemicals. Biological control is a

method that uses organisms which totally or partially inhibit or destroy pathogen populations (Agrios, 2005). Biocontrol agents are living organisms that require specific conditions to be effective; it is most desirable if these conditions are similar to those of the pathogen (Fernando, 2003). Theoretically, biocontrols applied at anthesis are able to overcome pathogens by aborting, curtailing or delaying germination of *F. graminearum* spores (Fernando, 2001). Effective biocontrols are able to reduce disease incidence and severity as well as minimizing DON concentration (Gilbert and Fernando, 2004). Biological controls weaken or destroy the pathogen by direct parasitism, competition for space and nutrients, production of antibiotics or toxins, production of enzymes able to attack cell wall components of the pathogen, and/or the ability to induce defence responses in the plants to name a few (Agrios, 2005).

Many strains of biological control agents have been identified as FHB disease suppressors, but timing of application and environmental conditions are very important. Gilbert and Fernando (2004) noted that biocontrol agents are desirable alternatives when the window of protection is narrow, however, much research needs to be done on each biocontrol agent to determine the dose, formulation and timing of application. Strains of *Bacillus spp.* such as strain AS 43.4 (Khan et al., 2001; Schisler et al., 2002), 43.3 (Khan et al., 2001; Schisler et al., 2002), Cohn strain H-08 (Schisler et al., 2002) have been shown as effective FHB biocontrols. Other biocontrols identified include *Cryptococcus* strain OH 182.9 (Fernando et al., 2002), *Microsphaerosis spp*. isolate P190A (Bujold et al., 2001), *Pseudomonas florescens* strains MKB 158 and MKB 249 (Khan et al., 2009), *Pseudomonas sp*. AS 64.4 (Khan et al., 2009), to name a few. In general, it is very difficult to make any definite conclusions about any of these potential biocontrols

because the potential differences in environmental conditions required for success of both the pathogen and biocontrol. Biocontrols are a promising control strategy of FHB but cannot not be solely relied upon at the present time.

3.0 COMPARISON OF THE EFFECTS OF 3-ADON AND 15-ADON CHEMOTYPES ON WHEAT LINES DIFFERING IN RESPONSE TO *FUSARIUM GRAMINEARUM*

3.1 Abstract

Fusarium head blight (FHB) caused by *Fusarium graminearum* produces a mycotoxin, deoxynivalenol (DON) which results in substantial losses in grain yield and quality. A shift in the chemotype population profile in Canada has shown that the frequency of the resident 15-acetyl DON (15-ADON) population while there has been a simultaneous decreased due to an increase in prevalence of the 3-acetyl DON (3-ADON) population, which has been reported to synthesize more DON. This study compared the effects of 13 3-ADON, 12 15-ADON *F. graminearum* isolates, and a mock-inoculated control, for disease progression, yield, fusarium-damaged kernels (FDK) and DON content on three wheat genotypes with known reactions to *F. graminearum*. Disease incidence and severity were measured every three days from the onset of symptom development to natural senescence. Yield was measured on whole plots, FDK were counted on a random sample from each plot to determine the proportion of infected kernels and DON was quantified using enzyme-linked immunosorbent assay (ELISA). Results showed that the isolates tested differed in levels of aggressiveness, although there was no consistent relative ranking of isolates between years. FDK and yield showed significant differences between chemotypes, with 3-ADON isolates producing higher mean FDK and lower average yields. No significant differences were found between chemotypes for area under the disease progress curve (AUDPC) incidence, severity or FHB index or DON, although 2008 results showed that DON accumulated in significantly higher levels for 3-ADON isolates. In 2009, 3-ADON isolates produced

more DON on average; however, the difference between chemotypes was not significant. The combined dataset did not show significant differences for DON accumulation between chemotypes. Within chemotypes, significant differences were found within 15-ADON isolates for all AUDPC measurements and yield. Isolate*genotype interactions were significant for all AUDPC measurements, yield and DON; however, there were no changes in ranking among the genotypes. These results indicate that there were different levels of aggressiveness in the isolates tested. No differences were seen within 3-ADON chemotypes which supports previous research suggesting a narrower genetic basis for this chemotype.

3.2 Introduction

Fusarium graminearum (Schwabe) (teleomorph *Gibberella zeae* (Schwein.) Petch), the causal agent of fusarium head blight (FHB) is one of the most destructive pathogens of wheat in the world. Of all of the *Fusarium* species, *F. graminearum* is the most common causal agent of FHB not only in North America, but also the world (Gilbert et al., 2001; Goswami and Kistler, 2004). FHB causes significant losses in both yield and quality (Kolb et al., 2001; Ludewig et al., 2005). Fusarium head blight is detrimental to end use quality as the pathogen produces a mycotoxin called deoxynivalenol (DON) (Tomczak et al., 2002; Bai et al., 1994; McMullen et al., 1997; Mesterhazy 1997; Parry et al., 1995). Miller et al. (1991) identified two chemotypes of DON: 3-acetyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON). 3-ADON chemotypes are DON producers that also make 3-ADON whereas 15-ADON chemotypes also produce DON but synthesize 15-ADON (Goswami and Kistler, 2004; Goswami and Kistler, 2005; Osborne et al., 2007; Ward et al., 2008). In North America,

the 15-ADON chemotype is the primary chemotype, whereas in Asia, the 3-ADON chemotype is more prevalent (Miller et al., 1991). However, recent research has shown that there has been a chemotype shift in eastern and central Canada from the 15-ADON chemotype to the 3-ADON chemotype (Ward et al., 2008). In 2004 in Manitoba, over 90% of the *Fusarium* species isolated were *F. graminearum* (Gilbert et al., 2010; Gilbert et al., 2009) with over 30% represented by the 3-ADON chemotype (Ward et al., 2008). The increased prevalence of the 3-ADON chemotype populations is a concern because the 3-ADON producers have an increased fitness advantage due to the increased DON production and vigour compared to the 15-ADON producers (Goswami and Kistler, 2004; Ward et al., 2008).

Fusarium graminearum is an extremely detrimental pathogen to wheat crops and development of resistant cultivars is a common goal in wheat breeding programs. One of the screening methods includes spray inoculation in field evaluations. Inoculum is usually applied as a liquid macroconidial suspension (Mesterhazy et al., 2005; Ludewig et al., 2005) and disease symptoms can be subsequently rated to evaluate resistance. Alternatively, infected straw or kernels, usually corn, are spread to incite infection where there is sufficient moisture in the field (Markell and Francl, 2003; Xue et al., 2006; McCartney et al., 2007). Ratings include disease incidence and severity, which can be used to determine percentage FHB index, and are done using a 0-100 scale with 0 showing no signs of infection to 100 showing complete infection (Mesterhazy et al., 2005).

Disease ratings are extremely valuable because they give an indication of potential yield losses, and toxin concentrations (Gilbert et al., 2001; Walker et al., 2001;

Wong et al., 1995; Ludewig et al., 2005). Once grain has been harvested, yield losses can be accurately measured but have been shown to have a close relationship to disease incidence/severity (Jiang et al., 2006; Ludewig et al., 2005). Fusarium damaged kernels (FDK) are also a reliable indicator of the amount of toxin accumulated in the grain (Gilbert et al., 2000; Mesterhazy et al., 2005; Wong et al., 1995). It is unlikely that breeders can directly measure the toxin accumulation in the grain on a large number of samples due to the cost and time associated with the test; therefore, other means for estimating toxin accumulation, such as FDK counts, are frequently used. For measurement of toxin concentrations enzyme-linked immunosorbent assay (ELISA), high performance liquid chromatography (HPLC), or gas chromatography-mass spectrometry (GC-MS) can be used (Jiang et al., 2006; Mesterhazy et al., 2005; Mirocha et al., 2003). ELISA is the most common method for DON quantification and is relatively quick (Mirocha et al., 2003). The ELISA method permits two antibodies to bind in synchrony to the DON molecules which then bind to a surface containing an enzyme which gives colour to the reaction which can then be used to quantify the amount of DON in each sample (Drolet et al., 1996; Mirocha et al., 2003).

Very little is known about the differences in the effects of the 3-ADON and 15-ADON chemotypes other than that 3-ADON isolates produce more DON than 15-ADON isolates (Ward et al., 2008). This study aims to determine if there is a difference in the interaction between *F. graminearum* and three spring wheat genotypes which differ in reaction to the pathogen, using isolates that differ in chemotype production and aggressiveness. Variables including area under the disease progress curve (AUDPC), yield, FDK, and DON will be used as points of comparison.

3.3 Materials and Methods

3.3.1 Isolates of *F. graminearum*

F. graminearum isolates were chosen to represent the Canadian population of the pathogen. Isolates were collected by R. Clear from the Grain Research Laboratory at the Canadian Grain Commission in 2006 from Fusarium-damaged kernels (FDK). Isolates were sampled from across Canada and across several crop districts in each wheat growing province. Samples were taken of both chemotypes in regions where both chemotypes were present, although the levels of aggressiveness of each isolate was unknown. Isolates were accessioned by the Agricultural Research Service (ARS) Culture Collection at the United States Department of Agriculture (Peoria, IL), to identify isolates by NRRL numbers (also known as Agricultural Research Service Culture Collection). A study done by (Ward et al., 2008) used a multilocus genotyping assay to identify each isolate as *F. graminearum sensu stricto* and (O'Donnell et al., 2004) identified the trichothecene chemotype of each isolate. In total, 13 3-ADON isolates and 12 15-ADON isolates were used in the present study. The isolates used are listed in Table 3.1. The chemotypes of each of the isolates were then confirmed at the University of Manitoba by the author, with further details and results presented in Appendix 7.7, specifically figures 7.1 and 7.2.

3.3.2 Genotype selection

Genotypes were selected based on their known reaction to FHB. CDC Teal was selected as the susceptible genotype, AC Cora as the intermediate resistant genotype and 93FHB37 as the resistant genotype (Figure 3.1). According to Seed Manitoba 2010 (2009), AC Cora is rated as fair or intermediate in reaction to FHB and CDC Teal is rated as very poor. AC Cora and CDC Teal are Canada Western Red Spring wheat cultivars.

93FHB37 is a line developed at Agriculture and Agri-Food Canada at the Cereal Research Centre in Winnipeg, Manitoba. The pedigree of this line is HY611/Ning8331 and has been shown to be resistant against FHB (McCartney et al., 2004).

Figure 3.1. Range in susceptibility of the genotypes used in the study. From left to right 93FHB37, AC Cora, CDC Teal.

3.3.3 Experimental design

The trials were arranged in a three replicate split plot design to allow differentiation between wheat genotypes and *F. graminearum* isolates. The main plot effect was *F. graminearum* isolate and wheat genotype was the sub plot effect. Main plots were separated by buffer plots of wheat cultivar Amazon. Amazon was chosen for the buffer plots because it is taller than the genotypes that were being tested, therefore

helping to reduce any potential inoculum drift among main plots. Figure 3.2 shows how the main plot and sub plot effects were separated.

Twenty-five isolates of *F. graminearum* were tested, 13 of which were 3-ADON producers and the balance were 15-ADON producers. There was one main plot per rep which was mock-inoculated with distilled water to act as a negative control. The experiment was conducted at the Point Research Station in Winnipeg, Manitoba for two growing seasons (2008 and 2009).

Plots were 1.5 m wide by 3 m long seeded at a rate of 1200 seeds per plot. Each replicate was split in half so that there were six blocks to the field. For a schematic layout, see Figure 3.3.

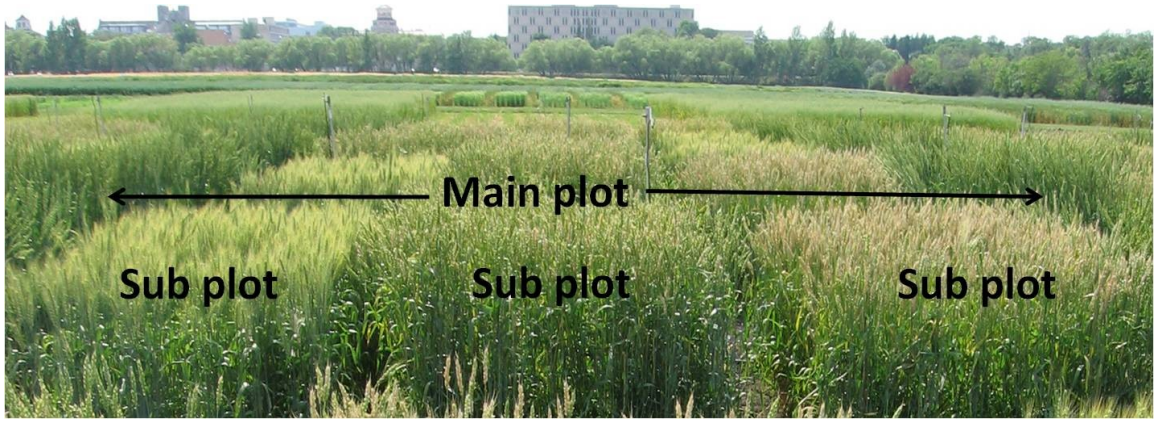

Figure 3.2 Separation of main plot and subplot effects in the experiment.

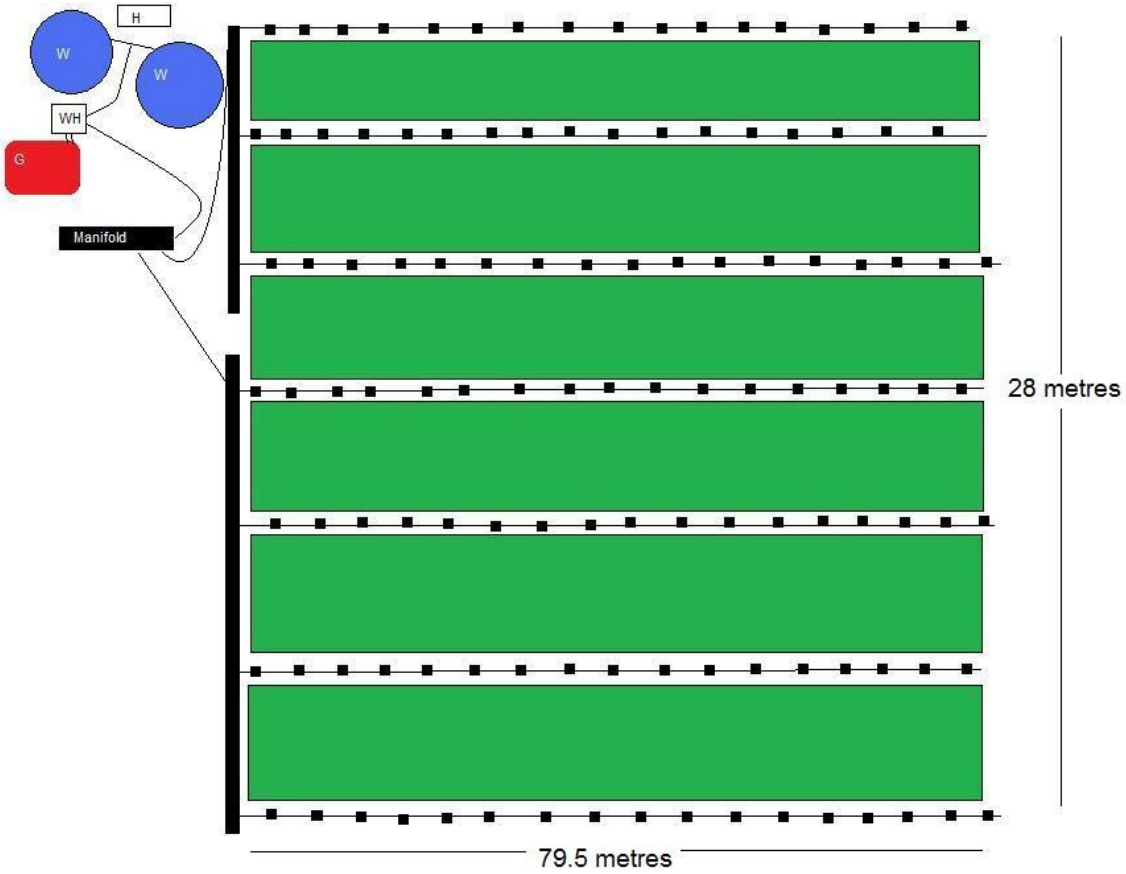

Figure 3.3: Schematic layout of field design (W= water tank; H=Hunter; WH=White house; G=Generator).

A misting system was used to provide favourable conditions for growth and colonization of the fungus to the wheat hosts (Figure 3.1). Two water tanks with a capacity of 1550L were attached to one another using 1.5 inch PVC pipe fitted with 1.5 inch ball valves. A line came off of the dual tank connection to attach to the white house which converted the electricity from the generator to pump the water to the manifold. Two valves from the manifold were used to split the water. The first manifold valve supplied water to three lines and the second manifold valve supplied water to the remaining four lines. Each line was fitted with a ¾ inch ball valve connected to ¾ inch hose which fed the misting nozzles that were supported by the metal risers. Each nozzle had a misting radius of 5m, therefore there were 17 riser/nozzle pairs per range. For each plot to be evenly misted, lines were run on either side of each range for a total of 7 lines. There were 17 riser/nozzle pairs per ¾ inch line for a total of 119 riser/nozzle pairs for the entire trial. A Hunter® controller (Hunter Industries, CA) was used to program the misting system cycle (Figure 3.2). The misting system was programmed to begin mist irrigation after inoculation for 10 minutes every hour for 10 hours each day until symptoms developed.

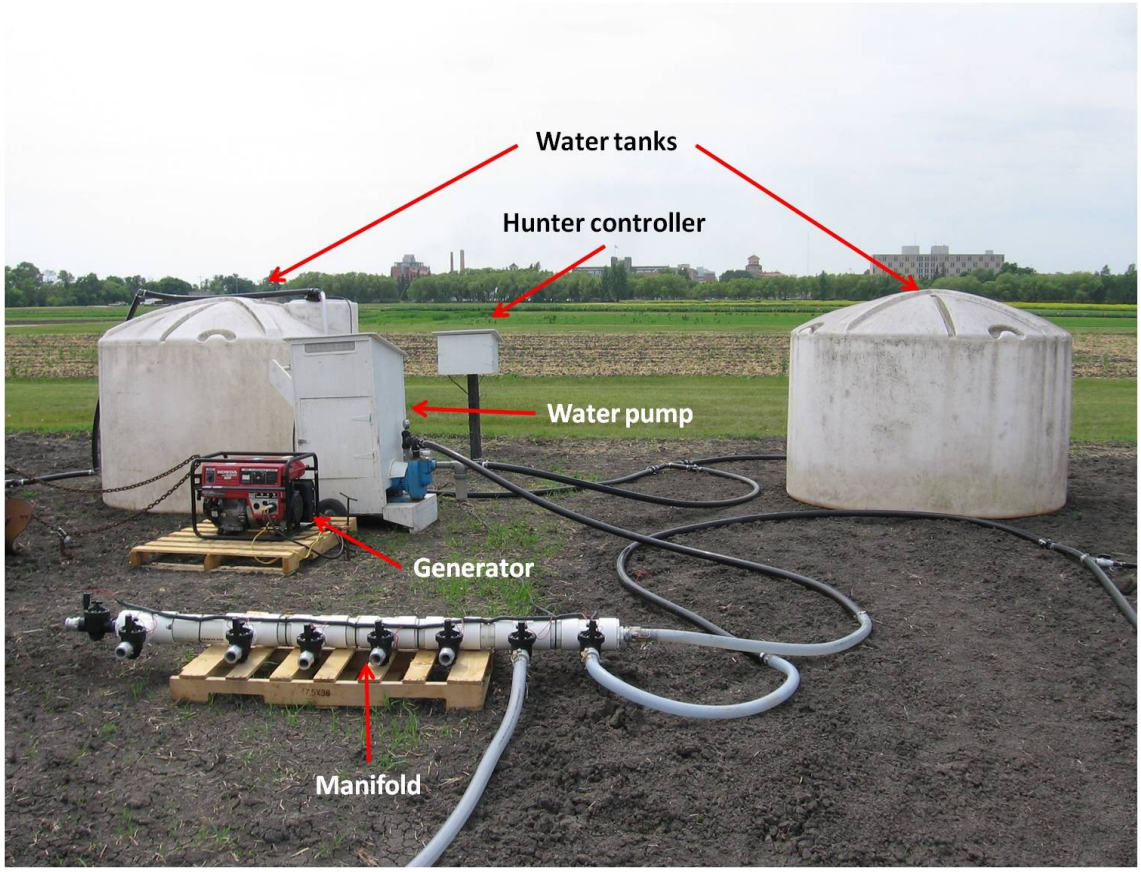

Figure 3.4: Photograph of the misting system, including water tanks, line connections, manifold and Hunter® programmer.

3.3.4 Inoculum production

Isolates were acquired on specific nutrient-poor agar (SNA) (see Appendix 7.2); isolate were incubated for 7 days under fluorescent light at room temperature to promote sporulation (Guo, 2008). Using aseptic techniques, one plug of each isolate was transferred mycelium side down to a fresh plate of potato dextrose agar (Difco Laboratories, MD) (see Appendix 7.1). These plates were incubated under ultraviolet (UV) light for seven days at room temperature. Single spore cultures of each isolate were made by pipetting 1mL of sterile distilled water into a microcentrofuge tube. Using

aseptic techniques, mycelia and spores were scraped from the plates aseptically and added to 1 mL sterile distilled water in a microcentrifuge tube. Tubes were then vortexed to liberate spores into the water. Approximately 0.25mL of the conidial solution was spread plated on water agar (WA) plates. Plates were sealed by applying parafilm to the outer edge and they were then incubated at 20^0C for 18-24 hours in the dark. After incubation, individual conidia and hyphae were cut out of the WA and transferred to a fresh PDA plates. These plates were incubated under UV light at room temperature for seven days.

To preserve the isolates for future use, plugs of the single spore cultures were transferred to fresh PDA plates lined with sterile filter paper. These plates were sealed with parafilm and put under the UV light bank for four days at room temperature. After incubation, filter papers covered in mycelia were removed from the plate and allowed to dry for 24 hours under sterile conditions in the laminar flow hood. Once papers were dried, papers were cut aseptically into $1cm^2$ square flakes and they were stored at -80^0C until further use. When needed, a single paper flake could be plated onto a fresh plate of PDA or SNA, and incubated under UV light for seven days at room temperature.

To produce liquid inoculum, 1.5 L of carboxymethyl cellulose (CMC) liquid media (Cappellini and Peterson, 1965) was prepared. In 2008, inoculum was prepared by adding 4-5 single spore culture plates of PDA to about 500mL of CMC media and streptomycin sulphate to a sterile blender. The mixture was blended until there were no large chunks of PDA. This mixture was added to a 2 L flask and the volume was made up to 1.5 L. A sterile #10 rubber stopper fitted with two 3/16" diameter glass tubes, one acting to aerate the inoculum and the other shorter tube acting as a waste tube which was

placed in the opening of each 2 L flask. Each glass tube was attached to tygon tubing so that the aeration tube could be attached to an external air supply (Hakko Air Pump, CA) and the waste tube could be hooked up to a waste beaker. The flasks of inoculum were set up under fluorescent light for seven days at room temperature. In 2009, this procedure was slightly modified by using roughly three SNA plates cut up into sections for each flask and dissolving the streptomycin sulphate in approximately 10mL of sterile distilled water.

After incubation, flasks of inoculum were aseptically strained through a triple layer of sterile cheesecloth into a sterile 1 L Kimax® bottle. Inoculum stock was stored at 4^0C until use, which never exceeded seven days. Inoculum stock concentrations were determined using a haemocytometer. Concentrations were calculated and adjusted to $5x10^4$ spores/mL. The amount of inoculum stock was measured out for each isolate and made up to 1 L with distilled water. Tween 20 was used as a surfactant and was added at a volume of 2 mL per 1L of inoculum. Bottles of inoculum were prepared on the day of inoculation.

3.3.5 Inoculation procedure

Inoculum was applied at a rate of 1 L per plot at 30 psi using a carbon dioxide (CO_2) backpack sprayer (R and D sprayer). The backpack sprayer was attached to a 6 nozzle boom with 20cm nozzle spacing. The nozzles were 8002 Teejet flat fan (i.e. 80 degree angle of spray and approximately 2 gallons per minute at 40psi).

A misting system was used to provide sufficient moisture and humidity for successful colonization of the pathogen on the host. The set up of the misting system was

described in section 3.3.3. The misting system was programmed to mist one hour after each inoculation for 10 minutes every hour for 10 hours.

3.3.6 Ratings

Upon symptom development, approximately five to seven days after inoculation, disease incidence and severity ratings were taken on a per plot basis. Disease incidence measured the spikes infected in the plot, on a scale of 0% indicating no infection in the plot to 100% indicating complete plot infection. Disease severity measured the average percentage of the spike which was infected, on a scale of 0% indicating no infection of the spikes to 100% indicating that spikes were completely infected. Fusarium head blight index (FHB index) was calculated for each plot by taking the product of incidence and severity divided by 100. Ratings were taken every three days until the control plots began to naturally senesce.

3.3.7 Yield measurements

Grain was harvested using a Wintersteiger small plot combine with the wind speed set very low to try to retain as many FDK as possible. Grain from individual plots was collected in cotton bags and air dried using a forced air dryer for at least seven days to equilibrate moisture. Grain samples were cleaned using a belt thresher and blower. The blower was set to low wind to ensure that only chaff was being removed and not any FDK. Yield was measured by weight, in grams, of cleaned grain from individual plots.

3.3.8 FDK evaluation

Fusarium damaged kernels were counted from a 100 seed sample from each plot. The FDK were separated from the healthy kernels and recorded as a percentage of kernels. A FDK was considered any seed that was shrivelled, had any mycelial growth or a chalky or pinkish colour, as per the Canadian Grain Commission (Government of Canada, 2009).

3.3.9 DON quantification

Deoxynivalenol was quantified at the Research Support Laboratory at the University of Guelph. A 10g grain sample from each plot was ground using a Romer® Mill (Model 2A). Deoxynivalenol was extracted using 50mL of deionized water, and then quantified using EZ-Quant® Vomitoxin ELISA kit from Diagnostix (www.diagnostix.ca) with a DON quantification limit of $0.5 mg kg^{-1}$.

3.3.10 Statistical Analysis

Area under the disease progress curve (AUDPC) was calculated for each of disease incidence, severity and FHB index by calculating the sum of the average ratings multiplied by the difference in growing degree days (GDD) as shown in Formula 3.1.

$$AUDPC = \Sigma\{[(r_n+r_{n+1})/2]*(GDD_{n+1}-GDD_n)\} \qquad \text{Formula 3.1}$$

Where r = rating.

Growing degree days were used instead of rating dates in order to account for differences in temperatures in 2008 and 2009, therefore, GDD gives a baseline for comparison of AUDPC between the two years. The formula used to calculate GDD was the average of

the maximum and minimum air temperatures for each day minus the base temperature of wheat which is 5^0C. The formula is shown below in Formula 3.2.

$$GDD = [(T_{min} + T_{max})/2]-T_{base} \qquad \text{Formula 3.2}$$

Where T_{min} = Minimum air temperature, T_{max} = maximum air temperature.

Analysis of variance (ANOVA) for AUDPC (based on GDD since anthesis), including incidence, severity and FHB index, yield, FDK and DON for each year and a combined analysis for the two years were performed using the "PROC GLM" procedure of the SAS software package (SAS Institute Inc., Version 9.2). A homogeneity test was conducted to ensure that the data could be combined over the two years. The model statement used in the combined analysis was variable = year block(year) isolate year*isolate year*block*isolate, genotype, isolate*genotype, year*genotype, year*isolate*genotype. Adjusted error terms were as follows: for isolate, block*isolate*year; for isolate*year, block*isolate*year; for year, block(year); for genotype, genotype*year.

Isolate effects were partitioned into: within 3-ADON isolates, within 15-ADON isolates, between chemotypes and control vs. inoculated plots. The reason for partitioning the sources of variation within isolate effects was to provide a comparison within each chemotype, between the chemotypes and between the control and inoculated plots. The model statement used in the combined analysis for analyzing variation within chemotypes was the same as in the complete analysis; however, the data was sorted "by chemotype". To compare chemotypes and the control versus the inoculated plots the main plot effect was recoded by chemotype or control versus inoculated to determine the

appropriate sum of squares and mean square. Adjusted error terms for analyzing variation within main plot effects were also the same as were used in the complete analysis. Correlations for all variables were performed using the “PROC CORR” procedure of the SAS software package.

Table 3.1 List of *F. graminearum* isolates collected from across Canada in 2006 used in field evaluations.

NRRL code	EQ code	Wheat Class	Provincial Origin	Luminex Chemotype
44613	A1-06-1	CWAD[1]	Alberta, Bow Island #1	15
44635	A2-06-1	CWRS[2]	Alberta, Brooks #1	3
44884	A4-06-5	CPSR[3]	Alberta, Provost #2	3
44886	A6-06-1	CWRS	Alberta, Westlock	3
44096	S1A-06-3	CWRS	Saskatchewan, Alameda	3
44097	S1A-06-4	CWRS	Saskatchewan, Carievale	15
44174	S3AN-06-1	CWRS	Saskatchewan, Eyebrow	15
44187	S3BS-06-1	CWRS	Saskatchewan, Bracken	3
44274	S8A-06-1	CWRS	Saskatchewan, Brooksby	3
44278	S8A-06-5	CWRS	Saskatchewan, Carrot River	15
44358	M2-06-1	CWRS	Manitoba, Brandon	3
44359	M2-06-2	CWRS	Manitoba, Carberry	15
44509	M8-06-2	CWRW[4]	Manitoba, Winkler	15
44512	M8-06-5	CWRS	Manitoba, Baldur	3
43897	ON-06-17	CERS[5]	Ontario, Oxford #1	15
45099	Q-06-10	CERS	Quebec	15
45100	Q-06-11	CERS	Quebec	3
45111	Q-06-22	CERS	Quebec	15
45112	Q-06-23	CERS	Quebec	3
45123	Q-06-34	CERS	Quebec	15
44963	NB-06-17	CERS	New Brunswick	3
44964	NB-06-18	CERS	New Brunswick	15
45038	NS-06-2	CERS	Nova Scotia	3
45039	NS-06-3	CERS	Nova Scotia	15

[1]Canada Western Amber Durum
[2] Canada Western Red Spring
[3]Canada Prairie Spring Red
[4] Canada Western Red Winter
[5] Canada Eastern Red Spring

3.4 Results and Discussion

3.4.1 Homogeneity Tests

Levene's test for homogeneity of variance were conducted on the combined 2008 and 2009 field experiment to determine whether data over the two years could be combined. Table 3.2 shows the results of Levene's homogeneity tests for the main plot (isolate), sub plot (genotype) and main plot by subplot interactions. As evident from Table 3.2, all variances for genotype and isolate by genotype interaction were significantly heterogeneous for all variables measured and the isolate effect was heterogeneous for the DON variable. Examination of reasons for heterogeneity of variances did not reveal a conclusive explanation for why variances were heterogeneous. As evident from tables of means for individual years, disease levels were substantially higher in 2009 than in 2008; as a result, variances are substantially larger. In addition, the difference in disease levels between the two years resulted in a larger spread between genotypes in 2009. Considering these factors, heterogeneity of variances were due to magnitude differences in the two years that the study was run which were then amplified by squaring these values when mean squares were calculated. Combining data that are considered heterogeneous can result in Type II error, i.e., accepting the null hypothesis when the null hypothesis is false. In other words, the implications for combining heterogenous data are that year by year differences could hide significant differences between variables.

Table 3.2: Significance (Pr > F) of Levene's homogeneity tests on the 2008 and 2009 dataset

	Isolate effects	Genotype effects	Isolate*Genotype effects
Yield	0.4920	0.0096	0.0004
AUDPCInc[1]	0.5780	<0.0001	<0.0001
AUDPCSev[2]	0.5653	<0.0001	<0.0001
AUDPCIdx[3]	0.1265	<0.0001	<0.0001
FDK	0.5200	<0.0001	<0.0001
DON	0.0005	0.0038	<0.0001

P > 0.05 indicate that variances were heterogeneous

[1]Area under the disease progress curve disease incidence
[2]Area under the disease progress curve disease severity
[3]Area under the disease progress curve FHB index

3.4.2 Disease progression/AUDPC

The 2008 and 2009 analyses of variance for AUDPC disease incidence (AUDPCInc), are shown in Tables 3.3 and 3.4, respectively. Within the 15-ADON isolates, there were significant differences in 2008, 2009 and in the combined analysis (Table 3.5). The range of values within the 15-ADON isolates was substantially larger in 2009 than in 2008. The isolate*genotype interaction was significant in 2008 (P=0.0150) but not in 2009 (P=0.1870), and when the data were combined there was a significant interaction (P<0.0001) (Table 3.5). These differences were due to differences in magnitude among genotypes when compared across isolates (Table 3.6). In other words, the relative ranking of genotypes remained the same regardless of the isolate, but the magnitude of the differences between the genotypes varied with the isolate used.

Table 3.3. 2008 Analysis of variance for area under the disease progress curve (incidence, severity and Fusarium head blight index), yield, Fusarium damaged kernels and deoxynivalenol.

		AUDPC[1] Incidence		AUDPC Severity		AUDPC FHB Index		Yield		FDK[2]		DON[3]	
Source of variation	**df**	**Mean Square**	**F value (significant)**	**Mean Square**	**F value (significant)**	**Mean Square**	**F value (significant)**	**Mean Square**	**F value (significant)**	**Mean Square**	**F value (significant)**	**Mean Square**	**F value (significant)**
Block	2	6.11 E 7	19.36 (*)	1.74 E 7	3.91 (*)	2.43 E 7	0.0013 (*)	2.35 E 5	17.6 (*)	1.96 E 3	6.84 (*)	1	0.19 (ns)
Isolate	25	6.49 E 7	6.35 (*)	3.84 E 7	3.86 (*)	3.08 E 7	3.56 (*)	2.11 E 5	5.36 (*)	507	1.55 (ns)	35	3.89 (*)
Within 3-ADON	12	1.68 E 7	1.65 (ns)	1.65 E 7	1.65 (ns)	9.61 E 6	1.10 (ns)	7.66 E 4	1.95 (ns)		-	31	3.43 (*)
Within 15-ADON	11	5.10 E 7	5.00 (*)	4.24 E 7	4.25 (*)	3.21 E 7	3.70 (*)	5.33 E 4	1.61 (*)		-	20	2.11 (*)
Between chemotypes	1	3.98 E 7	3.90 (*)	1.82 E 7	1.82 (ns)	1.55 E 7	1.79 (ns)	3.40 E 5	8.64 (*)		-	29	3.22 (*)
Control vs. inoculated	1	1.10 E 9	107.84 (*)	6.51 E 8	65.23 (*)	3.47 E 8	40.02 (*)	3.31 E 6	84.1 (*)		-	260	28.9 (*)
Block*Isolate	50	1.02 E 7	3.24 (*)	9.98 E 6	2.23 (*)	8.67 E 6	2.54 (*)	3.93 E 4	2.94 (*)	326	1.14 (ns)	9	2 (*)
Genotype	2	3.43 E 9	1087 (*)	2.67 E 9	596.8 (*)	2.23 E 9	652.7 (*)	1.33 E 7	996 (*)	2.65 E 4	92.45 (*)	1380	248 (*)
Isolate* Genotype	50	9.88 E 6	3.13 (*)	9.12 E 6	2.04 (*)	8.33 E 6	2.44 (*)	4.27 E 4	3.19 (*)	219	0.77 (ns)	9	1.6 (*)
Error	104	3.16 E 6		4.46 E 6		3.41 E 6				286		6	
Total	233												

[1] AUDPC = Area under the disease progress curve; [2] FDK = Fusarium damaged kernels; [3]DON = deoxynivalenol

Table 3.4. 2009 Analysis of variance for area under the disease progress curve (incidence, severity and Fusarium head blight index), yield, Fusarium damaged kernels and deoxynivalenol.

		AUDPC[1] Incidence		AUDPC Severity		AUDPC FHB Index		Yield		FDK[2]		DON[3]	
Source of variation	**df**	**Mean Square**	**F value (significant)**	**Mean Square**	**F value (significant)**	**Mean Square**	**F value (significant)**	**Mean Square**	**F value (significant)**	**Mean Square**	**F value (significant)**	**Mean Square**	**F value (significant)**
Block	2	8.09 E 7	9.24 (*)	2.27 E 7	6.65 (*)	7.97 E 6	2.53 (ns)	1.17 E 4	0.37 (ns)	2.97 E 3	19.26 (*)	150	11.63 (*)
Isolate	25	7.91 E 7	6.18 (*)	3.04 E 7	6.72 (*)	1.64 E 7	4.59 (*)	4.62 E 5	10.6 (*)	1.23 E 3	8.96 (*)	98	3.07 (*)
Within 3-ADON	12	2.12 E 7	1.65 (ns)	5.55 E 6	1.22 (ns)	5.61 E 6	1.57 (ns)	6.79 E 4	1.55 (ns)	1.83 E 2	1.33 (ns)	62	1.94 (ns)
Within 15-ADON	11	2.83 E 7	2.2 (*)	8.03 E 6	1.78 (ns)	8.26 E 6	2.31 (*)	1.36 E 5	3.11 (ns)	1.14 E 2	0.83 (ns)	48	1.52 (ns)
Between chemotypes	1	1.75 E 5	0.01 (ns)	3.40 E 5	0.08 (ns)	1.92 E 4	0.01 (ns)	8.34 E 3	0.19 (ns)	79	0.58 (ns)	112	3.50 (ns)
Control vs. inoculated	1	1.41 E 9	110 (*)	3.06 E 8	133 (*)	2.51 E 8	7.02 (*)	9.20 E 6	210.55 (*)	2.72 E 4	198.52 (*)	1070	33.61 (*)
Block* Isolate	50	1.28 E 7	1.46 (ns)	4.52 E 6	1.33 (ns)	3.57 E 6	1.14 (ns)	4.37 E 4	1.39 (ns)	1.37 E 2	0.89 (ns)	32	2.46 (*)
Genotype	2	3.07 E 9	350.23 (*)	3.45 E 9	1012 (*)	2.11 E 9	670 (*)	1.63 E 7	520.9 (*)	3.05 E 4	198 (*)	1250	97 (*)
Isolate* Genotype	50	1.08 E 7	1.23 (ns)	6.63 E 6	1.95 (*)	6.02 E 6	1.92 (*)	4.19 E 4	1.34 (ns)	1.27 E 2	0.82 (ns)	1	1.04 (ns)
Error	101	8.76 E 6		3.41 E 6		3.14 E 6		3.14 E 4		1.54 E 2		13	
Total	230												

[1] AUDPC = area under the disease progress curve; [2]FDK = Fusarium damaged kernels; [3]DON = deoxynivalenol

Table 3.5. Combined 2008 and 2009 Analysis of variance for area under the disease progress curve (incidence, severity and Fusarium head blight index), yield, Fusarium damaged kernels and deoxynivalenol.

Source	df	AUDPC[1] Incidence		AUDPC Severity		AUDPC FHB Index		Yield		FDK[2]		DON[3]	
		Mean Square	F value (significant)	Mean Square	F value (significant)	Mean Square	F value (significant)	Mean Square	F value (significant)	Mean Square	F value (significant)	Mean Square	F value (significant)
Year	1	4.17 E 7	0.55 (ns)	2.70 E 7	0.78 (ns)	1.16 E 8	6.46 (ns)	6.56 E 5	5.1 (ns)	1.61 E 5	65.57 (*)	7.47 E 3	98.98 (*)
Block (Year)	4	7.54 E 7	11.75 (*)	3.44 E 7	7.44 (*)	1.79 E 7	5.05 (*)	1.24 E 5	5.52(*)	2.46 E 3	11.15 (*)	75	8.23 (*)
Isolate	25	1.24 E 8	9.2 (*)	6.26 E 7	6.63 (*)	3.43 E 7	5.09 (*)	5.95 E 4	13 (*)	1.27 E 3	5.49(*)	86	4.21 (*)
Within 3-ADON	12	1.21 E 7	0.90 (ns)	6.47 E 6	0.69 (ns)	4.36 E 6	0.65 (ns)	4.17 E 4	1 (ns)	311	1.35 (ns)	30	1.47 (ns)
Within 15-ADON	11	3.77 E 7	2.81 (*)	2.05 E 7	2.17 (*)	1.83 E 7	2.71 (*)	8.79 E 4	2.11 (*)	65	0.28 (ns)	43	2.13 (*)
Between chemotypes	1	2.25 E 7	1.68 (ns)	6.73 E 6	0.71 (ns)	8.27 E 6	1.23 (ns)	2.27 E 5	5.48 (*)	1.43 E 3	6.19 (*)	127	6.26 (*)
Control vs. inoculated	1	2.51 E 9	186.54 (*)	1.25 E 9	132.72 (*)	5.94 E 8	88.4 (*)	1.18 E 7	284 (*)	2.59 E 4	112.05 (*)	1.19 E 3	58.43 (*)
Year*Isolate	25	3.13 E 7	2.33 (*)	2.06 E 7	2.18 (*)	1.50 E 7	2.23 (*)	1.33 E 5	3.19 (*)	360	1.56 (ns)	48	2.34 (*)
Year*Block* Isolate	100	1.34 E 7	2.09 (*)	9.44 E 6	2.04 (*)	6.73 E 6	1.89 (*)	4.15 E 4	1.85 (*)	231	1.05 (ns)	20	2.22 (*)
Genotype	2	6.76 E 9	38.85 (*)	5.83 E 9	114.27 (*)	4.39 E 9	93.05 (*)	2.95 E 7	243 (*)	5.57 E 4	37.47 (*)	2.62 E 3	231 (*)
Isolate* Genotype	50	1.42 E 7	2.21 (*)	1.05 E 7	2.27 (*)	9.96 E 6	2.8 (*)	5.35 E 4	2.39 (*)	169	0.77 (ns)	13	1.42 (*)
Year* Genotype	2	1.74 E 8	27.11 (*)	5.10 E 7	11.03 (*)	4.72 E 7	13.29 (*)	1.22 E 5	5.44 (*)	1.48 E 3	6.73 (*)	11	1.23 (ns)
Year*Isolate* Genotype	50	9.05 E 6	1.41 (ns)	6.42 E 6	1.39 (ns)	5.27 E 6	1.48 (*)	3.00 E 4	1.34 (*)	174	0.79 (ns)	9	1.00 (ns)
Error	205	6.42 E 6		4.63 E 6		3.55 E 6		2.24 E 4		221		9	
Total	464												

[1] AUDPC = area under the disease progress curve; [2] FDK = Fusarium damaged kernels; [3] DON = deoxynivalenol

Table 3.6. Means for AUDPC disease incidence, severity and fusarium head blight index in 2008 and 2009.

Isolate QC code	Chemotype	Genotype	AUDPCInc[1]		AUDPCSev[2]		AUDPCIdx[3]	
			2008 Mean	2009 Mean	2008 Mean	2009 Mean	2008 Mean	2009 Mean
A1-06-1	15	AC Cora	18994	11672	11456	7448	8569	3319
		93FHB37	6120	9819	6256	4096	1241	1391
		CDC Teal	22576	17681	19457	15120	15347	9205
S1A-06-4	15	AC Cora	15694	9129	9383	7448	6938	2510
		93FHB37	5253	6315	5356	3415	1189	1015
		CDC Teal	21446	18778	15772	15773	12658	10151
S3AN-06-1	15	AC Cora	9683	13701	5517	8039	3044	3874
		93FHB37	4873	6810	5016	3414	1141	998
		CDC Teal	13986	22290	11987	21273	7864	14328
S8A-06-5	15	AC Cora	11877	13540	6525	8623	3771	4271
		93FHB37	3341	5903	3443	4204	674	1024
		CDC Teal	18125	20410	13963	16892	10626	10790
M2-06-2	15	AC Cora	13823	14313	10252	7925	6269	4206
		93FHB37	4746	12471	4863	4789	1387	2134
		CDC Teal	19336	25684	18970	18803	13753	1484
M8-06-2	15	AC Cora	8945	11214	5782	10550	3005	4395
		93FHB37	3922	5147	4061	2926	1045	752
		CDC Teal	14613	19650	11989	17063	7971	10692
ON-06-17	15	AC Cora	7833	13440	5225	8619	2431	4529
		93FHB37	4943	8298	5065	3710	876	1322
		CDC Teal	13798	21423	10172	16993	6644	11815
Q-06-10	15	AC Cora	15924	14358	11510	8103	7987	4304
		93FHB37	5775	7359	5913	3813	1275	1223
		CDC Teal	24070	21259	23167	18441	18347	12751
Q-06-22	15	AC Cora	15938	12627	10407	7527	7422	3337
		93FHB37	5292	5937	5421	3443	1225	888
		CDC Teal	20444	18299	17036	16046	13392	10139
Q-06-34	15	AC Cora	12294	10415	10743	7118	6198	2882
		93FHB37	6898	7546	7051	3717	1768	1225
		CDC Teal	19509	18108	14100	17391	11073	10483
NB-06-18	15	AC Cora	15548	9832	11463	6291	7915	2414
		93FHB37	2942	8463	3084	3798	632	1374
		CDC Teal	22602	20539	19573	16963	15294	11178
NS-06-3	15	AC Cora	10567	13063	9714	9583	6656	4597
		93FHB37	5809	10877	5945	5291	1699	2270
		CDC Teal	20616	23715	16936	20922	13065	15332

Isolate	Chemotype	Genotype	AUDPCInc		AUDPCSev		AUDPCIdx	
			2008 Mean	2009 Mean	2008 Mean	2009 Mean	2008 Mean	2009 Mean
A2-06-1	3	AC Cora	9959	12362	6470	8743	3611	4149
		93FHB37	4577	8460	4685	4263	1022	1537
		CDC Teal	15960	23136	14681	19221	10036	13899
A4-06-5	3	AC Cora	16015	11423	10708	8410	7412	3883
		93FHB37	5047	8976	5176	4146	919	1597
		CDC Teal	20585	20721	18243	17125	13780	11884
A6-06-1	3	AC Cora	16069	14169	10120	7845	7063	3878
		93FHB37	4590	10144	4729	3865	985	1529
		CDC Teal	19157	19853	15411	16323	11580	10420
S1A-06-3	3	AC Cora	13413	14048	9368	8770	5574	4282
		93FHB37	6732	8046	6872	3813	1889	1216
		CDC Teal	18986	24678	17000	18905	12858	14131
S3BS-06-1	3	AC Cora	14329	9812	10192	8383	6586	3463
		93FHB37	7273	5958	7413	3170	1097	910
		CDC Teal	20455	16498	20255	13280	14331	7912
S8A-06-1	3	AC Cora	14506	14263	9071	8611	6176	4486
		93FHB37	5032	10465	5146	4249	1200	1679
		CDC Teal	18626	19167	14504	15528	11195	10497
M2-06-1	3	AC Cora	19283	10688	9947	7339	7860	2845
		93FHB37	5901	5990	6031	3237	1785	854
		CDC Teal	20245	20431	18206	18123	14283	11757
M8-06-5	3	AC Cora	14798	13571	8391	7870	5818	4071
		93FHB37	5413	4175	5523	3045	1544	586
		CDC Teal	21282	20733	18257	17043	14658	11584
Q-06-11	3	AC Cora	16741	11568	11013	8413	7758	3732
		93FHB37	5916	4988	6051	3428	1581	737
		CDC Teal	20358	20023	16752	15523	13092	10336
Q-06-23	3	AC Cora	15825	15037	8968	9552	6293	5056
		93FHB37	2517	7488	2647	3518	407	1107
		CDC Teal	17937	23668	13308	20301	9836	14684
NB-06-17	3	AC Cora	16609	13870	10742	9502	7771	4498
		93FHB37	6374	10695	6525	4333	1520	2065
		CDC Teal	23128	22600	21152	17484	16755	12335
NS-06-2	3	AC Cora	13058	14604	6829	8823	4559	4182
		93FHB37	5061	5473	5207	3170	1464	1032
		CDC Teal	19573	19085	17507	18014	12858	11177
MIN-1-1	3	AC Cora	14787	10978	9575	8945	6751	3845
		93FHB37	4843	6900	4965	3170	1206	1063
		CDC Teal	22256	19623	17493	17284	13967	11136

Isolate	Chemotype	Genotype	AUDPCInc		AUDPCSev		AUDPCIdx	
			2008 Mean	2009 Mean	2008 Mean	2009 Mean	2008 Mean	2009 Mean
CONTROL	n/a	AC Cora	1879	726	2298	684	277	80
		93FHB37	227	199	383	303	41	15
		CDC Teal	3069	1740	2418	3511	694	620
LSD			**2898**	**2780**	**2255**	**1733**	**1858**	**1665**

[1]Area under the disease progress curve for disease incidence
[2]Area under the disease progress curve for disease severity
[3] Area under the disease progress curve for Fusarium head blight index
Note: All sample sizes were equal to three except for NB-06-17 and M8-06-2 in 2009 where the sample size was equal to two.

Isolate*genotype interaction for all AUDPC variables was significant in 2008 (Table 3.3), 2009 (Table 3.4) and in the combined analysis (Table 3.5). This means that overall, the isolates responded differently on the genotypes; however there was no consistency in the ranking of the isolates indicating that the environmental conditions had a major impact on the performance of the isolates between years. As with AUDPC incidence (AUDPCInc) there was no change in relative ranking of the genotypes for AUDPC severity (AUDPCSev), however the magnitude of the differences between genotypes varied with the isolate used. The 2008 data showed significant differences within 15-ADON isolates, however this difference was not detected in 2009. A significant difference was detected within 15-ADON isolates when the data were combined. The combined analysis suggests that the 15-ADON isolates have more genetic variability than the 3-ADON isolates. This rationale is supported by Ward et al. (2008) who suggested that the 3-ADON chemotype was only recently introduced into Canada and therefore has a narrower genetic basis.

In the combined years' analysis for AUDPC FHB index (AUDPCIdx), there were no significant differences between chemotypes (P=0.2789) or within 3-ADON isolates

(P=0.8663), however there were significant differences within 15-ADON isolates (P=0.0051), (Table 3.5). Levene's test showed that isolate effects for AUDPCIdx were homogeneous (see Table 3.2). In the individual years as well as in the combined analysis, AUDPCIdx showed no significant differences for isolate*genotype. As for AUDPC Inc and AUDPCSev, there was no change in relative ranking of the genotypes for AUDPCIdx, however the magnitude of the difference among genotypes differed with the isolate used (Table 3.6). The block*isolate interaction was significant in 2008 but not significant in 2009. Other than the differences mentioned, the results from the AUDPCIdx analysis were identical to those in AUDPCInc and AUDPCSev. This is not unusual given that AUDPCIdx is a function of incidence and severity measurements.

Lack of consistency in the ranking of isolate means between years could be explained by differences in environmental conditions which may have affected disease progression. For instance, the mean temperature between flowering and the last rating date in 2008 was 19.3^0C, whereas in 2009 it was 17.4^0C. Disease progression was expressed on the basis of GDD to attempt to mitigate the effects of temperature differences between the two years. Typically daily GDD are calculated by subtracting the base temperature by the average of the maximum and minimum temperatures. In this study a base temperature of 5^0C (i.e. base temperature of wheat) was used because inoculations were done at the same physiological stage each year (i.e. 50% anthesis). However in the two years, different amounts of heat units were accumulated by the wheat plants. There is no base temperature or GDD equivalent for *F. graminearum* that could be used for this analysis. In 2009, the accumulated GDD required for symptom development were larger than in 2008, however, except for initial symptom development,

the shape of the curves of the two years are quite similar (Figure 3.3a-c). Even though the two years were quite different in terms of temperature and the amount of heat units accumulated by 50% anthesis, there was no effect on disease progression for any of the AUDPC's. In terms of disease progression, there were also no differences detected among chemotypes, although it seems as though there were slight differences between isolates in the two years as demonstrated by the significant year*isolate interaction. In terms of implications for farmers, it appears as though differences in temperature or growing degree days between years does not make any substantial differences as far as disease progression by the two *F. graminearum* chemotypes.

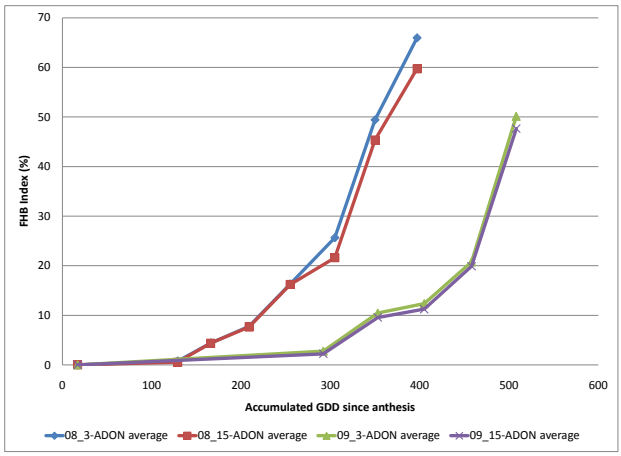

Figure 3.5a. Fusarium head blight index progression of the chemotype averages by year on AC Cora.

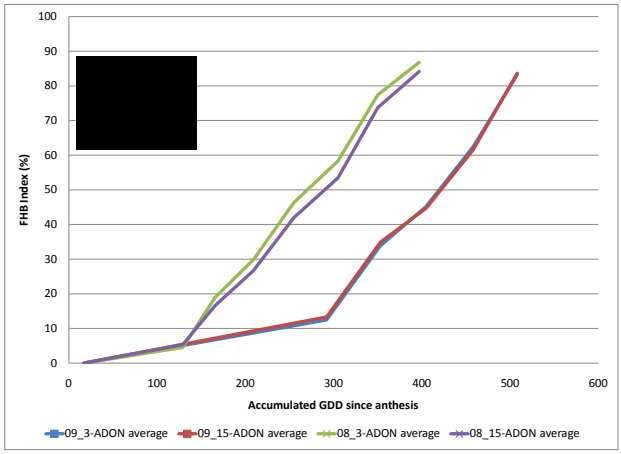

Figure 3.5b Fusarium head blight index progression of chemotype averages by year on CDC Teal.

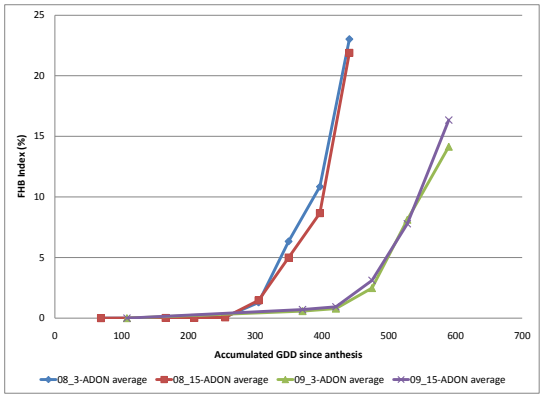

Figure 3.5c Fusarium head blight index progression of chemotype averages by year on 93FHB37.

In the combined year's analysis, AUDPCInc, AUDPCSev and AUDPCIdx showed significant block(year), isolate, within 15-ADON chemotype, control versus inoculated, year*isolate, year*block*isolate, genotype, isolate*genotype, and year*genotype effects (Table 3.5). Year, within 3-ADON chemotype, between chemotypes and year*isolate*genotype effects were not significant. The year*isolate*genotype interaction was only significant for AUDPCIdx. A significant isolate effect indicates that there were significant differences in the aggressiveness (i.e. disease causing ability as measured by disease progression) or levels of disease caused by the isolates.

Significant differences were also seen among genotypes in all analyses and for all variables measured, indicating that there were differences in disease progression for the genotypes. This was to be expected since the genotypes ranged in susceptibility to FHB (93FHB37 is resistant, AC Cora is moderately resistant and CDC Teal is susceptible). The relative rankings of genotypes remained the same: 93FHB37 had the smallest AUDPC values, AC Cora had intermediate values and CDC Teal had the largest AUDPC values, as seen in the tables of means in Table 3.7.

Table 3.7. Mean area under the disease progress curve for fusarium head blight incidence, severity and index for the three wheat genotypes in 2008 and 2009.

Genotype	**N[1]**	**AUDPCInc[2] Mean**	**AUDPCSev[2] Mean**	**AUDPCIdx[3] Mean**
AC Cora	156	12948	8478	4823
93FHB37	154	6196	4378	1216
CDC Teal	156	19472	16493	11732
LSD		**440.1**	**404.5**	**374.5**

[1] Sample size
[2] Area under the disease progress curve - disease incidence
[3] Area under the disease progress curve – disease severity
[4] Area under the disease progress curve – Fusarium head blight index

Significant year*isolate interactions indicate that the isolates performed differently in the two years, although there was no consistent relative ranking of isolates between years (Table 3.8). Appendix 7.5 shows AUDPC means for each rating for the combined analysis which also shows no consistency in ranking compared to the individual years. Inconsistent isolate ranking between the two years may indicate that isolates have different preferences for temperature and humidity for infection.

Table 3.8. AUDPC means for all isolates based on 2008 and 2009 data.

Isolate QC code	Chemotype	AUDPCInc[1] Means		AUDPCSev[2] Means		AUDPCIdx[3] Means	
		2008	**2009**	**2008**	**2009**	**2008**	**2009**
A1-06-1	15	15897	13057	12390	8888	8386	4639
S1A-06-4	15	14131	11407	10170	8879	6928	4559
S3AN-06-1	15	9514	14267	7506	10909	4016	6400
S8A-06-5	15	11114	13284	7977	9906	5024	5361
M2-06-2	15	12635	17489	11632	10506	7136	7068
M8-06-2	15	9160	12861	7277	11087	4007	5846
ON-06-17	15	8858	14387	5821	9774	3317	5889
Q-06-10	15	15256	14325	13530	10119	9203	6093
Q-06-22	15	13891	12288	10955	9005	7346	4788
Q-06-34	15	12900	12023	10631	9409	6346	4863
NB-06-18	15	13697	12945	11374	9018	7947	4989
NS-06-3	15	13830	15886	10865	11932	7140	7400
A2-06-1	3	10165	14653	8612	10743	4890	6528
A4-06-5	3	13882	13706	11375	9894	7370	5788
A6-06-1	3	13272	14722	10087	9344	6543	5276
S1A-06-3	3	13044	15591	11080	10496	6774	6543
S3BS-06-1	3	14019	10756	12620	8278	7671	4095
S8A-06-1	3	12721	14632	9574	9463	6190	5554
M2-06-1	3	15143	12370	11395	8566	7976	5152
M8-06-5	3	13831	12827	10724	9319	7340	5414
Q-06-11	3	1438	12196	11272	9121	7477	4935
Q-06-23	3	12093	15397	8308	11123	5512	6949
NB-06-17	3	15370	16350	12806	11203	8682	6829
NS-06-2	3	12564	13387	9848	10003	6294	5464
MIN-1-1	3	13962	12500	10678	9800	7308	5348
CONTROL	n/a	1725	888	1700	1499	337	238
LSD		**1899**	**2780**	**2256**	**1734**	**1859**	**1666**

[1] Area under the disease progress curve for disease incidence
[2] Area under the disease progress curve for disease severity
[3] Area under the disease progress curve for Fusarium head blight index
Note: Sample sizes were equal to 9 with the exception of M8-06-2 and NB-06-17 which were equal to 8.

3.4.3 Yield

In the individual year analyses, 2008 and 2009 showed similar results for isolate, within 3-ADON isolates, control versus inoculated plots, and genotype effects (Tables 3.3 and 3.4). The relative rankings for yield of the genotypes remained the same in the two years, i.e. 93FHB37 had the highest yield, AC Cora had an intermediate yield and CDC Teal had the lowest yield (Table 3.9). The variation about the mean was fairly consistent as well. Although there were no statistically significant differences in yield between the two years, on average yield was lower in 2009 than 2008 (Table 3.9). This may be explained by a higher frequency of FDK in 2009 than in 2008 (Table 3.14).

Table 3.9. Mean yield for AC Cora, CDC Teal and 93FHB37 in 2008, 2009, and combined over both years.

Year	Genotype	N	Mean (kg/ha)
2008	AC Cora	77	1349.12
2008	93FHB37	78	1851.13
2008	CDC Teal	77	1022.01
2009	AC Cora	78	1299.94
2009	93FHB37	76	1822.32
2009	CDC Teal	78	885.76
Combined	AC Cora	155	1324.37
Combined	93FHB37	154	1836.92
Combined	CDC Teal	155	953.45
2008 LSD = 36.89; 2009 LSD = 56.51; Combined LSD = 33.54			

As in the AUDPC analyses, there were significant differences for isolates, but the yield was also significantly different between chemotypes in the combined analysis. Table 3.10 shows a means comparison for isolate effects between years. There were significant differences for isolate*genotype interactions in 2008, but not in 2009 (Tables 3.3, 3.4). Based on the LSD values for each year, it is clear that there were differences

among isolates and genotypes in both years. Although there was a consistent ranking of genotypes within each year, the magnitude of the differences were not consistent (Table 3.10).

Table 3.10. Mean yield for all isolate*genotype combinations in 2008 and 2009.

Isolate QC code	Chemotype	Genotype	2008 Mean	2009 Mean	Combined mean
A1-06-1	15	AC Cora	1300.73	1301.33	1301.03
		93FHB37	1923.36	1767.66	1845.52
		CDC Teal	1019.23	876.00	947.61
S1A-06-4	15	AC Cora	1216.95	1537.33	1409.18
		93FHB37	1815.16	1947.33	1881.25
		CDC Teal	959.36	964.66	962.01
S3AN-06-1	15	AC Cora	1530.10	1210.00	1370.05
		93FHB37	1875.73	1738.66	1807.20
		CDC Teal	1229.60	708.33	916.84
S8A-06-5	15	AC Cora	1519.83	1171.00	1345.41
		93FHB37	1976.16	1991.66	1983.91
		CDC Teal	1021.03	840.33	930.68
M2-06-2	15	AC Cora	1304.70	1168.33	1236.51
		93FHB37	1885.70	1570.33	1728.01
		CDC Teal	1102.16	555.00	828.58
M8-06-2	15	AC Cora	1627.50	1257.66	1442.58
		93FHB37	1741.06	1825.50	1774.84
		CDC Teal	1236.33	812.00	1024.16
ON-06-17	15	AC Cora	1474.46	1447.33	1460.90
		93FHB37	1880.33	1826.00	1853.16
		CDC Teal	1072.26	847.66	959.96
Q-06-10	15	AC Cora	1158.23	115.00	1136.61
		93FHB37	1773.33	1786.33	1779.83
		CDC Teal	855.36	814.00	834.68
Q-06-22	15	AC Cora	1230.73	1374.33	1302.53
		93FHB37	1872.90	1851.66	1862.28
		CDC Teal	891.160	906.66	898.91
Q-06-34	15	AC Cora	1404.03	1389.66	1396.85
		93FHB37	1811.73	1914.66	1863.20
		CDC Teal	1045.86	1068.33	1054.60
NB-06-18	15	AC Cora	1352.86	1364.33	1358.60
		93FHB37	1997.10	1810.33	1903.71
		CDC Teal	997.23	855.66	926.45
NS-06-3	15	AC Cora	1269.76	967.33	1118.55
		93FHB37	1852.96	1590.00	1721.48
		CDC Teal	1070.83	732.00	901.41
A2-06-1	3	AC Cora	1592.23	1073.33	1332.78
		93FHB37	1807.43	1740.66	1774.05
		CDC Teal	1177.06	644.66	910.86

Isolate QC code	Chemotype	Genotype	2008 Mean	2009 Mean	Combined Mean
A4-06-5	3	AC Cora	1207.20	1163.00	1185.10
		93FHB37	1772.23	1697.00	1734.61
		CDC Teal	1069.16	690.66	879.91
A6-06-1	3	AC Cora	1288.33	1287.00	1287.66
		93FHB37	1900.43	1810.00	1855.21
		CDC Teal	934.46	868.66	901.56
S1A-06-3	3	AC Cora	1337.73	1260.66	1299.20
		93FHB37	1818.96	1615.66	1717.31
		CDC Teal	964.96	687.66	826.31
S3BS-06-1	3	AC Cora	1390.36	1117.66	1254.01
		93FHB37	1731.90	1900.33	1816.11
		CDC Teal	1017.46	964.66	991.06
S8A-06-1	3	AC Cora	1238.33	1141.66	1190.00
		93FHB37	1873.93	1702.00	1787.96
		CDC Teal	886.83	1191.33	1039.08
M2-06-1	3	AC Cora	1197.76	1486.00	1341.88
		93FHB37	1626.23	1828.66	1727.45
		CDC Teal	900.96	895.00	897.98
M8-06-5	3	AC Cora	1161.86	1138.00	1149.93
		93FHB37	1702.16	1876.33	1789.25
		CDC Teal	824.06	842.33	833.20
Q-06-11	3	AC Cora	1102.23	1247.00	1174.61
		93FHB37	1694.86	1810.00	1752.43
		CDC Teal	852.10	834.00	843.05
Q-06-23	3	AC Cora	1272.63	1284.33	1278.48
		93FHB37	2126.50	1920.33	2023.41
		CDC Teal	1051.50	830.00	940.75
NB-06-17	3	AC Cora	1350.06	1229.66	1289.86
		93FHB37	1899.73	1617.00	1786.64
		CDC Teal	795.73	728.33	762.03
NS-06-2	3	AC Cora	1419.13	1203.33	1311.23
		93FHB37	1800.33	1794.33	1797.33
		CDC Teal	907.73	876.00	891.86
MIN-1-1	3	AC Cora	1115.76	1475.66	125.71
		93FHB37	1878.83	1947.00	1912.91
		CDC Teal	814.53	840.66	827.60
CONTROL	n/a	AC Cora	1969.53	2387.66	2178.60
		93FHB37	2090.46	2433.66	2262.06
		CDC Teal	1944.60	2160.33	2052.46
LSD			**108.65**	**166.44**	**98.76**

Note: Sample sizes are equal to three with the exception of S1A-06-4 on AC Cora, S3AN-06-1 on CDC Teal in 2008 and S1A-06-4 and NB-06-17 on 93FHB37 in 2009 which had sample sizes equal to two.

There was a significant yield difference between chemotypes in 2008 and the combined analysis, but not in 2009. 3-ADON isolates resulted in lower yields on average than the 15-ADON isolates (Table 3.11). The difference in yield was sufficiently large to be significant in 2008 but not in 2009. There was no significant difference between years (Table 3.3), and isolate effects for yield were homogeneous (Table 3.2).

Table 3.11. Mean yield for year*chemotype combinations in 2008, 2009 and combined over both years.

Year	Chemotype	N	Mean
2008	3	117	1346
2008	15	106	1429
2009	3	116	1286
2009	15	107	1298
Combined	3	233	1316
Combined	15	213	1362
2008 LSD = 45.20; 2009 LSD = 53.76; Combined LSD = 35.02			

Isolate effects were partitioned out for the individual years and combined analyses (Table 3.10). There were no significant differences within 3-ADON isolates for any of the analyses indicating that inoculation by all of the 3-ADON isolates resulted in similar yields. Within the 15-ADON isolates, significant differences were detected in 2008, but not in 2009; however, in the combined analysis, there were significant differences within 15-ADON isolates.

The combined years' analysis for yield showed significant differences for all sources of variation tested except for year effects and within 3-ADON isolates (Table 3.5). A non-significant year effect means that the overall yields did not differ

significantly between years indicating that regardless of the differences in environmental conditions and the effects that this would have on pathogen development, yield was affected in the same way in both years. There was also no significant difference within the 3-ADON isolates which is similar to the results from the AUDPC analysis for incidence, severity and FHB index. The separate year's analysis showed no significant differences within the 3-ADON isolates either suggesting that regardless of external factors, 3-ADON isolates affected yield in a similar manner.

The results from this study indicate that *F. graminearum* isolates across Canada vary in how they affect yield of spring wheat. The combined analysis showed that there was a significant difference in the effect of the chemotypes on yield. The 3-ADON isolates on average resulted in lower yields than the 15-ADON isolates. Within the 3-ADON chemotype there were no significant differences in the effects on yield, however there were for the 15-ADON chemotype. Previous studies have investigated the westward chemotype shift in Canada, from a resident 15-ADON to the 3-ADON chemotype (Guo et al., 2006, Guo et al., 2008). If this shift continues west of Manitoba, farmers will experience greater average yield losses from 3-ADON isolates than they had been from 15-ADON isolates. This significant difference in yield losses affects not only the producers, but everyone who processes the grain, has a part in the end-uses of the grain, and consumers. It is important to attain a more complete understanding of how the 3-ADON isolates are able to substantially reduce yields and how to develop a solution for these potentially devastating results.

3.4.4 Fusarium damaged kernel evaluation

The combined years' analysis for FDK showed significant effects for year, block(year), isolate, between chemotypes, control versus inoculated plots, genotype and year*genotype effects (Table 3.5). The mean number of FDK was significantly higher in 2009 than 2008. The combined analysis showed that isolates significantly affected the number of recovered FDK. Isolate effects were significant in 2009 but not in 2008 (Tables. 3.3 and 3.4). This discrepancy could likely be due to the different environmental conditions in each year. The cooler conditions in 2009 provided on average a longer infection period before spike senescence which may have influenced the level of kernel damage. The combined analysis (Table 3.5) showed significant differences between the chemotypes while chemotype differences were detected in 2008 but not 2009 (Tables 3.3 and 3.4). A means comparison for chemotypes for each year as well as for the combined years showed there were no significant differences detected among isolates in 2008 or between chemotypes in 2009; however, in the combined analysis, there were significant differences between chemotypes (Table 3.12).

Table 3.12. Mean fusarium damaged kernels in 3-ADON and 15-ADON chemotypes in 2008, 2009 and combined over both years.

Level of year	Level of chemotype	N	Mean
2008	3	117	40.00
2008	15	108	34.33
2009	3	116	76.90
2009	15	106	75.67
Combined	3	233	58.52
Combined	15	214	54.81
2008 LSD = n/a; 2009 LSD = 3.06; Combined LSD = 2.69			

Similar to the other variables presented thus far, there was a significant difference among genotypes which was to be expected for all parameters due to the difference in levels of resistance of the genotypes tested (Table 3.13). As with the other variables presented, and again for FDK, the genotypes maintained a consistent relative ranking of the lowest numbers of FDK recovered on 93FHB37, the highest number recovered on CDC Teal and an intermediate number recovered on AC Cora.

Table 3.13. Average fusarium damaged kernels for year-genotype combinations in 2008 and 2009.

Level of year	Genotype	N	Mean
2008	AC Cora	77	38.1
2008	93FHB37	78	17.5
2008	CDC Teal	78	54.28
2009	AC Cora	77	82.29
2009	93FHB37	76	50.97
2009	CDC Teal	78	88.62
Combined	AC Cora	154	60.2
Combined	93FHB37	154	34.01
Combined	CDC Teal	156	71.45
2008 LSD = 5.38; 2009 LSD = 3.96; Combined LSD = 3.33			

In contrast to other variables discussed, there were no significant differences in isolate*genotype interactions for either 2008 or 2009, nor for the combined analysis. The relative ranking and magnitude of differences among genotypes was relatively constant with the different isolates used in each year indicating no significant isolate*genotype interactions (Table3.14). There was no significant year*isolate*genotype interaction in the combined analysis.

Table 3.14. Means of Fusarium damaged kernel (%)for isolate*genotype combinations for 2008, 2009, and combined over both years.

Isolate QC code	Chemotype	Genotype	2008 Mean	2009 Mean	Combined mean
A1-06-1	15	AC Cora	38.33	85.33	61.83
		93FHB37	18.66	59.66	39.16
		CDC Teal	46.00	93.66	69.83
S1A-06-4	15	AC Cora	32.00	81.66	56.83
		93FHB37	16.00	61.33	38.66
		CDC Teal	61.33	90.66	76.00
S3AN-06-1	15	AC Cora	39.66	88.66	64.16
		93FHB37	17.66	50.00	33.83
		CDC Teal	43.00	91.66	67.33
S8A-06-5	15	AC Cora	45.00	86.66	65.83
		93FHB37	14.00	38.00	26.00
		CDC Teal	35.00	94.00	64.50
M2-06-2	15	AC Cora	34.33	85.33	59.8
		93FHB37	17.00	55.66	36.33
		CDC Teal	48.00	94.33	71.16
M8-06-2	15	AC Cora	27.00	90.00	58.50
		93FHB37	13.00	39.50	23.60
		CDC Teal	43.66	92.00	67.83
ON-06-17	15	AC Cora	29.66	74.00	51.83
		93FHB37	28.33	52.00	40.16
		CDC Teal	45.00	95.00	70.00
Q-06-10	15	AC Cora	40.00	83.66	61.83
		93FHB37	17.33	46.00	31.66
		CDC Teal	55.66	90.66	73.16
Q-06-22	15	AC Cora	48.33	73.66	61.00
		93FHB37	11.67	52.66	32.16
		CDC Teal	61.00	78.66	69.83
Q-06-34	15	AC Cora	35.00	84.66	59.83
		93FHB37	23.33	45.33	34.33
		CDC Teal	53.33	84.33	68.83
NB-06-18	15	AC Cora	25.00	93.00	52.20
		93FHB37	8.00	55.00	31.50
		CDC Teal	57.33	92.66	75.00
NS-06-3	15	AC Cora	31.33	91.00	61.16
		93FHB37	13.33	54.66	34.00
		CDC Teal	62.66	93.00	77.83
A2-06-1	3	AC Cora	38.00	87.00	62.50
		93FHB37	25.33	57.00	41.16
		CDC Teal	55.00	95.33	75.16

Isolate QC code	Chemotype	Genotype	2008 Mean	2009 Mean	Combined Mean
A4-05-5	3	AC Cora	35.33	92.33	63.83
		93FHB37	28.00	65.66	46.83
		CDC Teal	49.00	96.00	72.50
A6-06-1	3	AC Cora	50.33	74.00	62.16
		93FHB37	21.00	53.00	37.00
		CDC Teal	51.00	93.66	72.33
S1A-06-3	3	AC Cora	40.33	92.30	66.33
		93FHB37	15.66	59.66	37.66
		CDC Teal	55.33	91.33	73.33
S3BS-06-1	3	AC Cora	24.33	74.00	49.16
		93FHB37	23.33	49.66	36.50
		CDC Teal	42.33	86.33	64.33
S8A-06-1	3	AC Cora	48.00	95.33	71.66
		93FHB37	8.00	60.33	34.16
		CDC Teal	68.66	72.33	70.50
M2-06-1	3	AC Cora	46.33	90.66	68.50
		93FHB37	10.66	54.00	32.33
		CDC Teal	50.33	93.33	71.83
M8-06-5	3	AC Cora	49.33	80.66	65.00
		93FHB37	16.33	49.66	33.00
		CDC Teal	50.66	88.00	69.33
Q-06-11	3	AC Cora	36.33	93.33	64.83
		93FHB37	22.33	45.66	34.00
		CDC Teal	68.00	92.33	80.16
Q-06-23	3	AC Cora	44.00	76.33	60.16
		93FHB37	18.66	57.66	38.16
		CDC Teal	49.00	90.66	69.83
NB-06-17	3	AC Cora	37.66	74.66	56.16
		93FHB37	21.66	38.50	28.40
		CDC Teal	80.33	87.33	83.83
NS-06-2	3	AC Cora	36.33	92.66	64.50
		93FHB37	12.66	53.00	32.83
		CDC Teal	67.00	94.66	80.83
MIN-1-1	3	AC Cora	64.00	89.33	76.66
		93FHB37	28.33	53.66	41.00
		CDC Teal	83.00	95.00	89.00
CONTROL	n/a	AC Cora	3.00	13.00	9.00
		93FHB37	4.66	10.00	7.33
		CDC Teal	29.66	37.33	33.50
LSD			**15.85**	**11.69**	**9.81**

3.4.5 Deoxynivalenol evaluation

Significant differences for DON were detected between and within both chemotypes in 2008 but not in 2009 (Tables 3.3 and 3.4). Table 3.15 shows that 3-ADON isolates produced on average more DON than 15-ADON isolates in both years and combined over years although the difference was not statistically significant in 2009.

Table 3.15. Means of deoxynivalenol accumulation by chemotypes for 2008 and 2009.

Year	Chemotype	N	Mean DON (ppm)
2008	3	117	6.61
2008	15	108	5.89
2009	3	116	15.21
2009	15	106	13.80
Combined	3	233	10.89
Combined	15	214	9.81
2008 LSD = 0.79; 2009 LSD = n/a; Combined LSD = 0.73			

This agrees with *in vitro* studies done by Ward et al. (2008) which showed that 3-ADON populations accumulated significantly more trichothecenes than isolates from 15-ADON populations. Year*isolate interactions were significant indicating that the isolates performed and ranked differently in each year. The isolate*genotype interaction was significant in the combined analysis as well as in 2008 (P=0.0227), but not in 2009 (P=0.2329) (Table 3.16). The 2009 DON results show that there is not always a consistent ranking of genotypes within each isolate. A possible reason for this could be that the high disease levels and high FDK levels in 2009 resulted in loss of some of the sample during harvest.

Table 3.16. Deoxynivalenol means for all isolate-genotype combinations for 2008, 2009 and combined over both years.

Isolate QC code	Chemotype	Genotype	2008 Mean	2009 Mean	Combined Mean
A1-06-1	15	AC Cora	6.40	11.69	9.05
		93FHB37	1.96	11.45	6.71
		CDC Teal	10.66	19.70	15.19
S1A-06-4	15	AC Cora	5.53	11.03	8.28
		93FHB37	2.10	11.70	6.90
		CDC Teal	10.20	21.18	15.69
S3AN-06-1	15	AC Cora	2.56	11.36	6.96
		93FHB37	3.03	12.10	7.56
		CDC Teal	5.90	17.14	11.52
S8A-06-5	15	AC Cora	3.83	14.01	8.92
		93FHB37	3.80	12.07	7.93
		CDC Teal	12.56	18.25	15.40
M2-06-2	15	AC Cora	5.23	12.62	8.92
		93FHB37	3.56	16.89	10.23
		CDC Teal	15.36	18.74	17.05
M8-06-2	15	AC Cora	1.26	9.78	5.52
		93FHB37	1.20	8.66	4.18
		CDC Teal	5.43	17.97	11.70
ON-06-17	15	AC Cora	3.70	10.06	6.88
		93FHB37	3.03	9.46	6.25
		CDC Teal	9.80	16.08	12.95
Q-06-10	15	AC Cora	6.56	11.09	8.83
		93FHB37	3.60	9.95	6.77
		CDC Teal	10.03	15.35	12.69
Q-06-22	15	AC Cora	6.83	8.73	7.78
		93FHB37	2.29	10.21	6.25
		CDC Teal	13.43	18.42	15.92
Q-06-34	15	AC Cora	4.13	11.58	7.85
		93FHB37	3.53	7.99	5.76
		CDC Teal	9.93	14.04	11.98
NB-06-18	15	AC Cora	5.40	14.78	9.15
		93FHB37	1.30	14.83	8.06
		CDC Teal	11.70	27.94	19.82
NS-06-3	15	AC Cora	5.36	12.27	8.81
		93FHB37	2.16	11.02	6.59
		CDC Teal	8.86	15.27	12.06

Isolate QC code	Chemotype	Genotype	2008 Mean	2009 Mean	Combined Mean
A2-06-1	3	AC Cora	3.00	18.53	10.77
		93FHB37	1.93	14.31	8.12
		CDC Teal	8.66	22.65	15.66
A4-06-5	3	AC Cora	5.70	14.27	9.99
		93FHB37	3.90	13.86	8.88
		CDC Teal	11.53	13.08	12.30
A6-06-1	3	AC Cora	2.20	12.65	7.43
		93FHB37	1.86	13.09	7.47
		CDC Teal	6.03	19.41	12.72
S1A-06-3	3	AC Cora	4.06	12.23	8.15
		93FHB37	3.10	7.26	5.18
		CDC Teal	11.90	20.63	16.26
S3BS-06-1	3	AC Cora	4.90	19.19	12.04
		93FHB37	3.20	14.12	8.66
		CDC Teal	8.96	25.67	17.32
S8A-06-1	3	AC Cora	9.26	13.70	11.48
		93FHB37	4.10	10.30	7.20
		CDC Teal	19.20	17.49	18.34
M2-06-1	3	AC Cora	7.33	10.83	9.08
		93FHB37	2.53	9.54	6.03
		CDC Teal	16.67	19.04	17.85
M8-06-5	3	AC Cora	6.43	11.73	9.08
		93FHB37	2.60	9.89	6.24
		CDC Teal	11.46	20.26	15.86
Q-06-11	3	AC Cora	6.06	15.80	10.93
		93FHB37	2.46	13.14	7.80
		CDC Teal	12.30	25.00	18.95
Q-06-23	3	AC Cora	4.20	11.26	7.73
		93FHB37	0.90	9.73	5.31
		CDC Teal	11.83	14.72	13.27
NB-06-17	3	AC Cora	5.76	14.00	9.88
		93FHB37	2.66	12.90	6.76
		CDC Teal	11.36	20.30	15.83
NS-06-2	3	AC Cora	4.23	12.83	8.53
		93FHB37	2.53	17.56	10.05
		CDC Teal	10.96	24.36	17.66
MIN-1-1	3	AC Cora	6.56	10.77	8.66
		93FHB37	3.13	7.37	5.25
		CDC Teal	12.50	18.43	15.46

Isolate QC code	Chemotype	Genotype	2008 Mean	2009 Mean	Combined Mean
CONTROL	0	AC Cora	0.63	2.75	1.69
		93FHB37	0.26	2.39	1.32
		CDC Teal	1.46	5.04	3.25
LSD			**1.98**	**3.28**	**2.00**

Note: Sample sizes were equal to three for individual years with the exception of M8-06-2, NB-06-17 and NB-06-18 in 2009 which were equal to two. In the combined analysis, sample sizes were equal to six.

DON measurements were done in ppm.

The year*genotype interaction was not significant in the DON analysis indicating that the genotypes responded to infection and accumulated DON in a similar manner (Table 3.17).

Table 3.17. Deoxynivalenol (DON) means for AC Cora, 93FHB37, and CDC Teal in 2008, 2009 and combined over both years.

Year	Genotype	N	Mean DON (ppm)
2008	AC Cora	78	4.89
2008	93FHB37	78	2.56
2008	CDC Teal	78	10.72
2009	AC Cora	77	12.26
2009	93FHB37	76	11.23
2009	CDC Teal	78	18.72
Combined	AC Cora	155	8.55
Combined	93FHB37	154	6.84
Combined	CDC Teal	156	14.72
2008 LSD = 1.98; 2009 LSD = 1.15; Combined LSD = 0.68			

The genotypes ranked consistently over years, as with all of the parameters tested. In this case, the lowest DON levels were seen in 93FHB37, the highest in CDC Teal, and intermediate levels in AC Cora.

The combined years' analysis for DON showed significant effects for year, block(year), isolate, within 15-ADON isolates, between chemotypes, control versus inoculated plots, year*isolate, year*block*isolate, genotype, and isolate*genotype (Table 3.5). As for FDK, DON levels were higher in 2009 than in 2008, suggesting that the cooler conditions in 2009 may have contributed to higher levels of DON accumulation although AUDPC values and Figures 3.3(a-c) show similar values between years. The isolates tested produced significantly different amounts of DON (Table 3.18) as seen for all parameters tested, indicating that the isolates varied in levels of disease causing ability, in this case, ability to produce DON.

Table 3.18. Deoxynivalenol means combined over 2008 and 2009.

MP QC code	**Chemotype**	**Mean DON (ppm)**
A1-06-1	15	10.31
S1A-06-4	15	8.68
S3AN-06-1	15	8.68
S8A-06-5	15	10.76
M2-06-2	15	12.07
M8-06-2	15	7.31
ON-06-17	15	8.69
Q-06-10	15	9.43
Q-06-22	15	9.98
Q-06-34	15	8.53
NB-06-18	15	12.53
NS-06-3	15	9.16
A2-06-1	3	11.52
A4-06-5	3	10.39
A6-06-1	3	9.21
S1A-06-3	3	10.29
S3BS-06-1	3	12.68
S8A-06-1	3	12.34
M2-06-1	3	10.99
M8-06-5	3	10.39
Q-06-11	3	12.56
Q-06-23	3	8.77
NB-06-17	3	11.06
NS-06-2	3	12.08
MIN-1-1	3	9.79
CONTROL	n/a	2.09
LSD		**1.99**

The combined years' DON analysis showed that there were significant differences between chemotypes and within 15-ADON isolates, however no significant differences were detected within 3-ADON isolates (Table 3.5).

3.4.6 Correlations among variables

Correlations were done for each year individually as well as the years combined on all of the variables measured. Each correlation was made with the treatment means (i.e., isolate*genotype) across replicates in order to give a better estimate of the strength of the correlation across genotypes.

Correlations separated based on chemotypes in 2008 showed similar correlation coefficients for each chemotype and were combined for both of the chemotypes. All correlation coefficients were quite strong (i.e. greater than 0.48). Correlations involving yield were all negative and significant indicating that yield was negatively affected by FDK, DON, and all AUDPC variables (Table 3.19).

Table 3.19. 2008 Correlation coefficients and significance for fusarium-damaged kernels, deoxynivalenol, area under the disease progress curve (disease incidence, severity and fusarium head blight index) for isolates on CDC Teal, AC Cora and 93FHB37.

	FDK[1]	DON[2]	AUDPCInc[3]	AUDPCSev[4]	AUDPCIdx[5]
DON	0.78[6] 0.79[7] 0.78[8]				
AUDPCInc	0.86 0.89 0.87	0.80 0.86 0.82			
AUDPCSev	0.80 0.84 0.80	0.83 0.89 0.85	0.91 0.94 0.93		
AUDPCIdx	0.84 0.86 0.84	0.85 0.89 0.87	0.96 0.97 0.96	0.99 0.99 0.99	
Yield	-0.88 -0.90 -0.89	-0.83 -0.86 -0.84	-0.97 -0.96 -0.96	-0.91 -0.89 -0.90	-0.94 -0.93 -0.94

[1] Fusarium damaged kernels
[2] Deoxynivalenol
[3]Area under the disease progress curve (disease incidence)
[4]Area under the disease progress curve (disease severity)
[5]Area under the disease progress curve (Fusarium head blight index)
[6]Top values are always for 3-ADON isolates
[7]Middle values are always for 15-ADON isolates
[8]Bottom values are always for all isolates

Note: All correlations are significant at the 0.01 level.

The 2009 correlations consistently showed that the combined chemotypes revealed higher correlation coefficients than either chemotype individually (Table 3.20).

Table 3.20. 2009 Correlation coefficients and significance for fusarium-damaged kernels, deoxynivalenol, area under the disease progress curve (disease incidence, severity and fusarium head blight index) and yield for isolates on CDC Teal, AC Cora and 93FHB37.

	FDK[1]	DON[2]	AUDPCInc[3]	AUDPCSev[4]	AUDPCIdx[5]
DON	0.51				
	0.55				
	0.78				
AUDPCInc	0.77	0.65			
	0.79	0.71			
	0.87	0.82			
AUDPCSev	0.78	0.70	0.97		
	0.79	0.70	0.96		
	0.80	0.85	0.93		
AUDPCIdx	0.72	0.69	0.97	0.99	
	0.74	0.70	0.97	0.99	
	0.84	0.87	0.96	0.99	
Yield	-0.86	-0.71	-0.93	-0.93	-0.90
	-0.90	-0.66	-0.94	-0.93	-0.91
	-0.88	-0.84	-0.96	-0.90	-0.94

[1] Fusarium damaged kernels
[2] Deoxynivalenol
[3]Area under the disease progress curve (disease incidence)
[4]Area under the disease progress curve (disease severity)
[5]Area under the disease progress curve (Fusarium head blight index)
[6]Top values are always for 3-ADON isolates
[7]Middle values are always for 15-ADON isolates
[8]Bottom values are always for all isolates

Notes: All correlations are significant at the 0.01 level.

Correlation coefficients for FDK-DON were all relatively high with the minimum correlation coefficient of 0.51 for 3-ADON isolates in 2009. It was evident that there was less variability in the relationship between FDK and DON at low FDK or DON levels, however, once FDK were greater than 40%, the strength of the relationship between FDK and DON began to deteriorate (Figure 3.4). The correlation coefficient for the 3-ADON producers is 0.74 and for the 15-ADON producers 0.72. The difference in

terms of slopes of the lines is 0.18 for 3-ADON producers and 0.17 for 15-ADON producers. These slope values indicate that that is the value of DON related to 1% FDK. The results from this correlation indicate that the chemotype shift has caused a substantial change in the prediction ratios currently used by the Canadian Grain Commission and that these ratios should be adjusted to 6:1 (FDK:DON) when FDK are measured on a kernel basis as opposed to weight.

Deoxynivalenol and FDK levels for 93FHB37 were relatively high considering that this genotype is resistant indicating that DON levels in particular, are much less predictable for this genotype than for AC Cora or CDC Teal (Table 3.13, 3.18). These relatively high FDK-DON correlation results are consistent with the findings of Mesterhazy et al. (2005) and Zhang et al. (2008). Within each chemotype, the correlation coefficients for 2008 (3-ADON = 0.78, 15-ADON = 0.79) were stronger than in 2009 (3-ADON = 0.51, 15-ADON = 0.55). One potential explanation for this discrepancy could be that 2008 showed significant differences both between and within chemotypes, however, in 2009, there were no significant differences between or within chemotypes. Considering this, a dataset which shows significant differences would likely have a stronger correlation than a dataset which was not significantly different. The 2009 experiment also showed higher FDK and DON levels than 2008, on average even though it was not statistically significant. Another explanation for this discrepancy could be that cooler temperatures result in poorer correlations between these two variables. The 2009 results showed much higher DON levels than 2008 and although FDK values were still higher in 2009, the difference was not as great as that between the corresponding DON levels. This discrepancy between correlations between years could be a concern for how

grain is graded. Currently, FDK are used to forecast the amount of DON in a sample. The results from this study indicate that differences in temperatures between years can seriously affect this correlation and thus, the forecasting system for DON might need to be re-evaluated in severe epidemic conditions such as those which were created in these experiments.

There were significant positive correlations between AUDPCinc—AUDPCSev (minimum correlation coefficient=0.91), AUDPCInc-AUDPCIdx (minimum correlation coefficient=0.95) and AUDPCSev-AUDPCIdx (minimum correlation coefficient=0.98) which was to be expected as the FHB index calculation includes incidence and severity ratings (Tables 3.19, 3.20, 3.21). The AUDPCInc-AUDPCSev correlation was significant and positive. AUDPCInc-yield correlation was negative and had a highly significant. It is intuitive that as disease incidence increases, yield should decrease due to the high prevalence of shrunken and light weight kernels. This result is in accordance with Ludewig et al. (2005) who found that yield was a reliable indicator of disease incidence.

Table 3.21. Combined 2008 and 2009 correlation coefficients and significance for Fusarium damaged kernels, deoxynivalenol, area under the disease progress curve (disease incidence, severity and Fusarium head blight index) and yield for isolates on CDC Teal, AC Cora and 93FHB37.

	FDK[1]	DON[2]	AUDPCInc[3]	AUDPCSev[4]	AUDPCIdx[5]
DON	0.81 0.84 0.82				
AUDPCInc	0.60 0.59 0.59	0.54 0.61 0.57			
AUDPCSev	0.50 0.49 0.50	0.50 0.54 0.52	0.93 0.94 0.93		
AUDPCIdx	0.45 0.43 0.44	0.46 0.51 0.48	0.95 0.96 0.95	0.98 0.98 0.98	
Yield	-0.66 -0.68 -0.67	-0.61 -0.64 -0.62	-0.95 -0.94 -0.94	-0.91 -0.90 -0.90	-0.90 -0.88 -0.89

[1] Fusarium damaged kernels
[2] Deoxynivalenol
[3]Area under the disease progress curve (disease incidence)
[4]Area under the disease progress curve (disease severity)
[5]Area under the disease progress curve (Fusarium head blight index)
[6]Top values are always for 3-ADON isolates
[7]Middle values are always for 15-ADON isolates
[8]Bottom values are always for all isolates

Notes: All correlations are significant at the 0.01 level.

For the 2008 and 2009 AUDPCSev-DON correlations, the correlations were also strong (minimum correlation coefficient = 0.70). When the two years were combined, the correlations were 0.52. At this level, one can not be completely confident that the differences are statistically or biologically significant. Studies done by Bai et al. (2001a) and Ludewig et al. (2005) found a strong correlation between disease incidence and FDK,

which is in accordance with the results of the present study which found that there were strong, highly significant correlations between these parameters, ranging from 0.59-0.89.

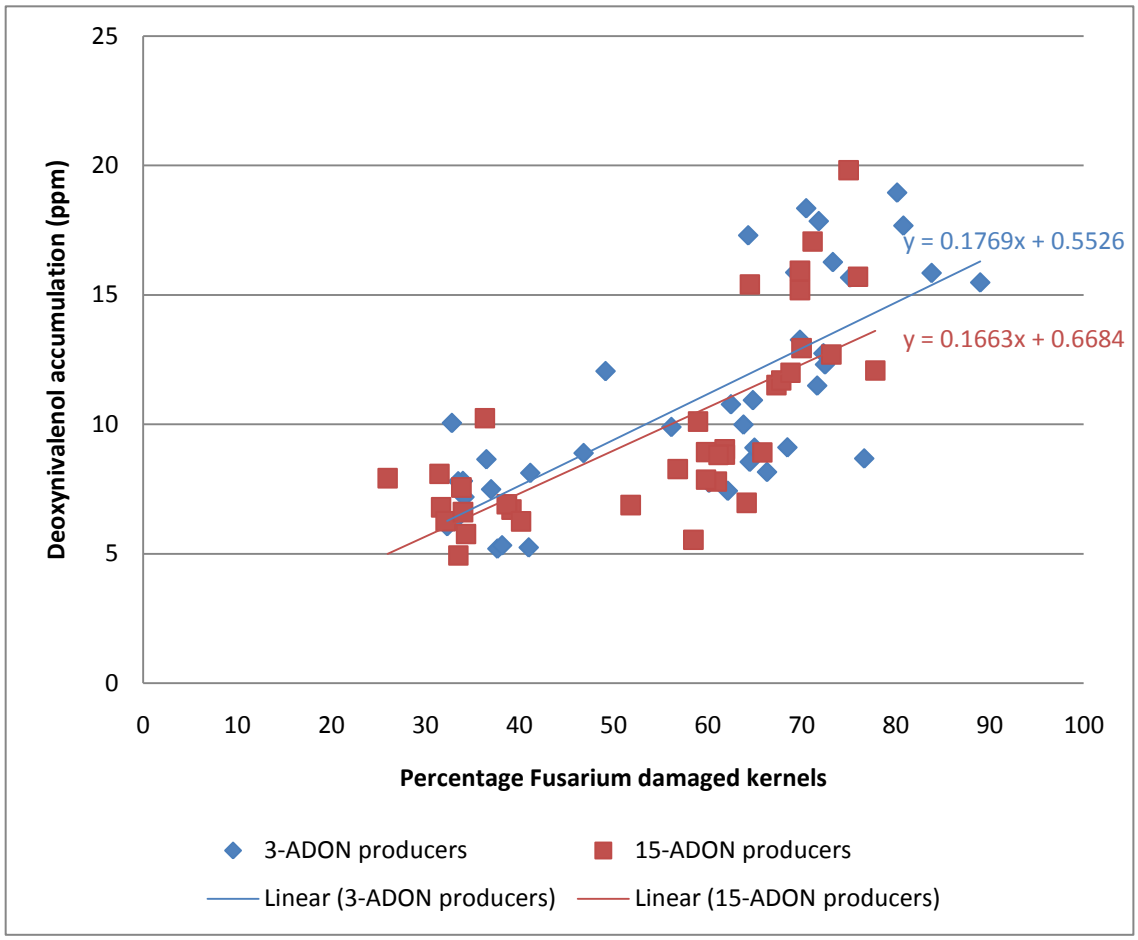

Figure 3.6 Relationship between fusarium-damaged kernels and deoxynivalenol based on combined years' means for isolate*genotype.

3.5 Conclusions

Results from this study provide convincing evidence that the recent introduction of the 3-ADON chemotype population into Manitoba has, and will cause, significant damage to wheat crops in epidemic years. This study confirms the results of previous studies that 3-ADON isolates produce more DON toxin on average than 15-ADON

isolates and provides some of the first evidence of 3-ADON isolates having a significant effect on disease progression, yield and proportions of FDK. 3-ADON isolates were shown to increase FDK and DON concentration and decrease yield.

Future work investigating chemotype differences of *F. graminearum* on agronomic and end use parameters should test the effects of more isolates of each chemotype in field experimentation to increase the robustness of the findings of the present study. It would also be interesting to include a study on the differences of colonization patterns of the chemotypes in order to determine if there are differences in the way the chemotypes infect susceptible hosts.

4.0 COMPARISON OF THE EFFECT OF 3-ADON AND 15-ADON CHEMOTYPES OF *F. GRAMINEARUM* ON 3BS AND 4B QTL LINES

4.1 Abstract

Fusarium head blight (FHB) of wheat is caused primarily by *Fusarium graminearum*. There have been very few sources resistance sources identified, but resistant cultivars are one of the key factors for controlling FHB epidemics. A chemotype shift has been detected in eastern Canada. Historically, the 15-acetyl deoxynivalenol (15-ADON) chemotype was the principle chemotype in Manitoba. However a 3-acetyl deoxynivalenol (3-ADON) chemotype, prevalent in Europe and Asia, has started to increase in frequency in eastern Canada. The 3-ADON chemotypes are considered to be more toxigenic than the 15-ADON chemotypes which could increase the risk of FHB to the Canadian wheat industry. The present study investigated the reaction to two *F. graminearum* isolates of seven lines selected for specific FHB quantitative trait loci (QTL), three with the 3BS QTL and four with the 4B QTL. Each QTL line was screened for reaction to a 3-ADON isolate and a 15-ADON isolate. Only one 3BS QTL line expressed resistance to fungal spread within the wheat spike. No differences in disease severity were detected between chemotypes tested in this study. This suggests that resistance genes respond similarly to the two chemotypes tested. Further work is required to confirm these results.

4.2 Introduction

Fusarium head blight (FHB) is devastating disease of wheat world-wide. The principal causal agent of FHB is *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* [Schwein.] Petch). Fusarium head blight affects all levels of the grain

industry from production to end-use. The pathogen is able to produce trichothecene toxins, specifically deoxynivalenol (DON) and acetylated derivatives 3-acetyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON). Until recently, only the 15-ADON chemotype was present in North America, however a recent study by Ward et al. (2008) demonstrated there has been a chemotype shift in eastern Canada from the 15-ADON chemotype to the 3-ADON chemotype.

Schroeder et al. (1963) described two types of resistance: resistance to initial infection (Type I resistance) and resistance to spread of infection within the spike (Type II resistance). They developed screening techniques to differentiate between the two types of resistance: spray inoculation to demonstrate Type I resistance, or lack thereof, and point inoculation to demonstrate Type II resistance, or lack thereof. Point inoculation has been widely used to identify Type II resistance (Bai et al., 1994; Gilbert et al., 2000) and is done by placing a droplet of macroconidial suspension on top of the stigma or injecting inoculum onto the floret via a hypodermic syringe or micropipette. Type II resistance is governed by active mechanisms which restrict pathogen growth from the point of inoculation through the spike tissues (Ribichich et al., 2001). Engle et al. (2003) determined that although point inoculation is good for detecting high levels of Type II resistance, it may be difficult to quantify Type II resistance due to variation in environmental factors. It is well known that resistance to FHB is a quantitative trait. Buerstmayr et al. (2009) postulated that more than 100 quantitative trait loci (QTL) for FHB resistance in wheat have been published. Bai et al. (1994) demonstrated that FHB resistance genes are located throughout the genome and are cultivar dependent. It is also well known that chromosome 3BS carries a gene with a major effect for FHB resistance

(Bai et al. 1999; Waldron et al. 1999; Ban et al. 2000; Anderson et al. 2001; Buerstmayr et al. 2002; Zhou et al. 2002; Bourdoncle et al. 2003; Buerstmayr et al. 2003; Guo et al. 2003; Liu and Anderson, 2003; Shen et al. 2003; Somers et al. 2003; Yang et al. 2003; Zhou et al. 2003; Liu et al. 2004; Paillard et al. 2004; Zhang et al. 2004; Zhou et al. 2004; Jia et al. 2005; Lemmens et al. 2005; Mardi et al. 2005; Yang et al. 2005; Chen et al. 2006; Cuthbert et al. 2006; Liu et al. 2006; Ma et al. 2006; Miedaner et al. 2006; Jiang et al. 2007; Klahr et al. 2007; Liu et al. 2007; McCartney et al. 2007; Abate et al. 2008; Yu et al. 2008; Zhang et al. 2010; Zhou et al. 2010). Several studies have shown that the 3BS QTL makes a significant contribution to both Types I and II resistance. A minor QTL is located on chromosome 4B and has been shown to lower disease severity (Buerstmayr et al. 1999; Waldron et al. 1999; Anderson et al. 2001; Somers et al. 2003; Steiner et al. 2004; Jia et al. 2005; Yang et al. 2005; Lin et al. 2006; Liu et al. 2007; McCartney et al. 2007; Abate et al. 2008). The objective of this study was to explore the potential differences between chemotype effects on disease severity and fusarium-damaged kernels (FDK) for different QTL lines.

4.3 Materials and Methods

4.3.1 Isolate selection

Isolates were selected based on 2008 and 2009 area under the disease progress curve (AUDPC) FHB index, 2008 FDK and 2008 DON levels from experiments in Chapter 3. One 3-ADON isolate (S8A-06-1) and one 15-ADON isolate (M2-06-2) were chosen (refer to table 3.1 for more information about these isolates). These isolates were

both highly aggressive in terms of disease progression and were relatively consistent between years.

4.3.2 Line selection

The QTL lines used in this experiment were developed at the Eastern Cereal and Oilseeds Research Centre in Ottawa, Ontario. The donor parent, HC374, was developed from the cross: Wuhan/Nyubai, and the elite parent, 98B69*L47, was developed from the cross: Augusta/HW Alpha//3*BW252) (Somers et al., 2005). The donor parent was the female in the cross and developed from doubled haploid lines carrying specific FHB resistance QTL (Somers et al., 2003; Yang et al., 2003). The crosses made to develop the QTL lines were HC374*3/98B69*L47 followed by two backcrosses to the elite parent and one selfing generation to produce a BC_2F_2 generation (Somers et al., 2005).

Leaf tissue samples were taken from 20 QTL lines and screened with a series of markers that span the two QTL regions to confirm the QTL in each line. The haplotypes of the QTL lines were examined and lines that tested positive for only one FHB QTL were selected. From these results, four 4B and three 3BS QTL lines were selected for evaluation (Table 4.1). Information regarding the forward and reverse primers of these markers can be found in Appendix 7.4. Following analysis of the results from the experiment, additional markers were run on the 3BS lines to determine if there were differences in presence or absence of markers. These additional markers included barc133, sts163, sts138 and sts142 as per Cuthbert et al. (2006).

Table 4.1. Results from marker tests for each QTL from leaf tissue samples.

		point 2D	flanking 2D	flanking 2D	point 2D	point 2D	point 3BS	point 3BS	point 3BS	flanking 3BS	point 3BS	flanking 4B	point 4B	flanking 4B	flanking 5A	point 5A	flanking 5A
ID	**QTL**	**wmc 245**	**gwm 608**	**wmc18**	**cfd 73**	**cfd 233**	**UMN 10**	**sts66**	**sts80**	**gwm 533**	**gwm 493**	**wmc 710**	**wmc238**	**gwm 149**	**gwm 293**	**wmc705**	**gwm 304**
1-14	3BS	a	a	a	a	a	b	b	b	b	b	a	0	a	a	a	a
1-41	3BS	a	a	a	a	a	b	b	b	b	b	a	a	a	a	a	a
1-44	3BS	a	a	a	a	a	b	b	b	b	b	a	a	a	a	a	a
1-1	4B	a	a	a	a	a	a	a	a	a	a	b	b	b	a	a	a
1-12	4B	a	a	a	a	a	a	a	a	a	a	b	b	b	a	a	a
1-21	4B	a	a	a	a	a	a	a	a	a	a	b	b	b	a	a	a
1-87	4B	a	a	a	a	a	a	a	a	a	a	b	b	b	a	a	a
1-9	non 4B,3BS QTL	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
1-10	non 4B,3BS QTL	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a

Note: ‘a’ indicates the absence of the marker in question; ‘b’ indicates the presence of the marker in question.

4.3.3 Experimental design

The plants were arranged in a completely random design. In total, there were 9 experiments. Experiments 1-7 contained only the lines containing single QTLs for 3BS (1-14, 1-41, 1-44); for 4B (1-1. 1-12. 1-21, 1-87). Three to five spikes per plant were inoculated with one isolate of *F. graminearum* differing in chemotype, thus giving a total of 4-5 plants (each with three to five spikes) inoculated per isolate (Table 4.1). Experiment 8 included each of the QTL lines as well as 2 QTL null-lines (1-9 and 1-10). Null lines were excluded from experiments 1-7 due to unavailability of seed at that time. Similar to experiments 1-7, three to five spikes were inoculated per plant in experiment 8. Experiment 9 included only CDC Teal plants in order to provide a completely susceptible comparison by which to compare the QTL and null lines. Again with experiment 9, inoculations were performed on three to five spikes per plant. In all experiments, four plants per QTL line were inoculated with each isolate and one plant per QTL line was mock inoculated with distilled water. In total, experiment 9 had 16 plants inoculated with the 3-ADON isolate, 16 plants inoculated with the 15-ADON isolate and 8 plants mock-inoculated with distilled water (Table 4.2).

Table 4.2: Summary of lines tested within each experiment.

	QTL lines							QTL null lines		
Experiment	**1-1**	**1-12**	**1-14**	**1-21**	**1-41**	**1-44**	**1-87**	**1-9**	**1-10**	**CDC Teal**
1	x	x	x	x	x	x	x	-	-	-
2	x	x	x	x	x	x	x	-	-	-
3	x	x	x	x	x	x	x	-	-	-
4	x	x	x	x	x	x	x	-	-	-
5	x	x	x	x	x	x	x	-	-	-
6	x	x	x	x	x	x	x	-	-	-
7	x	x	x	x	x	x	x	-	-	-
8	x	x	x	x	x	x	x	x	x	-
9	-	-	-	-	-	-	-	-	-	x

3BS lines: 1-14, 1-41 and 1-44; 4B lines: 1-1, 1-12, 1-21 and 1-87

4.3.4 Inoculum production

Inoculum was prepared as described in section 3.3.4 according to the 2009 preparation method.

4.3.5 Inoculation procedure

Inoculation was done by point inoculation according to (Cuthbert 2008). Three to five spikes per plant were inoculated once individual spikes were close to 50% anthesis. Inoculations were performed every other day. Before inoculation, awns were cut off to make inoculation easier and to ensure that the glassine bag would fit over the spikes. Inoculation points were one third down from the top of the spike. Two florets in a spikelet were inoculated by injecting 10µL of a macroconidial suspension adjusted to $5x10^4$ spores/mL between the lemma and palea of a floret. Following inoculation, a glassine bag was placed over the spike to increase humidity. The bags were left on the spikes for 48 hours and then removed.

4.3.6 Disease severity ratings

Severity ratings were done visually, as described in section 3.3.6. In brief, the ratings started at the onset of symptom development and continued until natural senescence.

4.3.7 Fusarium-damaged kernel evaluation

Fusarium-damaged kernel evaluation was done as described in section 3.3.8 although the proportion was based on the number of infected kernels per plant if this number was less than 100. Seeds from all inoculated spikes per plant were pooled together for FDK counts.

4.4 Results and Discussion

4.4.1 Disease Severity progression

Due to the variation between the disease severity ratings of some of the spikes within each plant, means of the terminal severity ratings (i.e., last rating before onset of natural senescence) were calculated using all inoculated spikes of each plant.

Levene's homogeneity tests for experiments 1-7 as shown in Table 4.3, indicate that line and line*chemotype variation are heterogeneous, although chemotype variation is homogenous.

Table 4.3: Levene's test for homogeneity of terminal severity and fusarium-damaged kernel variance of deviations from group means from experiments 1-7.

Source of variation	df	Terminal Severity			Fusarium damaged kernels		
		Mean Square	F-value	Pr>F	Mean Square	F-value	Pr>F
Line	6	662	5.07	<0.0001	1769	15.43	<0.0001
Chemotype	1	96	0.52	0.4707	1800	11.49	0.0008
Line*Chemotype	13	384	2.91	0.0005	860	7.94	<0.0001

The implications of combining data with heterogeneous or unequal variances are that either the samples are dependent on one another or, that the population is not normally distributed.

Individual ANOVAs are presented in Table 4.4 for experiments 1-7. The ANOVA for experiments 8 and 9 are presented in Table 4.5 and Table 4.6, respectively (recall experiment 9 only contains CDC Teal, so only chemotype differences were tested, since lines were identical). The significant differences among experiments may be attributed to the fact that there were temperature and humidity differences when each of the experiments was run, particularly when comparing the earlier experiments to the later ones. Although there were significant differences among experiments for terminal severity ratings, all experiments except for 4, 5, and 7 showed significant differences between QTL lines; all experiments except for 2 showed no significant differences between *F. graminearum* chemotypes; and all experiments except for 4 and 5 showed no significant differences for line*chemotype interactions. All experiments except for 3, 7 and 8 showed non-significant differences within 4B QTL lines, and all experiments except for 6 and 7 showed significant differences within 3BS QTL lines. Experiment 9 shows no significant difference between chemotypes.

Table 4.4. Analysis of variance for terminal disease severity ratings for individual experiments 1-7.

Source of variation	df	Experiment 1			Experiment 2			Experiment 3			Experiment 4			Experiment 5			Experiment 6			Experiment 7		
		MS[1]	F[2]	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F
Line	6	4472	16.8	<0.0001	1884	9.46	<0.0001	3346	15.18	<0.0001	291	0.64	0.6964	291	0.64	0.6964	1195	3.59	0.0058	549	2.33	0.05
Within 3BS	2	9980	32	<0.0001	4078	16	<0.0001	8220	62	<0.0001	-	-	-	-	-	-	550	1.23	0.3155	128	0.55	0.5859
Within 4B	3	744	3.29	0.0399	655	4.05	0.0165	465	1.62	0.2111	-	-	-	-	-	-	1367	5.52	0.005	986	4.14	0.0169
Chemotype	1	692	2.6	0.1126	1029	5.17	0.0276	29	0.13	0.7206	45	0.1	0.7556	45	0.1	0.7556	463	1.39	0.2451	186	0.79	0.3794
Line*Chemotype	6	191	0.72	0.636	210	1.06	0.4022	386	1.75	0.1323	1084	2.39	0.0444	1084	2.39	0.0444	298	0.89	0.5084	180	0.76	0.604
Error	54	266			199			220			454			454			333			235		
Total	67																					

[1]Mean Square
[2]F-value
Note: Experiment 2 error=47, total=60; Experiment 3 error = 42, total = 55; Experiments 4 and 5 error = 43, total = 56; Experiment 6 error = 42, total = 55; Experiment 7 error = 41, total = 45

Table 4.5. Analysis of variance for terminal disease severity ratings in experiment 8.

Source of variation	df	Mean Square	F value	Pr>F
Line	8	5099	23.35	<0.0001
Within nulls	1	413	1.06	0.3113
Within 3BS	2	16995	248	<0.0001
Within 4B	3	401	3.36	0.0361
Null vs. 3BS	1	1024	1.14	0.2898
Null vs. 4B	1	4444	17.38	<0.0001
Chemotype	1	300	1.37	0.2454
Line*Chemotype	8	131	0.6	0.7758
Error	70	218		
Total	87			

Table 4.6. Analysis of variance for terminal severity ratings in experiment 9.

Source of variation	df	Mean Square	F value	Pr>F
Chemotype	1	32	1.83	0.1860
Error	30	17.47		
Total	31			

The ANOVA for the combined experiment 1-7 dataset examining terminal severity ratings demonstrated a significant difference among experiments and lines, and within 3BS and 4B lines (Table 4.7). However, chemotype and line*chemotype effects were not significantly different (Table 4.7). No significant differences between chemotypes for ability to cause disease were detected by other researchers (Gilbert et al., 2001; Gilbert et al., 2010). No significant differences for line*chemotype interactions were reported by Gilbert et al. (2001). Table 4.7 shows that there are significant differences detected among experiments which may be attributed to the differences in temperature and humidity when experiments were run. Considering the significant difference among experiments, combining data from experiments 1-7 results in Type II error, i.e., accepting the null hypothesis when the null hypothesis is false. In other words,

the implications for combining heterogenous data are that experiment by experiment differences could hide significant differences between variables.

Table 4.7. Analysis of variance for terminal severity ratings of experiments 1-7.

Source of variation	**df**	**Mean square**	**F value**	**Pr>F**
Experiment	6	1344	3.69	0.0014
Line	6	7297	20.04	<0.0001
Within 3BS	2	18621	42.27	<0.0001
Within 4B	3	902	3.18	0.0250
Chemotype	1	188	0.51	0.4734
Line*Chemotype	6	517	1.42	0.2054
Error	389	364		
Total	408			

Unfortunately, due to the significant differences detected among experiments, no concrete conclusions regarding the stability of FHB resistance QTLs can be made from this experiment. In four of the eight experiments, there were significant differences among 3BS QTL lines, and in five of the eight experiments, there were significant differences among 4B QTL lines. It is evident that the differences among the lines were not large with the exception of line 1-44 carrying the 3BS QTL (Table 4.8). Due to the fact that there were no consistent results within the 3BS QTL, a known resistance QTL to FHB, more markers were run on the lines containing the 3BS QTL (1-14, 1-41 and 1-44). The additional markers that were run were identified in Cuthbert et al. (2006) and included barc133, sts163, sts138, and sts142. There were no differences among the lines for these markers. Therefore, differences in interval profile cannot be used to explain the differences among the lines.

The majority of the QTL lines tested had similar terminal severity ratings, with the exception of line 1-44 which had a significantly lower terminal severity rating and the largest standard deviation (Table 4.8).

Table 4.8. Terminal disease severity rating means and standard deviations for QTL line effects in experiments 1-7.

Level of Line	QTL	Number of plants	Mean (%)	Standard deviation
1-1	4B	61	70.3	18.3
1-12	4B	58	78.5	16.7
1-21	4B	56	75.8	15.9
1-87	4B	60	71.6	18.7
1-14	3BS	58	79.8	21.9
1-41	3BS	58	76.3	16.8
1-44	3BS	58	47.1	26.1
LSD = 6.94				

Table 4.9. Terminal disease severity rating means and standard deviations for chemotype effects in experiments 1-7.

Chemotype	Number of plants	Mean (%)	Standard deviation
3-ADON	206	70.6	22.3
15-ADON	203	72.0	21.6

Table 4.10. Terminal disease severity rating means and standard deviations for line*chemotype effects in experiments 1-7.

Level of Line	Level of chemotype	Number of plants	Mean (%)	Standard deviation
1-1	3	31	68.7	18.0
1-1	15	30	71.9	18.7
1-12	3	29	76.8	17.8
1-12	15	29	80.2	15.7
1-14	3	29	83.4	19.5
1-14	15	29	76.2	24.0
1-21	3	28	70.9	17.4
1-21	15	28	80.7	12.7
1-41	3	29	78.9	16.8
1-41	15	29	73.8	16.8
1-44	3	30	44.5	24.0
1-44	15	28	49.9	28.3
1-87	3	30	71.7	20.2
1-87	15	30	71.5	17.5

Tables 4.11-4.13 show the terminal severity ratings for line, chemotype and line*chemotype effects in experiments 8 and 9. There was no significant difference within or between QTL lines with the exception of line 1-44 which had a lower terminal severity rating compared to any of the lines tested in the experiment (Table 4.11). Experiment 8 results also show that the QTL null lines have lower terminal disease severity ratings than any of the lines containing a QTL. A possible explanation for this could be that individual QTLs, with the exception of line 1-44 are ineffective at resisting disease spread. CDC Teal values in Table 4.11 are clearly higher than any of the lines containing QTLs. Terminal severity ratings for chemotypes in experiments 8 and 9 showed that there was no significant difference between chemotypes in either experiment

(Table 4.12). There was no consistency in which chemotype produces higher disease severity and that there was no significant difference in the disease causing ability of the two chemotypes (Table 4.13). Again, line 1-44 showed a fairly strong resistance to the spread of infection (i.e. Type II resistance) indicating that this line is more effective at reducing disease by 3-ADON isolates.

Table 4.11. Terminal disease severity ratings for line effects in experiments 8 and 9.

Line	**QTL**	**N**	**Mean (%)**	**Standard deviation**
1-1	4B	8	91.00	11.47
1-12	4B	8	93.25	5.33
1-21	4B	8	93.87	8.88
1-87	4B	8	80.75	15.41
1-14	3BS	8	97.87	2.74
1-41	3BS	8	86.62	10.25
1-44	3BS	8	16.25	14.67
1-9	Null	16	76.37	15.34
1-10	Null	16	69.19	22.26
LSD			**13.90**	
CDC Teal	n/a	32	96.50	4.24

Table 4.12. Terminal disease severity rating for chemotype effects in experiments 8 and 9.

Experiment	**Chemotype**	**N**	**Mean (%)**	**Standard deviation**
8	3-ADON	44	75.59	28.44
8	15-ADON	44	79.09	22.79
9	3-ADON	16	97.50	2.73
9	15-ADON	16	95.50	5.24

Table 4.13. Terminal disease severity ratings for line*chemotype effects in experiments 8 and 9.

Line	QTL	Chemotype	N	Mean (%)	Standard deviation
1-1	4B	3	4	89.50	10.66
1-1	4B	15	4	92.50	13.69
1-12	4B	3	4	93.50	5.25
1-12	4B	15	4	93.00	6.21
1-21	4B	3	4	97.00	6.00
1-21	4B	15	4	90.75	11.05
1-87	4B	3	4	77.00	11.83
1-87	4B	15	4	84.50	19.41
1-14	3BS	3	4	98.50	1.91
1-14	3BS	15	4	97.25	3.59
1-41	3BS	3	4	83.25	14.22
1-41	3BS	15	4	90.00	3.55
1-44	3BS	3	4	5.50	5.25
1-44	3BS	15	4	27.00	12.90
1-9	Null	3	8	76.75	15.07
1-9	Null	15	8	76.00	16.64
1-10	Null	3	8	66.87	26.85
1-10	Null	15	8	71.50	18.14
CDC Teal	n/a	3	16	97.50	2.73
CDC Teal	n/a	15	16	95.50	5.224

4.4.2 Fusarium damaged kernel evaluation

Levene's test for homogeneity revealed that all parameters: QTL line, *F. graminearum* chemotype as well as the interaction between the line and chemotype were heterogeneous (Table 4.3).

All experiments, with the exception of experiment 4, showed significant differences among QTL lines (Table 4.14). All experiments showed significant differences within the 3BS QTL lines with the exception of experiment 6. No experiments showed significant differences within the 4B QTL lines with the exception

of experiments 6 and 7. Only experiments 2 and 8 showed significant chemotype effects; the remainder did not.

There were significant differences between null and 4B lines, which can be explained by the fact that the null lines have lower terminal severity levels than the 4B lines. No difference was detected between null and 3BS lines, which is likely because the null terminal severity levels are low and so are the 3BS line means, especially due to line 1-44 which shows a fairly good resistance level. Table 4.15 also shows that there was no significant difference between chemotypes used in experiment 9.

Table 4.14 Analysis of variance for fusarium damaged kernel counts for experiments 1-7.

Source of variation	df	Experiment 1			Experiment 2			Experiment 3			Experiment 4			Experiment 5			Experiment 6			Experiment 7		
		MS[1]	F[2]	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F	MS	F	Pr>F
Line	6	2868	12.28	<0.0001	982	4.42	0.0013	1284	4.13	0.0024	594	1.81	0.1211	1024	6.24	<0.0001	752	5.07	0.0005	529	4.1	0.0026
Within 3BS	2	7800	38.86	<0.0001	1826	5.92	0.0098	3412	5.8	0.0114	-	-	-	2776	8.64	0.0023	240	2.95	0.0781	397	6.34	0.0088
Within 4B	3	234	0.9	0.452	133	0.81	0.4994	54	0.53	0.6656	-	-	-	47	0.99	0.4131	883	4.5	0.0117	770	4.38	0.0135
Chemotype	1	836	3.58	0.0638	1488	6.69	0.0129	13	0.04	0.8389	223	0.68	0.4143	257	1.57	0.2178	448	3.03	0.0891	109	0.85	0.3615
Line* Chemotype	6	335	1.44	0.2179	161	0.73	0.6312	101	0.33	0.9187	529	1.61	0.1679	9	0.06	0.999	163	1.1	0.3785	112	0.87	0.5252
Error	54	233			222			311			328			164			148			128		
Total	67																					

[1] Mean Square
[2]F-value
Note: Experiment 2 error = 47, total = 60; experiment 3, 4 and 5 error = 42, total = 55; experiment 6 error = 43, total = 56; experiment 7 error = 41, total = 54

Table 4.15. Analysis of variance for fusarium damaged kernel counts for experiments 8 and 9.

	Experiment 8				Experiment 9			
Source	**df**	**Mean Square**	**F value**	**Pr>F**	**df**	**Mean Square**	**F value**	**Pr>F**
Line	8	3032	13.24	<0.0001		-	-	-
within nulls	1	1830	6.11	0.0198		-	-	-
within 3BS	2	7155	30.99	<0.0001		-	-	-
within 4B	3	206	1.42	0.2619		-	-	
Null vs. 3BS	1	929	1.59	0.2125		-	-	-
Null vs. 4B	1	86583	494.58	<0.0001		-	-	-
Chemotype	1	2316	10.11	0.0022	1	72.00	3.52	0.0705
Line*Chemotype	8	176	0.77	0.6292		-	-	-
Error	70	229			30	20.47		
Total	87				31			

Although significant differences were detected among experiments, all experiments showed significant differences were detected among lines and chemotypes (Table 4.16). A possible explanation for the differences detected between the experiments could be that there were temperature and humidity differences between the experiments, particularly when comparing earlier experiments to later experiments. Significant differences were also detected among QTL lines, within lines containing the 3BS QTL but not within lines containing the 4B QTL.

Table 4.16. Analysis of variance for combined Fusarium damaged kernel dataset from experiments 1-7.

Source of variation	**df**	**Mean square**	**F value**	**Pr>F**
Rep	6	1572	6.08	<0.0001
Line	6	3867	14.94	<0.0001
Within 3BS	2	10221	31.46	<0.0001
Within 4B	3	344	1.74	0.1601
Chemotype	1	2434	9.41	0.0023
Line*Chemotype	6	424	1.64	0.1347
Error	389	258		
Total	408			

There was a marked difference among 3BS QTL lines, for example, line 1-44 had a mean value of 67.9, whereas lines 1-14 and 1-41 had mean values of 92.9 and 89.4, respectively (Table 4.17). It is evident that the significant difference within the 3BS QTL lines is due to the significant difference between line 1-44 compared to lines 1-14 and 1-41. No significant differences were detected among 4B lines (Table 4.17) where all 4B lines (1-1, 1-12, 1-21 and 1-87) have similar FDK mean values. *F. graminearum* chemotypes also show significant differences, which is supported in Table 4.15 with chemotype A (3-ADON chemotype) producing lower mean values of FDK than

chemotype B (15-ADON chemotype). Line*chemotype interactions were not considered to be significant in the combined FDK data.

Table 4.17. Combined Fusarium damaged kernel means and standard deviations for line effects in experiments 1-7.

Level of line	QTL	N	Mean	Standard deviation
1-1	4B	61	84.5	14.4
1-12	4B	58	86.9	15.5
1-21	4B	57	90.4	13.4
1-87	4B	60	85.8	14.9
1-14	3BS	57	92.9	10.2
1-41	3BS	58	89.4	14.8
1-44	3BS	58	67.9	28.8
LSD			**5.85**	

Table 4.18 shows that there is a significant difference between chemotypes in the combined data for experiments 1-7, with the 15-ADON isolate resulting in significantly more FDK than the 3-ADON isolate.

Table 4.18. Combined Fusarium damaged kernel means and standard deviations for chemotype effects in experiments 1-7.

Level of chemotype	N	Mean	Standard deviation
3-ADON	206	82.9	20.3
15-ADON	203	87.9	16.0
LSD		**3.13**	

There were significant differences in the numbers of FDK that each line produced. It is also evident that line 1-44, which carries the 3BS QTL, had significantly lower FDK levels than the other QTL lines (Table 4.19).

Table 4.19. Combined fusarium-damaged kernel means and standard deviations for line*chemotype effects in experiments 1-7.

Level of line	QTL	Level of chemotype	N	Mean	Standard deviation
1-1	4B	3	31	81.3	16.5
1-1	4B	15	30	87.8	11.2
1-12	4B	3	29	87.9	11.4
1-12	4B	15	29	86.0	18.9
1-21	4B	3	28	86.1	17.2
1-21	4B	15	29	94.6	6.4
1-87	4B	3	30	83.2	18.3
1-87	4B	15	30	88.3	10.0
1-14	3BS	3	29	91.9	11.4
1-14	3BS	15	28	93.9	8.9
1-41	3BS	3	29	89.4	15.1
1-41	3BS	15	29	89.4	14.6
1-44	3BS	3	30	61.1	29.8
1-44	3BS	15	28	75.1	26.3

There was no significant difference among lines containing QTLs compared to the QTL null lines, with the exception of line 1-44 which appeared to show partial resistance (Table 4.20). Table 4.21 shows that there is a significant difference between the isolates that were used in this experiment. The 15-ADON isolate produces significantly more FDK than the 3-ADON isolate. The interaction, as shown in Table

4.22, shows that for the most part, the 15-ADON isolate produces more FDK on average than the 3-ADON isolate within lines, with the exception of lines 1-12 and 1-21. Table 4.22 again shows that line 1-44 demonstrated resistance by having less FDK than any other line. Similarly to Table 4.13, the 15-ADON isolate seems to have more negative effects than the 3-ADON isolate, in this case, ability to produce FDK. The results for experiment 8 are similar to the combined results from experiments 1-7.

Table 4.20. Combined Fusarium damaged kernel means and standard deviations for line effects in experiment 8.

Line	QTL	N	Mean	Standard deviation
1-1	4B	8	87.25	15.94
1-12	4B	8	88.13	6.44
1-21	4B	8	94.5	7.09
1-87	4B	8	82.13	17.02
1-14	3BS	8	87.75	17.46
1-41	3BS	8	76.88	17.30
1-44	3BS	8	31.38	17.36
1-9	Null	16	81.13	15.56
1-10	Null	16	66.00	18.96
LSD			**14.23**	

Table 4.21. Combined Fusarium damaged kernel means and standard deviations for chemotype effects in experiment 8.

Chemotype	N	Mean	Standard deviation
3-ADON	44	71.39	26.59
15-ADON	44	81.75	16.21
LSD		**6.44**	

Table 4.22. Combined Fusarium damaged kernel means and standard deviations for line*chemotype effects in experiment 8.

Line	QTL	Chemotype	Mean	Standard deviation
1-1	4B	3	77.75	18.37
1-1	4B	15	96.75	3.77
1-12	4B	3	88.50	5.25
1-12	4B	15	87.75	8.30
1-21	4B	3	95.25	8.22
1-21	4B	15	93.75	6.94
1-87	4B	3	76.75	21.43
1-87	4B	15	87.50	11.81
1-14	3BS	3	82.25	24.94
1-14	3BS	15	93.25	2.98
1-41	3BS	3	71.50	24.55
1-41	3BS	15	82.25	4.27
1-44	3BS	3	16.75	5.43
1-44	3BS	15	46.00	10.16
1-9	Null	3	77.00	20.57
1-9	Null	15	85.25	7.55
1-10	Null	3	61.23	23.25
1-10	Null	15	70.75	13.32

Note: Sample sizes of QTL lines were 4 and the null lines were 8.

There were no significant differences between chemotypes on CDC Teal (Table 4.23).

Table 4.23. Fusarium damaged kernel means and standard deviations for chemotype effects in experiment 9.

Line	QTL	Chemotype	Mean	Standard Deviation
CDC Teal	n/a	3	93.00	6.01
CDC Teal	n/a	15	96.00	2.19

Note: Sample sizes of lines were equal to 16; no significant differences between chemotypes.

4.5 Conclusion

Based on the results from this experiment, the majority of the individual experiments showed significant differences among lines, with significant differences within 3BS QTL lines but no differences within 4B QTL lines, and no significant differences between chemotype or line*chemotype interactions. Both disease severity and FDK counts showed significant differences among experiments which temperature and humidity differences at the times that each of the experiments were run may account for. Although significant differences were detected among experiments, the majority of the experiments generated similar data for each variable tested which was in accordance with the results from the combined dataset.

The experiment identified line 1-44, which caries the 3BS QTL, as a line which consistently shows partial Type II resistance. The differences detected within the 3BS QTL lines was due to line 1-44 compared to the other lines carrying the 3BS QTL as well as the null lines. Based on the QTL region selected, line 1-44 most likely carries *Fhb1*, however further work would be required to confirm this.

Results from this study also suggest that single QTLs are not effective at conferring resistance to FHB. While genes with "major" effects may be located on 3BS and 4B, alone they do not confer resistance and need minor genes with additive effects to obtain better resistance.

Further work on the effect of *F. graminearum* chemotype on lines containing different FHB resistance QTL is required. This would provide more robust results than the present study. DON analysis for this study would also be beneficial, especially to see

if there is a positive correlation between FDK counts and DON content. DON analysis was not feasible in this study due to small sample sizes. A comparison between the two types of FHB resistance might help to draw more conclusive results regarding specific resistance genes in the specific host-isolate interaction. A field and/or controlled environment evaluation of the QTL line/*F. graminearum* isolates from the present study would also provide an interesting comparison from the results found.

5.0 GENERAL DISCUSSION

FHB is one of the most serious diseases affecting wheat production worldwide. The pathogen *F. gramimnearum* produces the mycotoxin deoxynivalenol (DON) which reduces grain yield and quality. The recent detection of an acetylated derivative of *F. graminearum* to Canada, the 3-acetyl DON (3-ADON) chemotype, which is known to be more toxigenic than the resident 15-acetyl DON (15-ADON) chemotype, has spurred many questions in the grain industry as to the ramifications of increased levels of 3-ADON chemotype in the environment. The 3-ADON chemotype has historically been more prevalent in Asia, whereas the 15-ADON chemotype has usually been found in North America (Miller, et al., 1991).

The first study reported in chapter 3, compared several isolates of each chemotype on wheat genotypes of known reaction to FHB in order to determine if differences could be detected both between the chemotypes but also within each of the chemotypes. Significant differences were detected within the 15-ADON isolates but no significant differences within 3-ADON isolates across all variables tested. A lack of significant differences within the 3-ADON isolates that were tested show that there is a narrower genetic pool in this chemotype compared to the 15-ADON chemotype population. Considering that the 3-ADON chemotype was introduced into Canada in the last 30 years (Ward et al., 2008), it appears, from the results of the present study, that there is less genetic variation within the 3-ADON chemotype population in Canada. The study done by (Ward et al., 2008) suggested that observation of the rapid chemotype shift signifies that the 3-ADON population has a selective advantage over 15-ADON population. In addition, Guo et al. (2008) demonstrated this shift in Manitoba. In the present study,

differences were detected between chemotypes for yield, DON accumulation and FDK measurements but not for disease progression. The implications of these results mean that although there is no difference in disease progression between chemotypes, the 3-ADON chemotype had greater negative effects on yield, and resulted in higher DON accumulation and FDK levels. Considering yield is the most important trait to the farmer and the grain industry, the rapid chemotype profile shift towards the 3-ADON chemotype could be a serious concern for the grain industry in Canada unless resistant cultivars and better control methods can be implemented.

Correlations of tested parameters showed that there was a strong positive correlation between FDK and DON as also demonstrated by (Mesterhazy et al., 2005; Zhang et al., 2008). Currently the Canadian Grain Commission uses FDK as a percentage by weight as a predictor for DON content in grain samples at a 1:1 ratio. It was thought that the presence of the 3-ADON chemotype may affect this ratio, and the results from the present experiment support this, when FDK are measured by kernel number rather than as a percentage by weight. A scatter plot of the two variables showed that the correlation was stronger at lower FDK and DON levels, however, when the levels of either variable increased, the strength of the correlation began to deteriorate. The correlation coefficients showed a moderately strong relationship between FDK and DON, however, the slope of the line of best fit indicates that the relationship may follow a 6:1 (FDK:DON) ratio better. It seems as though the relationship of DON to FDK depends primarily on the isolate-genotype interaction and that there is specificity in this interaction. The results consistently showed that there were stronger correlations for 15-ADON isolates for each genotype tested, and the difference between chemotypes was

significantly different. Results from this study show that the chemotype shift in Canada have caused prediction ratios to change to 6:1 for FDK:DON (when FDK are measured by kernel number) and that this prediction ratio is strongest at FDK levels below 40%.

The second study examined differences in Type II disease progression and FDK levels in wheat lines with two known resistance QTLs and the reaction of the chemotypes on the different lines. The QTLs used were 3BS, a major QTL for FHB resistance (Waldron et al., 1999; Chen et al., 2000; Gupta et al., 2000; Anderson et al., 2001; Kolb et al., 2001; Zhou et al., 2002b; Shen et al., 2003; Somers et al., 2003; Yang et al., 2003), and 4B, a minor QTL for FHB resistance (Waldron et al., 1999; Somers et al., 2003; McCartney et al., 2007). Just one of the 3BS lines tested in the experiment demonstrated partial resistance to FHB in terms of terminal disease severity and FDK levels. Additional 3BS markers were tested, and all markers tested were present in all 3BS lines. No significant differences were detected between chemotypes for terminal severity on any of the lines tested; however chemotype differences were detected for FDK levels, with the 15-ADON isolate displaying on average more FDK than the 3-ADON isolate. The 15-ADON isolate producing more FDK than the 3-ADON isolate is contradictory to the results found in chapter 3. A possible explanation for this is that in this particular case, based on the isolates chosen, that the 15-ADON isolate, although not displaying significantly different terminal severity ratings than the 3-ADON isolate, is able to cause more damage to the seed. More research such as comparing accumulated DON levels of these two isolates used in this study would be advantageous. To the author's knowledge, no studies have been published regarding chemotype comparisons on FHB resistance

QTLs; therefore, no comparisons between the present study can be made to previous research.

In summary, the recent chemotype shift in Canada towards a more aggressive 3-ADON chemotype will continue to pose risks to grain, especially in epidemic years. Additional testing will be required in years where there are high FDK levels as this study showed that the current standards cannot precisely predict DON levels in extreme epidemic situations. More research on the chemotype shift in Canada will aid in the understanding of the 3-ADON chemotype as well as provide insight into better control strategies.

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7.0 Appendices

Appendix 7.1Potato dextrose agar (PDA) recipe

39g PDA (Difco Laboratories, MD)

1L distilled Water

Dissolve PDA in water and autoclave at 120^0C for 20-25 minutes.

Appendix 7.2 Specific Nutrient-Poor Agar or Spezieller Nährstoffarmer Agar (SNA) recipe (Nireberg, 1981)

1.0g KH_2PO_4

1.0g KNO_3

0.5g $MgSO_4$-$7H_2O$

0.5g KCl

0.2g Glucose

0.2g Sucrose

20g Agar

1L distilled water

Dissolve nutrients in water and autoclave at 120^0C for 20-25 minutes.

Appendix 7.3 Carboxymethyl Cellulose Media (CMC) recipe (Cappellini and Peterson, 1965)

1.50g NH_4NO_3

1.50g KH_2PO_4

0.75g $MgSO_4$-$7H_2O$

1.50g Yeast extract

22.50g CMC

1.5L distilled water

0.38g streptomycin sulphate

Dissolve nutrients and CMC in water and autoclave at 120^0C for 20-25 minutes. Once cooled, add streptomycin sulphate to autoclaved media.

Appendix 7.4 Marker name, chromosome location, forward and reverse primers and reference source for markers used to verify Quantitative trait loci (QTL).

Marker	QTL	Forward primer (5'-3')	Reverse primer (5'-3')	Reference
cfd233	2D	GAATTTTTGGTGGCCTGTGT	ATCACTGCACCGACTTTTGG	http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker
cfd73	2D	GATAGATCAATGTGGGCCGT	AACTGTTCTGCCATCTGAGC	http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker
wmc18	2D	CTGGGGCTTGGATCACGTCATT	AGCCATGGACATGGTGTCCTTC	http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker
wmc245	2D	GCTCAGATCATCCACCAACTTC	AGATGCTCTGGGAGAGTCCTTA	http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker
gwm608	2D	ACATTGTGTGTGCGGCC	GATCCCTCTCCGCTAGAAGC	Roder et al. (1998)
umn10	3B	CGTGGTTCCACGTCTTCTTA	TGAAGTTCATGCCACGCATA	Liu et al (2008)
sts80	3B	AGAAGAAGGAAGCCCCTCTG	GCCATGTCTTTTGTGCCTTT	Liu and Anderson (2003)
gwm533	3B	AAGGCGAATCAAACGGAATA	GTTGCTTTAGGGGAAAAGCC	Roder et al. (1998)
gwm493	3B	TTCCCATAACTAAAACCGCG	GGAACATCATTTCTGGACTTTG	Roder et al. (1998)
sts163	3B	TTCATGGACGAGTACGACGA	AAGGTTGCCATTGCTCTCAC	Liu and Anderson (2003)
sts138	3B	CAAGATCAAGAAGGCCAAGC	AGGTACACCCCGTTCTCGAT	Liu and Anderson (2003)
sts142	3B	CGAGTACTACCTCGGCAAGC	CATAGAATGCCCCGAAACTG	Liu and Anderson (2003)
barc133	3B	AGCGCTCGAAAAGTCAG	GGCAGGTCCAACTCCAG	http://www.scabusa.org
sts66	3B	AGTCAGGCGAAGAGCGATAA	AGCACTGCACAATGAGCATC	Liu and Anderson (2003)
wmc238	4B	TCTTCCTGCTTACCCAAACACA	TACTGGGGGATCGTGGATGACA	http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker
wmc710	4B	GTAAGAAGGCAGCACGTATGAA	TAAGCATTCCCAATCACTCTCA	http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker
gwm149	4B	CATTGTTTTCTGCCTCTAGCC	CTAGCATCGAACCTGAACAG	Roder et al. (1998)
wmc705	5A	GGTTGGGCTCCTGTCTGTGAA	TCTTGCACCTTCCCATGCTCT	http://wheat.pw.usda.gov/cgi-bin/graingenes/browse.cgi?class=marker
gwm293	5A	TAGTGGTTCACATTGGTGCG	TCGCCATCACTCGTTCAAG	Roder et al. (1998)
gwm304	5A	AGGAAACAGAAATATCGCGG	AGGACTGTGGGGAATGAATG	Roder et al. (1998)

Appendix 7.5 Area under the disease progress curve (AUDPC) means for incidence, severity and index for all isolates based on the 2008 and 2009 combined dataset.

Isolate	Chemotype	N[1]	AUDPCInc[2] Mean	AUDPCSev[3] Mean	AUDPCIdx[4] Mean
A1-06-1	15	18	14477	10639	6215
S1A-06-4	15	18	12769	9524	5743
S3AN-06-1	15	18	11890	9207	5208
S8A-06-5	15	18	12199	8942	5193
M2-06-2	15	18	15062	10933	7102
M8-06-2	15	17	10902	9070	4872
ON-06-17	15	18	11622	8297	4603
Q-06-10	15	18	14791	11824	7647
Q-06-22	15	18	13089	9980	6067
Q-06-34	15	18	12462	10020	5605
NB-06-18	15	18	13321	10196	6468
NS-06-03	15	18	14858	11398	7270
A2-06-1	3	18	12409	9677	5709
A4-06-5	3	18	13794	10634	6579
A6-06-1	3	18	13997	9715	5909
S1A-06-3	3	18	14317	10788	6658
S3BS-06-1	3	18	12387	10449	5883
S8A-06-1	3	18	13677	9518	5872
M2-06-1	3	18	13756	10480	6564
M8-06-5	3	18	13329	10021	6377
Q-06-11	3	18	13266	10197	6206
Q-06-23	3	18	13745	9715	6230
NB-06-17	3	17	15831	12051	7810
NS-06-2	3	18	12976	9925	5878
MIN-1-1	3	18	13231	10239	6328
CONTROL	0	18	1307	1599	288
LSD			**1296**	**1191**	**1103**

[1]Sample size
[2]Area under the disease progress curve for disease incidence
[3]Area under the disease progress curve for disease severity
[4]Area under the disease progress curve for Fusarium head blight index

Appendix 7.6 Overview of inoculation method comparison experiment

7.6.1 Introduction

Many experiments have been performed attempting to differentiate between screening procedures for Types I and II resistance (i.e., resistance to initial infection and resistance to spread of infection, respectively) with limited success. Type II resistance is typically evaluated in greenhouse studies (Kolb et al., 2001) due to the ease of the screening procedures and repeatability of results. A cultivar is type I resistant if it is resistant to spray inoculation but susceptible to point inoculation and is type II resistant if it is susceptible to spray inoculation but resistant to point inoculation (Schroeder and Christensen, 1963). The present experiment attempted to investigate if there were consistent significant differences in screening procedures for Types I and II resistance.

7.6.2 Materials and Methods

Two separate experiments were conducted after the first experiment was not showing repeatable results. The first experiment used 6 spring wheat lines including AC Cora, AC Vista, 5602HR, AC Barrie, 93FHB37 and CDC Teal, 2 isolates of *F. graminearum*, Min-1-1 (3-ADON producer) and M5-06-8 (15-ADON producer) as well as a water-inoculated control (3 plants per wheat line). Thirty-six plants of each line were used. There were three inoculation methods used in this experiment, which were: spray inoculation followed by 24 hours in a humidity chamber; spray inoculation followed by 48 hours under a polyethylene bag; and point inoculation followed by 48 hours under a polyethylene bag. One to two spikes were inoculated per plant and severity ratings were taken at the onset of disease symptoms, every three days, until natural

senescence. In summary, there were 18 plants per isolate or 6 plants per isolate per treatment.

The second experiment used three spring wheat lines: CDC Teal, AC Cora and 93FHB37 and only one *F. graminearum* isolate, Min-1-1 (3-ADON producer) and a water-inoculated control (3 plants per wheat line). Thirty-nine plants of each line were used. The same three inoculation methods were used as in the first experiment, with the exception of the bags. In the second experiment, glassine bags were used in place of polyethylene bags. Similar to the first experiment, one to two spikes per plant were inoculated and rated for disease symptoms at the onset of disease symptoms, every third day, until natural senescence. In summary, there were 12 plants per treatment.

7.6.3 Results

The results from the first experiment showed variable infection rates within treatments. The treatments involving 48 hours under a polyethylene bag resulted in an incredibly rapid onset of symptoms and polyethylene bags created an environment that was too hot and humid resulting in the spikes reaching maximum disease severity prematurely. Possible sources of error in this experiment include that the isolates could have been too aggressive, there was too much inoculum applied in the spray treatments, and it was too humid under the polyethylene bag. Measures to mitigate these sources of error were to apply less inoculum on the spray treatments (i.e. only spray each head for 3-5 seconds), use glassine crossing bags instead of polyethylene bags so that there was some air exchange to decrease humidity and reduce heat within the bags.

The results from the second experiment again showed variable infection rates within treatments, displaying both head to head variation, within the same plant, as well as plant to plant variation, within treatments.

7.6.4 Conclusions

Results from these two experiments show that more favourable results might be obtained from screening wheat lines for *F. graminearum* in growth chambers instead of a greenhouse. Growth chambers are able to control temperature and humidity which was likely the reason why repeatable results could not be obtained between plants or within treatments. Engle et al. (2003) and Del Ponte et al. (2007) were able to get repeatable results with placing inoculated plants in a greenhouse, however, Fernandez et al. (2005), Dufault et al. (2006) and Bai et al. (2001) returned plants to the same temperature conditions used prior to inoculation. A study done by Miedaner et al. (2003) which compared spray and point inoculation, although in a field setting, inoculated using both methods on the same day so that legitimate comparisons between treatments could be made.

Appendix 7.7 Chemotype confirmation study

7.7.1 Introduction

To confirm the chemotype of each isolate that was used in the field study, multiplex polymerase chain reactions (PCR) were run on fungal mycelium of each isolate. The PCR products of the multiplex PCR were then analysed on a electrophoresis gel to visually confirm results.

7.7.2 Materials and Methods

Single spore cultures of each isolate (Table 3.1) were grown under UV light for 7 days. The mycelium from each plate was then scraped off using a sterile laboratory spatula and put into a sterile 1.5mL microcentrofuge tube. Each tube was covered with a layer of parafilm. The parafilm was perforated once using a sterile needle. The mycelia were then freeze-dried for 24 hours. After freeze drying mycelia was pulverized using sterile toothpicks to which 600µl of 1x TAE buffer (2% 50x TAE [242g Tris, 57.1mL glacial acetic acid, 100mL 0.5 EDTA ph 8.0] diluted in double distilled water) was added. This solution was crushed again using a sterile pestle driven by an electric motor. To this solution, 140µl of 5M NaCl and 70µl of 65^0C 10% CTAB (10g CTAB, 0.7M (14mL 5M) NaCl dissolved in 100mL double distilled water) was added and vortexed. These samples were incubated at 65^0C for 20 minutes. Six hundred microlitres of phenol:chloroform:isoamylalcohol (25:24:1) was then added and centrifuged at 10 000 rpm for 15 minutes. The supernatant was transferred to a new 1.5mL microcentrofuge tube and the previous step was repeated twice. In order to precipitate the DNA, 1000µl of 100% ethanol and 80 µl of 5M NaCl were added to the supernatant and centrifuged at 10 000rpm for 10 minutes. The supernatant from the centrifuged sample was discarded and 200µl of 80% ice cold ethanol was added to wash the pellet in order to remove the salts in the precipitated DNA. The ethanol was poured off and the pellet was allowed to air dry for 20 minutes. The dry pellet was then re-dissolved in 200µl of sterile distilled water. To remove RNA, RNAse was added and the sample was incubated at 37^0C for 30 minutes. The DNA was run on a 1% agarose gel. Once the presence of genomic DNA

had been confirmed from this gel, the DNA concentration was measured using a spectrophotometer. Each sample was adjusted to 10ng/µl using sterile distilled water.

After DNA had been adjusted to 10ng/µl, the PCR reaction contained 2.5µl 10x PCR buffer (Invitrogen Canada Inc., Burlington, ON), 2.5µl dNTPs, 1.0µl mM $MgCl_2$, 1.25µl of each for 4 primers used, 1.0µl DNA 0.1µl *Taq* DNA polymerase (Invitrogen Canada Inc., Burlington, ON) and 12.90µl sterile distilled water.

Fusarium graminearum chemotypes were identified using the multiplex PCR marker developed by (Ward et al., 2002). The four primers used in the PCR were 3CON (5-TGGCAAAGACTGGTTCAC-3), 3D15 (5-ACTGACCCAAGCTGCCATC-3), 3D3A (5-CGCATTGGCTAACACATG-3), 3NA (5-GTGCACAGAATATACGAGC-3). They produced a 610-bp fragment for the 15-ADON chemotype, and a 243-bp fragment for the 3-ADON chemotype. The multiplex PCR cycle that was used was 95^0C for 5minutes (1x), 94^0C for 30 seconds 52^0C for 30 seconds 72^0C for 1 minute (45x), 72^0C for 8 minutes (1x), 4^0C infinitely. After the samples had been in the PCR cycle, the PCR products were run on a 2% agarose gel supplemented with 3µl ethidium bromide. Five microlitres of PCR products and 2µl of loading buffer were added to each well. The gel was run at 80 volts for 30 minutes.

7.7.3 Results

The results of the PCR products run on the 2% agarose gel are presented in Figure 7.1 (isolates 1-10) and 7.2 (isolates 11-25 plus control). The green line represents 200bp on the ladder and the red line represents 600bp. 15-ADON isolates show bands at 610bp

whereas 3-ADON isolates show bands at 243bp. When cross referenced with table 3.1, all of the isolate chemotypes were found to have been properly identified.

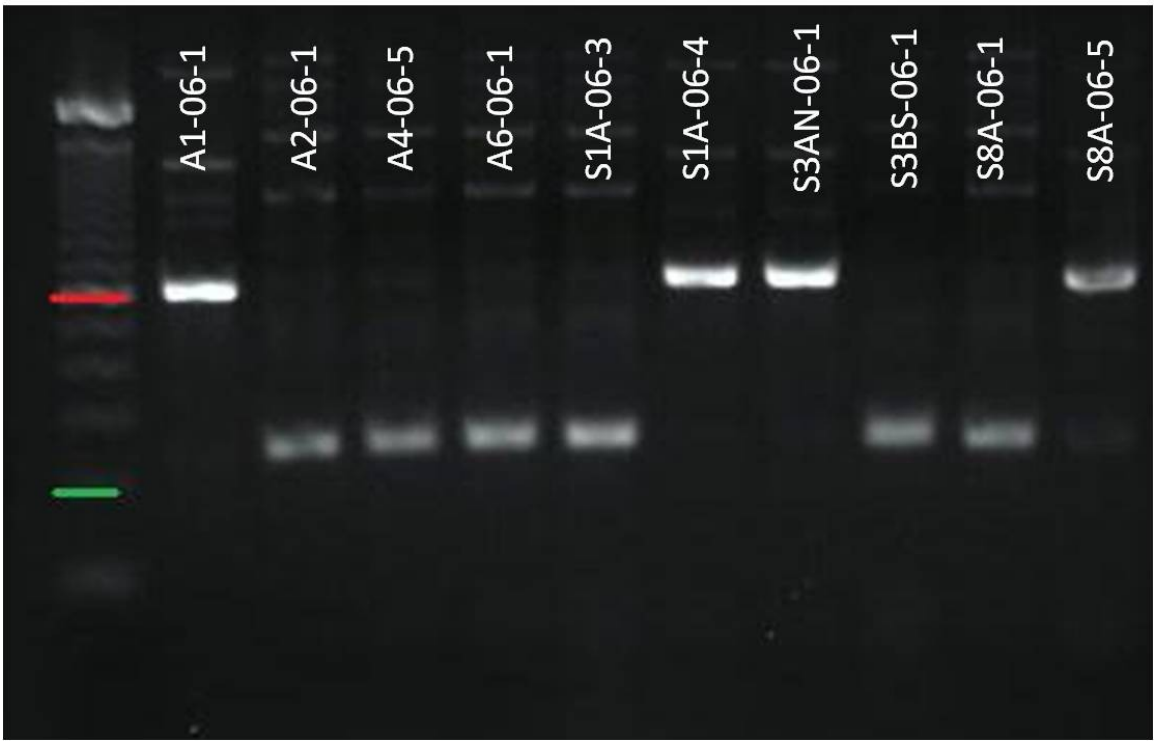

Figure 7.1 Isolate multiplex PCR products run on 2% agarose gel.

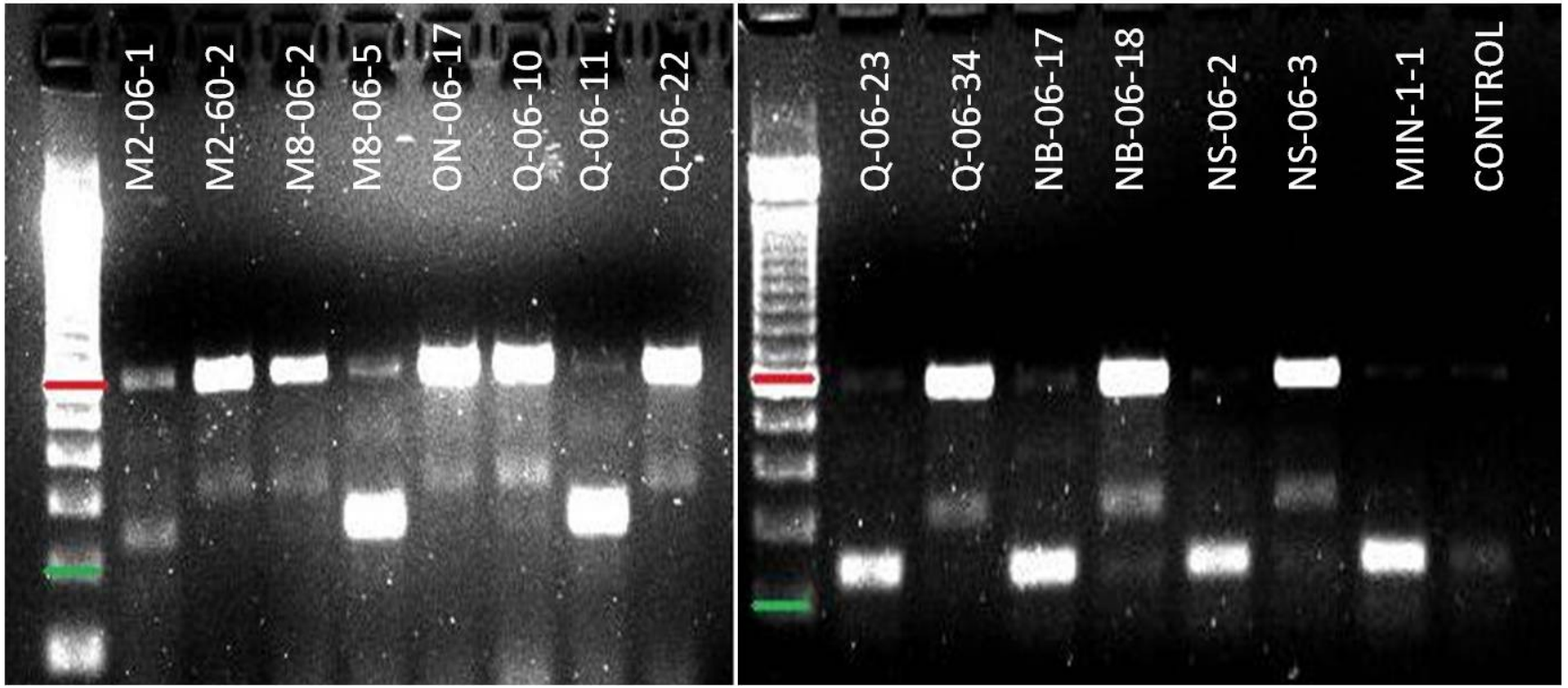

Figure 7.2 Isolates plus control multiplex PCR products run on 2% agarose gel.

7.7.4 Conclusions

In conclusion, multiplex PCR confirmted the isolates' chemotypes were determined to be labelled correctly.