The Effect of Plant Growth Regulators on Fusarium Head Blight Infection in Spring Wheat (*Triticum aestivum* L.) by *Fusarium graminearum*

By

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Abbreviation	Description
3-ADON	3-acetyldeoxynivalenol
15-ADON	15-acetyldeoxynivalenol
ACC	1-aminocyclopropane-1-carboxylic acid
ANOVA	Analysis of variance
C8	Carbon 8
CCC	Chlormequat chloride
CDP	Copalyl diphosphate
CMC	Carboxymethyl cellulose
CNHR	Canada Northern Hard Red
CPSR	Canada Prairie Spring Red
CWRS	Canada Western Red Spring
DMI	Demethylation inhibitor
DON	Deoxynivalenol
ET	Ethylene
FDK	Fusarium damaged kernel
FGSC	*Fusarium graminearum* species complex
FHB	Fusarium head blight
GA	Gibberellin
GAI	Gibberellin insensitive
GID1	GA-INSENSITIVE DWARF1
GS	Growth stage
I	Intermediate Resistance
INC	Incidence
JA	Jasmonic acid
LTA	Long term average
MR	Moderate Resistance
NIV	Nivalenol
PGR	Plant growth regulator
qPCR	Quantitative real time polymerase chain reaction
QTLs	Quantitative trait loci
R	Resistance
RGA	Repressor of GA1
ROS	Reactive oxygen species
SCR	SARECROW
SDHI	Succinate dehydrogenase inhibitor
SEV	Severity
SNA	Spezieller Nährstoffarmer agar
SNP	Single-nucleotide polymorphism
TKW	Thousand kernel weight
TWT	Test weight

Abstract

Lee, Younyoung, MSc., The University of Manitoba, 2021. The Effect of Plant Growth Regulators on Fusarium Head Blight Infection in Spring Wheat (*Triticum aestivum* L.) by *Fusarium graminearum.*

Professor: Dr. Anita Brule-Babel.

Fusarium head blight (FHB) is an important fungal disease of wheat predominantly caused by *Fusarium graminearum*. FHB infection in cereal crops leads to yield loss and decreased grain quality due to accumulation of mycotoxins such as deoxynivalenol. Many highly FHB resistant wheat cultivars are tall and are prone to lodging. Application of plant growth regulators (PGRs) to tall cultivars with the highest level of FHB resistant could prevent wheat from lodging and overcome the negative association between shorter plants and FHB susceptibility. The objectives of this study were to investigate the effect of PGRs on FHB infection and anther retention in spring wheat cultivars. Two commercially available PGRs, Manipulator™ and Ethrel™, were applied to five spring wheat (*Triticum aestivum* L.) cultivars which differed in height, FHB resistance level and semi-dwarfing alleles. Results from this study showed Ethrel™ reduced plant height under field and controlled environments conditions. Under field conditions, PGRs did not influence anther retention and FHB infection in spring wheat. In contrast, in controlled environment conditions, Manipulator™ decreased anther retention, while Ethrel™ increased anther retention. Treatment with Manipulator™ showed conflicting results on the accumulation of *F. graminearum* DNA in the spike. The Ethrel™ treatment showed that accumulation of *F. graminearum* DNA is genotype specific. Overall, the performance of PGRs on height, anther retention and FHB infection were influenced by environmental conditions.

1 Chapter 1.0 General Introduction

Wheat (*Triticum aestivum*) is one of the most important carbohydrate sources in the world (Xu et al. 2020). During the Green Revolution in the 1960s, introduction of semi-dwarf wheat substantially increased wheat yield in the world by reducing lodging (Hedden 2003). Among these semi-dwarf cultivars, the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* originated from 'Norin 10' (Gilbert and Haber 2013) and are gibberellin (GA) insensitive. They encode truncated DELLA proteins that reduce sensitivity to GA in plants, resulting in a semi-dwarf phenotype (Buerstmayr and Buerstmayr 2016). More than 70% of modern cultivars have either one of both of the semi-dwarfing alleles *Rht-B1b* or *Rht-D1b* (Miedaner and Voss 2008; Buerstmayr and Buerstmayr 2016).

Gibberellins are phytohormones that control the growth and development of plants such as height and filament extension. DELLA proteins play a role as the central regulators of the gibberellin (GA) signalling pathway. They repress the GA responses in wheat (Nelson and Steber 2016). When bioactive GA is present, DELLA is degraded, thereby GA response is turned on. However, truncated DELLA resulting from semi-dwarfing alleles in the plant abates GA-induced degradation of DELLA proteins by preventing GA and GA receptors from binding DELLA proteins, resulting in short plant. Gibberellins also act as regulators of plant disease resistance and susceptibility by stimulating degradation of the DELLA proteins (Navarro et al. 2008; Bari and Jones 2009; Saville et al. 2012). DELLA proteins not only affect the jasmonic acid and ethylene (ET) pathway to enhance resistance to necrotrophs (Navarro et al. 2008), but also are able to lower reactive oxygen species (ROS) (Achard et al. 2008). Increasing tolerance against necrotrophs results from postponing cell death through repressing the accumulation of ROS by DELLA.

Fusarium head blight (FHB) is an important fungal disease of cereal crops caused by several *Fusarium* spp. It is mainly caused by *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* Schwein (Petch)] in temperate regions throughout the world. Fusarium head blight causes substantial losses of yield and results in lower end use quality and downgrading of grain due to presence of *Fusarium* damaged kernels and the accumulation of deoxynivalenol (DON) (McMullen et al. 1997; Windels 2000; Shaner 2003). Fusarium head blight can infect cereal crops and forage grasses and is one of the greatest threats to wheat production across Western Canada. Management of FHB has been a challenge. Agronomic practices, including the use of fungicides, can be used to protect crops from FHB. However, they do not provide complete FHB control. Breeding for FHB resistance cultivar has led to development of cultivars that have moderate to intermediate resistance to FHB and it is a reliable method to protect wheat from FHB when combined with suitable agronomic management practices. Currently, there are limited numbers of highly FHB resistant wheat cultivars available in the market. Only two wheat cultivars are classed as highly FHB resistant in Canada; one is AAC Tenacious from the Canada prairie red spring wheat class and Emerson from the Canada western red winter wheat class (Seed Manitoba 2018). Even though there are highly FHB resistance wheat cultivars, resistance is often associated with undesirable traits such as tall plant height. Semi-dwarf wheat cultivars are preferred by growers because shorter cultivars have higher harvest index, are easier to harvest, have less risk of lodging and tend to produce higher yield under intensive management. However, semi-dwarf plants are associated with higher FHB infection (Draeger et al. 2007; Miedaner and Voss 2008; Srinivasachary et al. 2009; Liu et al. 2013; Lu et al. 2013; Buerstmayr and Buerstmayr 2015, 2016). Because short plants are closer to the inoculum source and have compact canopies which can retain higher humidity, shorter plants provide favorable conditions for FHB development. In spite of that,

spray inoculation directly on wheat spikes still showed higher FHB infection in short cultivars. Hence, higher frequency of anther retention has been suggested as one mechanism that explains higher FHB infection in semi-dwarf cultivars (Buerstmayr and Buerstmayr 2016).

Anther retention means anthers are trapped between the lemma and palea in the floret. Retained anthers provide a surface on which *Fusarium* spores can land and enter the spike, contributing to initial infection (Strange et al. 1972; Skinnes et al. 2010; Buerstmayr and Buerstmayr 2015, 2016; He et al. 2016). Anther retention has been highly correlated with FHB susceptibility (Buerstmayr and Buerstmayr 2015, 2016; He et al. 2016). As a quantitative trait, anther retention is controlled by multiple minor genes and is affected by environment (Buerstmayr and Buerstmayr 2016). Although it has the characteristics of a quantitative trait, anther retention has high heritability (Skinnes et al. 2010; Muqaddasi et al. 2017b; Steiner et al. 2017). Selection against anther retention could provide breeders a fast and accurate screening process for breeding FHB resistance cultivar. The *Rht* loci have an impact on anther retention and the loci of *Rht-B1* and *Rht-D1* overlap with quantitative trait loci for anther retention on chromosomes 4BS and 4DS, respectively (Xu et al. 2020).

By affecting the level of phytohormone in the plant, plant growth regulators (PGRs) are used to control the growth of plants. In wheat, increasing stem thickness and reducing the plant height to decrease risk of lodging are the primary reasons for using PGRs. To achieve these goals, the ET or GA pathways are the main target. Two main groups of PGRs are currently available: GA inhibitors and ET releasing compounds (Rademacher 2016). Chlormequat chloride is one of the GA inhibitors which is widely used in Canada. It inhibits the early steps of GA biosynthesis leading to short plants. Ethephon is an ET releasing compound. Ethylene plays a role in the GA and auxin pathways to alter plant growth. Therefore, use of plant growth regulators (PGRs) would

allow growers to choose tall, more FHB resistant, cultivars and counteract the effect of plant height by using PGRs. However, there is a potential that PGRs could affect FHB susceptibility of spring wheat because they affect the sensitivity of GA in plant, either directly or indirectly.

To provide answers for this potential problem, the objectives of this project were 1) to determine the effect of the PGRs, Manipulator™ (GA inhibitor) and Ethrel™ (ET releasing compound), on FHB infection and anther retention in five spring wheat cultivars that vary in semi-dwarfing alleles, height and level of FHB resistance and examine the interaction of the PGRs with semi-dwarfing alleles, *Rht-B1b* and *Rht-D1b* in the field, and 2) to investigate the effect of PGRs on anther retention and the accumulation *F. graminearum* DNA in controlled environment conditions.

2 Chapter 2.0 Literature Review

2.1 Wheat

2.1.1 History and general information

Wheat (*Triticum aestivum*) is one of the most important staple food crops worldwide and is grown widely around the world (Xu et al, 2019). The processed wheat grain is used to produce noodles, pasta, bread, and other baked goods; it can serve also as a functional ingredients because of the its unique gluten protein fraction (Shewry and Hey 2015). As a source of carbohydrate, wheat is considered the most important food source. Other than carbohydrates, wheat contains protein, fiber, vitamins especially B vitamins, minerals, lipids, and phytochemicals (Shewry and Hey 2015; Igrejas et al. 2020). After world war II and with population growth, global wheat production has gradually increased (Igrejas et al. 2020). The green revolution, along with advances in agronomic and genetic knowledge, contributed to increased wheat production. Since 1961, despite only requiring a 5.7% increase in world wide production area, wheat production has increased by 244% (Food and Agriculture Organization of the United Nations 2020). As one of top 10 wheat producers, the total cultivated land for wheat in Canada decreased by 5.75% from 10Mha to 9.6Mha, while wheat production increased by 319% from 7.7Mt to 32.3Mt since 1961 (Food and Agriculture Organization of the United Nations 2020). In Canada, approximately 95% of wheat production is in Western Canada; 44% in Saskatchewan, 33% in Manitoba, and 16% in Alberta (Hayes 2019).

Wheat is a member of the *Poaceae* family with seven chromosome pairs (Peng et al. 2011). Wheat has three different subgenomes; A, B, and D. Depending on the number of subgenomes wheat has, it can be a diploid (AA), tetraploid (AABB) or hexaploid (AABBDD) (Willenborg and Van Acker 2008; Peng et al. 2011). Hexaploid wheat is the most commonly grown wheat throughout the world and is also known as common or bread wheat (Willenborg and Van Acker

2008). Hexaploid bread wheat can be divided into two types based on growth habit; spring wheat and winter wheat.

2.1.2 Spring wheat

Spring wheat generally does not have a vernalization requirement and is sown in the spring and harvested in late summer, or early fall, in Canada. Time to maturity of spring wheat is shorter than that of winter wheat and is approximately four months. Spring wheat generally has lower yield than winter wheat, but higher protein levels.

Based on the functional characteristics of the grain, Canadian wheat varieties are categorized into 14 classes by the Canadian Grain Commission. Among the 14 classes, eight classes are spring wheat (Canadian grain commission 2018). In 2019, about 79% of wheat produced in Canada was spring wheat (Statistis Canada 2020). Spring wheat production is challenged by many diseases. Leaf, stem and stripe rust and Fusarium head blight are priority diseases for wheat breeding in Canada.

2.2 Fusarium head blight (FHB)

2.2.1 FHB and history

Globally, Fusarium head blight (FHB) is one of the most devastating diseases of cereal crops such as barley (*Hordeum vulgare*), corn (*Zea mays*), rye (*Secale cereale*) and wheat (*Triticum aestivum*). This fungal disease ultimately results in yield loss, end-use quality degradation, and mycotoxin contamination in the grain, and thus greatly threatens both food and feed safety. Fusarium head blight in wheat is caused by several *Fusarium* species including *F. avenaceum, F. culmorum, F. graminearum and F. poae* (Mesterházy 1995; Steiner et al. 2017; Amarasinghe et al. 2019). Among the many *Fusarium* species that cause FHB, the predominant

causal agent in North America is *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.) Petch] (Shaner 2003; Gilbert et al. 2010; Tamburic-Ilincic et al. 2015).

Fusarium head blight was first described as wheat scab in 1884, in England (Smith 1884). The first occurrence of FHB in Western Canada was reported in 1923 in Manitoba (Clear and Patrick 1990). Reports of FHB were sporadic in Canada until the 1980s when multiple *Fusarium* species were identified on grain samples from Ontario, Manitoba and Saskatchewan (Clear and Patrick 2000; Shaner 2003). The first major FHB epidemic in the upper Great Plains, including Manitoba, Minnesota, North Dakota, and South Dakota occurred in 1993 and resulted in an estimated $ 1 Billion USD loss with severe yield and quality reduction (McMullen et al. 1997). In Manitoba alone, yield loss in wheat was estimated to be $704 million USD (McMullen et al. 1997). Loss of grain quality was also problematic for producers. In addition, *F. graminearum* infected grain has high concentrations of the mycotoxin DON, which reduces marketability and price. Losses incurred by wheat producers alone approximated $86 million due to DON in 1993 (McMullen et al. 1997). Several factors contributed to the 1993 FHB outbreaks; above normal rainfall during anthesis, the prevalence of minimum tillage, limited or absence of FHB resistant cultivars, and short crop rotation intervals between susceptible host crops. (McMullen et al. 1997; Clear and Patrick 2000; Windels 2000). After 1993, severe FHB epidemics in the upper Great Plains recurred in successive years (McMullen et al. 1997; Shaner 2003). Economic losses to wheat producers in the United States and Canada during the 1990s due to FHB are estimated at over $ 3 billion USD (Windels 2000). During the 2000s, Manitoba had more major FHB epidemics. For example, Western Canada had one of the worst FHB epidemics in 2016. The average percentage of Canada Western Red Spring (CWRS) wheat samples containing *Fusarium* damaged kernels (FDK) was 90.1% (incidence) and the average FDK within samples was over 1% (severity)

in Manitoba alone (Canadian grain commission 2021). The Canadian Grain Commission sets the tolerance ranges from 0.25% of FDK in samples for number one grade to 1.5% of FDK for number three grade and 4% FDK for feed grade for CWRS wheat (Canadian grain commission 2021). Hence, with over 1% FDK in most grain samples, growers experienced huge economical losses in 2016. In Alberta, losses in the CWRS class due to FHB in 2016 were 12.8 million Canadian dollars with 32.8% incidence and 0.59% severity in grain samples (Komirenko 2018). Therefore, total losses from FHB for all wheat classes in 2016 is assumed to be higher than for the CWRS class alone. Generally, FHB has caused losses from 50 million to $300 million Canadian dollars annually since the early 1990s (Government of Alberta 2021). Although advances made in managing FHB and DON over the last decade have been able to reduce economic losses from this disease, FHB and DON continue to cause significant economic losses. In order to minimize FHB, it is necessary to understand the disease cycle of *Fusarium* spp.

2.2.2 FHB disease cycle

Fusarium spp. undergo only one disease cycle per growing season (Gilbert and Fernando 2004; Skelsey and Newton 2015). *Fusarium* spp. function as saprotrophs, biotrophs, and necrotrophs as they progress through the disease cycle. As a saprotroph, *Fusarium* spp. overwinter on infected crop residues from the previous crop and produce sexual and asexual spores (Figure 2.1). Three types of asexual spores which are microconidia, macroconidia, chlamydospores are also developed from conidiophores, sprodochium, the hyphae and macroconidia, respectively (Dweba et al. 2017). Asexual spores are dispersed by rain-splash or wind, thus they can travel only short distances (Osborne and Stein 2007). When the weather warms, perithecia develop and discharge sexual ascospores (Gilbert and Haber 2013). Ascospores are discharged into the air and can travel extensive distances in the order of kilometers (Gilbert and Fernando, 2004; Osborne and

Stein, 2007). Release of asexual spores and sexual spores often overlap with the anthesis stage of wheat. During anthesis, primary infection occurs when the ascospores and conidia land on the florets of susceptible wheat spikes. The optimal temperature for germination of both spores is between 25 C° and 30 C° and higher moisture levels help *F. graminearum* to germinate, penetrate and infect wheat spikes (Osborne and Stein 2007). Once the host is infected, *Fusarium* becomes biotrophic; fungal hyphae penetrate directly into the glume, palea, or lemma to enter the floret, and travel to the ovary. When the fungal hyphae have successfully colonized the plant, they can invade neighboring florets and infect other kernels. Hyphae then spread into neighboring spikelets through vascular bundles. Following this, the pathogen becomes necrotophic and takes nutrients from the plant leading to development of unhealthy kernels. After harvest, fungi continue to grow on plant residues and become dormant during the winter. After overwintering, the disease cycle repeats again (Gilbert and Fernando 2004).

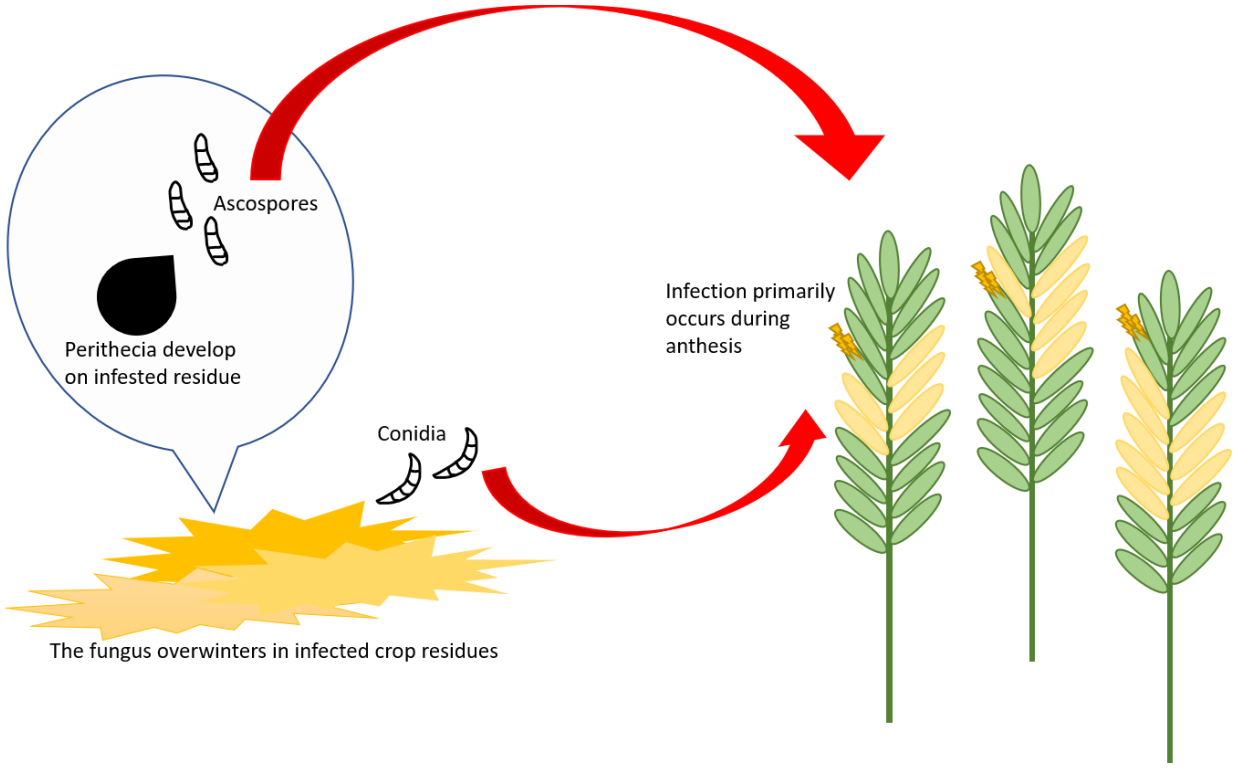

Figure 2.1. A simplified disease cycle of *F. graminearum* (Scheale III and Bergstrom 2003).

2.2.3 Symptoms

Fusarium head blight symptoms in wheat first appear as water soaked brownish discoloration at the base of the glumes and then the spikelet becomes bleached in colour (Bushnell et al. 2003). As the fungus spreads vertically through the vascular bundles, this discoloration extends into the rachis and neighboring spikelet within the same spike. When environmental conditions are highly favorable for FHB development, orange-pink colored sporodochia or bluish-black perithecia develop on the base of the glumes. Anthesis occurs first in the spikelets near the middle of the wheat spike and then proceeds from there to the top and base of the spike. As a result, FHB symptoms usually begin in the middle of the spike and progress outwards. Infected wheat spikes show premature bleaching/whitening of tissues as FHB infection proceeds (McMullen et al. 2012b; Valverde-Bogantes et al. 2020).

When the fungus infects the wheat spike during anthesis, kernel growth becomes stunted, resulting in *Fusarium* damaged kernels (FDK); these kernels are typically white, light weighted, shriveled, and have a hardened chalky texture (McMullen et al. 2012b; Gilbert and Haber 2013). Sometimes the infected kernels have salmon colored discoloration (Gilbert and Fernando 2004). Since wheat is more vulnerable and susceptible to FHB infection during anthesis, the degree of kernel damage decreases the longer the post-anthesis period before infection occurs.

2.2.4 Mycotoxins

Mycotoxins are secondary metabolites produced by fungal species that are toxic to plants, animals, and humans. *Fusarium graminearum* produces two main classes of mycotoxins, zearalenone and trichothecenes, of which the latter are the predominant mycotoxins associated with FHB (Valverde-Bogantes et al. 2020). The trichothecenes are eukaryotic protein synthesis inhibitors (McCormick et al. 2011; Foroud et al. 2019a) that are resistant to degradation by high temperature and thus will remain after cooking (Sudakin 2003). During the FHB infection process, mycotoxins accumulate in the kernels of infected spikes resulting in reduction of grain quality and posing a significant risk to food and feed safety. In plants, trichothecenes accumulate in, and cause necrosis of infected tissues. Furthermore, they may function as important aggressiveness and virulence factors in some plant hosts (Foroud et al. 2019, McCormick et al. 2011). In humans and animals, ingestion of trichothecenes is implicated in immune problems, vomiting, anorexia, and even death at high doses (Chen et al. 2019).

Trichothecenes can be split into four groups; types A, B, C, and D. Only type A and B trichothecenes are associated with FHB (Alexander et al. 2011). All trichothecenes share a tricyclic skeleton with epoxide function, and can be classified by patterns of oxygenation and acetylation of the skeleton (Alexander et al. 2011; McCormick et al. 2011). The difference between

trichothecene type A and B is that type A has an ester bond at the carbon 8 (C8) position, whereas type B has a ketone at the C8 position (Alexander et al. 2011, Leonard and Bushnell 2003).

2.2.4.1 Trichothecene type B

Type B trichothecenes are considered the most important toxins because they are most commonly produced by members of the *F. graminearum* species complex (FGSC), and are identified in association with FHB-infected crops in North America (Valverde-Bogantes et al. 2020). In addition, DON in the trichothecene type B class is the primary mycotoxin in *Fusarium* infected grain in North America and is more phytotoxic than type A trichothecenes in wheat (Amarasinghe et al. 2019). Deoxynivalenol (DON) acts as a virulence factor in wheat and facilitates the spread of the fungus in the plant and subsequent symptom development. (Bai and Shaner 2004). Deoxynivalenol in grain decreases starch and protein quality. Furthermore, ingestion of DON is toxic to both animals and human. There are advisory limits for DON in cereals for human and animal consumption in many countries (Table 2.1).

Table 2.1. Maximum allowable limits of deoxynivalenol (DON) for human consumption in different countries and organizations.

Country	Maximum Limits for DON (µg/kg)	Food commodities
Canada	2000	Unprocessed soft white wheat in non-staple foods
	1000	Baby foods (Government of Canada 2020)
United States	1000	DON on finished wheat products (FDA 2010)
Japan	1100	Wheat (Anukul et al. 2013)
China	1000	Wheat flour and breakfast cereals (Ye 2015)
Colombia	100 – 1750	Food (Salazar-González et al. 2020)
Europe Union	500	Cereal products
	750	Flour used as raw materials (Anukul et al. 2013)
OECD	500 - 1750	Cereals, wheat and bread (Directorate et al. 2018)

Fusarium graminearum isolates are categorized into three different chemotypes; 3-ADON, 15-ADON, and NIV; in type B trichothecenes. Different genes control the trichothecene biosynthetic pathway and are used to characterize different chemotypes (Foroud et al. 2012; Malihipour et al. 2012). The 3-ADON chemotype produces deoxynivalenol (DON) and 3-acetyldeoxynivalenol (3-ADON). The 15-ADON chemotype produces DON and 15-acetyldeoxynivalenol (15-ADON). The NIV chemotype produces nivalenol (NIV) and its acetylated derivatives. In general, NIV isolates are less aggressive than DON producing isolates on some cereal crops and accumulate less trichothecene in cereal grains than the DON producing isolates (Foroud et al. 2012). Among type B trichothecene chemotypes, *F. graminearum* isolates are classified into either 3-ADON or 15-ADON chemotypes in North America, thus, until recently, only DON and its acetylated derivatives 3-ADON and 15-ADON have been found as trichothecene type B mycotoxins in North America (Gilbert and Haber 2013). However, new emerging NIV isolates has been reported in USA, and 15-ADON chemotypes have been replaced by 3-ADON chemotypes in some regions of North America (Amarasinghe et al. 2019).

In general, 3-ADON isolates grow faster and produce higher levels of DON and macroconidia than 15-ADON isolates (Gilbert et al. 2010; Foroud et al. 2012; Amarasinghe et al. 2019; Valverde-Bogantes et al. 2020). Also, plants inoculated with 3-ADON isolates were reported to have higher DON accumulation than those inoculated with 15-ADON isolates (Gilbert et al. 2010; Foroud et al. 2012). Therefore, 3-ADON isolates have been reported as more aggressive than 15-ADON isolate.

2.2.4.2 Trichothecenes type A

Type A trichothecenes include the T-2 and HT-2 toxins. They are less toxic in plants than in humans and animals (Mesterházy 2003). Ingestion of type A trichothecenes can cause fetal conditions such as alimentary toxic aleukia in humans and animals (Foroud et al. 2019a).

Recently, two new emergent groups of Type A trichothecenes, NX-2 and NX-3 from isolates of *F. graminearum* were reported in the northern USA and southern Canada (Varga et al. 2015; Kelly et al. 2016; Valverde-Bogantes et al. 2020). NX-2 is similar to 3-ADON, but lacks the ketone at the C8 position and its deacetylated form (NX-3) is equivalent to DON except for the ketone at the C8 position (Varga et al. 2015). The NX-2 chemotype is presumed to have evolved from a type B ancestor, because of recent natural selection and its limited distribution in North America (Kelly et al. 2016). As new toxins, NX-2 has the potential to escape glycosylation, and NX-3 has similar potency to inhibit protein synthesis as DON (Varga et al. 2015), it is important to keep monitoring the type A trichothecenes. Furthermore, there could be the potential for additional population shifts in the future because of climate change and global warming (Valverde-Bogantes et al. 2020). Monitoring changes in the pathogen population is critical to ensure breeders are using the right genes to breed for resistance to FHB and that growers utilize the right FHB control strategies.

2.2.5 FHB Control strategies

Fusarium head blight control strategies can be divided into four different management methods: cultural, chemical, biological, and genetic resistance. Successful management of FHB cannot be fully achieved by a single strategy. Because FHB is a complex disease, it requires an integrated pest management approach.

2.2.5.1 Cultural Strategies

Most cultural strategies to control FHB focus on reducing exposure of the crops to inoculum at anthesis by disturbing the disease cycle. As *F. graminearum* overwinters on crop residue and produces spores in the spring, conventional tillage can be used to invert the topsoil which buries crop residues and reduces potential inoculum build-up. However, studies have shown inconsistent results on the relationship between conventional tillage and FHB infection. Miller et al. (1998) indicated conventional tillage reduced FDK in wheat. In addition to this, Dill-Macky and Jones (2000) found moldboard plowed plots had lower FHB incidence and severity than no-till plots. However, Lori et al. (2009) found no significant difference between the impact of tillage and no tillage on FHB. Currently reduced tillage has been recommended to prevent soil erosion and reduce energy costs. Utilization of FHB resistant cultivars and consideration of environment conditions and disease pressure are more important factors to reduce FHB than applying tillage alone (Miller et al. 1998; Lori et al. 2009). Burning of infected crop residues and mechanical chopping of crop residues have been used to remove inoculum and promote decomposition of residues (Dill-Macky 2008; McMullen et al. 2012b). Crop rotation between host crops of *F. graminearum* and non-host crops provide time for degradation of infected crop residues and reduces the amount of infected residue for pathogen overwintering (Gilbert and Haber 2013). Rotating wheat with non-host crops such as clover, alfalfa, canola, and soybean reduced FHB infection in wheat (Dill-Macky and Jones 2000; Pirgozliev et al. 2003; McMullen et al. 2012b). Controlling host weeds could also reduce potential FHB inoculum (Pirgozliev et al. 2003). Early crop harvest can reduce further fungal growth on wheat spikes prior to harvest. The fraction of FDK in the harvested crop can be reduced by adjusting screen sizes or increasing combine airflow in order to remove the smaller and lighter FDK during harvest (McMullen et al. 2012b).

2.2.5.2 Chemical strategies

Control of FHB and DON with fungicides began in 1997 (McMullen et al. 2012b). This is the main approach for controlling FHB in many regions in the world. Available fungicides suppress FHB and DON, rather than eliminating FHB completely. The most widely used class of fungicides for the control of FHB and DON are demethylation inhibitor (DMI) fungicides (McMullen et al. 2012b). Demethylation inhibitors such as tebuconazole, metconazole, and prothioconazole provide consistent control of FHB and DON accumulation by preventing cell membrane formation of the fungus (Zhang et al. 2009). Other fungicide classes are the quinone inhibitor fungicides. The quinone inhibitors, such as strobilurin, block fungal respiration. However, strobilurins are not recommended for control of FHB and DON because they have been shown to increase DON level in grain (Chen et al. 2019; Bolanos-Carriel et al. 2020). Another fungicide class is succinate dehydrogenase inhibitor (SDHI). A new SDHI, pydiflumetofen, inhibits an enzyme involved in cell respiration. This is currently registered in Canada for protecting wheat mainly against FHB. In China, pydiflumetofen provided good control of FHB by inhibition of mycelial growth and conidium germination *in vitro* and in the field (Hou et al. 2017; Sun et al. 2020). In Canada, pydiflumetofen-containing fungicide reduced DON concentration by more than 50% (Xia et al. 2021).

Applying fungicide at the optimal timing is important for management of FHB. Application of fungicides just prior to anthesis, at anthesis, or up to six days after anthesis, when FHB infection is most favorable, has been recommended to achieve excellent FHB control (Mesterhazy 2003; McMullen et al. 2012b; Chen et al. 2019). Long term intensive application of the same fungicide can lead to fungicide resistance to certain modes of action in the pathogen. It has been reported that there are some triazole fungicide (DMI class) resistant isolates in *F. graminearum* (Yin et al.

2009; Spolti et al. 2014). Once the fungal populations develop resistance to a particular fungicide mode of action, there will be fewer fungicides available for growers to control FHB effectively. Therefore, rotating the right fungicide class at recommended timing of application is crucial for control FHB.

2.2.5.3 Biocontrol strategy

Biocontrol is an environmentally friendly way to manage FHB; specifically, it plays an important role in organic cereal production. Biocontrol agents are living, non pathogenic microorganisms that disrupt the pathogen's life cycle. Several fungi, bacteria, yeasts, and earthworms have been shown to be antagonistic against *F. graminearum*. The major modes of actions of these organisms are competition for nutrients, induction of localized resistance, and production of antifungal metabolites (McMullen et al. 2012b).

As a fungal antagonist of *F. graminearum*, Strain ACM941 of *Clonostachys rosea* showed potential suppression of *F. graminearum* in greenhouse studies (Xue et al. 2009; Gilbert and Haber 2013). However, using *Clonostachys rosea* as a biocontrol agent did not show consistent results in field studies. Xue et al (2009) and Gilbert and Haber (2013) showed possible suppression of *F. graminearum* using *C. rosea*, whereas Nowakowski (2018) indicated *C. rosea* did not show suppression of *F. graminearum* in the field.

Cryptococcus spp. are yeast antagonists of *F. graminearum* and work by competing for nutrients with the pathogen. Schisler et al. (2011) demonstrated that *Cryptococcus* spp. significantly reduced FHB disease severity in controlled environments; however, the results were discordant with field studies (Khan et al. 2004; Schisler et al. 2011; Gilbert and Haber 2013).

Bacillus spp. are the most commonly investigated biocontrol bacterial agents (Gilbert and Haber 2013). They produce antifungal metabolites to repress the growth of DON-producing fungal

species and reduce DON accumulation in the cereal host (Gilbert and Haber 2013). Although *Bacillus* spp. inhibited growth of *F. graminearum* in vitro, results under field conditions were equivocal (Zhao et al. 2014; Palazzini et al. 2016).

Different earthworm species such as *Aporrectodea caliginosa* and *Lumbricus terrestris* function as secondary decomposers to reduce *Fusarium* biomass and DON in wheat residues (Wolfarth et al. 2011, 2016). Despite earthworm's ability to reduce *Fusarium* inoculum, their use as a control agent is limited as there are no economical ways to produce high numbers of them. Many earthworm species such as *A. caliginosa* and *L. terrestris* are native to Europe, and may be incompatible with tilling and other agronomic practices (Wolfarth et al. 2011; Shekhovtsov et al. 2016; Klein et al. 2017). In addition to that, biocontrol may be a useful strategy for FHB control, but its adoption and success are limited by environment conditions, dose of the antagonist, and the wheat cultivar. To date, no biocontrol agent has been shown to reduce FHB to levels comparable with a fungicide. A combined management approach involving resistance cultivars, tillage, and crop rotation should provide better FHB control.

2.2.5.4 Breeding FHB resistant cultivar

The development of FHB resistant cultivars is the most effective, sustainable, and economical approach to control the disease. Growing FHB resistant cultivars can reduce economic costs by reducing chemical applications; this reduced need for chemicals has the added benefit of being more ecologically conscientious. Growing FHB resistance cultivars will reduce losses of yield and quality. However, because the level of resistance available in commercial cultivars is limited, FHB resistant cultivars must be used in combination with other control strategies to maximize the efficacy. Breeding of FHB resistant cultivars combined with other desired agronomic traits remains a gargantuan challenge as the resistance trait is quantitative; multiple

genes each with small effect are involved in the control of resistance, and these genes are susceptible to environmental factors. Furthermore, genetic sources of FHB resistant are limited and many available FHB sources possess undesired agronomic traits such as tall height, small spikes, and late maturity (Bai and Shaner 2004; Thambugala et al. 2020). For example, short plant statue is a desirable agronomic trait as the plant's energy contributes to grain yield rather than vegetative growth; thus short plants typically produce higher yield and have a reduced risk of lodging. However, short plants are closer to the *Fusarium* inoculum source and tend to have denser leaf growth: this growth pattern is conducive to *Fusarium* growth as it reduces air circulation and increases humidity (Buerstmayr and Buerstmayr 2016). As resistant accessions are associated with undesired agronomic traits, it is difficult to combine elite cultivars with FHB resistance (Bai and Shaner 2004; Foroud et al. 2019a). Moreover, the functions underlying FHB resistance genes are still largely unknown despite identification of more than 550 quantitative trait loci (QTLs) covering the whole genome of wheat (Fabre et al. 2020).

2.2.5.4.1 Types of FHB resistance

Five types of FHB resistance are documented (I to V) (Foroud et al. 2019a). Types I and II resistances are the most commonly studied and deployed by breeding programs. Type I resistance reduces initial infection. The assessment of type I resistance is estimated as a percentage of diseased spikes (Bai and Shaner 2004; Foroud et al. 2019a). Type II is resistance to the spread of infection within the infected spike. Type II resistance is evaluated by point inoculation, where spores are injected into single florets and resistance is quantified as a percentage of diseased spikelets within infected spikes (Mesterházy 2003). Type I resistance tends to be less stable and more affected by non genetic factors compared to type II resistance. Type I resistance can only be detected under low epidemic conditions as under high disease pressure all, or most, spikes will

show some level of infection (Bai and Shaner 2004). In addition, excellent type I resistance germplasm is scarce and measuring type I resistance is difficult (Xue et al. 2011). QTL for Type I and II resistance have been commonly identified using field studies. Effective field control requires both type I and II resistance (Bai and Shaner 2004). Type III resistance is resistance to kernel infection. Even if initial infection occurs, the degree of kernel infection can differ based on the level of type III resistance (Mesterházy 1995, 2003). Type IV resistance is tolerance against FHB and trichothecenes; regardless of the presence of disease, tolerant wheats maintain yield (Mesterházy 1995, 2003). Type V resistance is resistance to accumulation of trichothecenes in the grain. Depending on the mechanism of preventing trichothecenes in the grain, type V resistance is further subdivided into class 1 and class 2. In class 1, plants have the ability to degrade or detoxify trichothecenes, whereas in class 2, plants limit trichothecenes accumulation in their tissues (Foroud et al. 2019a). Breeding efforts towards improved type I and II resistance can be facilitated through the use of marker assisted selection.

2.2.5.4.2 Quantitative trait loci and resistance sources for FHB

Molecular markers help to find more precise information on the number and location of quantitative trait loci (QTLs); locations within the genome that contain genes contributing a particular quantitative trait (Collard et al. 2005). The deployment of molecular markers and marker assisted selection can facilitate plant breeding by selecting more efficiently for desirable traits such as selecting resistance genes from different sources and incorporating them into elite cultivars (Bai and Shaner 2004).

The *Fhb1* resistance QTL has been the most widely used FHB resistance QTL for wheat globally. It is derived from the Chinese spring wheat cultivar ‘Sumai-3’ and is located on the short arm of chromosome 3BS (Kazan and Gardiner 2018). *Fhb1* is associated with type II and V

resistance which are resistance to spreading fungus and accumulation of trichothecenes in the grain, respectively. It enhances the ability of the plant to convert DON into DON-3-glycoside, a non toxic product to the plant (Steiner et al. 2017; Foroud et al. 2019a). As an improved cultivar, Sumai-3 has good combining ability for both FHB resistance and yield traits; furthermore, Sumai-3 and its derivative cultivars have excellent type II resistance with consistent resistance to most *F. graminearum* isolates in the world (Bai and Shaner 2004). Sumai-3 has other major QTLs for FHB resistance as well; *Fhb2* on chromosome 6BS, and *Qfhs.ifa-5A* on chromosome 5A (Cuthbert et al. 2007; Steiner et al. 2017). *Fhb2* confers increased type II resistance (Cuthbert et al. 2007), while *Qfhs.ifa-5A* is mainly associated with type I and type V resistance (Steiner et al. 2017). Carrying *Fhb1* or *Qfhs.ifa-5A* alone reduced DON content on average by 59% and 43%, respectively, and when both *Fhb1* and *Qfhs.ifa-5A* were present, DON content was reduced by 79% (Foroud et al. 2019a). In North America, the practical deployment of major resistance QTL for FHB consists of *Fhb 1,* which is in 50% of FHB resistance wheat cultivars, and *Qfhs.ifa-5A* which is present in 5 to 10% of resistant cultivars (Steiner et al. 2017).

The Chinese wheat landrace, 'Wangshuibai', has a high level of FHB resistance. It has resistance QTL *Fhb 4* on chromosome 4B and *Fhb 5* on chromosome 5A (Xue et al. 2010, 2011). Both QTL mainly confer type I resistance. However, combining resistance genes from Wangshuibai into elite lines has been difficult because *Fhb 4* and *Fhb 5* are negatively related with desirable agronomic traits (Bai and Shaner 2004; Xue et al. 2010, 2011).

2.2.5.4.3 Passive resistance factors

Morphological and phenological traits such as plant height, anther retention, floral traits, and the presence of awns contribute to passive resistance for FHB (Gilbert and Haber 2013; Steiner et al. 2017; Buerstmayr et al. 2020). Furthermore, many FHB resistance QTL co-localize with

QTL for plant height and anther retention (Xue et al. 2010; Liu et al. 2013; Petersen et al. 2017; Steiner et al. 2017, 2019; Foroud et al. 2019a). Although shorter plants are closer to the inoculum source and provide favorable conditions for FHB infection, they also carry semi-dwarfing alleles *Rht-B1b, Rht-D1b* or *Rht 8c* which are associated with increased FHB susceptibility (Buerstmayr et al. 2020). The presence or absence of awns are also associated with FHB resistance (Gaikpa et al. 2020) as awns can capture airborne or splashed *Fusarium* spores, thus, increasing the chances of exposing the spike to inoculum (Mesterházy 2003). Generally, awned cultivars have an 80% higher disease incidence than awnless cultivars (Mesterházy 2003; Liu et al. 2013). Floral traits also contribute to FHB resistance. Narrow and short floral openings can promote anther retention by trapping anthers between the lemma and palea. Retained anthers provide a path for the fungus to enter and infect the host (Buerstmayr et al. 2020); therefore narrow and short floral openings can increase FHB infection by facilitating the establishment of fungus through retained anthers. Furthermore, wheat cultivars with retained anthers are more susceptible to FHB (Mesterházy 2003; Liu et al. 2013; Steiner et al. 2017; Buerstmayr et al. 2020). Visual selection for morphological and phenological traits is a rapid and cost effective way to phenotype for FHB resistance in breeding programs.

2.3 Interaction between wheat and FHB

2.3.1 Semi-dwarfing gene

With the Green Revolution in the 1960s, three major dwarfing alleles were introduced into wheat; GA insensitive mutant semi-dwarfing alleles at the *Reduced height* (*Rht-B1* and *Rht-D1*) loci on chromosomes 4B and 4D, and GA sensitive alleles *Rht8* loci on chromosome 2D (Hedden 2003; Gilbert and Haber 2013). Together, these genes have contributed to an increase in grain yields by increasing harvest index and reducing lodging. To date, one or more of the semi-dwarfing alleles, *Rht-B1b, Rht-D1b* and *Rht8c* of which *Rht8c* represents a small proportion, are found in

more than 70% of modern wheat cultivars worldwide (Miedaner and Voss 2008; Guedira et al. 2010; Buerstmayr and Buerstmayr 2016; Mo et al. 2018).

The GA insensitive semi-dwarf *Rht* loci on chromosomes 4B and 4D (*Rht-B1b* and *Rht-D1b*) are the most commonly used semi-dwarf mutants in modern wheat cultivars since the Green Revolution. They are originally from the Japanese cultivar 'Norin 10' (Gilbert and Haber 2013). They not only affect plant height, but also modulate spikelet fertility and grain number per spike, leading to dramatic increases in yield (Miedaner and Voss 2008; Buerstmayr et al. 2020). Mutant GA insensitive *Rht* alleles encode single-nucleotide polymorphism (SNP) mutations in the gene encoding DELLA protein that cause reduced sensitivity to gibberellins, resulting in reduced height (Buerstmayr and Buerstmayr 2016; Muqaddasi et al. 2017b; Steiner et al. 2017). Although semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* have been used to generate short cultivars, those which contain *Rht-B1b* or *Rht-D1b* tend to be susceptible to FHB. The *Rht-B1b* and *Rht-D1b* alleles are associated with decreasing type I FHB resistance (Gilbert and Haber 2013; Liu et al. 2013). Interestingly, the presence of the *Rht-D1b* allele had strongly increased FHB severity, whereas the presence of the *Rht-B1b* allele increased resistance against FHB severity, rendering plants with *Rht-B1b* less susceptible than those with *Rht-D1b* (Miedaner and Voss 2008; Liu et al. 2013; Lu et al. 2013; Buerstmayr and Buerstmayr 2016).

The GA sensitive gene *Rht8* on chromosome 2D is closely linked to the photoperiod insensitivity gene *Ppd-D1* (Gilbert and Haber 2013; Chen et al. 2016; Islam et al. 2016). This *Rht8* is derived from the Japanese cultivar 'Aka Komugi' (Gilbert and Haber 2013). Because QTL for *Rht8* coincide with *Ppd-D1*, they often occur together (Miedaner and Voss 2008; Gilbert and Haber 2013; Würschum et al. 2017). However, *Ppd-D1* itself has pleiotropic effects on plant height, and height reduction by *Ppd-D1* is greater than by *Rht8* (Miedaner and Voss 2008; Würschum et al.

2017). The semi-dwarfing allele *Rht8c* and the photoperiod insensitive allele *Ppd-D1a* are commonly used in areas which have hot and dry summers. *Ppd-D1* contributes to Increased yield by shortening the plant's life cycle, reducing plant height, and modifying both the number of grains per spikelet and the length of the grain filling period (Miedaner and Voss 2008). *Rht8c* and *ppd-D1a* show less adverse effects on FHB susceptibility than the GA insensitive genes (Miedaner and Voss 2008; Liu et al. 2013; Buerstmayr et al. 2020).

The three different dwarfing genes influence FHB susceptibility differently. Since the effect of the different semi-dwarfing alleles on plant height is approximately the same, factors other than height may cause differences in FHB susceptibility. Many studies indicate anther retention is one of the factors that affects differences in FHB severity between semi-dwarfing alleles (Skinnes et al. 2010; Lu et al. 2013; Buerstmayr and Buerstmayr 2015, 2016; He et al. 2016; Steiner et al. 2017).

2.3.2 Anther retention

Anther retention indicates anthers are retained in the floret while anther extrusion means the anthers are extruded outside the floret during anthesis. Thus, low anther extrusion is the same as high anther retention. Anthers provide a surface for attachment and entry, as well as nutrients, for the fungus, thereby retained anthers within spikelets increase susceptibility to FHB by decreasing type I resistance (Strange et al. 1972; Buerstmayr and Buerstmayr 2015, 2016; He et al. 2016). Anther retention has been shown to be highly correlated with FHB susceptibility (Buerstmayr and Buerstmayr 2015, 2016; He et al. 2016). Anther retention is a phenotypically diverse and continuous trait which suggests it is quantitative in nature. Many studies indicated highly accurate selection of anther retention is possible since this trait has high heritability (Skinnes et al. 2010; Muqaddasi et al. 2017b; Steiner et al. 2017). Anther retention is a quantitative

trait which means it is controlled by several loci with minor effect (Lu et al. 2013; Buerstmayr and Buerstmayr 2016; Muqaddasi et al. 2017b; Steiner et al. 2017). Lu et al. (2013) and Steiner et al. (2017) stated two of the three QTLs for anther retention overlapped with QTL for FHB susceptibility and five QTLs for anther extrusion overlapped with QTL for other FHB traits. Furthermore, the QTL for anther extrusion on 5AS and 5Ac are closely linked in the *Fhb5* region (Xu et al. 2020). Interestingly, the *Rht* loci also influence anther retention. Quantitative trait loci (QTLs) for anther retention on 4BS and 4DS are linked with *Rht-B1* and *Rht-D1*, respectively (Xu et al. 2020). *Rht-D1b* showed a stronger impact on percentage of anther retention than *Rht-B1b* (Lu et al. 2013; Buerstmayr and Buerstmayr 2016). This might be the reason why both semi-dwarfing alleles have different degrees of FHB susceptibility (Buerstmayr and Buerstmayr 2016). Since the *Rht-B1b* and *Rht-D1b* semi-dwarfing alleles affect the sensitivity of the plant to GA and GA regulates cell elongation rather than cell division in stamen development and filament extension, it could affect the development of shorter filaments (Buerstmayr and Buerstmayr 2016). Hence, reduced length of filaments promotes anther retention.

In addition to GA, Jasmonic acid (JA) also contributes to the last step of stamen development such as filament elongation and anther dehiscence (Peng 2009; Wilson et al. 2011; Marciniak and Przedniczek 2019). Gibberellin promotes JA production for stamen development by initiating the degradation of DELLA proteins (Marciniak and Prezdniczek 2019) which means they act synergistically during stamen development (Qi et al. 2014). Inhibiting one of the JA and GA pathways resulted in short filament in Arabidopsis (Cheng et al. 2009; Qi et al. 2014).

2.3.3 Phytohormones

Phytohormones are growth regulators which not only affect the growth and development of plants, but also play a role in plant defence against biotic and abiotic stresses (Egamberdieva et al. 2017).

2.3.3.1 Gibberellins and its function

Gibberellins are phytohormones that regulate the growth and development of plants through their influence on height, flower induction, pollen development, pollen tube growth, stamen development, filament extension, anther development and retention. Mechanistically, these phenotypes arise from the action of GA on cell proliferation, differentiation, and expansion, as well as the direction of cell elongation (Claeys et al. 2014; Hedden and Sponsel 2015; Muqaddasi et al. 2017b). Gibberellins are transported by diffusion through the xylem and phloem. The central regulators of the GA signalling pathway are the proteins of the DELLA family. The proteins of the DELLA family are known as growth inhibitors because they are key intercellular repressors of GA-dependent processes such as seed germination and growth (Daviere and Achard 2013; Nelson and Steber 2016). The DELLA family contains an N terminal regulatory domain and a C terminal functional domain which include three genes; GA insensitive (GAI), Repressor of GA1 (RGA), and SARECROW (SCR) which are called together as GRAS (Figure 2.2) (Nelson and Steber 2016).

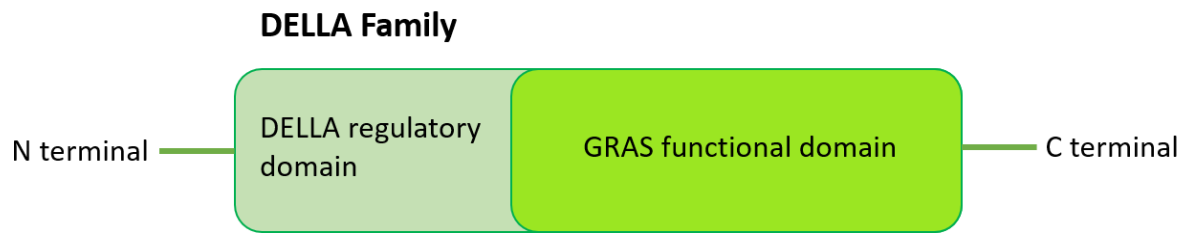

Figure 2.2. Schematic Diagram of DELLA family (Nelson and Steber 2016; Thomas et al. 2016).

In the absence of bioactive GA, GA responses are repressed by DELLA proteins (Figure 2.3). In the presence of bioactive GAs, GAs bind to the receptor GA-INSENSITIVE DWARF1 (GID1), and form a GA-GID1-DELLA trimeric complex by binding on the N terminal of the DELLA family (Figure 2.3). This complex is responsible for DELLA degradation and de-repression of GA responses. Thus, DELLA proteins prevent plant growth, while GAs promote plant growth by overcoming DELLA-mediated growth restraint (Daviere and Achard 2013). Plants with semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* encode premature stop codons in the DELLA proteins preventing the N terminal of the DELLA proteins from being bound by the GID1-GA complex (Thomas 2017). Hence, this mutation abates the GA-induced degradation of DELLA and keeps the DELLA repressing GA-responsive growth and development.

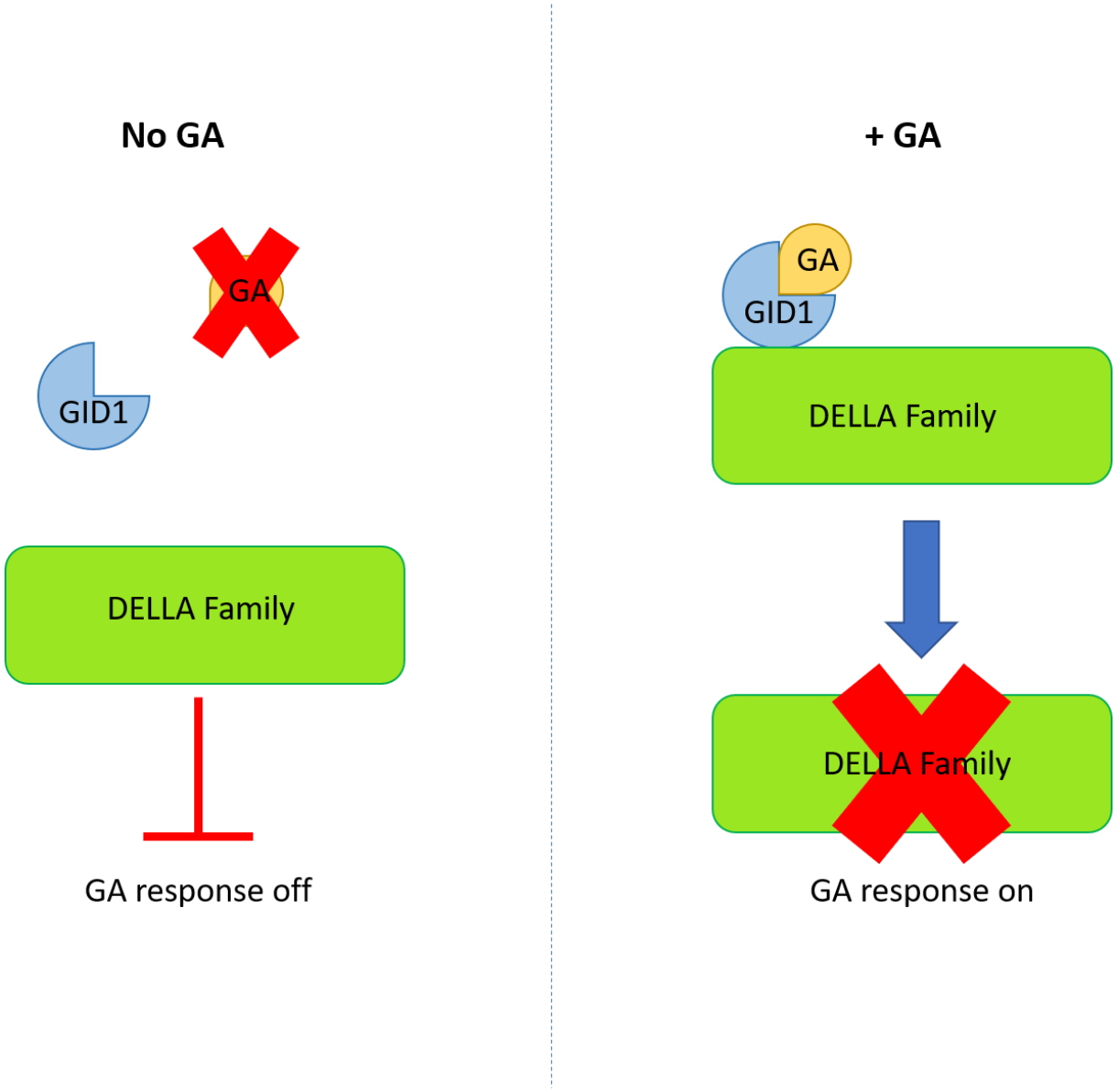

Figure 2.3. Model of GA signalling on plants. In the absence of GA, DELLA proteins are stable and repress GA response (left). In the presence of GA, GA binds GID1 followed by binding DELLA protein. DELLA protein will then be degraded (right) (Nelson and Steber 2016)

Gibberellins also play roles in the regulation of plant disease resistance and susceptibility (Bari and Jones 2009). In Arabidopsis, GA enhances resistance to biotrophs and susceptibility to necrotrophs by stimulating degradation of the DELLA protein (Navarro et al. 2008). The DELLA proteins activate the JA/ethylene (ET) pathway to increase resistance to necrotrophs (Navarro et al. 2008). Furthermore, the DELLA proteins have the ability to lower reactive oxygen species (ROS) which are often generated in response to stress (Achard et al. 2008). By repressing the accumulation of ROS, DELLA proteins contribute to delayed cell death and promote tolerance against necrotrophs (Achard et al. 2008). In wheat, the DELLA protein plays a role in a similar manner to that in Arabidopsis by promoting susceptibility to biotrophs and resistance to necrotrophs (Saville et al. 2012).

2.3.3.2 Ethylene and its function

Ethylene (ET) is considered to be a multifunctional phytohormone, since it affects diverse aspects of plant growth, development, and senescence (Iqbal et al. 2017). As ethylene is a gaseous hormone, it is transported by gaseous diffusion. Depending on the concentration of ET, timing of application, and the plant species, ethylene either promotes or inhibits plant growth by regulating cell size, cell elongation, and cell division (Dugardeyn et al. 2008; Schaller 2012). However, the most prominent roles of ET are inhibition of stem elongation, increase in stem thickening, and inducing fruit ripening (Dugardeyn et al. 2008). Ethylene affects DELLA proteins by inhibiting GA synthesis. It inhibits GA synthesis, thus it stabilizes DELLA protein which makes plants insensitive to GA (Ross et al. 2016; Iqbal et al. 2017). Hence, ET and GA interact with each other (Schaller 2012; Bakshi et al. 2015; Ross et al. 2016). Furthermore, ET participates in crosstalk with auxin to inhibit elongation by reducing auxin synthesis and mobility in stem tissue (Caldwell et al. 1988; Ramburan and Greenfield 2007; Wiersma et al. 2011; Vaseva et al. 2018).

Ethylene is also well known as a major defense hormone against various necrotrophic pathogens (Chen et al. 2009). Ethylene and JA operate in concert to facilitate defence mechanisms in response to necrotrophs and are required for induced systemic resistance (Bari and Jones 2009; Foroud et al. 2019b). On the other hand, ET facilitates the development of disease symptom by regulating senescence, thereby, promoting cell death (Li and Yen 2008; Chen et al. 2009; Wang et al. 2018). For necrotrophic pathogens, cell death would benefit the pathogen survival.

2.4 Plant growth regulators

2.4.1 Functions

Plant growth regulators (PGRs) are exogenous phytohormone supplements containing natural or synthetic compounds used to modify the growth of plants. They are not phytotoxic but influence the plant's hormonal status. In cereal crops, PGRs are used to reduce plant height and increase stem thickness to prevent lodging. As a result, proper PGR application can contribute to maximizing yield potential, especially under intensive management. The gibberellin and ET pathways are targeted by PGRs in cereal crops. There are currently two main groups of cereal plant growth regulators, anti-GA products and ET releasing compounds (Rademacher 2016). Both groups aim to reduce plant height and thicken stems to reduce the risk of lodging and yield loss.

Gibberellin biosynthesis inhibitors alter plant growth. The active ingredients chlormequat chloride (CCC) and trinexapac-ethyl are GA biosynthesis inhibitors available in Canada for use in cereals. Chlormequat chloride is the most widely used PGR in cereal production. It reduces GA production in the early steps of GA biosynthesis by blocking Copalyl diphosphate (CDP) synthase and *ent*-Kaurene synthase (Figure 2.4). Trinexapac-ethyl inhibits soluble 2-oxoglutarate-dependent dioxygenases involved in the late steps of GA synthesis (Figure 2.4). Therefore, both chemicals reduce cell elongation and cell division, resulting in compact plants (Rademacher 2016).

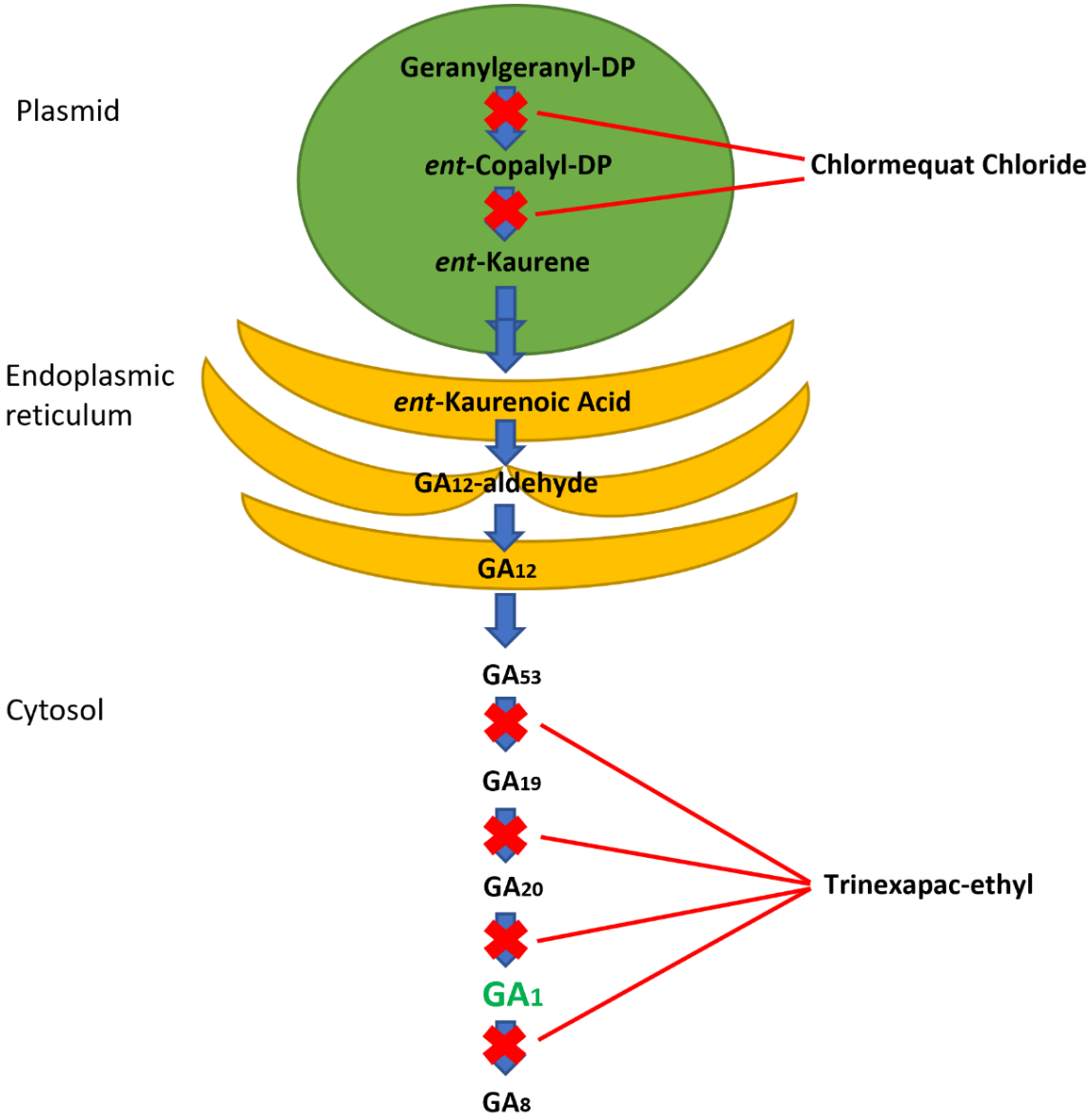

Figure 2.4. Biosynthesis of gibberellin producing biologically active GA1 and points of inhibition by plant growth regulator active ingredients (Rademacher 2016).

Ethephon acts as exogenous hormone that increases endogenous ET in the plant. It affects not only the ET pathway, but also the pathways that are inter-related with the GA pathway and auxin pathway (Wiersma et al. 2011; Sun et al. 2016; Iqbal et al. 2017). Ethylene and GA are considered as antagonists of each other. The application of exogenous ET represses the expression of GA metabolism genes (Dugardeyn et al. 2008; Iqbal et al. 2017). In addition, ET limits auxin activity in the stem by inhibiting auxin transport in the vascular tissue (Wiersma et al. 2011; Vaseva et al. 2018; Lee et al. 2021). Consequently, applying ethephon could lead to shorter plants in the opposite way as GA and auxin do.

2.4.2 Manipulator™ and its impact on FHB

Manipulator™ is an anti-GA PGR product. It contains CCC as an active ingredient. As an inhibitor of the formation of GA precursor, application of Manipulator™ results in a reduction in plant height and increased stem thickening. The window for the application of manipulator is between Zadoks growth stage (GS) 12 and 39 (Zadoks et al. 1974). However, between Zadoks GS 30 (beginning of stem elongation) and 32 (first to second node formation) is the ideal timing for the most effective results.

In the field, application of the plant growth regulator CCC increased not only *Fusarium spp* fungi, but also zearalenone concentration in the grain without artificial inoculation (Mankevičiene et al. 2008). Chlormequat chloride increased *Fusarium* infection on both spikelets and seeds (Fauzi and Paulitz 1994). Since CCC inhibits GA production in the plant, lower levels of GA in the plant could be one reason why the plant has high infection of FHB. These results are in line with Buhrow et al.'s (2016) finding which indicated that application of GA on wheat decreases FHB symptoms.

2.4.3 Ethrel™ and its impact on FHB

Ethrel™ is an ET releasing PGR product. Its active ingredient is an ethephon. The application of Ethrel™ results in elevated ET concentrations in plant tissue. This causes an inhibition in cell elongation and, thus, a reduction in plant height (Rajala et al. 2002). The window for the application of Ethrel™ is narrow, with optimal application between Zadoks GS 37 and 45 (flag leaf just visible to boot just swollen).

The direct role of ET against FHB is ambiguous. While some reports indicate that application of ET enhanced susceptibility of wheat spikes to *F. graminearum* (Nicholson et al. 2008; Chen et al. 2009), other publications indicated that ET played a positive role in FHB resistance in wheat (Li and Yen 2008; Foroud et al. 2019b). Furthermore, other researchers demonstrated that chemical treatment with ethephon did not have any impact on wheat FHB resistance level (Sun et al. 2016).

3 Chapter 3.0. Evaluation of the role of plant growth regulators on Fusarium head blight infection in spring wheat (*Triticum aestivum* L.) by *Fusarium graminearum* under field conditions

3.1 Abstract

The fungal disease Fusarium head blight (FHB) threatens Canadian wheat production with reduced yield, decreased end use grain quality, and often leads to the accumulation of deoxynivalenol, a fungal toxin that poses a threat to food and feed safety of the crop. The main causal pathogen of FHB in the Canadian prairies is *Fusarium graminearum.* Cultivars with the best FHB resistance often possess undesirable agronomic traits such as tall height and a propensity for lodging. To counteract these effects, FHB resistant cultivar must be used together with other control strategies to maximize efficacy of overall profitability. The application of plant growth regulators (PGRs) may enable farmers to grow the most resistant cultivars while controlling plant height to minimize lodging risk. Short wheat plants appear to be more susceptible than their taller counterparts to FHB. Plant height in wheat is genetically controlled by some semi-dwarfing alleles that alter the plant's sensitivity to gibberellins (GAs). The presence of semi-dwarfing alleles increases the frequency of anther retention which may contribute to FHB susceptibility by providing an initiation site for infection. In this study, five spring wheat cultivars that differed in level of FHB resistance, height, and semi-dwarfing alleles were tested to determine the effect of PGRs, specifically Manipulator™ and Ethrel™, on height, anther retention and FHB resistance level and the interactions between them in Winnipeg and Carman, Manitoba in 2019 and 2020. Combined field results showed that Ethrel™ significantly reduced plant height. Both PGRs did not affect anther retention and FHB resistance level of tested cultivars under dry conditions. There were significant interactions between variables, but they were relatively small compared to the main treatment and cultivars. Based on the results of this study, producers could benefit from the higher levels of FHB resistance often associated with tall cultivars and use PGRs to manage plant height and lodging without

increased risk of FHB. The information from this study will identify new breeding targets for breeders to directly assist in the development of Canadian spring wheat cultivars with improved FHB resistance and provide growers alternative ways to control FHB without sacrificing potential yield due to lodging.

3.2 Introduction

Wheat (*Triticum aestivum L.*) is one of the important staple food crops and is grown worldwide. As world population grows, food security issues have been increased. Wheat yield and quality must be enhanced to maintain food security. Fusarium head blight (FHB) is a fungal disease mainly caused by *Fusarium* species. In North America, *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.) Petch] is the predominant FHB pathogen (Gilbert and Haber 2013). Wheat infected by *F. graminearum* causes tremendous economic losses through reduction of yield and end-use quality of the grain, and accumulation of mycotoxins such as deoxynivalenol (DON) that pose a threat to food and feed safety (Buhrow et al. 2016). Losses in Canada caused by FHB have been estimated to be $50 to $300 million annually since the early 1990s (Government of Alberta 2021).

Breeding for resistance to FHB has increased the number of cultivars with moderate to intermediate resistance to FHB (Seed Manitoba 2018). Nevertheless, most cultivars with better FHB resistance tend to be taller, which makes them more difficult to adopt in regions with high moisture and higher lodging potential. Generally, growers prefer shorter cultivars, since shorter cultivars are not prone to lodging and tend to produce higher yield under intensive management. However, research has shown that short plants tend to have higher levels of FHB than taller plants. There are two possible explanations for this problem. One explanation is that shorter plants are closer to the inoculum source from crop residues and are thus exposed to higher levels of inoculum

and humidity that promote disease development (Hilton et al. 1999; Buerstmayr et al. 2009). Despite this explanation, research using spray inoculation directly on the spike shows that the association between higher FHB and the shorter plants exists even when all spikes receive the same quantity of inoculum (Mwaniki 2017). Therefore, the proximity to inoculum and humidity cannot fully explain why shorter plants are more susceptible to FHB. Another explanation for higher FHB susceptibility of short plants is that they carry one or more semi-dwarfing alleles. One of the possible reasons that plants carrying semi-dwarfing alleles are more susceptible to FHB may be that the *Rht-B1b* and *Rht-D1b* semi-dwarfing alleles affect the plant's sensitivity to gibberellins (GAs), and shorten the plants by affecting cell elongation, including shortening the filament of the anthers. This may result in anther retention during flowering. Retained anthers between the lemma and palea provide a surface for the fungus to land on, and nutrients to support fungal grow which allows the fungus to enter the floret (Buerstmayr and Buerstmayr 2016). Thus, retained anthers constitute a preferred target for initial infection (Buerstmayr and Buerstmayr 2015).

There are two main FHB resistance types that can be evaluated in the field; type I and type II. Type I resistance represents resistance to initial infection (Bai and Shaner 2004; Foroud et al. 2019a). Type II resistance indicates resistance to the spread of infection within the infected spike. Since retained anthers facilitate initial establishment of the fungus, it reduces type I resistance. With high heritability of anther retention, selection for low anther retention could be efficient and would have implications for FHB resistance in wheat breeding programs. Anther retention is a quantitative trait (Skinnes et al. 2010; Muqaddasi et al. 2017a; Steiner et al. 2017; Xu et al. 2020) and has been highly correlated with FHB susceptibility (Buerstmayr and Buerstmayr 2015, 2016; He et al. 2016). Quantitative trait loci for anther retention on chromosomes 4DS and 4BS overlap with *Rht-B1* and *Rht-D1* (Xu et al. 2020). This indicates higher FHB susceptibility of semi-dwarf

plants seems to be not only due to short plant height, but that effects on anther retention also contribute to FHB susceptibility.

More than 70% of current wheat cultivars grown in the world have one or both of the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* (Buerstmayr and Buerstmayr 2016; Mo et al. 2018). The semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* have contributed to a huge increase in grain yields by substantially reducing height, thereby increasing harvest index and reducing lodging under intensive management. In the wild type plant, bioactive GAs bind to its receptor, GIBBERELLIN INSENSITIVE DWARF1 (GID1), leading to degradation of DELLA proteins by the 26 proteasome (Saville et al. 2012; Daviere and Achard 2013). However, the *Rht-B1* and *Rht-D1* loci encode DELLA proteins which act as key signaling proteins for GA response in semi-dwarf plants. A base mutation in the coding sequence of the semi-dwarfing alleles encodes a premature stop codon which translates into truncated DELLA proteins. This represses GA-responsive growth by inhibiting GA and GID1 from interacting with DELLA proteins, thus GA induced degradation of DELLA proteins is not possible and DELLA proteins repress GA response growth and development (Hedden 2003; Buerstmayr and Buerstmayr 2016; Muqaddasi et al. 2017b; Steiner et al. 2017). As a result, accumulation of DELLA proteins in semi-dwarf plants is expected to be higher than the wild type (Saville et al. 2012). The DELLA proteins also play roles in the regulation of plant disease resistance and susceptibility (Achard et al. 2008; Navarro et al. 2008; Saville et al. 2012). They enhance resistance to necrotrophs and susceptibility to biotrophs by affecting the balance of jasmonic acid (JA) and salicylic acid in the plant (Saville et al. 2012). In contrast, GA improves resistance to biotrophs and susceptibility to necrotrophs by mediating degradation of DELLA proteins (Navarro et al. 2008).

As a plant defence hormone against necrotrophic pathogens, ethylene (ET) works with JAs to promote defence mechanisms in response to necrotrophs and systemic resistance (Bari and Jones 2009; Chen et al. 2009; Foroud et al. 2019b). Ethylene also controls plant height by inhibition of shoot cell elongation (Dugardeyn et al. 2008; Ross et al. 2016; Iqbal et al. 2017). Ethylene signaling affects the GA-GID1-DELLA mechanism by decreasing the level of bioactive GA and stimulating DELLA protein buildup (Achard et al. 2003; Iqbal et al. 2017). By participating in DELLA-mediated growth inhibition, ET affects plant growth (Iqbal et al. 2017). Furthermore, ET inhibits auxin biosynthesis and movement to prevent plant growth (Strydhorst et al. 2018; Vaseva et al. 2018). Due to ET's ability to impede plant growth, ET signalling is one of the pathways to be utilized for plant growth regulators (PGRs).

Plant growth regulators are natural or synthetic compounds used to control or modify the growth of plants by altering the plant's hormonal status. Plant growth regulators are used to reduce plant height and increase stem thickness, thus improve lodging resistance in cereal crops. Also, reducing plant height by application of PGRs makes harvesting of tall cultivars easier and increases harvest index. Since cereal yields can be reduced from 7 to 35 percent from lodging (Strydhorst et al. 2018), the application of PGRs can have benefits for maximizing yield potential through improving crop standability. Plant growth regulators for cereal crops exploit either GA or ET pathways. Reduction of plant height is achieved by either of two main groups of PGRs; GA biosynthesis inhibitors and an ET-releasing compound. Manipulator™ contains the active ingredient chlormequat chloride (CCC) which is one of the GA biosynthesis inhibitors. It blocks an early phases in GA biosynthesis resulting in less cell elongation and cell division in plants (Rademacher 2000; Hedden and Sponsel 2015). Manipulator™ manipulates apical dominance of the plant, thus it makes plants produce shorter, thicker, and stronger stems for improved lodging

resistance. Manipulator™ can be applied to spring, winter, and durum wheat between Zadoks GS 12 to 39 which are from the two-leaf stage to the flag leaf collar visible stage (Zadoks et al. 1974). Ethrel™ contains ethephon as an active ingredient. As an ET releasing compound, ethephon elevates the level of ET in the plant. Ethrel™ influences plant growth by accelerating fruit ripening and maturity, and reducing lodging in spring and winter wheat. Ethrel™ can be applied on spring and winter wheat between Zadoks GS 37 to 45 which are between the early flag leaf emergence stage to the swollen-boot stage (Zadoks et al. 1974). The effects of both PGRs are specific to plant species, cultivar, and environmental conditions (Strydhorst et al. 2018). Also, the optimal timing for application is critical for ensuring successful results.

Theoretically, both Manipulator™ and Ethrel™ reduce plant height by reducing cell elongation. If filaments are shortened through use of PGRs and anther retention is increased, it is possible that these PGRs may lead to an increase in FHB infection in wheat. If PGRs do not affect anther retention and have no impact on FHB infection, breeders can have more options to breed FHB resistant wheat and growers can utilize PGRs with FHB resistance cultivars to maximize productivity and reduce the potential risk of FHB infection. Thus, the roles of both PGRs on FHB in wheat need to be determined. The objectives of this study were to determine the effect of the PGRs, Manipulator™ (GA inhibitor) and Ethrel™ (ET releasing compound), on five spring wheat cultivars that differ in their levels of FHB resistance and height, and to determine the effect of PGRs on anther retention and FHB in spring wheat cultivars under field conditions.

3.3 Materials and methods

3.3.1 Plant materials

Five commercial spring wheat genotypes were chosen to represent a range of end-use classes and differences in height, level of FHB resistance, and semi-dwarfing alleles. The five commercial spring wheat genotypes and their characteristics are listed in Table 3.1.

Table 3.1. List of five spring wheat cultivars used in field experiments conducted in Winnipeg and Carman, Manitoba in 2019 and 2020 with wheat end-use class, height, FHB resistance level and the presence of semi-dwarfing alleles.

Cultivars	**End-use classes**	**Height (cm)[a]**	**Resistance to FHB[a]**	**Semi-dwarfing Alleles (*Rht-B1* or *Rht-D1*)[b]**
AAC Tenacious	Canada Prairie Spring Red (CPSR)	Tall (101)	Resistance (R)	None
AAC Penhold	CPSR	Short (71)	Moderate Resistance (MR)	*Rht-D1b*
AAC Brandon	Canada Western Red Spring (CWRS)	intermediate (81)	MR	*Rht-B1b*
AAC Cameron	CWRS	Tall (94)	Intermediate Resistance (I)	None
Prosper	Canada Northern Hard Red (CNHR)	Intermediate (84)	I	*Rht-B1b*

[a]Seed Manitoba 2018 [b]Dr. Santosh Kumar from Agriculture and Agri-Food Canada – Brandon Research and Development Centre

3.3.2 Plant Growth Regulators

Commercially available plant growth regulators Manipulator™ (620 g/L of Chlormequat chloride) developed by Taminco US LLC and Ethrel™ (240 g/L of Ethephon) manufactured by Bayer CropScience Inc were used in field experiments.

3.3.3 Field experimental design and treatments

Field trials were conducted in Winnipeg and Carman, Manitoba in the growing seasons of 2019 and 2020. Each field trial was a split plot design with four replicates. The main plot effect (Main treatment) was the combination of plant growth regulator treatments and FHB inoculation (Table 3.2). The subplot effect was five different cultivars, AAC Tenacious, AAC Cameron, AAC Penhold, AAC Brandon, and Prosper. AGROBASE Generation II® software [Agronomix., Winnipeg] was used to randomly assigning treatments to each plot.

Table 3.2. Main and subplot treatments for each experiment conducted in Carman and Winnipeg, Manitoba in 2019 and 2020.

Main treatments		**Subplot treatments**
Ethrel™	FHB inoculation	AAC Tenacious AAC Penhold AAC Brandon AAC Cameron Prosper
Ethrel™	no FHB inoculation	
Manipulator™	FHB inoculation	
Manipulator™	no FHB inoculation	
Control	FHB inoculation	
Control	no FHB inoculation	

Each plot represented a single cultivar combined with a PGR and FHB treatment. The size of each plot was 3 meters with six rows spaced 17 cm apart. Plots were sown at a seeding rate of 1200 seeds/plot. A total of 120 plots (6 treatments x 5 cultivars x 4 replicates) were at each experimental site in each growing season. Each main plot was separated with a buffer plot (3 m x 6 rows spaced 17 cm apart) of a tall wheat cultivar, Amazon, to prevent wind drift of PGR and FHB applications onto non-target plots.

In 2019 and 2020, field trials were located at the University of Manitoba's Ian N. Morrison Research Station in Carman, Manitoba and the University of Manitoba's Fort Gary campus (the Point), Winnipeg, Manitoba. In 2019, the trial in Carman was sown on May 8th, and the trial in

Winnipeg was sown on May 17th. In 2020, the Carman and Winnipeg field trials were sown on May 6th and May 19th, respectively. Phenotypic data were collected on individual plots over both growing seasons.

Soil tests were taken at each field site to determine soil fertility. To determine the available nitrogen, composite samples were taken from 0 to 15 cm and 15 to 60 cm. Most of the nitrogen was applied as broadcast fertilizer without incorporation using 46-0-0 to adjust available nitrogen to a target of 150kg of Nitrogen/ha for both locations in 2019 and 2020. In addition to broadcast fertilization, 11-52-0 fertilizer was incorporated with the seed at seeding time at a rate of 35 kg/ha.

3.3.4 Seedling stand density

Prior to counting the number of seedlings, one meter of the two middle rows within each plot was measured and marked with flags. Seedling numbers for each plot were determined by counting the number of plants in one meter sections of the two middle rows in each plot before seedlings started tillering in each growing season in 2019 and 2020. The number of seedlings counted in the two rows were used to calculate the plant density as plant number per meter squared using following equation:

$$\text{Seedling stand density} = \frac{\text{The number of seedlings in the two one meter rows} * 9}{4\ \text{m}^2}$$

3.3.5 Plant growth regulator application

For plots treated with Manipulator™ (620 g/L of Chlormequat choride), it was applied to the plants at the Zadoks GS 30 at the recommended rate of 1.8L/ha as a single application. For plots treated with Ethrel™ (240 g/L of Ethephon), it was applied at Zadoks GS 37 to 45 for plants at the recommended rate of 1.25 L/ha. CO_2 powered back-pack sprayers with a six-nozzle boom at 30 psi air pressure were used for both plant growth regulator applications.

3.3.6 Fungal inoculum preparation and inoculation

Four different isolates of *F. graminearum* were obtained in 2015 from Dr. Maria Antonia Henriquez (Morden Research and Development Center of Agriculture and Agri-Food Canada): the 3 acetyl-deoxynivalenol chemotype for isolates HSW-15-39 and HSW-15-87, and the 15 acetyl-deoxynivalenol chemotype for isolates HSW-15-27 and HSW-15-57.

A section of a single spore isolation on potato dextrose agar media was transferred onto a Spezieller Nährstoffarmer agar (SNA) (Nirenberg 1981) media plate (20 mL media/plate), and plates were placed under a UV light at room temperature for one week. Subsequently, the SNA media was sliced and transferred into flasks of liquid carboxymethyl cellulose (CMC) (Tuite 1969) media (1.5 L/flask) and placed under UV light with aeration for another week to produce macroconidia. The culture was strained through sterile cheesecloth into sterile glass bottles and kept at 4^{o}C. Macroconidia concentration of each culture was determined visually under a microscope using a hemocytometer. To prepare spray inoculum, equal macroconidia counts of each isolate were combined in a one litre bottle and distilled water was used to adjust the concentration to produce one litre of spray inoculum at a final concentration to 50,000 macroconidia/mL. Tween 20 [VWR international., Edmonton] was added as a surfactant to each bottle at a rate of 4 ml/L.

All FHB inoculated plots were inoculated twice. Plots were inoculated with 1 liter of inoculum when 50% of the plants in the plot were at anthesis (Zadoks GS 65) and then three days later using CO_2 powered back-pack sprayers with a six-nozzle boom at 30 psi air pressure. After each inoculation, overhead mist irrigation was used to maintain humidity and promote development of FHB. Mist irrigation was turned on one hour after inoculation for 5 min every 90

minutes for 12 hours in Carman. In Winnipeg, misted irrigation was applied for 10 min every 60 minutes for 12 hours.

3.3.7 Wheat spike collection for evaluation of anther retention

Twenty spikes per plot were collected five to seven days post anthesis and stored at -20°C until data could be collected for anther retention. Samples were defrosted before counting the retained anthers. Any anthers that were found to be located within florets or trapped between the lemma and palea were considered retained anthers. The retained anthers in the primary and secondary florets of four spikelets in the central portion of the spike were counted by opening florets up. The percentage of anther retention was calculated as the number of retained anthers divided by maximum number of anthers times 100%.

3.3.8 Phenotypic disease and physical characteristic evaluation in field

Plot were visually evaluated for FHB disease incidence and severity 18 to 21 days after the first inoculation. Disease incidence was measured as the percentage of infected spikes in the plot and disease severity was measured as the percentage of infected spikelets within infected spikes. These values were used to calculate the FHB index. The FHB index was calculated by incidence times severity and divided by 100.

After the crop was fully headed out, the number of spikes were counted in two one meter of middle rows within the plot previously marked with flags.

Plant height for each plot was measured using a 2-meter ruler prior to harvest. Five different plants within a plot were randomly picked and measured from the soil surface to the end of spike without the awn. The final heights recorded were taken from an average of five height measurements for each plot.

Lodging was rated before harvesting and lodging rating was calculated from the percentage portion of the plot affected by lodging multiplied by the lodging scale of 1-9, in which 1 = no lodging and 9 = completely lodged. Lodging occurred only in the Carman 2020 trial after high wind with severe rainfall on 30th June 2020.

3.3.9 Phenotypic evaluation postharvest

Plots were harvested with a plot combine [Classic Plot combine, WINTERSTEIGER., Saskatoon]. In 2019, plots in Carman and Winnipeg were harvested on August 19th and 30th, respectively. Harvesting in Winnipeg 2019 was delayed because of rainfall. In 2020, the Carman trial was harvested on August 24th and the Winnipeg trial was harvested on August 26th. To prevent loss of *Fusarium* damaged kernels (FDK), the wind speed of the combine was reduced from normal by 30%. Harvested seeds from each plot were stored in cotton bags and placed on forced air-drying beds for three days to dry all seed samples. A blower was used to remove straw and chaff from the samples and unthreshed spike segments in samples were threshed by hands to ensure all FDK were completely separated from the lemma and palea. After that, a dockage tester [Carter Day International Inc., USA] was used to further clean samples of undesired weed seeds. Grain yield for each plot was measured at this point by weighing the clean samples. Finally, all the samples were stored in paper bags. A seed counter [Model U, International Marketing and Design Corp., USA] was used to count one thousand kernels from each plot. The one thousand kernels samples were weighed for each plot to determine thousand kernel weight. Test weight for each plot was measured by filling a half litre cylinder using a filling hopper and stand. As per the protocol from the Canadian Grain Commission, the weight in grams from the 0.5 L was converted to kg/hL (Canadian grain commission 2019).

To determine FDK and DON for all plots, a 50g sub sample of seed from each plot was sent to SGS Biovision in 2019 and Central *Testing* Laboratory Ltd in 2020. For FDK analysis, the samples were divided into a working portion using a Boerner divider and a minimum of 10 grams of grain was examined to remove possible *Fusarium* affected kernels. All the FDK were inspected under 10 times magnification to check for fibrous mold. *Fusarium* damaged kernel was measured as a percentage of the total sample by weight of *Fusarium* affected kernel. For analyzing DON, each 50 g sample was ground and thoroughly mixed. Five grams of ground sample were added to 100 ml of distilled water. Using ultra-turrax, the sample was blended and filtered through Whatman No.1 filter. 50ul of the filtrate per well was used for the quantitative determination of DON content using enzyme-linked immunosorbent assay (ELISA) with RODASCREEN®FAST DON kit [R-Biopharm AG., Germany].

To determine protein content in the grain, 400 g of samples from each plot from the 2019 and 2020 field trials were sent to the Central Testing Laboratory Ltd for analysis using near-infrared analyzer [FOSS., Denmark] on the whole grain. A scoop of sample of grain was poured into the top of the hopper and levelled with the top of the hopper. The analyzer ran 10 determinations and detected the moisture and protein content of the sample. The protein content was reported on a dry matter basis.

3.3.10 Weather conditions in Carman and Winnipeg during the growing season Data

Weather data from Winnipeg were taken from the Point weather station at the University of Manitoba. Carman's weather data were taken from the Environment Canada weather station at the Ian N. Morrison Research Farm, Carman. Weather data included daily average temperatures (°C) and precipitation (mm) for the 2019 and 2020 growing season from May to August. From the data, line and bar graphs of the daily average temperature (°C) and precipitation (mm) for each

location in 2019 and 2020 were created in Microsoft Excel® 2016. Long term (23 and 24-year) average data of growing seasons (from May to August) for monthly average temperature and total precipitation were obtained from Environment Canada historical data (from 1996 to 2019) at Carman and Winnipeg stations. Weather data from 2019 was compared with the average monthly temperature and total precipitation of growing seasons from 1996 to 2018. Weather data from 2020 was compared with the average monthly temperature and total precipitation of growing seasons from 1996 to 2019.

3.3.11 Data analysis

Statistical analyses were conducted using SAS software version 9.4 [SAS institute Inc., USA]. Analysis of variance was conducted on all response variables; plant density, spike density, height, anther retention, protein, test weight, thousand kernel weight, yield, FHB index, incidence, severity, FDK and DON using PROC Mixed.

Analyses of variance were performed on all response variables for each site year as well as the combined site years. In the single site year model, the treatment, genotypes and treatment*genotype as fixed effects. Rep and treatment*rep were listed as random effects. (Tables A.12 to A.23).

The data from four site years were evaluated using PROC Univariate to see whether data from the different site years could be combined. The distribution of the residuals suggested that the four site years of data could be combined together through visual inspection of normality. The model statement listed the treatments, genotypes, and the interaction of treatment*genotype as fixed effects. Environment, rep(environment), environment*treatment, environment*genotype, and environment*treatment*genotype were considered random effects. Tukey means comparison test was used for comparisons of means for main effects and their interactions.

Eta squared was calculated as described by Brown (2008) to determine the proportion of the variation for each response variable, treatments, genotypes, and the interaction of treatment*genotype, environment, rep(environment), environment*treatment, environment*genotype, and environment*treatment*genotype (Brown 2008). Contrasts between main effect treatments were done using PROC Mixed.

Pearson's correlation coefficients between response variables; yield, thousand kernel weight, test weight, anther retention, height, FHB index, incidence, severity, FDK and DON were generated using PROC Corr in SAS version 9.4.

3.4 Results

3.4.1 Weather

Plant growth regulators were applied in June 2019 and June 2020. Generally, total precipitation in June 2019 was lower than in June 2020; Carman 2019 had 37.9 mm, Winnipeg 2019 had 47.23 mm, Carman 2020 had 70.7 mm and Winnipeg 2020 had 59.18 mm (Figures 3.1 to 3.4). Inoculation started in early July in 2019 and 2020. Over the month of July in 2019, the total precipitation was higher in Winnipeg (101.86 mm) than Carman (57.4 mm), while total precipitation was slightly higher in Carman (54 mm) than in Winnipeg (44.33 mm) in 2020. Overall, Winnipeg had slightly higher average temperatures than Carman in July, with 2020 having higher temperatures than 2019.

According to the long term average (LTA) data from Environment Canada, Carman 2019 trial had 70% of the normal total precipitation based on the 23-year average of growing seasons (from May to August) (Table A.1). Winnipeg 2019 had 72% of LTA precipitation. Carman 2020 trial had 64% of LTA precipitation. Winnipeg 2020 trial had 69% of LTA precipitation. May 2020

in Winnipeg was the driest month and July 2019 in Winnipeg was the wettest month among growing seasons.

It was hotter in 2020 than in 2019. Based on historical data of the LTA, June and July in 2019 and 2020 at both locations recorded higher temperatures than the LTA (Table A.2). Among the experimental seasons, June 2020 in Winnipeg had the hottest month followed by June 2020 in Carman and in Winnipeg. Generally, Winnipeg 2020 was hotter than other experimental environments.

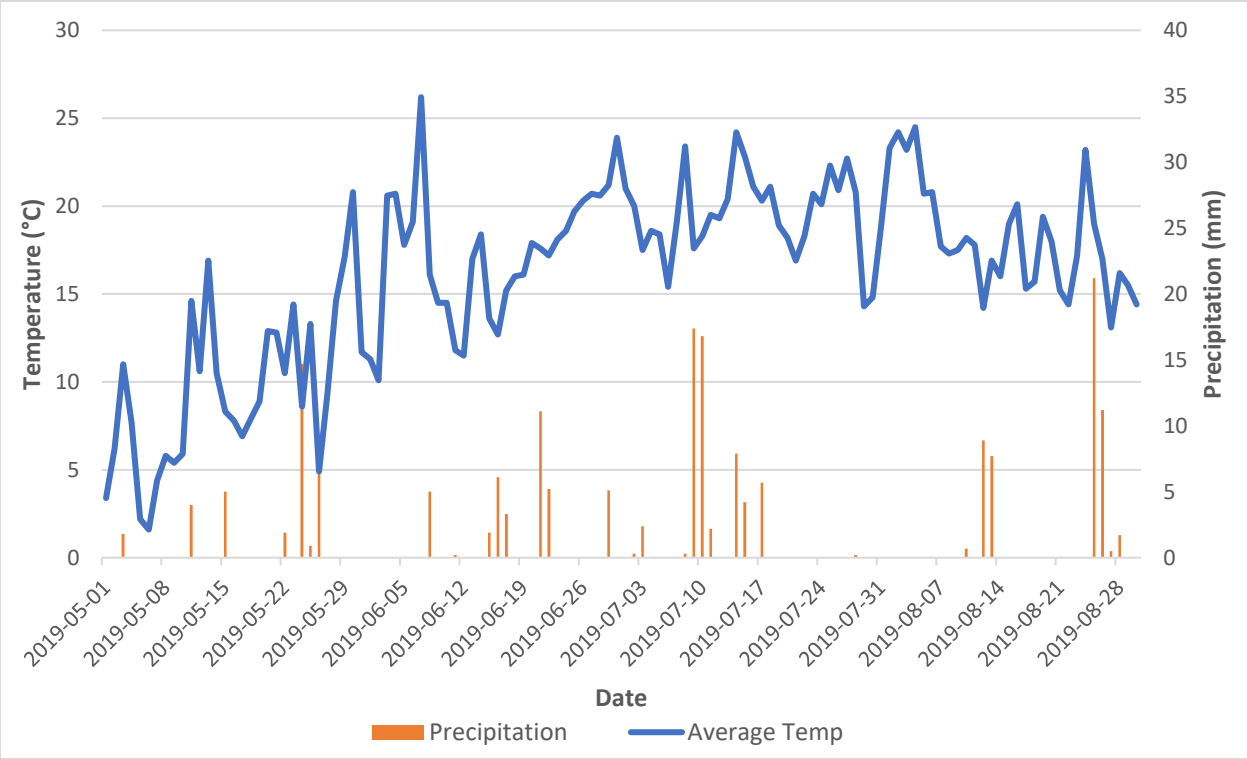

Figure 3.2. Daily precipitation (mm) and average temperature (°C) data for the Carman trial site from May 1st 2019 to August 30th 2019.

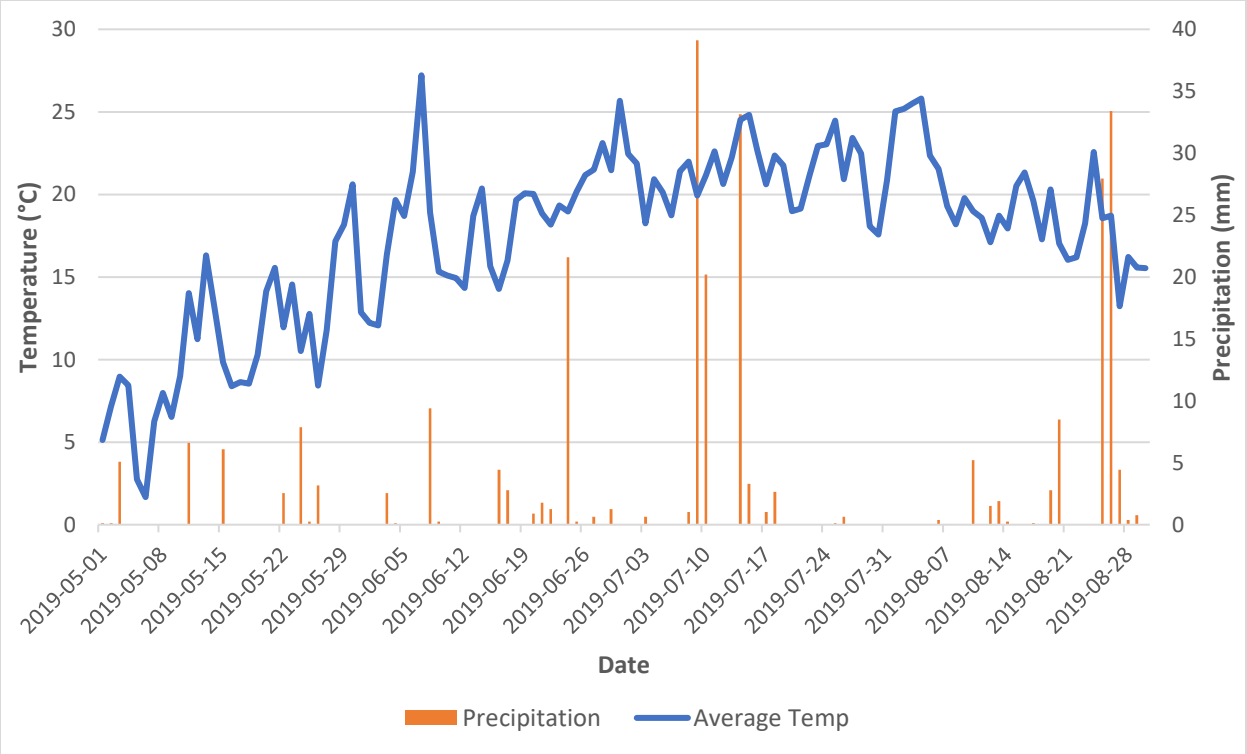

Figure 3.1. Daily precipitation (mm) and average temperature (°C) data for the Winnipeg trial site from May 1st 2019 to August 30th 2019.

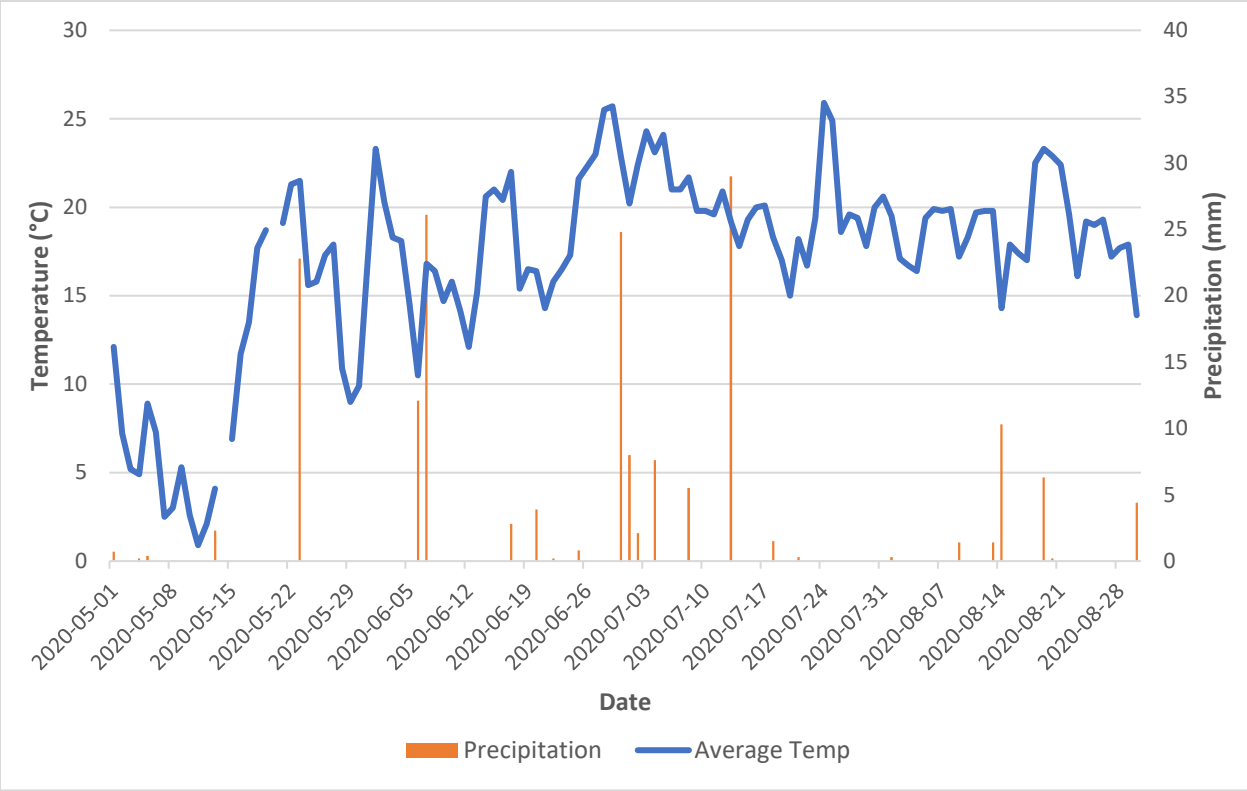

Figure 3.3. Daily precipitation (mm) and average temperature (°C) data for the Carman trial site from May 1st 2020 to August 30th 2020.

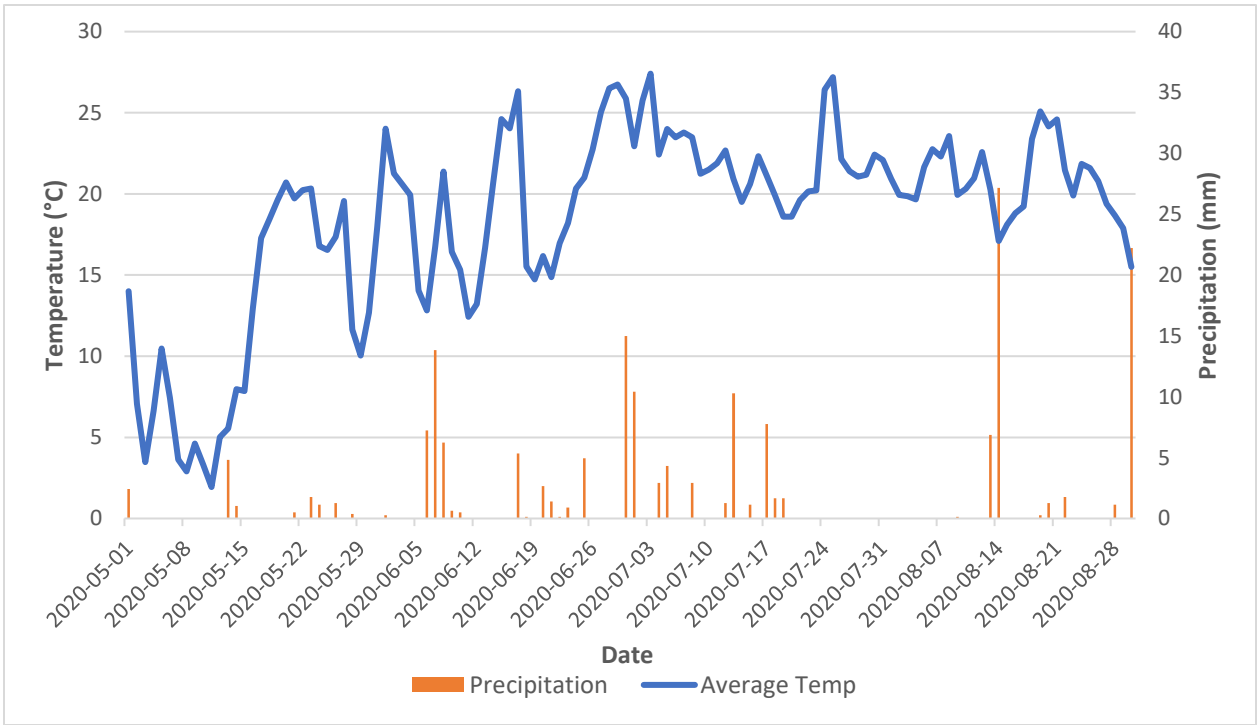

Figure 3.4. Daily precipitation (mm) and average temperature (°C) data for the Winnipeg trial site from May 1st 2020 to August 30th 2020.

3.4.2 Analysis of variance for agronomic traits

Analyses of variance showed there were significant differences among main treatments for height, protein content, test weight, thousand kernel weights, and yield (Table 3.3 and Table 3.4). Main treatment did not affect anther retention. Cultivars had a significant effect on all variables except thousand kernel weight and yield. Treatment*cultivar interactions were significant for anther retention, protein content, test weight, thousand kernel weight, and yield. There were significant environment effects in all agronomic traits except anther retention and thousand kernel weight. Significant environment*treatment*cultivar interaction was observed for height, anther retention, test weight, thousand kernel weight, and yield.

3.4.3 Effect of main plot treatments on agronomic traits

There was a significant difference in height for PGR treatment (Table 3.5). Ethrel™ treatment reduced height significantly compared to the control. Plants treated with Manipulator™ were intermediate in plant height to those treated with Ethrel™ and the control, but Manipulator™ treated plants were not significantly different from the other two treatments (Table 3.5).

The protein content of inoculated treatments differed from those of uninoculated treatments (Table 3.5). Inoculation led to higher protein content than the uninoculated treatments. There were no differences in protein content associated with PGR treatment.

Inoculated treatments showed significantly lower test weight, thousand kernel weight and yield compared to uninoculated treatments (Table 3.5). There were no differences in these traits associated with PGR treatment. In addition to this, there was no main treatment effect on anther retention (Table 3.5).

3.4.4 Effect of cultivars on agronomic traits

Plant density and spike density had similar trends among cultivars (Table 3.6). AAC Tenacious had the highest plant and spike density. AAC Penhold had the lowest plant density and spike density.

AAC Tenacious was the tallest cultivar (91.3 cm) followed by AAC Cameron (86.5 cm), Prosper (75.3 cm), and AAC Brandon (71.4 cm). AAC Penhold was the shortest cultivar (64.9 cm) (Table 3.6).

Percentage of anther retention for AAC Brandon was 54.5%, which was the highest percentage anther retention (Table 3.6). AAC Tenacious had the lowest percentage anther retention (20.3%). Percentage anther retention for Prosper, AAC Cameron, and AAC Penhold were lower than AAC Brandon and higher than AAC Tenacious, but they were not statistically different from AAC Brandon and AAC Tenacious.

The highest protein content (17.95%) was observed in AAC Brandon (Table 3.6). It was followed by AAC Cameron (17.24%), AAC Penhold (17.12%), Prosper (16.50%) and AAC Tenacious (15.21%). Statistically, AAC Cameron and AAC Penhold had the same protein content.

AAC Tenacious had the highest test weight (80.8 kg/hL) (Table 3.6). Test weight for AAC Brandon (79.5 kg/hL) was the second highest, but it was not statistically different from the test weights for AAC Tenacious, AAC Cameron (78.4 kg/hL) and Prosper (77.9 kg/hL). AAC Penhold had the lowest test weight which was 77.7 kg/hL, but was not significantly different from AAC Cameron and Prosper.

Table 3.3. Combined analysis of variance for agronomic traits: plant density, spike density, height, anther retention, and protein content from four test environments (Carman and Winnipeg for 2019 and 2020).

Source of Variation	DF[a]	Plant density		Spike density		Height		DF	Anther retention		Protein	
		MS[b]	P[c]	MS	P	MS	P		MS	P	MS	P
Main treatment	5	6022.86	0.3319	5762.10	0.6331	386.49	0.0002	5	50.28	0.4358	3.96	0.0402
Cultivar	4	12587	0.0023	89032	0.0071	11122	<.0001	4	14917	0.01	98.94	<.0001
Treatment*Cultivar	20	1926.91	0.1369	3729.43	0.442	27.01	0.0647	20	237.08	<.0001	1.00	<.0001
Environment	3	240853	<.0001	595240	<.0001	6569.57	<.0001	3	6067.89	0.1479	108.60	<.0001
Rep(Environment)	12	10485	<.0001	5123.54	0.129	115.19	<.0001	12	169.17	<.0001	3.35	<.0001
Env*Treatment	15	4788.88	0.0002	8254.25	0.0124	38.03	0.0099	15	48.88	0.6496	1.27	<.0001
Env*Cultivar	12	1594.16	0.3067	14997	0.0001	391.63	<.0001	12	2758.98	<.0001	1.18	<.0001
Env*Trt*Cultivar	60	1331.07	0.9927	3615.80	0.3929	16.16	0.0166	60	59.52	0.0023	0.26	0.9225
Error	342	2251.07		3458.60		10.89		338	35.33		0.35	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table 3.4. Combined analysis of variance for agronomic traits: test weight, thousand kernel weight, and yield from four test environments (Carman and Winnipeg for 2019 and 2020).

Source of Variation	Test weight			Thousand kernel weight			Yield		
	DF[a]	MS[b]	P[c]	DF	MS	P	DF	MS	P
Main Treatment	5	336.62	<.0001	5	923.39	<.0001	5	62382676	<.0001
Cultivar	4	157.01	0.0003	4	62.24	0.1136	4	1792049	0.547
Treatment*Cultivar	20	25.19	<.0001	20	56.01	<.0001	20	2822119	<.0001
Environment	3	239.25	<.0001	3	113.69	0.2013	3	40039713	0.0019
Rep(Environment)	12	4.49	<.0001	12	11.65	<.0001	12	1598283	<.0001
Env*Treatment	15	5.66	0.005	15	39.96	<.0001	15	3228830	<.0001
Env*Cultivar	12	12.30	<.0001	12	26.56	<.0001	12	2236946	<.0001
Env*Trt*Cultivar	60	2.21	<.0001	60	5.79	0.0007	60	398862	<.0001
Error	336	0.96		338	3.23		329	177850	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table 3.5. Least square means for agronomic traits: height, protein, test weight, thousand kernel weight (TKW), and yield for the different main treatments tested across cultivars from pooled data from four test environments (Carman and Winnipeg for 2019 and 2020).

Main treatment		Height (cm)	Protein (%)	Test weight (Kg/hL)	TKW (g)	Yield (Kg/ha)
Ethrel™	FHB inoculation	75.3 B	17.08 A	77.3 B	29.7 B	3236.2 B
Ethrel™	no FHB inoculation	75.4 B	16.62 B	81.0 A	35.7 A	4673.0 A
Manipulator™	FHB inoculation	77.6 AB	16.89 A	76.7 B	30.3 B	3132.4 B
Manipulator™	no FHB inoculation	78.4 AB	16.70 B	80.5 A	36.5 A	5018.2 A
Control	FHB inoculation	79.9 A	17.01 A	76.9 B	30.8 B	3161.4 B
Control	no FHB inoculation	80.6 A	16.52 B	80.7 A	37.1 A	4757.7 A

Means followed by same letter in a column are not significantly different at p=0.05 based on the Tukey means comparison test.

Table 3.6. Least square means for agronomic traits: plant density, spike density, height, anther retention, protein and test weight for cultivars tested across different main treatments from pooled data from four test environments (Carman and Winnipeg for 2019 and 2020).

Cultivar	Plant density (Seedlings/m^2)	Spike density (spikes/m^2)	Height (cm)	Anther retention (%)	Protein (%)	Test weight (Kg/hL)
AAC Brandon	224.1 A	490.9 A	71.4 BC	54.5 A	17.95 A	79.5 AB
AAC Cameron	223.2 A	465.8 AB	86.5 A	33.9 AB	17.24 B	78.4 BC
AAC Penhold	203.9 B	421.1 B	64.9 C	31.3 AB	17.12 B	77.7 C
AAC Tenacious	235.5 A	493.6 A	91.3 A	20.3 B	15.21 D	80.8 A
Prosper	217.9 AB	490.4 A	75.3 B	40.8 AB	16.50 C	77.9 BC

Means followed by same letter in a column are not significantly different at p=0.05 based on the Tukey means comparison test.

3.4.5 Analysis of variance for disease traits

Combined analyses of variance showed that there were significant differences among main treatments, cultivars, main treatment*cultivar interactions and all interactions with environment for all disease variables; FHB index, incidence, severity, FDK and DON (Table 3.7). Environment effects were significant for FHB index, incidence, severity, and DON.

3.4.6 Effect of main treatments on disease traits

Disease traits variables from inoculated with and without PGRs were significantly higher than uninoculated with and without PGRs (Table 3.8). All uninoculated plots showed little disease for all FHB variables. There was a trend that treatments that were inoculated with FHB combined with the Manipulator™ application showed higher FHB index (42.9%), disease severity (53.5%), FDK (5.88%), and DON (9.75 ppm) than the inoculated without any PGR control treatment. The Ethrel™ plus inoculation treatment showed a tendency of having lower FHB index (34.6%), disease incidence (64.4%), disease severity (47.7%), FDK (5.39 %), and DON (8.90 ppm) compared to the inoculated without any PGR control treatment. However, PGR treatments did not statistically differ from no PGR treatment for disease traits within the inoculated and uninoculated treatments.

3.4.7 Effect of cultivars on disease traits

Fusarium head blight resistance cultivar AAC Tenacious consistently gave the lowest values for all disease trait parameters and was significantly different from the other cultivars for FHB index, disease incidence, FDK and DON (Table 3.9). Prosper had the highest FHB index followed by AAC Penhold, AAC Cameron and AAC Brandon, but these were not significantly different from each other. The highest disease incidence was observed in AAC Brandon. Prosper had the second highest incidence followed by AAC Penhold and AAC Cameron. Prosper had the

highest severity followed by AAC Penhold, AAC Cameron and AAC Brandon. AAC Brandon was not statistically different from AAC Tenacious for disease severity. AAC Penhold had the highest FDK and Prosper had the second highest FDK, but they were not statistically different. AAC Cameron and AAC Brandon followed them. There were no significant differences in FDK between AAC Brandon, AAC Cameron and Prosper. Prosper had the highest DON content followed by AAC Penhold, AAC Brandon, and AAC Cameron. There were no statistical differences between them.

Table 3.7. Combined analysis of variance for disease traits: Fusarium head blight (FHB) index, incidence, severity, *Fusarium* damaged kernel (FDK), deoxynivalenol (DON) content from four test environments (Carman and Winnipeg for 2019 and 2020).

Source of Variation	DF[a]	FHB Index		Incidence		Severity		FDK		DON	
		MS[b]	P[c]	MS	P	MS	P	MS	P	MS	P
Main treatment	5	37211.00	<.0001	104791.00	<.0001	49887.00	<.0001	703.08	<.0001	2016.91	<.0001
Cultivar	4	10456.00	0.0001	14423.00	<.0001	13685.00	0.0016	267.53	0.0001	584.71	0.0004
Treatment*Cultivar	20	2068.29	<.0001	2584.01	<.0001	1645.73	<.0001	50.70	<.0001	121.89	<.0001
Enviroment	3	5390.04	0.0435	5855.71	0.0238	13811.00	0.0046	171.75	0.0645	625.57	0.0294
Rep(Environment)	12	235.41	<.0001	120.13	0.0012	229.53	0.0015	11.60	<.0001	18.61	<.0001
Env*Treatment	15	1036.19	<.0001	1114.55	<.0001	895.51	<.0001	41.90	<.0001	127.39	<.0001
Env*Cultivar	12	672.33	<.0001	487.08	0.0005	1574.29	<.0001	17.66	0.0006	48.19	0.0004
Env*Trt*Cultivar	60	154.97	<.0001	136.67	<.0001	211.54	<.0001	5.09	0.0016	13.18	<.0001
Error	338	46.79		43.09		84.17		2.96		4.57	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table 3.8. Least square means for disease traits: Fusarium head blight (FHB) index, incidence, severity, *Fusarium* damaged kernel (FDK), deoxynivalenol (DON) content for the different main treatments tested across cultivars from pooled data from four test environments (Carman and Winnipeg for 2019 and 2020).

Main Treatment		FHB Index (%)	Disease Incidence	Disease Severity	FDK (%)	DON (ppm)
Ethrel™	FHB inoculation	34.6 A	64.4 A	47.7 A	5.39 A	8.90 A
Ethrel™	no FHB inoculation	0.0 B	0.7 B	4.0 B	0.10 B	0.17 B
Manipulator™	FHB inoculation	42.9 A	69.6 A	53.5 A	5.88 A	9.75 A
Manipulator™	no FHB inoculation	0.5 B	1.4 B	5.8 B	0.15 B	0.10 B
Control	FHB inoculation	42.1 A	70.1 A	51.6 A	5.54 A	9.55 A
Control	no FHB inoculation	0.4 B	1.3 B	4.8 B	0.13 B	0.11 B

Means followed by same letter in a column are not significantly different at p=0.05 based on the Tukey means comparison test.

Table 3.9. Least square means for disease traits: Fusarium head blight (FHB) index, incidence, severity, *Fusarium* damaged kernel (FDK), deoxynivalenol (DON) content for cultivars tested across different main treatments from pooled data from four test environments (Carman and Winnipeg for 2019 and 2020).

Cultivar	Resistance Level[a]	FHB Index (%)	Disease Incidence	Disease Severity	FDK (%)	DON (ppm)
AAC Brandon	MR[b]	20.2 A	43.3 A	23.3 AB	2.69 B	5.16 A
AAC Cameron	I[c]	23.1 A	35.6 A	34.4 A	2.70 B	4.91 A
AAC Penhold	MR	26.6 A	38.8 A	35.8 A	4.74 A	5.36 A
AAC Tenacious	R[d]	2.1 B	13.1 B	8.7 B	0.28 C	0.76 B
Prosper	I	28.3 A	42.0 A	37.2 A	3.91 AB	7.61 A

Means followed by same letter in a column are not significantly different at p=0.05 based on the Tukey means comparison test. [a]Seed Manitoba 2018, [b]MR=Moderate Resistance, [c]I=Intermediate Resistance, and [d]R=Resistant.

3.4.8 Effect of Interactions

There were significant experiment*treatment*cultivar interactions for height, anther retention, test weight, thousand kernel weight, yield and all disease trait variables. These interactions for height and anther retention were likely caused by different environmental conditions such as drought and growing temperature (Table A.1). Significant three way interactions in test weight, thousand kernel weight, yield and all disease traits are mainly explained by differences in disease pressure among the environments (Tables A.28 to A.31). Furthermore, most of interactions between treatment and experiment are mainly due to differences in magnitude between treatments across environments. In some environments, there were no significant differences, but when there were significant differences, the trends were similar across the environments (Tables A.24 to A.31).

3.4.9 Proportion of total variation

Generally, the main treatment effect contributed to the highest proportion of variation for all disease traits; DON, FDK, Severity, Incidence, FHB index and sample weight variables; yield, thousand kernel weight (TKW), and test weight (TWT) (Figure 3.5). Comparisons of main treatments were done using contrasts (Tables A.3 to A.11). It revealed depending on the presence of inoculation, disease traits and sample weight variables showed different results. Thus, the presence or absence of inoculation strongly affected disease traits and sample weight variables. Cultivar contributed to the highest proportion of variation for protein, anther retention and height. Therefore, protein, anther retention and height were influenced mostly by genotypes. Although treatment*genotype interactions from combined data were statistically significant for all variables, the proportion of variance attributed to the interactions was relatively small compared to the main effects of treatment and cultivars. More than 10 % of total variation was caused by environmental

effect in height, anther retention, protein content, TWT and yield. Especially, approximately 33% of variation in protein content was affected by environment.

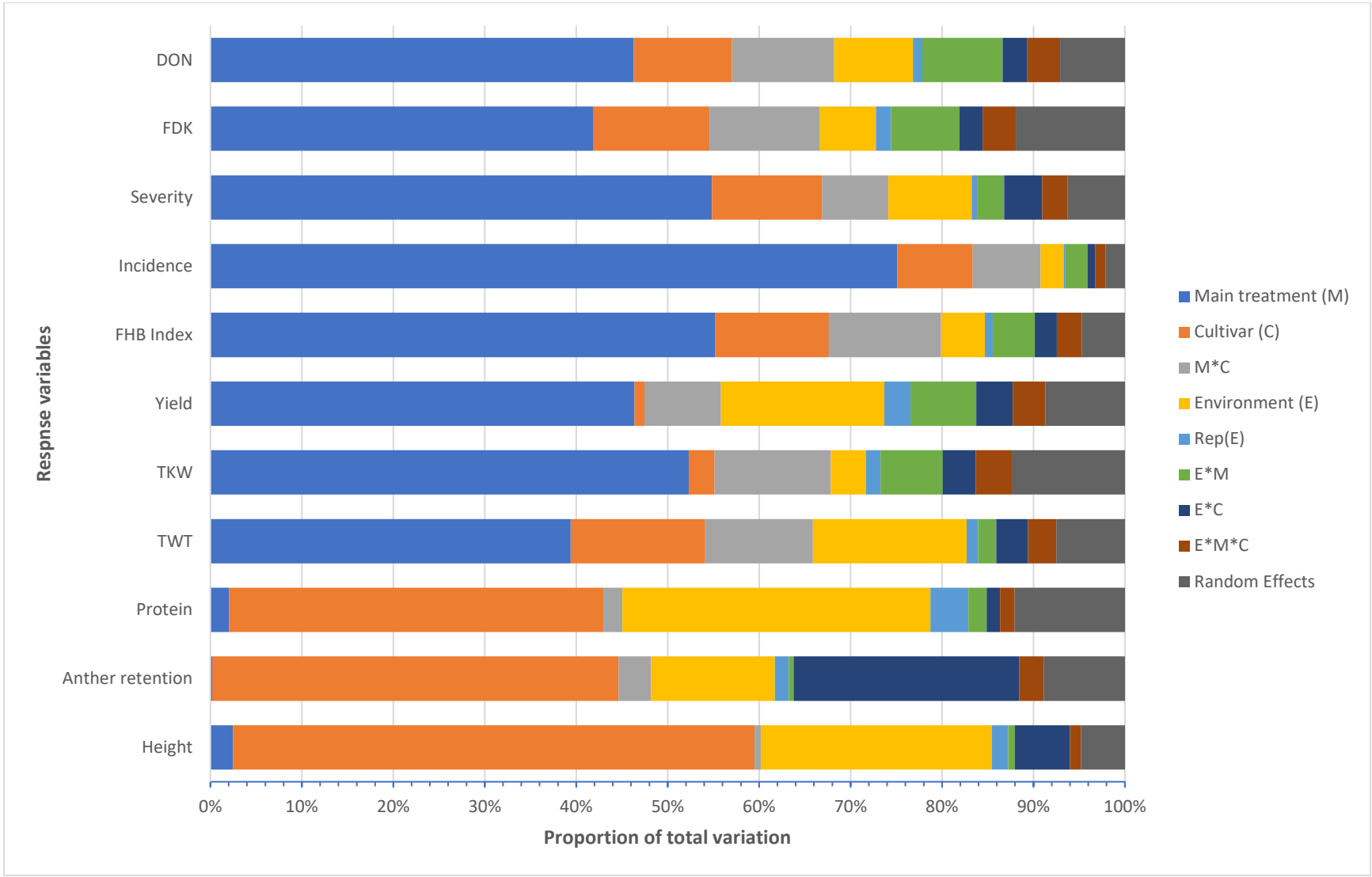

Figure 3.5. Proportion of total variation allocated to the main treatments and cultivars and their interactions for each response variable. Eta squared was calculated by adding all the sums of squares then dividing the sums of squares for each of the effects, interactions, and the residual by that total to indicate the relative proportion of total variation explained by each factor in the model (Brown 2008).

3.4.10 Correlation between the measured variables

Most of measured variables were significantly correlated with each other except height and thousand kernel weight (TKW), anther retention and yield, and height and DON content (Table 3.10). Height was negatively correlated with anther retention and FHB index, severity, incidence, and FHB. Significant negative correlations were observed between anther retention and test weight (TWT), and anther retention and TKW. Anther retention was positively correlated with FHB index, severity, incidence, FDK and DON. Test weight, TKW, and yield were positively correlated with all sample weight variables and negatively correlated with all disease variables. In Carman 2020, lodging showed significant positive correlation only with height.

Table 3.10. Pearson's correlation coefficients between height, anther retention (AR), test weight (TWT), thousand kernel weight (TWK), yield, FHB index, severity (SEV), incidence (INC), *Fusarium* damaged kernels (FDK), deoxynivalenol (DON), and lodging for combined experiments conducted in Carman and Winnipeg in 2019 and 2020.

	Height	AR	TWT	TKW	Yield	FHB index	SEV	INC	FDK	DON
AR	-0.20 <.0001 470									
TWT	0.10 0.0378 468	-0.16 0.0007 468								
TKW	0.06 0.1777 470	-0.21 <.0001 470	0.77 <.0001 468							
Yield	0.33 <.0001 461	0.07 0.1404 461	0.51 <.0001 460	0.61 <.0001 461						
FHB index	-0.13 0.0052 470	0.20 <.0001 470	-0.89 <.0001 468	-0.79 <.0001 470	-0.63 <.0001 461					
SEV	-0.20 <.0001 470	0.12 0.0079 470	-0.78 <.0001 468	-0.75 <.0001 470	-0.70 <.0001 461	0.88 <.0001 470				
INC	-0.11 0.0211 470	0.20 <.0001 470	-0.83 <.0001 468	-0.76 <.0001 470	-0.57 <.0001 461	0.93 <.0001 470	0.80 <.0001 470			
FDK	-0.14 0.0021 470	0.15 0.0016 470	-0.84 <.0001 468	-0.75 <.0001 470	-0.60 <.0001 461	0.83 <.0001 470	0.78 <.0001 470	0.74 <.0001 470		
DON	-0.08 0.1002 470	0.15 0.0013 470	-0.80 <.0001 468	-0.74 <.0001 470	-0.61 <.0001 461	0.84 <.0001 470	0.84 <.0001 470	0.72 <.0001 470	0.89 <.0001 470	
Lodging*	0.18 0.0463 118	-0.05 0.5677 118	-0.13 0.1707 118	-0.03 0.7323 118	-0.10 0.2855 114	0.17 0.0690 118	0.18 0.0555 118	0.12 0.2131 118	0.07 0.4547 118	0.14 0.1375 118

Note: top row indicates correlation coefficients (r), second row indicates possibility, and last row indicates sample size (n) in the cell. *Represents only Carman 2020 trial.

3.5 Discussion

The average monthly temperatures in June and July 2019 and 2020 at Winnipeg and Carman were at least 1% and as high as 10% higher than the long term average of the growing seasons (from May to August). Total precipitations during the growing seasons were 28% (2019) to 36% (2020) lower than the long term average of growing seasons. These weather data indicated that the two experimental seasons of 2019-2020 were hot and dry. In general, plants were shorter than expected especially during the 2019 growing season and the effect of PGRs was less than expected due to hot and dry weather. After inoculation, field plots were under mist irrigation to provide favorable conditions for FHB development. The development of FHB was successful in all inoculated plots. Uninoculated plots had a low level of FHB from natural inoculum.

According to the results from this study, Ethrel™ and Manipulator™ did not affect plant density and spike density. Lack of effect on plant density is expected as PGRs had not been applied prior to measurement of plant density. Since spike density was highly dependent on plant density, lack of effect of PGR on spike density is expected. Differences in plant density and spike density were mainly determined by cultivar. Their relative ranking in plant density and spike density were similar. This suggests choice of cultivar is more important than relying on PGRs to increase tillers and spike density.

In this study, plant growth regulators were applied during June 2019 and 2020. Combined data showed Ethrel™ significantly reduced plant height by about 5 cm, while Manipulator™ slightly reduced plant height by approximately 2 cm. The response from PGRs can be affected by other factors such as weather conditions, rate and timing of application, and wheat cultivars (Strydhorst et al. 2018). Labelling from both PGRs indicated that they should not be applied under stress conditions such as drought and excessive heat. According to the long term average for

growing season precipitation, total precipitation for May and June in 2019 was only about half of normal precipitation or less, which is considered as severely dry. It was not as dry in June 2020 compared to June 2019, but both locations in June 2020 had lower total precipitation than the long term average of growing seasons for total precipitation. In addition to that, the average temperature of June 2019 and 2020 were 3% to 11% higher than the long term June average temperature. These dry and hot conditions might explain why PGRs did not reduce plant height as much as expected. Especially, height data in 2019 showed PGR treatments had less effect on plant height than in 2020. The experimental mean of plant height in Carman 2019 was about 20 cm shorter than in Carman 2020 and those in Winnipeg 2019 was about 8 cm shorter than those in Winnipeg 2020 (Table A.24). Overall plant height in 2019 was shorter than in 2020. With dryer conditions in 2019, plants were already compact and short. A combination of unsuitable environmental conditions for PGRs could have contributed to poor height reduction. Ethrel™ was more effective in reducing plant height than Manipulator™ across cultivars. The effective of height reduction by PGRs depended on cultivar (Clark and Fedak 1977; Strydhorst et al. 2018). Because three of cultivars have GA insensitive semi-dwarfing allele, inhibiting GA production by Manipulator™ to decrease plant height might be unsuccessful compared to Ethrel™. The active ingredient of Ethrel™, ethephon, enhances levels of ET in plant. Ethylene modulates DELLA proteins to be more resistant to the effect of GA (Achard et al. 2003). This stabilization of DELLA proteins by ethylene prevents the plant from growing (Achard et al. 2003; Dugardeyn et al. 2008; Iqbal et al. 2017). Also, ET affects growth hormone auxin signaling for inhibiting plant growth by simulating the breakdown of apical dominance (Wiersma et al. 2011; Strydhorst et al. 2018; Vaseva et al. 2018). Since ET inhibits plant growth through both the GA pathway and auxin pathway, Ethrel™ could be more effective in reducing plant height than Manipulator™.

The cultivar height means across treatments showed the same relative ranking reported in Seed Manitoba 2018. AAC Penhold, AAC Brandon, and Proser were shorter than AAC Tenacious and AAC Cameron because AAC Penhold, AAC Brandon, and Prosper had semi-dwarf alleles. Lack of a treatment by cultivar interaction suggests that all cultivars responded to PGRs in a similar manner.

Throughout the 2019 and 2020 growing seasons in Winnipeg and Carman, PGRs did not show any significant effect on anther retention. The most significant variation in anther retention was due to cultivars. Semi-dwarfing cultivars, which are GA insensitive, AAC Brandon, Prosper and AAC Penhold, showed higher anther retention. Since GAs regulate cell elongation, semi-dwarf plants may have shorter anther filaments which enhances anther retention (Buerstmayr and Buerstmayr 2016). The loci for the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* are linked with QTL for anther retention on chromosomes 4B and 4D, respectively (Xu et al. 2020). In this study, anther retention was negatively correlated with height (Table 3.10). This result agreed with previous studies that showed that semi-dwarf cultivars have higher anther retention than taller cultivars (Buerstmayr and Buerstmayr 2016; He et al. 2016; Steiner et al. 2017; Xu et al. 2020). The tallest cultivar AAC Tenacious had the lowest anther retention, while the second tallest cultivar, AAC Cameron, showed slightly higher anther retention than AAC Tenacious and similar levels of anther retention with AAC Penhold and Prosper. The lowest anther retention was for the highly FHB resistance cultivar AAC Tenacious, indicating that low anther retention could be one of the factors that contributes to FHB resistance in AAC Tenacious. Furthermore, there were positive correlations between anther retention and disease variables which indicated that anther retention plays a role in FHB susceptibility (Table 3.10). Disease incidence and anther retention had higher correlation (r=0.2) compared to other combinations of disease variables and anther

retention. This would be expected because retained anther give a surface for fungal pathogens to easily invade the floret, consequently, increasing initial infection (Strange et al. 1972; Buerstmayr and Buerstmayr 2015, 2016).

Protein content was affected by the main plot treatment in the combined data. Evaluation of this effect showed that the main reason for differences was due to differences between the inoculated and uninoculated treatments (Table A.3). Protein content of wheat grain is represented as a percentage of the total grain. Thus, protein content in infected kernels is higher than in healthy kernel because infected kernels are smaller, as confirmed by the thousand kernel weight and test weight values. Therefore, even if there is the same total protein in infected kernels and healthy kernels, percent protein is higher in the smaller FHB infected grains. Cultivars had a significant effect on protein content. This may be mainly due to different end use wheat classes of the cultivars. The Canadian Grain Commission groups wheat cultivars depending on their functional characteristics. In this study, the five cultivars were from three different end use classes; Canada Western Red Spring (CWRS), Canada Prairie Spring Red (CPSR), and Canada Northern Hard Red (CNHR). The Canada Western Red Spring class is known for its high protein content (Canadian Cereals 2019). Canada Prairie Spring Red has medium protein content. Canada Northern Hard Red has a broad range of protein content between the CWRS and CPSR classes. Protein results from this study corresponded to the end use class of the cultivars such that CWRS wheat cultivars AAC Brandon and AAC Cameron had higher protein content than CPSR cultivars AAC Tenacious and AAC Penhold. The protein content of Prosper from the CNHR class was between the range of the CWRS and CPSR cultivars.

The main plot treatment had a significant effect on sample weights represented by test weight, thousand kernel weight, and yield. Simple contrasts determined that the significant treatment effect was due to differences between inoculated and uninoculated treatments, and not because of PGR applications (Tables A.3 to A.5). Since Fusarium head blight infected kernels are smaller and lighter than uninfected kernels (McMullen et al. 2012a; Gilbert and Haber 2013), samples from FHB inoculated plots had lower test weight, thousand kernel weight, and yield. Plant growth regulators are primarily for improving lodging resistance. When PGRs prevent wheat from lodging, yield could be higher than non PGR treated wheat. However, because of hot and dry conditions, plants were shorter than usual, and were not at risk of lodging from wind and rain events, except in the Carman 2020 trial. It has been reported that PGRs did not affect grain yield in the absence of lodging (Wiersma et al. 2011). In this study, although there was severe wind and rainfall on 30th June 2020 which causing lodging in Carman 2020 trial, the correlation of lodging and yield in Carman 2020 data did not show a significant relationship (Table 3.10). According to lodging data from Carman 2020 (Table A.26), plants without PGR treatment showed higher lodging ratings because PGRs reduced plant height and might thicken the stem of plant, thereby lowering the chance of lodging (Table A.24). Stem lodging is often caused by weather events such as high speeds wind and heavy rain. Since taller plants have longer stems, wind and rain place more force on the stems which can lead to stem breakage or buckling (Berry et al. 2003; Rademacher 2016). Therefore, shorter plants are less prone to lodging. The correlation between height and lodging supports that there was significant positive relationship between them (Table 3.10). Cultivar was the main factor contributing to lodging because all tested cultivars had different heights. As explained earlier, taller cultivars such as AAC Cameron had a higher lodging rating and the shortest cultivar AAC Penhold had the lowest lodging rate (Table A.27).

There were significant differences for test weight among the cultivars. The highest test weight was observed in FHB resistant cultivar AAC Tenacious. The moderately FHB resistance cultivar AAC Penhold had the lowest test weight. In this study, this phenomenon was associated with FHB susceptibility in wheat cultivars. The relative ranking of tested cultivars for test weight was the same as for FDK and similar with other disease traits. This is because FDK are light weighted, thus, when density of grain is measured, more FDK in a specific volume resulted in lower test weight. Moreover, the correlation between test weight and all disease variables including FDK showed highly significant negative relationship (Table 3.10). Therefore, AAC Penhold, which was the most susceptible cultivar to FHB in this study, had the lowest test weight.

Combined data for disease traits showed that PGRs did not affect FHB index, incidence, severity, FDK, and DON. Based on contrasts, the difference between main treatments was caused mainly by differences between inoculated and uninoculated treatments (Table A.7 to 11). Ethrel™ slightly reduced all disease traits compared to the control, but this was not statistically significant. This result agrees with findings from Sun et al (2016) and Fauzi and Paulitz (1994). Sun et al (2016) found that application of ethephon did not have any impact on the FHB resistant level of wheat in greenhouse conditions. Fauzi and Paulitz (1994) reported the application of ethephon showed no effect on incidence of spikelet infection and seed infection in dry and wet field conditions when spray inoculation was used (Fauzi and Paulitz 1994). However, when there was wet weather and infested corn was used for inoculation, ethephon increased incidence of spikelet infection (Fauzi and Paulitz 1994). Unlike Ethrel™, Manipulator™ cause a slight increase for all disease traits except incidence, but it was not significantly different from the control. This result is consistent with findings from Fauzi and Paulitz (1994) that showed that application of chlormequat chloride did not affect incidence of seed infection and spike infection in dry weather or wet weather when

spray inoculation was used. These results suggest that Ethrel™ and Manuipulator™ did not change the FHB susceptibility in wheat.

As the most FHB resistant cultivar, the mean from AAC Tenacious across all treatments showed the lowest values for all of disease variables over different environments. AAC Brandon is rated as moderately resistance to FHB, but had the highest incidence. This could be because of anther retention, where AAC Brandon had the highest anther retention. Anther retention affects type I resistance for initial FHB infection (Lu et al. 2013; Buerstmayr and Buerstmayr 2015; He et al. 2016), because anthers trapped between the lemma and palea enhance fungal growth and initial infection (Buerstmayr and Buerstmayr 2016). For severity, AAC Brandon ranked as the second lowest among tested cultivars. Previous research demonstrated that semi-dwarfing alleles, *Rht-B1b* and *Rht-D1b*, significantly decreased resistance to initial infection, but *Rht-B1b* significantly increased type II resistance (Srinivasachary et al. 2009; Buerstmayr and Buerstmayr 2016). Moreover, Saville et al. (2012) suggested that lines with DELLA accumulation are more susceptible to the initial infection, but more resistant to spreading infection within the spike. In the present study, results from the moderately resistant cultivar AAC Brandon agreed with findings that *Rht-B1b* contributes to type II resistance, but hinders type I resistance. Although AAC Brandon had a higher disease incidence, it may have strong type II resistance to overcome weak type I resistance. In addition to that, one of AAC Brandon parents 'ND 744' which has high level resistance to FHB provides a good genetic background for FHB resistance in AAC Brandon (Mergoum et al. 2005). Except for AAC Brandon, cultivars with semi-dwarfing alleles, AAC Penhold and Prosper, were generally more susceptible to FHB in the present study. Although AAC Penhold and Prosper were not significantly different from other cultivars except AAC Tenacious on all of disease variables, they had slightly higher values for all of disease variables than other

tested cultivars. For Prosper, incidence was higher than severity, which could be due to semi-dwarfing allele *Rht-B1b*. AAC Penhold which has *Rht-D1b* as the semi-dwarfing allele also showed lower incidence than severity, but it showed less reduction of severity compared to semi-dwarf cultivars with *Rht-B1b*. Even though AAC Brandon and AAC Penhold are at the same FHB resistance level (Seed Manitoba 2018), AAC Penhold appeared to be more susceptible to FHB than AAC Brandon based on higher disease severity, FDK and DON than AAC Brandon. This suggested that the semi-dwarfing alleles, *Rht-B1b* and *Rht-D1b*, have different effects on FHB. Buerstmayr and Buerstmayr (2016) indicated different degrees of disease severity caused by both semi-dwarfing alleles may be because *Rht-D1b* had a stronger impact on anther retention than *Rht-B1b*. However, in this study, AAC Brandon had higher anther retention than AAC Penhold (Table A.25).

AAC Penhold is rated as moderately resistance to FHB. However, in this study it tended to have higher disease values than AAC Cameron which is rated as an intermediate resistant cultivar (Seed Manitoba 2018). AAC Penhold was registered at Canadian Food Inspection Agency in 2014 and it is derived from the cross '5700PR/HY644-BE//HY469' (Cuthbert et al. 2017b). HY644-BE is the source of FHB resistant, and it has a moderate resistance level (Cuthbert et al. 2017b). According to cultivar descriptions for AAC Penhold and AAC Cameron, they were evaluated for FHB at Carman in 2011 and 2012 (Fox et al. 2016; Cuthbert et al. 2017b). In 2011, AAC Cameron was rated as susceptible and AAC Penhold was rated as intermediate while in 2012, AAC Cameron and AAC Penhold were rated as moderately resistance and intermediate, respectively. This suggests both cultivars were vulnerable to environmental effect, and it can be speculated that AAC Penhold and AAC Cameron may have similar levels for FHB resistance depending on environmental conditions. In addition to that, there have been some changes in how cultivars are

classified in terms of determining resistance levels of FHB since 2016 (Pers. Comm. Dr. Anita Brule-Babel, 2021, University of Manitoba). With the new FHB evaluation system, cultivars are rated for not only on index, but also DON. The combined rating of index and DON accumulation may affect the overall cultivar rating. For example, AAC Cameron might have been rated as a moderately resistance cultivar based on index, but it accumulated more DON thereby it might be registered as intermediate resistance level of FHB. Also, *Fusarium graminearum* isolates for FHB testing have changed since 2018. AAC Penhold might be rated as moderately resistance based only on disease symptoms with old isolates. How FHB ratings have been applied through the registration system may affect the rankings today with use of modern isolates of *F. graminearum* and with DON included in the analysis. Therefore, during the 2019 and 2020 field seasons in Winnipeg and Carman, AAC Penhold showed higher FHB disease levels than the intermediate cultivar AAC Cameron suggesting that AAC Penhold may not be moderately resistance to new populations of *F. graminearum*, thereby new evaluations may be required to accurately report FHB resistance level.

The present study clearly showed that height, anther retention, protein content, test weight and yield are quantitative traits that are affected by not only genotypes, but also environment. Because each environment was different in terms of temperature and precipitation during the experiments (Figure 3.1 to 4 and Table A.1 to 2), mean plant heights in 2019 were generally shorter than those of 2020 (Table A.25). Experimental means for anther retention, test weight, and yield varied depending on the environment (Table A.25 to 27). Particularly, protein content was highly influenced by environment (Figure 3.5). Although protein content depended primarily on genotype in this study, it is well known as one of the traditional traits that is strongly influenced by environmental factors such as drought and post-anthesis temperature (Triboï et al. 2003).

Therefore, the significant environment effect was caused by big differences of weather and disease level between Winnipeg and Carman in 2019 and 2020.

This research determined how Manipulator™ and Ethrel™ affect FHB, plant height, and yield of spring wheat, and the interaction between semi-dwarfing alleles and PGRs. In this study, application of PGRs did not affect FHB in all tested spring wheat cultivars that differed in the level of resistance to FHB and plant height. Ethrel™ significantly reduced plant height and Manipulator™ slightly reduced plant height. Both PGRs did not have a significant effect on anther retention, test weight, thousand kernel weight, and yield. Cultivars with semi-dwarfing alleles tended to have higher anther retention. There was a different impact on FHB resistance depending on type of semi-dwarfing alleles. Cultivars with *Rht-B1b* showed stronger resistance to FHB than the cultivar with *Rht-D1b* at the same FHB resistance level from Seed Manitoba 2018. The level of disease variables; FHB index, incidence, severity, FDK and DON in tested cultivars showed the same rankings as reported in Seed Manitoba 2018, except AAC Penhold.

This research provides important information for producers, especially in the higher moisture regions of the prairies where FHB epidemics are most prevalent. These are the regions that have the highest yield potential and risk of lodging under intensive management and would benefit most from PGR application. Since there was no significant effect of both PGRs on FHB, growers could choose to grow the taller FHB resistance cultivars and control height with PGRs to reduce the risk of yield losses due to lodging. However, growers will need to weigh the cost of PGRs verses the potential gain in yield and quality in terms of value when using PGRs in tall FHB resistant cultivars. It may be possible for growers to benefit from the genetics of FHB resistance and use intensive management methods without sacrificing potential yield due to lodging.

Implementation of PGRs to control plant height would also allow breeders to select the lines with the highest FHB resistance without focussing so closely on plant height.

4 Chapter 4.0. The effect of the two plant growth regulators Manipulator™ and Ethrel™ on anther retention in five spring wheat (*Triticum aestivum L.*) genotypes.

4.1 Abstract

Gibberellins (GAs) regulate the growth and development of plants, including height and stamen development. Semi-dwarf wheats with either the *Rht-B1b* or *Rht-D1b* allele are insensitive to GA. Deficiency in GA concentration in plants can cause abnormal stamen development such as failure of filament elongation and reduction in anther size which could promote anther retention. Because anther retention is correlated with higher FHB infection, this could be the reason why semi-dwarf wheat is associated with higher FHB susceptibility. Plant growth regulators (PGRs) alter plant growth and development using either the GA pathway or ethylene (ET) pathway. In cereal production, PGRs are used to produce shorter, thicker, and stronger stems which reduce the risk of lodging. With the recent increased interest in PGRs, it is important to understand whether PGRs affects anther retention in wheat with and without semi-dwarfing alleles. In this study, five spring wheat cultivars that differed in FHB resistance level, height, and the presence of semi-dwarfing alleles were used to investigate the interaction between PGRs and anther retention along with interaction between PGRs and semi-dwarfing alleles. A no PGR control, the anti-gibberellin product Manipulator™, and the ET releasing product Ethrel™ were tested under controlled environments using five spring wheat cultivars (AAC Brandon, AAC Cameron, AAC Penhold, AAC Tenacious, and Prosper). Both PGRs reduced plant height in general. Manipulator™ reduced anther retention while Ethrel™ increased anther retention. The interaction between PGRs and anther retention was genotype dependant. The interaction between PGRs and semi-dwarfing alleles showed Ethrel™ was more effective to reduce height for cultivars with the *Rht-B1b* allele. The height of cultivar with *Rht-D1b* was not affected by either PGR. Generally, there was a tendency of genotypes with higher FHB resistance level to exhibit less anther retention.

4.2 Introduction

Gibberellins (GAs) are a family of phytohormones that regulate growth and developmental processes in plants. DELLA proteins regulate the downstream GA signalling pathway (Mo et al. 2018) and function as growth inhibitors because they repress growth in the absence of bioactive GA (Daviere and Achard 2013; Nelson and Steber 2016; Mo et al. 2018). Bioactive GAs are bound to the receptor GA-INSENSITIVE DWARF1 (GID1) and form the GA-GID1-DELLA complex through conformational changes at the N terminus of GID1 (Mo et al. 2018). This complex leads to DELLA degradation through a proteosome pathway (Gao and Chu 2020) thus targets of DELLA are released and initiate downstream GA-mediated growth responses.

With the introduction of dwarfing traits into wheat cultivars, the 'Green Revolution' of the 1960s successfully boosted the yield of wheat under high fertilizer input (Hedden 2003): plants with short and strong stalks were able to support the weight of the grain and thus minimized yield loss due to lodging. Dwarfing traits can be introduced into the plant by the presence of semi-dwarfing alleles. The semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* are the major dwarfing alleles deployed in over 70% of current wheat cultivars in the world (Mo et al. 2018). Both semi-dwarfing alleles originated from the Japanese cultivar 'Norin 10' and are located on chromosomes 4B and 4D, respectively (Hedden 2003; Gilbert and Haber 2013). A base substitution within the coding sequence of the semi-dwarfing alleles encodes a premature stop codon which translates into a truncated DELLA protein. This prevents GID1-GA from binding to DELLA proteins leading to GA insensitive plants with reduced height (Hedden 2003; Buerstmayr and Buerstmayr 2016; Muqaddasi et al. 2017b; Steiner et al. 2017). Although semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* have contributed to higher yield in optimal environments, these alleles have been associated with cultivar susceptibility to Fusarium head blight (FHB) (Gilbert and Haber 2013; Liu et al. 2013;

Mo et al. 2018). Fusarium head blight is a serious fungal disease of cereals including wheat. Infection of wheat by FHB results in a reduction in both grain yield and quality. Shorter wheat cultivars tend to have higher FHB infection. There are two possible theories that explain higher FHB susceptibility of short plants. First is that shorter plants are physically closer to the inoculum source and provide a favorable environment within the canopy for *Fusarium* growth by reducing air circulation and increasing humidity (Buerstmayr and Buerstmayr 2016). Another is that shorter plants have a higher frequency of anther retention which might reduce *Fusarium* resistance.

Anther retention is a quantitative trait which is under the control of several loci and is affected by environment, but has high heritability (Skinnes et al. 2010; Muqaddasi et al. 2017b; Steiner et al. 2017). In wheat, short stamen filaments promote retention of anthers in the floret. Anthers that are trapped between the palea and lemma facilitate initial infection by *Fusarium* spp by providing an entry point and nutrition for the fungus. Previous research indicated anther retention was associated with increased susceptibility to FHB (Buerstmayr and Buerstmayr 2015, 2016; He et al. 2016). Because anther retention is highly heritable (Skinnes et al. 2010; Lu et al. 2013; Buerstmayr and Buerstmayr 2015), selection for wheat lines with low anther retention could be a good strategy for FHB resistance wheat breeding. Quantitative trait loci for anther retention have been identified on chromosomes 4BS and 4DS, and overlap with the *Rht-B1* and *Rht-D1* loci (Xu et al. 2020). This suggests the negative effects of *Rht-B1* and *Rht-D1* on FHB infection is not only a function of reduced plant height, but may be due to effects on anther retention.

Gibberellins (GAs) are key phytohormones that modulate stamen development including filament elongation and anther dehiscence (Plackett et al. 2011; Marciniak and Przedniczek 2019). Specifically, GA stimulate filament elongation through increased cell elongation (Marciniak and

Przedniczek 2019). During the late stage of stamen development, controlled concentration levels of GA are essential to regulate proper anther development and function of the male reproductive organs (Plackett et al. 2011; Marciniak and Przedniczek 2019).

Ethylene (ET) contributes to flower development. Changes in the levels of ET induce or delay flower development (Iqbal et al. 2017). Since ET is a key phytohormone that controls plant aging (Iqbal et al. 2017), ET plays a role in anther dehiscence (Koning 1983). Moreover, as a multifunctional phytohormone, ET contributes to plant height regulation by inhibiting shoot cell expansion (Dugardeyn et al. 2008; Ross et al. 2016; Iqbal et al. 2017). Ethylene signaling influences the GA-GID1-DELLA mechanism by reducing bioactive GA levels, causing increased accumulation of DELLA proteins (Achard et al. 2003; Iqbal et al. 2017). By mitigating DELLA-mediated growth inhibition, ET is able to prevent plant growth (Iqbal et al. 2017). In addition, ET acts through crosstalk with the growth hormone auxin to reduce plant growth by limiting auxin synthesis and mobility in stem tissues (Caldwell et al. 1988; Ramburan and Greenfield 2007; Wiersma et al. 2011; Vaseva et al. 2018). Because of this, ET is exploited as one of methods for plant growth regulators (PGRs) in cereal crops.

Synthetic formulations affecting phytohormones have been developed to modify plant growth (Rademacher 2016). In cereal crops, PGRs are used to reduce plant height and increase stem thickness thereby reducing the risk of lodging. As a result, PGRs make managing and harvesting of the crop easier. Other than that, the application of PGRs has benefits for root development such as providing better root anchorage, and possible improvement for grain quality. The PGRs which target the GA or ET pathways have been used to improve standability in cereal crops (Rajala et al. 2002; Rademacher 2016; Strydhorst et al. 2018). Manipulator™ is a PGR that

inhibits early steps in GA biosynthesis. Ethrel™ is a PGR that makes plants release ET. Appropriate use of PGRs can maximize the yield potential by improving the morphology of the plant to make the crop more conducive to high yields. Because PGRs modulate stem elongation by inhibiting biosynthesis of GA or releasing ET, it is important to understand if anther retention is affected by their applications. To address these questions, the objectives of this study were to explore the interaction between PGRs and anther retention, and examine interactions between PGRs and semi-dwarfing alleles in different spring wheat cultivars.

4.3 Materials and Methods

4.3.1 Plant material and experimental design

Two controlled environment experiments were conducted in a completely random design with four replicates for Trial 1 and five replicates for Trial 2. Trial 1 was conducted in Fall 2019 and Trial 2 was conducted in Fall 2020. The same growth room was used for both trials. Growth room conditions were set to 23°C for 16 hours with light and 18°C for 8 hours in the dark. Each replicate consisted of one plant per cultivar assigned to each of the three treatments: Manipulator™, Ethrel™, and no PGR control.

Five cultivars (AAC Tenacious, AAC Penhold, AAC Brandon, AAC Cameron, and Prosper) were used in the study. The designated end-use quality class, height, the level of FHB resistance, and the presence of semi-dwarfing alleles for each cultivar are presented in Table 4.1. Plants were initially grown in root trainers (30 x 23 mm and 125mm deep) with Sunshine® soil mix #4 [Sungro® Horticulture., USA]. When they reached Zadoks growth stage (GS) 12 (Zadoks et al. 1974), they were transplanted into 150 x 180 mm pot. Slow release fertilizer (13-12-12) [Master Plant-Prod Inc., Brampton] was applied at 15 ml/pot the day following transplanting. After transplanting, plants were fertilized every three weeks with a water-soluble fertilizer (20-20-20) [Even spray & Chemicals LTD., Winnipeg] diluted to a rate of 15 ml of fertilizer to 4 litres of water.

Table 4.1. List of five cultivars used in controlled environment experiments with wheat end-use class, height, FHB resistance level and the presence of semi-dwarfing alleles.

Cultivars	Wheat Classes	Height (cm)[a]	Resistance to FHB[a]	Semi-dwarfing Alleles (*Rht-B1* or *Rht-D1*)[b]
AAC Tenacious	Canada Prairie Spring Red (CPSR)	Tall (101)	Resistance (R)	None
AAC Penhold	CPSR	Short (71)	Moderate Resistance (MR)	*Rht-D1b*
AAC Brandon	Canada Western Red Spring (CWRS)	Intermediate (81)	MR	*Rht-B1b*
AAC Cameron	CWRS	Tall (94)	Intermediate Resistance (I)	None
Prosper	Canada Northern Hard Red (CNHR)	Intermediate (84)	I	*Rht-B1b*

[a]Seed Manitoba 2018 and [b]Dr. Santosh Kumar from Agriculture and Agri-Food Canada – Brandon Research and Development Centre

4.3.2 Plant growth regulators application

Manipulator™ (620 g/L of Chlormequat chloride) was applied to the plants at Zadoks GS 30 at a rate of 1.8L/ha in a spray chamber model #2 manufactured by Taminco US LLC. Ethrel™ (240 g/L of Ethephon) was applied to the plants at the Zadoks GS 37 to 45 at a rate of 1.25/ha in the spray cabinet. Rates and timing were determined based on the label recommendations. Both PGRs were diluted to the recommended concentration with distilled water. For the control, nothing was applied.

4.3.3 Height measurement

Plant height was determined prior to sample collection by measuring with a 2-meter ruler the height of all individual spikes sampled on each plant from the soil surface in the pot to the tip of the spike (excluding awns). Plant height was measured only in Trial 2.

4.3.4 Spike collection for anther retention

Anthesis date was recorded for each spike in the plant and was defined by when the first anther was visible. Five spikes per plant were collected five days after the anthesis date. The number of retained anthers in the primary and secondary floret from the middle of four alternate spikelets in each spike was determined by opening them up (Figure 4.1). Any anthers that were inside the floret or trapped between the lemma and palea were considered retained anthers. Thus, a total of eight florets for each spike was used to determine the number of retained anthers. The percentage of retained anthers per spike was calculated by using the following equation:

$$\text{Percentage anther retention} = \frac{\text{The number of retained anthers}}{\text{The maxium number of anthers}} * 100\%$$

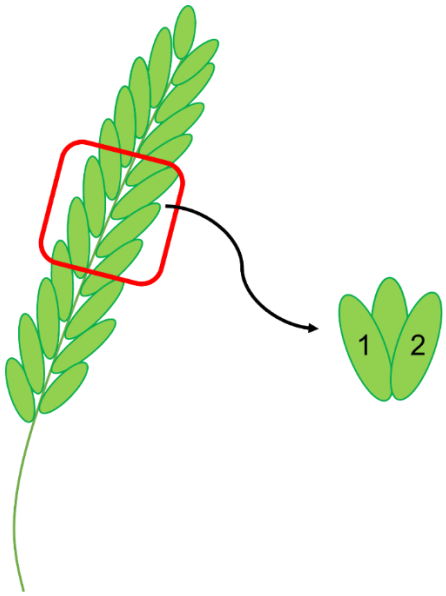

Figure 4.1. Schematic diagram of the location of florets sampled in the wheat spike. A total of four alternating spikelets in the center of spike were selected to determine percentage anther retention. Primary and secondary florets for each sampled spikelet (four spikelets per spike) were opened and retained anthers were counted.

4.3.5 Data analysis

Statistical analyses were performed using SAS software version 9.4 [SAS institute Inc., USA]. Analysis of variance was conducted on the percentage of anther retention using PROC Mixed. Levene's tests for homogeneity of variance was conducted using PROC GLM. Levene's test revealed that the data from the two trials could not be combined. In addition to that, the distributions of the error variances were visually observed using PROC Univariate and it also suggested that two trials could not be pooled, thus, trial data were analyzed and presented separately. The model statement listed the treatments, genotypes, and the interaction of treatment*genotype as fixed effects. Tukey means comparison test was used for comparisons of means for main effects and their interactions. To meet the assumptions for analysis of variance,

the data were transformed by using square root transformation which improved skewness, kurtosis and Shapiro-wilk value.

Height data for Trial 2 was analyzed using SAS software version 9.4 [SAS Institute Inc., USA] with PROC Mixed. The model statement set the fixed effects for the treatments, cultivars, and the treatment*cultivar interaction. Tukey means comparison test was used to compare main effect and interaction means.

A Pearson's correlation coefficient between anther retention and plant height in Trial 2 was generated using PROC Corr in SAS version 9.4.

4.4 Results

4.4.1 Analysis of variance for anther retention

Table 4.2 shows the analyses of variance for percentage anther retention in Trials 1 and 2. Both trials showed that there were significant differences among treatments and cultivars for the anther retention. Only Trial 2 had a significant interaction between treatment and cultivar (Table 4.2).

Table 4.2. Analysis of variance for percentage anther retention in Trial 1 and Trial 2 using square root transformation.

Source of variation	Trial 1			Trial 2		
	DF[a]	MS[b]	P[c] value	DF	MS	P value
PGR[d] treatment	2	16.86	0.0007	2	86.41	<.0001
Cultivar	4	145.39	<.0001	4	224.61	<.0001
PGR treatment*Cultivar	8	1.59	0.6839	8	22.93	<.0001
Error	280	2.24		357	2.45	

[a] DF=Degrees of Freedom, [b]MS=Mean square, [c]P=Probability, and [d]PGR=Plant growth regulator.

4.4.2 Effect of PGR on anther retention

For both trials, there were significant differences in anther retention for PGR treatment. In Trial 1, anther retention for the Manipulator™ treatment was 10.09%, compared to 14.89% for the control (Table 4.3). Anther retention for the Ethrel™ treatment (15.39%) was not significantly different from the control and was significantly higher than the Manipulator treatment in Trial 1. Anther retention in Trial 2 was two to three times higher than in Trial 1. In Trial 2, there were significant differences among all PGR treatments for anther retention, where Manipulator™ had the lowest anther retention (22.68%) followed by the control (28.31%) and Ethrel™ (41.08%) (Table 4.3).

Table 4.3. Least square means of percentage anther retention of plant growth regulator treatments (Control, Ethrel™, and Manipulator ™) across five cultivars in Trial 1 and 2.

PGR treatment	Anther retention (%)	
	Trial 1	Trial 2
Control	14.89 A	28.31 B
Ethrel™	15.39 A	41.08 A
Manipulator™	10.09 B	22.68 C

Means followed by same letter in a column are not significantly different at p=0.05 based on the Tukey means comparison test.

4.4.3 Effect of cultivar on anther retention

Significant differences in anther retention for cultivars was observed in both Trials 1 and 2. In Trial 1, AAC Brandon, which has the semi-dwarfing allele *Rht-B1b*, had the highest percentage anther retention (29.48%) among cultivars (Table 4.4). AAC Cameron with no semi-dwarfing alleles had the second highest anther retention percentage which was 27.57%, but it was not statistically different from the percentage of anther retention of AAC Brandon. AAC Tenacious

had 8.9% anther retention which was significantly lower than AAC Brandon and AAC Cameron, but not different from AAC Penhold and Prosper. AAC Penhold which has the semi-dwarfing allele *Rht-D1b* showed statistically similar percentage anther retention to AAC Tenacious and Prosper. For Prosper which has the semi-dwarfing allele *Rht-B1b*, it had the lowest percentage of anther retention.

Trial 2 showed different cultivar rankings for percentage anther retention than Trial 1. AAC Brandon had the highest rate of anther retention (Table 4.4) where more than half the spikelets (58.56%) contained anthers. AAC Cameron and Prosper had similar percentage of anther retention which were 38.84% and 35.56%, respectively. The cultivar AAC Penhold, had the second lowest anther retention among cultivars at 20.37%. The lowest percentage of anther retention (9.77%) was observed in AAC Tenacious.

Table 4.4. Least square means of percentage anther retention of cultivars (AAC Brandon, AAC Cameron, AAC Penhold, AAC tenacious, and Prosper) across plant growth regulator treatments in Trial 1 and 2.

Cultivar	Anther retention (%)	
	Trial 1	Trial 2
AAC Brandon	29.48 A	58.56 A
AAC Cameron	27.57 A	38.84 B
AAC Penhold	6.04 BC	20.37 C
AAC Tenacious	8.90 B	9.77 D
Prosper	4.58 C	35.56 B

Means followed by same letter in a column are not significantly different at p=0.05 based on the Tukey means comparison test.

4.4.4 Interaction between cultivar and PGR for anther retention

Analysis of variance showed no significant interaction between cultivar and PGR treatment in Trial 1, but there was a significant interaction in Trial 2 (Table 4.2). The main reason for the interaction between cultivar and PGR treatment was a change in ranking among the cultivars under different PGR treatments (Figure 4.2). Relative rankings of AAC Penhold and Prosper changed considerably with PGR treatments (Figure 4.2). For example, Prosper had the third lowest anther retention for the control and Manipulator™ treatments, but the highest anther retention when treated with Ethrel™. AAC Penhold also had higher anther retention when treated with Ethrel™ compared to the control and Manipulator™ treatments. Anther retention when Ethrel™ was used with AAC Penhold or Prosper was about doubled that of the control. AAC Brandon and AAC Tenacious had similar anther retention across PGR treatments, while AAC Cameron had a slightly higher anther retention when treated with Ethrel™, compared to the control and Manipulator™ treatments. In addition to that, the lowest anther retention for AAC Penhold occurred when it was treated with Manipulator™.

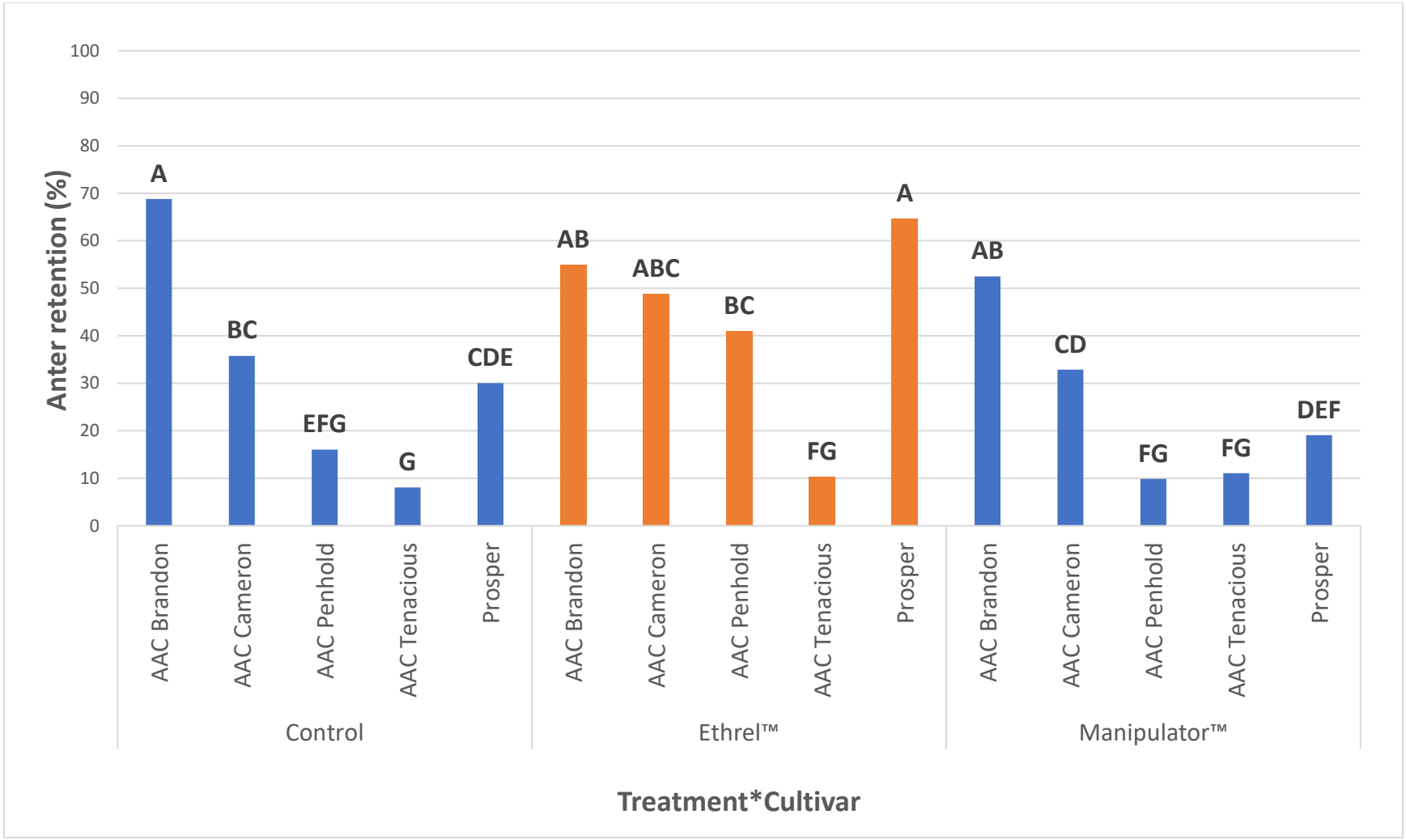

Figure 4.2. Interaction between plant growth regulator (PGR) treatments (Control, Ethrel™, and Manipulator™) and cultivars (AAC Brandon, AAC Cameron, AAC Penhold, AAC Tenacious, and Prosper) on anther retention (%) in Trial 2. Bars indicate the percent anther retention for each treatment combination. Bars denoted by the same letter are not significantly different p=0.05 based on the Tukey means comparison test.

4.4.5 Analysis of variance for plant height in Trial 2

Analysis of variance for plant height indicated that there were significant differences between PGR treatments, cultivars, and the interaction between PGR treatment and cultivar (Table 4.5).

Table 4.5. Analysis of variance for the plant height (cm) in Trial 2.

Source of Variation	DF[a]	Mean Square	P[b] value
PGR[c] treatment	2	2293.85	<.0001
Cultivar	4	4986.60	<.0001
PGR treatment*Cultivar	8	231.04	<.0001
Error	358	31.93	

[a] DF=Degrees of Freedom, [b]P=Probability, and [c]PGR= Plant growth regulator

Both Ethrel™ and Manipulator™ significantly reduced the plant height by more than 5cm compared to the control (Table 4.6). The relative height of cultivars followed the same ranking as reported in Seed Manitoba 2018 (Table 4.7). AAC Tenacious was the tallest cultivar, followed by AAC Cameron, Prosper, AAC Brandon and AAC Penhold.

Cultivar plant height rankings were similar across all PGR treatments, but the magnitude of the differences between the cultivars was not the same across treatments. Ethrel™ significantly reduced the height of AAC Brandon and Prosper, while application of Manipulator™ did not cause a significant reduction in plant height of these cultivars (Figure 4.3). Both of AAC Brandon and Prosper carry the *Rht-B1b* semi-dwarf allele. For AAC Cameron and AAC Tenacious, application of Ethrel™ and Manipulator™ decreased plant height in a similar manner. AAC Cameron and AAC Tenacious do not carry any semi-dwarfing alleles. Although there was a slight reduction in plant height when Ethrel™ or Manipulator™ were applied to AAC Penhold, plant height was not significantly different from the control. AAC Penhold carries the *Rht-D1b* semi-dwarfing allele.

Table 4.6. Least square means of plant height (cm) with plant growth regulator treatments (Control, Ethrel™, and Manipulator™) in Trial 2.

PGR Treatment	Height (cm)
Control	70.25 A
Ethrel™	62.25 B
Manipulator™	63.56 B

Means followed by same letter in a column are not significantly different at p=0.05 based on the Tukey means comparison test.

Table 4.7. Least square means of plant height (cm) with cultivars (AAC Brandon, AAC Cameron, AAC Penhold, AAC Tenacious, and Prosper) in Trial 2.

Cultivar	Height (cm)
AAC Brandon	62.54 C
AAC Cameron	68.99 B
AAC Penhold	54.16 D
AAC Tenacious	76.29 A
Prosper	64.78 C

Means followed by same letter in a column are not significantly different at p=0.05 based on the Tukey means comparison test.

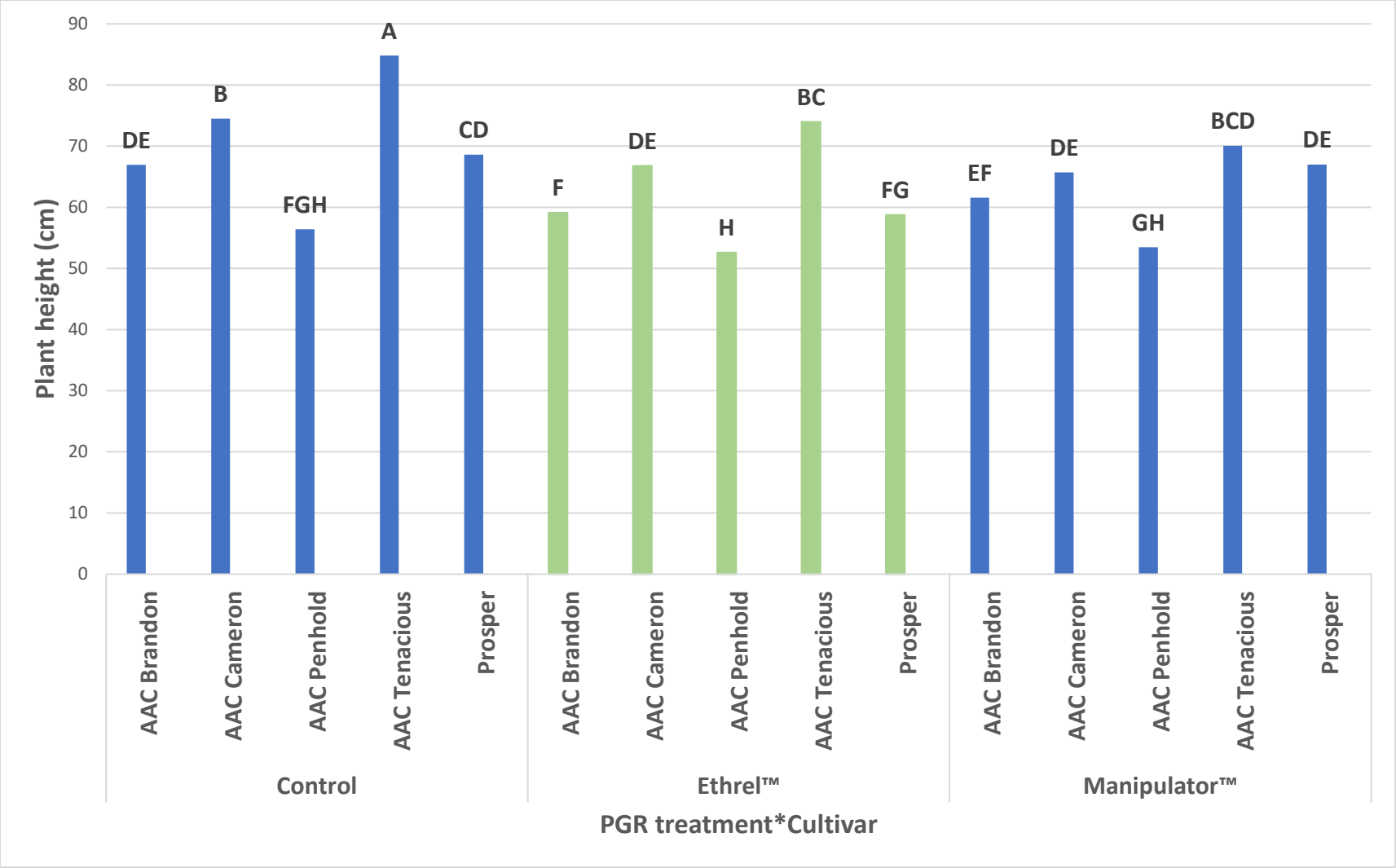

Figure 4.3. Interaction between plant growth regulator (PGR) treatments (Control, Ethrel™, and Manipulator™) and cultivars (AAC Brandon, AAC Cameron, AAC Penhold, AAC Tenacious, and Prosper) on the plant height (cm) in Trial 2. The height of the bar represents the plant height for each treatment by cultivar combination. Bars denoted by the same letter are not significantly different at p=0.05 based on Tukey means comparison test.

4.4.6 Correlation of percentage of Anther retention and plant height in Trial 2

The percentage of anther retention were negatively correlated with plant height (-0.28, $p < 0.0001$, $n=372$).

4.5 Discussion

Selection against anther retention may be a proxy for FHB resistance when breeding wheat cultivars. Phytohormones such as GA play important roles to regulate stamen development. With benefits of using PGRs, interest in PGR has increased. Because PGRs target the GA pathway, it is essential to understand how PGRs affect anther retention in wheat breeding against FHB.

The effect of Ethrel™ application on anther retention was similar across the two trials: the treatment tended to increase anther retention in Trial 1, although the effect was not statistically significant, and significantly increased anther retention in Trial 2. These results agree with those of Bennett and Hughes (1972) and Campbell et al. (2001) who reported ET promoted anther retention in wheat. Both studies applied exogenous ET to wheat plants which resulted in the abnormal development of anthers and led to the failure of anther extrusion and dehiscence. Of note, the treated plants produced smaller anthers and pollen to enhance anther retention (Bennett and Hughes 1972). Therefore, smaller anthers have a higher chance to be retained inside of the floret compared to the bigger anthers. Ethylene represses cell elongation through crosstalk with the GA pathway and auxin pathway (Caldwell et al. 1988; Ramburan and Greenfield 2007; Dugardeyn et al. 2008; Wiersma et al. 2011). Although anther size in this study was not measured, the Ethrel™ treatment could have had an impact on shortening the lodicules and stamen filaments in addition to decreasing the size of anthers which consequently encourages anther retention by trapping the tissue between the lemma and palea, in addition to reducing plant height. The magnitude of effect of ET on plant development is dependant on environmental factors such as light, temperature,

oxygen and carbon dioxide concentrations (Klassen and Bugbee 2004). Although growth rooms are designed to provide a controlled environment, it is not possible to entirely replicate the exact same condition across trials. Variation in growth room conditions such as light intensity and gas concentration across the room and between experiments can influence the reproducibility of experiments (Measures et al. 1973; Potvin et al. 1990; Porter et al. 2015). This unavoidable variation, therefore, likely explains the different results obtained in Trial 1 and Trial 2, despite both experiments being conducted in same the growth room. Nonetheless, results of the two trials suggest that Ethrel™ application affects anther retention in wheat and further research is warranted to dissect the underlying mechanism.

Gibberellins, the target of the PGR product Manipulator™, are important plant hormones that contribute to stamen development and cell elongation in plants. During the last step of stamen development, stamen filaments begin to elongate and anther tissue rapidly degenerates to dehisce anthers (Marciniak and Przedniczek 2019). The amount of GA in the plant during this phase is critical. The active ingredient of Manipulator™, Chlormequat chloride (CCC), inhibits the GA synthesis pathway through blocking *ent*-copalyl diphosphate-synthase and *ent*-Kaurene synthase in early steps of GA biosynthesis (Rademacher 2016). As inappropriate GA levels in the plant cause abnormal development of anthers and aberrant elongation of filament (Marciniak and Przedniczek 2019), Manipulator™ may cause abnormal filament elongation and anther development. In this study, the application of Manipulator™ significantly decreased the percentage anther retention compared to the control in both trials. This is very interesting result because alterations in GA levels in the plant by Manipulator™ were predicted to lead to abnormal filament elongation and anther development, thereby increasing anther retention. One possible reason for lower anther retention caused by Manipulator™ could be that the active ingredient of

Manipulator™, chlormequat chloride, breaks down before filament elongation happened, since application of Manipulator™ was performed at an early growth stage; Zadoks GS 30. Clark and Fedak (1977) reported barley treated with chlormequat chloride showed increased plant height. This was due to the temporary cessation, or general decline of GA synthesis, by chlormequat chloride (Clark and Fedak 1977). During the temporary cessation, GA precursors accumulated in the plant. As soon as the chlormequat chloride degrades, GA synthesis resumes and excess GA precursors produce high levels of active GAs resulting in higher plant height (Strydhorst 2014). Elevated levels of GA precursors from deferment of GA synthesis may promote elongation of anther filaments leading to lower anther retention in this study. Therefore, further research is required to measure GA concentrations in the floret to monitor changes in GA levels.

Plant height was significantly reduced by Ethrel™ and Manipulator™ in Trial 2. Ethrel™ and Manipulator™ reduced overall plant height by the same amount. However, unexpectedly, they showed the opposite result in anther retention; Ethrel™ increased anther retention while Manipulator™ decreased anther retention. Higher anther retention by Ethrel™ is thought to be due to reduced cell extension of the filament. In theory, if the PGR reduced plant height, it may have the same effect on the filament. Hence shorter filaments would be expected to cause higher anther retention. However, Manipulator™ decreased anther retention. The effect of Manipulator™ was different on plant height and anther retention. This suggested that, although GA can be produced in almost the entire plant, different organs and cells have a different extent of GA synthesis. In Arabidopsis and rice, different responses to GA were reported for certain organs and cell types (Dugardeyn et al. 2008). Another possibility might be timing of Manipulator™ application. As mentioned earlier, application of Manipulator™ at Zadoks GS 30 reduced plant height, but early application may have facilitated break down of chlormequat chloride, thus GA synthesis resumes

with excess GA precursors at the phase of filament elongation. Thus, this could explain why Manipulator™ only reduced stem elongation, not filament elongation.

AAC Brandon had the highest anther retention in both Trials. This may be due to the presence of the semi-dwarfing allele *Rht-B1b*. Because gibberellins control cell elongation, GA insensitive semi-dwarf plants may have shorter anther filaments which results in anthers being retained within the spikelets (Buerstmayr and Buerstmayr 2016).

Interestingly, although AAC Cameron did not have any semi-dwarfing alleles, it ranked second highest for anther retention among the cultivars tested and was not statistically different from AAC Brandon for anther retention in Trial 1. In Trial 2, AAC Cameron also had the second highest anther retention, but was statistically lower than AAC Brandon. AAC Cameron rated as intermediate for FHB resistance, but it had lower anther retention than AAC Brandon which is rated as moderate resistance. This indicated that the differences in FHB resistance level cannot be determined by only considering anther retention between these two cultivars

AAC Tenacious, Prosper and AAC Penhold had different rankings among cultivars in terms of the percentage of anther retention in Trial 1 and Trial 2. This discrepancy is likely due to environmental variation between the experiments; as anther retention is a quantitative trait, changes in the environment can confound the accuracy of the phenotyping. Also, the cultivar means represent the means across all PGR treatments. Since cultivars responded differently to the PGR, looking at the means across all PGR treatments could explain the discrepancy of ranking.

With the exception of AAC Brandon, FHB resistance ratings of the cultivars reflected the level of anther retention in Trial 2 where lower anther retention was associated with higher FHB resistance. Generally, cultivars with little or no anther retention tend to have low FHB infection

(Buerstmayr and Buerstmayr 2015; Wanguimwaniki 2017). Therefore, it can be concluded that low anther retention may contribute the level of FHB resistance of cultivars.

Plant height was measured only in Trial 2. The correlation between plant height and anther retention revealed a significant negative relationship. Plant height is controlled by cell elongation and cell division. If the plant is short, it may also have smaller anthers, and shorter anther filaments which may lead to higher anther retention. Buerstmayr and Buerstmayr (2016) also found highly negative correlation between height and anther retention in winter wheat populations.

Overall, the percentage of anther retention in Trial 2 was approximately twice that of Trial 1. The nature of quantitative trait is highly influenced by environment. Because it was impractical to have identical environments between trials, minor genes affecting anther retention could react differently to environment, resulting in different percentages of anther retention in Trial 1 and Trial 2. Therefore, more environments under the different temperature and light intensity would be required to evaluate anther retention despite the fact that anther retention is highly heritable.

Data on Trial 2 showed AAC Penhold or Prosper with Ethrel™ treatment led to a significant increase in anther retention compared to treatment with Manipulator™. Both AAC Penhold and Prosper have semi-dwarfing alleles *Rht-D1b* and *Rht-B1b,* respectively. Because semi-dwarf plants are already insensitive to GA, anther retention of semi-dwarf plants could be more sensitive to application of Ethrel™ than application of Manipulator™. Unlike AAC Penhold and Prosper, AAC Brandon containing *Rht-B1b* did not appear to have higher anther retention when Ethrel™ was applied. This may be due to different sensitivity to ET in different cultivars (Campbell et al. 2001).

Taken together, application of the PGR Ethrel™ significantly increased anther retention and reduced plant height in spring wheat. In contrast, the PGR Manipulator™ reduced both anther retention and plant height. The wheat genotypes had different degrees of anther retention in the absence of PGR treatment. Different responses of anther retention among the cultivars in Trial 1 and 2 confirm the quantitative nature of anther retention and the susceptibility of the trait to environmental influence. Treatment of semi-dwarfing cultivars Prosper and AAC Penhold with Ethrel™ increased anther retention compared to the control, while treatment of the semi-dwarfing cultivar AAC Brandon with Ethrel™ slightly decreased in anther retention, but it was not significantly different from the control. This suggests sensitivity to ET in cultivars could be different, despite the presence of the same semi-dwarfing allele. Because at least part of the growth inhibition by ET is modulated through the destabilization of DELLA protein directly and also decreasing auxin synthesis and its movement in stem tissues (Caldwell et al. 1988; Achard et al. 2003; Ramburan and Greenfield 2007; Wiersma et al. 2011), GA-insensitive cultivars could be affected by Ethrel™ more effectively than Manipulator™.

AAC Brandon is a very interesting cultivar because it has a moderate level of resistance to FHB and had the highest anther retention in this study. AAC Brandon comes from the cross 'Superb/CDC Osler//ND 744' (Cuthbert et al. 2017a). Among the parents of AAC Brandon, ND 744 has high level resistance to FHB. ND 744 is derived from the cross 'ND 2831/Parshall//ND 706' (Mergoum et al. 2005). The FHB resistance source of ND 2831 is inherited from a cross with 'Sumai-3'. Sumai-3 is the most widely used for a source of resistance to FHB in the world and its derivative cultivars have excellent resistance to the spread of infection (type II) within the infected spike (Bai and Shaner 2004). Because anther retention can increase initial infection by *Fusarium*, AAC Brandon may have strong type II FHB resistance to overcome potential issues caused by

anther retention. Therefore, it is possible that this strong type II FHB resistance contributes to the moderate level of resistance to FHB in AAC Brandon in spite of its higher anther retention.

Application of PGRs to tall cultivars with the highest level FHB resistant could be used to counteract the negative effects of FHB susceptibility associated with shorter plants. If anther retention is affected by PGR application, this could also affect FHB resistant level in cultivars. Therefore, with increased interest in PGRs, it is useful to understand if anther retention is affected by PGR applications. The information in this study is important for growers to decide whether using taller FHB resistant cultivar with PGRs to manage lodging risk would be useful. The results from this study ascertain the effect of PGRs on anther retention and plant height and showed there was interaction between PGRs and semi-dwarfing alleles. In the present study, Manipulator™ did not increase anther retention in general, but Ethrel™ did. The effect on plant height by PGRs was different depending on the semi-dwarfing alleles. Both PGRs reduced plant height for plants with no semi-dwarfing alleles, while Ethrel™ reduced plant height for the cultivars with the *Rht-B1b* allele, but Manipulator™ did not affect plant height in these cultivars. Neither PGR significantly affected plant height of the cultivar with the *Rht-D1b* allele. Therefore, growers need to adjust their management when they apply PGR on wheat. For example, based on the type of PGR, anther retention could be increased, thereby growers need to consider the type of PGR and genotype of wheat. In addition to this experiment, inoculation of *Fusarium* can be applied to determine FHB infection severity related to anther retention and semi-dwarfing alleles in the future research. Finally, anther filament length and anther size can be measured in order to determine how PGRs affect these traits.

5 Chapter 5.0. The effect of the plant growth regulators Manipulator™ and Ethrel™ on the accumulation of *Fusarium graminearum* DNA in spring wheat (*Triticum aestivum* L.) spikes

5.1 Abstract

Fusarium head blight (FHB) is a devastating fungal disease of cereal crops caused mainly by *Fusarium graminearum.* It decreases yield and quality of the grain. Height in wheat (*Triticum aestivum* L.*)* is associated with FHB infection. This could be because of the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b*. Both semi-dwarfing alleles influence gibberellin (GA) response to make plants short. Some plant growth regulators (PGRs) also affect the GA pathway, either directly or indirectly. As use of PGRs has increased with intensive management, it is essential to examine whether PGRs affect FHB development in wheat. This study evaluated the effect of PGRs, Manipulator™ and Ethrel™, on the accumulation of *F. graminearum* DNA in the spike and plant height of spring wheat cultivars that differ in height, semi-dwarfing genes, and FHB resistance. Two controlled environment studies were conducted using five spring wheat cultivars (AAC Brandon, AAC Cameron, AAC Penhold, AAC Tenacious, and Prosper), and three PGR treatments (Manipulator™, Ethrel™ and no PGR control).

Ethrel™ significantly reduced ($p<0.001$) the fungal DNA accumulation in Trial 1 and plant height in both trials. For wheat cultivars with the semi-dwarfing allele *Rht-B1b,* Prosper and AAC Brandon, spraying Ethrel™ significantly decreased the fungal DNA accumulation in both trials. For cultivars without the semi-dwarfing *Rht-B1b* allele, application of Ethrel™ increased fungal DNA accumulation in the spike in both trials. This indicated Ethrel™ effects are different among genotypes. Results for application of Manipulator™ were variable and inconsistent for fungal DNA accumulation in the two trials. Significant differences between Trial 1 and Trial 2 accounted for variation and conflicting results. AAC Penhold which has the semi-dwarfing allele *Rht-D1b*

responded in the opposite direction for fungal DNA accumulation in Trial 1 and Trial 2 when it was sprayed with Manipulator™. This suggested that Manipulator™ may interact differently with *Rht-D1b* in different environments. Overall, the results presented here show that the role of PGRs in the accumulation of fungal DNA in the spike is different depending on the genotype of wheat. Further experiments are required to understand the specific interaction between PGRs and wheat genotype.

5.2 Introduction

Fusarium head blight (FHB), caused by *Fusarium* spp., is one of the most important fungal diseases of cereal crops. In North America, *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.) Petch] is the predominant agent. Fusarium head blight causes loss of yield and grain quality and threatens food and feed safety by producing mycotoxins, including deoxynivalenol (DON), in the grain. Resistance to Fusarium head blight in wheat is a quantitative trait and is influenced by environment. Research has shown that semi-dwarfing alleles from the *Rht-B1* and *Rht-D1* loci in wheat are associated with increased susceptibility to FHB (Buerstmayr and Buerstmayr 2016). The *Rht-B1* and *Rht-D1* loci are located on chromosomes 4B and 4D, respectively. Both semi-dwarfing alleles from the *Rht-B1* and *Rht-D1* loci are associated with higher FHB disease incidence during the initial infection stage (Gilbert and Haber 2013; Liu et al. 2013). The presence of the *Rht-D1b* allele also increased disease spread within the spike tissues while the presence of the *Rht-B1b* allele did not (Miedaner and Voss 2008; Srinivasachary et al. 2009; Liu et al. 2013; Lu et al. 2013; Buerstmayr and Buerstmayr 2016). Semi-dwarf plants are preferable to growers since semi-dwarf plants produce less straw to thresh and have better lodging tolerance; the latter enables optimum nutrient management and substantial yield increases. The *Rht-B1* and *Rht-D1* loci encode DELLA proteins which function in gibberellin (GA) response; the

Rht-B1b and *Rht-D1b* semi-dwarfing alleles encode truncated DELLA proteins which render the plants insensitive to GA, thus resulting in a semi-dwarf phenotype.

Plant growth regulators (PGRs) are naturally present in plants and PGRs are also referred to as synthetic compounds that modify plant growth and development by altering hormonal activity in the plant. Currently, PGRs are used in cereal crops primarily to prevent lodging. Three commercial PGR formulations are available to wheat producers in Canada: Manipulator™ [Taminco US LLC., USA] and Moddus® [Syngenta Canada Inc., Guelph] are GA inhibitors, while Ethrel™ [Bayer CropScience Inc., Calgary] is an ethylene (ET) releasing product. The active ingredient for Manipulator™ is chlormequat chloride (CCC) which directly inhibits the biosynthesis of GA (Hedden and Sponsel 2015; Rademacher 2016). The active ingredient for Ethrel™ is ethephon which elevates levels of ethylene (ET) in the plant. Endogenous ET represses production of bioactive GAs and causes an increase in DELLA protein accumulation (Achard et al. 2003; Dugardeyn et al. 2008; Iqbal et al. 2017). In addition to that, ET interacts with auxin by supressing synthesis and movement of auxin in the stem tissue (Caldwell et al. 1988; Wiersma et al. 2011; Vaseva et al. 2018). When applied singly, these PGR products induce morphological changes in the plant by reducing cell elongation and thickening stems; however, because of crosstalk and interaction between GA, ET and auxin, the disruption of one may inadvertently result in unpredictable secondary effects.

Studies of the effect of CCC application or ethephon application have produced conflicting results. For example, Mankevičiene et al. (2008) reported that use of CCC and ethephon together, or spraying CCC twice, once at an early stage and once at a late stage of winter wheat, increased the content of *Fusarium* spp fungi in the grains, significantly increased zearalenone, and slightly

increased DON concentration in grains in the field condition without artificial inoculation. On the other hand, a field study showed that both PGRs, ethephon and CCC, had a significant effect on spikelet and seed infection caused by *F. graminearum* under wet field conditions using ascospore inoculation, while there was no effect of both PGRs on spikelet and seed infection when macroconidia inoculation of spikes at anthesis was used on the red spring wheat cultivar 'Max' (Fauzi and Paulitz 1994). Under greenhouse conditions, the application of the active ingredients CCC or ethephon, did not significantly affect on FHB progression (Fauzi and Paulitz 1994). Therefore, the role of PGRs and its mechanisms on FHB inhibition are largely unclear and represent a knowledge gap in the field.

Exogenous applications of GA and ET exhibited either a positive or negative effect on FHB infection in wheat, depending on the study (Li and Yen 2008; Chen et al. 2009; Buhrow et al. 2016; Sun et al. 2016; Foroud et al. 2018; Haidoulis and Nicholson 2020). Treatment with exogenous GA and inoculum of *F. graminearum* in the FHB-susceptible wheat cultivar 'Fielder' at anthesis resulted in a reduction of both FHB disease symptoms and the accumulation of DON in isolated grains compared to inoculation of the pathogen alone without the PGR (Buhrow et al. 2016). In another study, GA treatments on the FHB resistant cultivar 'Sumai-3' and the FHB susceptible landrace 'Y1193-6' did not show a noticeable impact on their FHB resistance or susceptibility (Li and Yen 2008). The effects of exogenous application of ET on FHB are more ambiguous. The application of ethephon did not affect FHB in Sumai-3, but it did induce FHB resistance in Y1193-6 (Li and Yen 2008). Ethylene enhancers, ethephon and ET precursor 1-aminocyclopropane-1-carboxylic acid (ACC), increased resistance to *F. graminearum* in FHB susceptible cultivars (Foroud et al. 2019b). In contrast, wheat treated with ethephon enhanced susceptibility of wheat to *F. graminearum* infection (Chen et al. 2009). Additionally, the application of ET precursor ACC

significantly increased susceptibility of Purple false brome (*Brachypodium distachyon*) to FHB (Haidoulis and Nicholson 2020). In another study, ethephon treatment failed to have any impact on the FHB in the highly FHB resistant wheat landrace 'Wangshuibai' and FHB susceptible mutant 'NAUH117' (Sun et al. 2016).

In considering the current body of empirical evidence on the effect of PGRs and exogenous application of phytohormones on FHB, the relationship between the two remains equivocal. With increasing interest in the use of PGRs on high yield wheat cultivars to reduce lodging and increase yield, research to explore the effects of PGRs on FHB and its mechanisms is warranted.

Quantitative real time polymerase chain reaction (qPCR) is very sensitive and rapid tool for detecting and quantifying *Fusarium* species in infected samples. In particular, it allows for differentiation of species and offers an efficient estimation of individual species. Because *F. graminearum* is the most important trichothecene producing *Fusarium* species in North America, using qPCR to estimate fungal contamination of samples provides highly specific and relatively fast quantification of individual species.

Therefore, the objectives of this study were to determine the effect of two PGRs, Manipulator™ and Ethrel ™, on plant height and to quantify the accumulation of *F. graminearum* DNA in the wheat spikes of different spring wheat cultivars. Furthermore, the study aimed to validate the interaction between the semi-dwarfing alleles *Rht-B1b* and *Rht-D1b* with PGRs and FHB in spring wheat cultivars.

5.3 Materials and methods

5.3.1 Plant material and experimental design

The experiment was conducted in a completely random design with three replicates inside a growth room. The whole experiment was repeated as Trial 1 and Trial 2. Growth room conditions were set to 23°C for 16 hours with light and 18°C for 8 hours without light. Each replicate consisted of one plant per cultivar assigned to each of the three treatments: Manipulator™, Ethrel™, and control.

Five cultivars, AAC Tenacious, AAC Penhold, AAC Brandon, AAC Cameron, and Prosper, were used in the study. The designated end-use quality class, height, level of FHB resistance, and the presence of semi-dwarfing alleles for each cultivar are presented in Table 5.1. Plants were grown in root trainers (14 x 3.5 x 2.5 cm per one cell) with Sunshine® soil mix #4 [Sungro® Horticulture., USA]. When they reached Zadoks growth stage (GS) 12 (Zadoks et al. 1974), they were transplanted individually into 15 x 18 cm pots. Slow release fertilizer (13-12-12) [Master Plant-Prod Inc., Brampton] was applied the day following transplanting at 15 ml/pot. Water-soluble fertilizer (20-20-20) [Even spray & Chemicals LTD., Winnipeg] was diluted at a rate of 15 ml/ 4 L of water and applied to each plant every three weeks.

Table 5.1. List of five cultivars used in each controlled environment experiment with wheat end-use class, height, FHB resistance level and the presence of semi-dwarfing alleles.

Cultivars	Wheat Classes	Height (cm)[a]	Resistance to FHB[a]	Semi-dwarfing Alleles (*Rht-B1* or *Rht-D1*)[b]
AAC Tenacious	Canada Prairie Spring Red (CPSR)	Tall (101)	Resistance (R)	None
AAC Penhold	CPSR	Short (71)	Moderately Resistance (MR)	*Rht-D1b*
AAC Brandon	Canada Western Red Spring (CWRS)	Intermediate (81)	MR	*Rht-B1b*
AAC Cameron	CWRS	Tall (94)	Intermediate Resistance (I)	None
Prosper	Canada Northern Hard Red (CNHR)	Intermediate (84)	I	*Rht-B1b*

[a]Seed Manitoba 2018 and [b]Dr. Santosh Kumar from Agriculture and Agri-Food Canada, Brandon Research and Development Centre

5.3.2 Plant growth regulator application

Manipulator™ (620 g/L of Chlormequat choride) was applied to the plants at Zadoks GS 30 at a rate of 1.8L/ha in the spray chamber model #2 manufactured by Taminco US LLC. Ethrel™ (240 g/L of Ethephon) was applied to the plants at the Zadoks GS 37 to 45 at a rate of 1.25 L/ha in the spray cabinet. Both PGRs were diluted with distilled water. PGR treatments were applied following manufacturer's suggested rate. For the control treatment, nothing was applied.

5.3.3 Inoculum preparation and inoculation

Four different isolates of *F. graminearum* were obtained in 2015 from Dr. Maria Antonia Henriquez (Morden Research and Development Center of Agriculture and Agri-Food Canada). Two isolates were the 3 acetyl-deoxynivalenol chemotype (HSW-15-39 and HSW-15-87), and two isolates were the 15 acetyl-deoxynivalenol chemotype (HSW-15-27 and HSW-15-57).

Inoculum for each isolate was prepared by taking a section of a single spore isolation on potato dextrose agar media and transferring it onto a Spezieller Nährstoffarmer agar (SNA) (Nirenberg 1981) media plate (20mL media/plate). Plates were placed under a UV light at room temperature for one week. Subsequently, the SNA media was sliced and transferred into flasks of liquid carboxymethyl cellulose (CMC) (Tuite 1969) media (1.5 L/flask) and placed under UV light with aeration for another week to produce macroconidia. The culture was strained through sterile cheesecloth into sterile glass bottles and kept at 4^{o}C until use. Macroconidia concentration of each culture was determined visually under a microscope using a hemocytometer. To prepare spray inoculum, equal macroconidia counts of each isolate were combined in a one litre bottle and distilled water was used to adjust the concentration to produce one litre of spray inoculum at a final concentration of 50,000 macroconidia/mL. Tween 20 [VWR international., Edmonton] was added as a surfactant to each bottle at a rate of 4ml/L.

Anthesis date was recorded for each spike in the plant and was defined by visible extrusion of the first anther in the spike. When 50% of spikelets had extruded anthers, the spike was sprayed with inoculum using a hand sprayer. Each spike was sprayed with three pumps until whole spike was wet. Three spikes per plant were inoculated. After inoculation, spikes were covered with a glycine bag for two days to maintain humidity.

5.3.4 Height measurement

Plant height was determined prior to sample collection by measuring with a 2-meter ruler the height of all individual spikes sampled on each plant from the soil surface in the pot to the tip of the spike (excluding awns).

5.3.5 Spike collection

Inoculated spikes were collected 14 days post inoculation, flash frozen immediately in liquid nitrogen, and stored at -80°C for further DNA extraction.

5.3.6 DNA extraction

Whole spikes were ground to a fine powder with liquid nitrogen in a sterile mortar and pestle. Approximate 0.90 g of fine powder of each sample was transferred into a 1.5 ml tube. DNA extraction was performed using the Qiagen plant mini kit [QIAGEN., USA] following the manufacturer's protocol.

Fungal DNA from HSW-15-39 served as a reference for downstream *F. graminearum* quantification. Fungal DNA was isolated from liquid cultures of HSW-15-39 using an extraction protocol provided by Dr. Maria Antonia Henriquez (Pers. Comm. Dr. Maria Antonia Henriquez, 2021, Agriculture and Agri Food Canada). The extraction protocol was a modification of the Qiagen plant mini kit protocol. Briefly, fungal cultures in CMC were centrifuged for 10 min at 12000 rpm to form a pellet of the mycelia. Approximately 0.7 g of mycelia was ground and

dispersed in 1000 µL TES extraction buffer (NaCl, Tris pH7.5, EDTA pH8 SDS, and H_2O). 2 µL of RNase (10 mg/mL) and 2 µL of proteinase K were added. After incubation at 65°C for 45min, 500 µL of 7.5 M ammonium acetate was added and the samples were incubated at room temperature for 5 min. After centrifugation for 10 min at 12000 rpm, DNA was isolated with chloroform: isoamyl alcohol (24:1). Another round of centrifuge was done for 10 min at 12000 rpm, the supernatant was collected, and isopropanol was added to the supernatant. After adding isopropanol into the samples, the isolated DNA was subsequently precipitated and purified using the Qiagen plant mini kit. DNA concentration was determined using a Nanodrop™2000 [Thermo Scientific™., USA] and stored at -20^{o}C until use.

5.3.7 Quantitative real time PCR

Quantitative real time polymerase chain reaction (qPCR) was used to quantify fungal DNA in the inoculated wheat spikes. A primer pair specific to *F. graminearum* (translation elongation factor 1α gene) was used: FgramB379 (CCATTCCCTGGGCGCT) and FgramB411 (CCTATTGACAGGTGGTTAGTGACTGG) (Nicolaisen et al. 2009). Quantitative real time PCR reactions were performed in 96 well plates using 20 µl reactions consisting of 10 µl 2x PowerUp™ SYBR™ Green Master Mix [Applied Biosystems., USA], 250 nM of each primer, 40 ng template DNA, and nuclease free water as required. Each reaction was performed in technical triplicate for all samples. Reactions were run using a CFX96™ Real-Time System [Bio-Rad Laboratories (Canada) Ltd., Mississauga] using the following protocol 2 min at 50 °C; 95 °C 10 min; 40 cycles of 95 °C for 15 s and 62 °C for 1 min followed by dissociation curve analysis at 60 to 95 °C (Nicolaisen et al. 2009). A standard curve of *F. graminearum* DNA from isolate HSW-15-39 was included on each plate for quantification of fungal DNA in infected spike samples. The standard curve consisted of a ten-fold dilution series spanning from 50 ng to 500 fg of pure fungal DNA.

The amount of fungal DNA in each sample was calculated using the linear equation derived from Cq values of the standard curve.

5.3.8 Data analysis

Statistical analyses were performed using SAS software version 9.4 [SAS institute inc., USA]. Analysis of variance was carried out on the accumulation of fungal DNA using PROC Mixed. Levene's tests for homogeneity of variance was performed using PROC GLM. Levene's test determined that the data could not be pooled. In addition to that, PROC Univariate visual observation of the distributions of the residual variances also suggested that trials could not be pooled, thus data from the two trials were analyzed and presented separately. The model statement listed the treatments, genotypes, and the interaction of treatment*genotype as fixed effects. Tukey means comparison test was used for comparisons of means for main effects and their interactions. To meet assumptions for analysis of variance, the data were transformed by using square root transformation for Trial 2 to improve skewness, kurtosis and Shapiro-wilk values.

Height data for both Trial 1 and 2 were analyzed using SAS software version 9.4 [SAS Institute Inc., USA] with PROC Mixed. Levene's test for homogeneity of variance was conducted using PROC GLM. Levene's test indicated that data could be pooled. Results from the trials were pooled and analyzed using PROC Mixed. The model statement listed the treatments, genotypes and the treatment*genotype interaction as fixed effects. Trial, trial*treatment, trial*genotype, and trial*treatment*genotype were used as random effects. Tukey means comparison test was used for comparisons between main treatments and between genotypes. Eta squared was calculated as described by Brown (2008) and was used to ascertain the proportion of variation in height contributed by the treatments, genotypes and treatment*genotype interaction, trial, trial*treatment, trial*genotype, and trial*treatment*genotype.

A Pearson's correlation coefficient between the fungal DNA accumulation and the plant height in Trial 1 and Trial 2 were generated using PROC Corr in SAS version 9.4.

5.4 Results

5.4.1 Analysis of variance for fungal DNA accumulation

Analysis of variance showed significant PGR treatment, cultivar, and PGR treatment*cultivar effects for the accumulation of fungal DNA in both trials (Table 5.2).

Table 5.2. Analysis of variance for fungal DNA (ng) accumulation in the Trial 1 and Trial 2. Trial 2 was transformed by square root.

Source of variation	Trial 1			Trial 2		
	DF[a]	MS[b]	P[c] value	DF	MS	P value
PGR[d] treatment	2	109.98	<.0001	2	1.11	<.0001
Cultivar	4	302.23	<.0001	4	22.81	<.0001
PGR treatment*Cultivar	8	32.01	<.0001	8	0.92	<.0001
Error	120	4.87		120	0.083	

[a] DF=Degrees of Freedom, [b]MS=Mean square, [c]P=Probability, and [d]PGR=plant growth regulator.

5.4.2 Effect of PGR on fungal DNA accumulation

Plant growth regulator treatment affected accumulated fungal DNA in the spike for both trials. The Manipulator™ treatment significantly increased fungal DNA (6.63 ng) in the spike while the Ethrel™ treatment reduced fungal DNA accumulation (3.51 ng) in the spike compared to the control (4.87 ng) in Trial 1 (Table 5.3). The accumulation of fungal DNA in spikes treated with Manipulator™ in Trial 1 was more than three times higher than in Trial 2. In Trial 2, Manipulator™ significantly reduced the accumulation of fungal DNA (2.01 ng) compared to the control (2.58 ng). Ethrel™ application slightly lowered the fungal DNA (2.58 ng) in the spike, but was not statistically different from the control treatment in Trial 2.

Table 5.3. Least square means of fungal DNA (ng) in spike of plant growth regulator (PGR) treatments (Control, Ethrel™, Manipulator™) with three replicates across five cultivars in Trial 1 and Trial 2.

PGR treatment	Fungal DNA (ng)	
	Trial 1	Trial 2
Control	4.87 B	2.99 A
Ethrel™	3.51 C	2.58 A
Manipulator™	6.63 A	2.01 B

Means followed by same letter in a column are not significantly different at p=0.05 based on the Tukey means comparison test.

5.4.3 Effect of cultivar on fungal DNA accumulation

The accumulation of fungal DNA in the spike was significantly different, depending on cultivars in both Trial 1 and 2 (Table 5.4). In Trial 1, two moderately resistant cultivars AAC Brandon and AAC Penhold, along with the intermediately resistant cultivar AAC Cameron showed similar levels of fungal DNA accumulation. The intermediate resistant cultivar Prosper had the highest fungal DNA accumulation whereas the resistant cultivar AAC Tenacious had the lowest fungal DNA accumulation for both trials. In Trial 2, AAC Cameron was the second highest for fungal DNA accumulation followed by AAC Brandon. AAC Cameron had slightly higher fungal DNA accumulation than AAC Brandon, but they were not statistically different. Fungal DNA accumulation for AAC Penhold was the second lowest among cultivars.

Table 5.4. Least square means of fungal DNA (ng) in spikes of cultivars, AAC Brandon, AAC Cameron, AAC Penhold, AAC Tenacious, and Prosper, with Fusarium head blight resistance level across plant growth treatments in Trial 1 and Trial 2.

Cultivar	FHB Resistance Level[a]	Fungal DNA (ng)	
		Trial 1	Trial 2
AAC Brandon	MR[b]	4.93 B	2.55 BC
AAC Cameron	I[c]	4.12 B	3.14 B
AAC Penhold	MR	5.75 B	2.16 C
AAC Tenacious	R[d]	0.45 C	0.06 D
Prosper	I	9.76 A	8.01 A

Means followed by same letter are not significantly different at p=0.05 based on the Tukey means comparison test. [a]Seed Manitoba 2018, [b]MR=Moderate Resistance, [c]I=Intermediate Resistance, and [d]R=Resistant.

5.4.4 Interaction between cultivar and PGR for fungal DNA accumulation

Analysis of variance showed there were significant interactions between cultivar and PGR treatment in both trials (Table 5.2) The interaction between cultivar and PGR treatment was mainly caused by a changes in the relative differences in magnitude among the cultivars under different PGR treatments (Figure 5.1 and Figure 5.2). Within the PGR treatments, the trends of the fungal DNA accumulation among the cultivars were similar.

In Trial 1, across all treatments AAC Tenacious had the lowest fungal DNA accumulation and Prosper had the highest fungal DNA accumulation (Figure 5.1). There were slight differences in relative ranking of fungal accumulation among the cultivars AAC Brandon and AAC Cameron. AAC Penhold showed large differences in fungal DNA accumulation among the different PGR treatments with much higher accumulation with the application of Manipulator™ compared to the control or application of Ethrel™. Prosper had significantly

higher fungal DNA accumulation with the application of Manipulator™ compared to application of Ethrel™.

In Trial 2, the lowest fungal DNA accumulation was observed in AAC Tenacious, and the highest fungal DNA accumulation was observed in Prosper across all treatments (Figure 5.2). There were significant changes in relative ranking of fungal accumulation among the cultivars AAC Brandon, AAC Cameron and AAC Penhold, depending on the PGR treatments. AAC Brandon showed less accumulation of fungal DNA with the application of Ethrel™ and slightly less accumulation of fungal DNA with the application of Manipulator™ compared to the control. AAC Cameron showed much higher fungal DNA accumulation with the Ethrel™ compared to the control or application of Manipulator™. AAC Penhold had much lower accumulation with application of Manipulator™ compared to the control or application of Ethrel™.

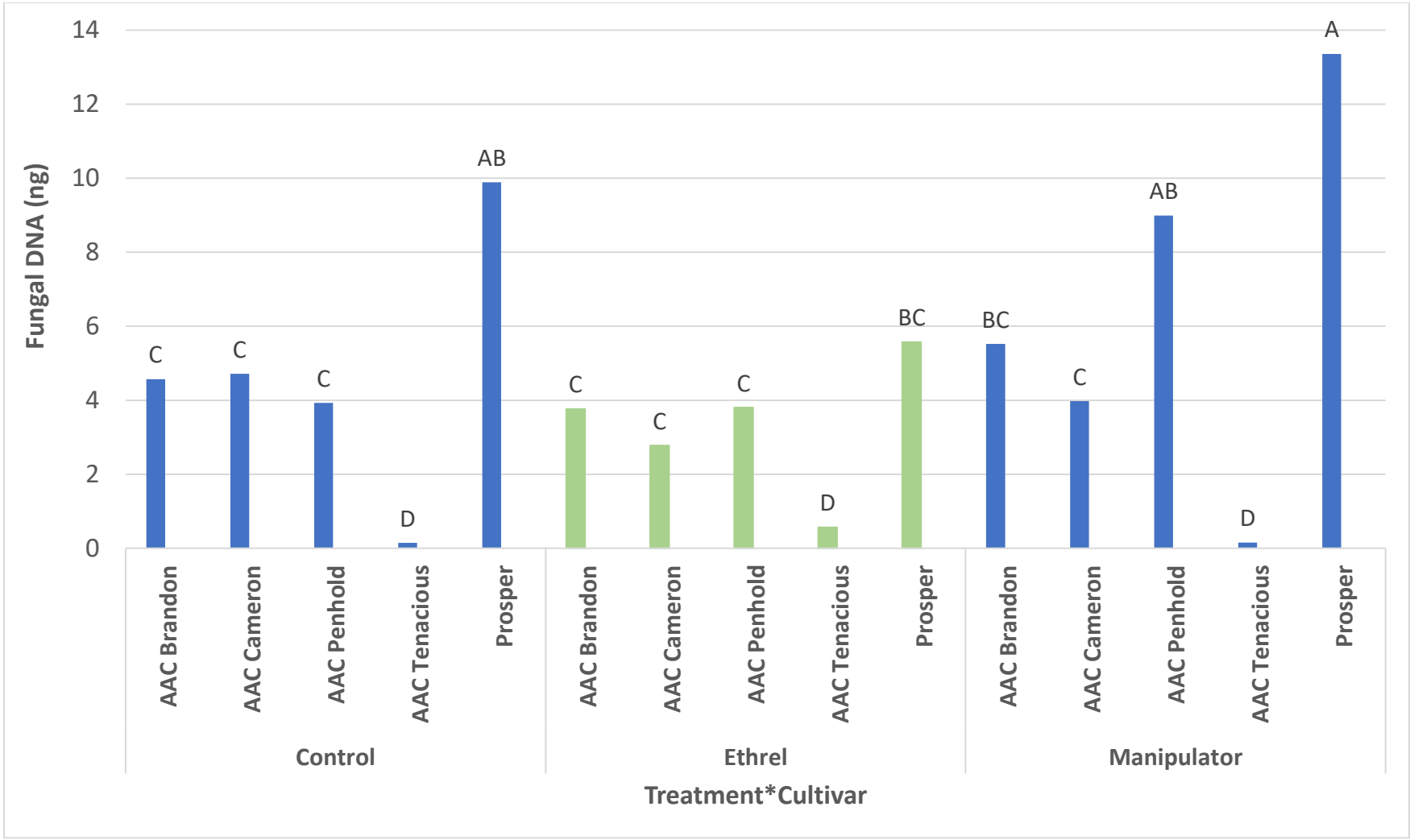

Figure 5.1. Interaction between plant growth regulator (PGR) treatments (Control, Ethrel™, and Manipulator™) and cultivars (AAC Brandon, AAC Cameron, AAC Penhold, AAC Tenacious, and Prosper) on fungal DNA (ng) per spike in Trial 1. Bars indicate the accumulation of fungal DNA (ng) for each treatment combination. Bars denoted by the same letter are not significantly different at p=0.05 based on the Tukey means comparison test.

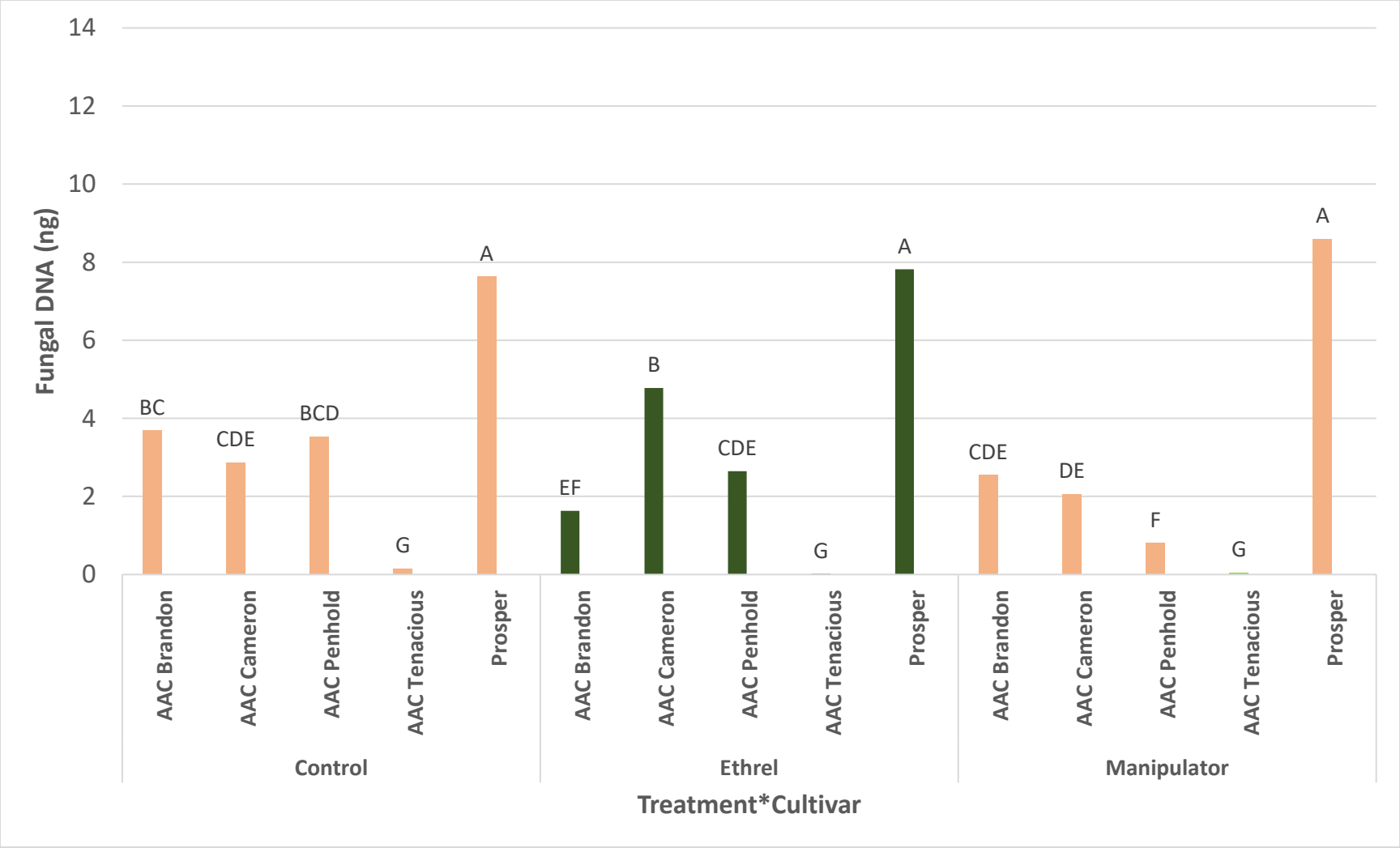

Figure 5.2. Interaction between plant growth regulator (PGR) treatments (Control, Ethrel™, and Manipulator™) and cultivars (AAC Brandon, AAC Cameron, AAC Penhold, AAC Tenacious, and Prosper) on fungal DNA (ng) per spike in Trial 2. Bars indicate the accumulation of fungal DNA (ng) for each treatment combination. Bars denoted by the same letter are not significantly different at p=0.05 based on the Tukey means comparison test.

5.4.5 Plant height

Analysis of variance showed there were significant PGR treatment and cultivar effects on plant height (Table 5.5). However, no interaction between treatment and cultivar was observed. The plant heights in Trial 1 and Trial 2 were not significantly different from each other. There was a significant trial*PGR treatment*cultivar interaction.

Based on proportion of variation explained, cultivar contributed more than 60% to the total variation while PGR treatment and interaction between PGR treatment and cultivar contributed 3% and 4% each to the total variation (Figure 5.3). With 60% of contribution to the total variation, cultivar mainly caused differences in the height. The residual contributed 21% of the total variation, indicating that a lot variation in the trial was not accounted for in the model. Trial*PGR treatment*cultivar interaction attributed 3% to the total variation of height. The significant three way interaction was probably due to behavior of cultivars across of combinations of treatment and cultivar between the trials.

Table 5.5. Combined analysis of variance for plant height (cm) in Trials 1 and 2.

Source	DF[a]	Mean Square	Pr > F
PGR[b] treatment	2	379.74	0.0294
Cultivar	4	3919.79	0.0034
PGR treatment*Cultivar	8	125.49	0.2432
Trial	1	151.88	0.3895
Trial*Treatment	2	11.51	0.8607
Trial*Cultivar	4	137.57	0.2171
Trial*PGR treatment*Cultivar	8	75.32	0.0006
Residual	240	20.95	

[a]DF=Degrees of Freedom, and [b]PGR=Plant growth regulator.

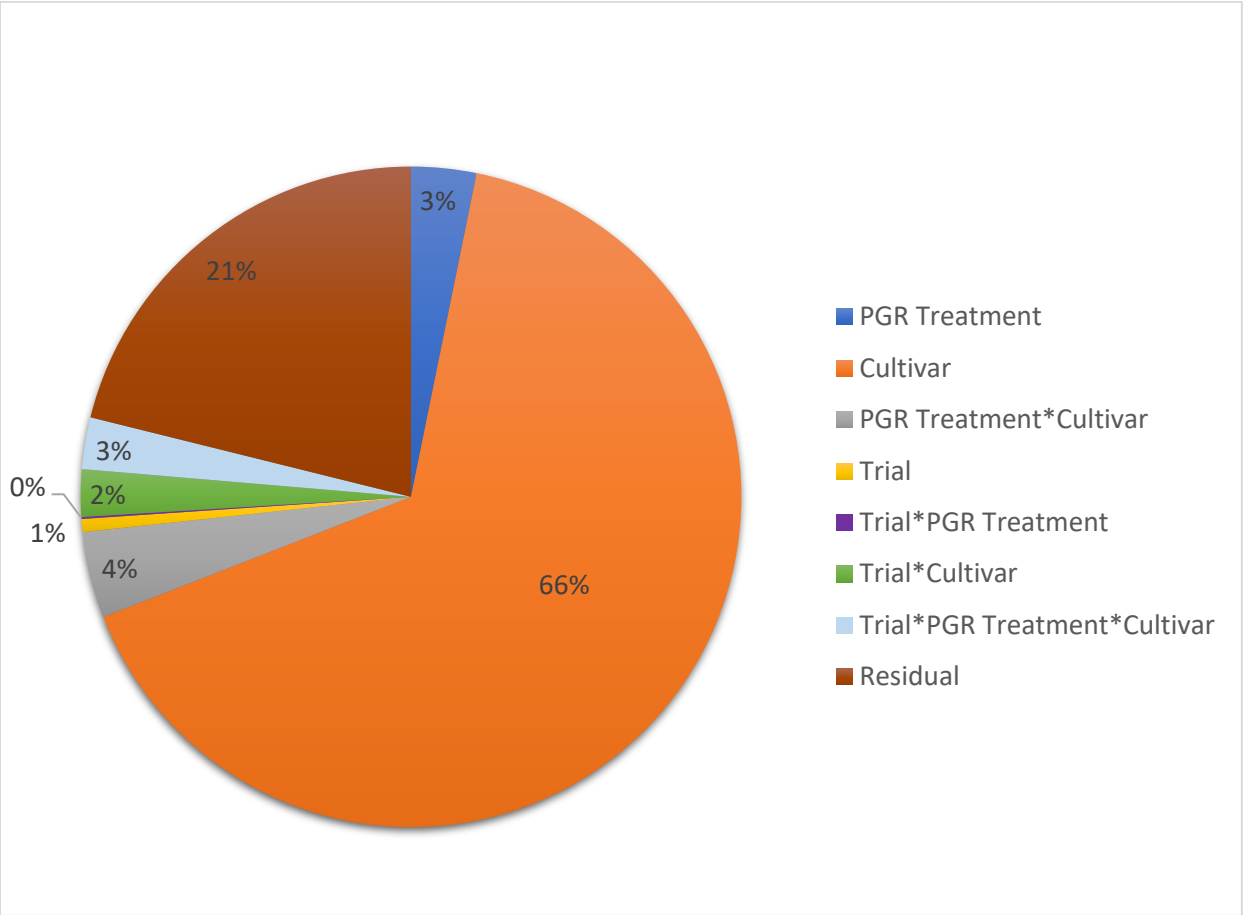

Figure 5.3. Proportion of total variation allocated to the main effects and cultivars and their interactions for each response variables. Eta squared was calculated by adding all the sums of squares then dividing the sums of squares for each of effects, interactions, and the residual by that total to indicate the relative proportion of variation explained by the effects (Brown 2008).

PGR treatment affected plant height. Ethrel™ significantly reduced plant height compared to the control treatment (Table 5.6). Manipulator™ lowered the plant height, but it was not statistically different than the control treatment or the Ethrel treatment. AAC Cameron had the tallest plants, followed by AAC Tenacious, AAC Brandon, and Prosper (Table 5.7). The plant heights of AAC Brandon and Prosper were not significantly different from each other. AAC Penhold was the shortest cultivar among the cultivars, but was not significantly different from AAC Brandon and AAC Penhold.

Table 5.6. Least square means of plant height (cm) with plant growth regulator treatments, Control, Ethrel™, Manipulator™, across five cultivars in the combined Trial 1 and Trial 2.

Plant growth regulator treatment	Height (cm)
Control	74.95 A
Ethrel™	70.95 B
Manipulator™	73.76 AB

Means followed by same letter in a column are not significantly different at p=0.05 based on the Tukey means comparison test.

Table 5.7. Least square means of plant height (cm) of cultivars (AAC Brandon, AAC Cameron, AAC Penhold, AAC Tenacious, and Prosper) across PGR treatments in combined Trial 1 and 2.

Cultivar	Height (cm)
AAC Brandon	71.24 BC
AAC Cameron	86.04 A
AAC Penhold	62.99 C
AAC Tenacious	75.86 B
Prosper	69.97 BC

Means followed by same letter in a column are not significantly different at p=0.05 based on the Tukey means comparison test.

5.5 Discussion

The lack of consistently effective FHB control strategies makes FHB a significant threat to the wheat production. Growing wheat cultivars with strong resistance to FHB could be the most reliable and consistent method to control FHB when they are combined with other FHB control strategies. However, wheat cultivars in the market that have both strong resistance to FHB and desirable agronomic traits are limited. While growers prefer to grow semi-dwarf cultivars of wheat, most FHB-resistant cultivars tend to be tall. With growing interest in the use of PGRs to reduce height and increase lodging tolerance, an understanding of how these compounds affect the interaction between FHB and wheat cultivars is necessary. In this study, different spring wheat cultivars with varying levels of FHB resistance, height, and semi-dwarfing gene combinations were used to examine the role of PGRs in the accumulation of *F. graminearum* in the spike.

This study showed that the accumulation of fungal DNA followed the level of resistance to FHB of the cultivars such that the intermediate resistant FHB cultivar Prosper had the highest fungal DNA accumulation and the resistant cultivar AAC Tenacious had the lowest. According to Seed Manitoba (2018), Prosper is an intermediate height spring wheat cultivar and AAC Tenacious is a tall spring cultivar. Even though AAC Cameron was the tallest cultivar during these studies, it had more than 10 times the fungal DNA accumulation than AAC Tenacious, indicating the resistance level of the cultivar contributed greater to the accumulation of fungal DNA in the spike than height.

The results presented in this study suggested that Ethrel™ not only reduced the plant height, but also tended to lower fungal DNA accumulation in the spike except the case of AAC Cameron in Trial 2. Since Ethel™ has ethephon as an active ingredient, it induces increases in ET level in the plant. Previously, the work of Li and Yen (2008) found that exogenous application of ethephon

plays positive roles in increasing FHB resistance in susceptible cultivars; these results mirrored those of Foroud et al. (2019) which found ET enhancers such as ethephon and ACC improved resistance, especially type II (spread within the spike) resistance, in the susceptible cultivars and did not change the level of FHB resistance or susceptibility in the resistant cultivars. Collectively, these works match the observations in this study. Ethrel™ with Prosper or AAC Brandon significantly reduced fungal accumulation compared to the control treatment. Both Prosper and AAC Brandon have the semi-dwarfing allele *Rht-B1b.* The *Rht-B1b* alleles encodes mutant forms of DELLA proteins, which repress GA-responsive growth (Saville et al. 2012). DELLA proteins have the ability to regulate plant and pathogen interactions. The presence of DELLA proteins contributes to resistance against necrotrophic pathogens through their influence on the jasmonic acid and ET signaling pathways (Navarro et al. 2008; Harberd et al. 2009). Moreover, the DELLA proteins play a role in the reduction of reactive oxygen species accumulation. This role contributes to delayed cell death and promotes tolerance against necrotrophic pathogens (Achard et al. 2008). Taken all together, DELLA proteins contribute a huge role in regulating plant growth in response to internal cues and external biotic and abiotic stresses (Sun 2010). Since ET inhibits GA synthesis which stabilizes DELLA proteins (Ross et al. 2016; Iqbal et al. 2017), spraying Ethrel™ elevates the levels of ET in the semi-dwarf wheat and it could have a synergetic effect with DELLA proteins for increasing resistance to FHB. In addition, ET could act as one of the defence hormones in the plant and result in host resistance (Haidoulis and Nicholson 2020). Ethylene plays a role with jasmonic acid in reducing disease development caused by necrotrophic pathogens (Li and Yen 2008). On the other hand, application of Ethrel™ to AAC Cameron increased the accumulation of fungal DNA. AAC Cameron is an intermediate resistant cultivar with no semi-dwarfing gene. Ethylene may play different roles in mediating the response to FHB depending on genotype. For

example, application of ethephon did not significantly change in Sumai-3 FHB reaction, but it did increase FHB resistance in Y1193-6 (Li and Yen 2008). In another study, ET enhancers increased resistance to *F. graminearum* in FHB susceptible cultivars (Foroud et al. 2019b). These results show that differences in genotype may affect important factors for ET response to FHB. According to Chen et al. (2009), enhancing ET levels significanlty increased colonization and conidial production of *F. graminearum* in wheat. Since ET accelerated plant senescence, it could lead to cell wall softening or dissolving and promoting cell death to offer favorable conditions for the pathogen to invade the plant (Li and Yen 2008). Another possibility is that since ET promotes susceptibility to biotrophs and Ethrel™ was applied before inoculation, this influences mostly the biotrophic phase of *F. graminearum* infection and colonization (Haidoulis and Nicholson 2020). These could be the reason why AAC Cameron with application of Ethrel™ had significantly higher fungal accumulation compared to the control or Manipulator™ application. Overall, our results using Ethrel™ as one of the PGRs for investigating *F. graminearum* DNA accumulation indicate that the role of Ethrel™ in *F. graminearum* accumulation is genotype dependent.

The lack of consistency in the effect of Manipulator™ was due to levels of variation between the two trials performed and the nature of the quantitative trait for FHB resistance. In addition, the interaction between Manipulator™ and AAC Penhold had contradictory results. Although growth rooms are an excellent instrument to control environment for research, it is very difficult to re-enact identical environments between experiments because of variability between repetitions within a chamber (Potvin and Tardif 1988). Many reports have mentioned that various factors in growing conditions such as humidity, spectral changes with light over time, gas concentration, schedule of watering, among other reasons, cause differences between experiments (Measures et al. 1973; Potvin and Tardif 1988; Potvin et al. 1990; Porter et al. 2015). Potvin and

Tardif (1988) identified three main sources of variation in growth chambers: chamber effect, time effect, and an interaction between chamber and time effect. Since the same growth room was used for Trial 1 and 2, time effect and interactions between trials and time could have influenced the results of this study. Time effect is caused by uncontrolled factors such as batch of soil or fertilizer and watering schedule. Interaction of effects of a particular chamber at a given time can be influenced by fluctuations of light intensity as bulbs age. Because a single growth room does not produce an identical environment for each trial, different environments could affect the GA signalling pathway (Kamiya and García-Martínez 1999; Achard et al. 2006; Harberd et al. 2009) and the FHB resistance gene in wheat cultivars. Resistance to Fusarium head blight in wheat is a quantitative trait. Quantitative traits are strongly affected by environments. Differences in environment produced in the growth room could affect the accumulation of fungal DNA in wheat cultivars. For example, moderately resistant cultivar AAC Penhold with application of Manipulator™ significantly increased fungal DNA accumulation compared to the control in Trial 1, while it decreased fungal DNA accumulation compared to the control in Trial 2. This phenomenon indicated that the possibilities that the fungal DNA accumulation in infected spikes of cultivars were more vulnerable to environment factors when Manipulator™ was applied, there were variations in application of Manipulator™ between the trials, or the batch of *F. graminearum* between the trials had variation. The differences in correlation between height and fungal DNA accumulation supports that there was the variation in *F. graminearum* between trials (Table A.32).

Manipulator™ has the active ingredient chlormequat chloride (CCC). As a gibberellin biosynthesis inhibitor, CCC participates in early steps of GA biosynthesis by blocking CDP-synthase and *ent*-Kaurene synthase (Rademacher 2016). AAC Penhold has the semi-dwarfing allele *Rht-D1b.* Unlike *Rht-B1b*, *Rht-D1b* may interact differently with Manipulator™. This could

be supported by different effects of the semi-dwarfing genes on type II (spread within the spike) resistance in wheat. Both *Rht-B1b* and *Rht-D1b* reduced the type I (disease incidence) resistance, whereas *Rht-B1b* increased resistance against FHB severity, while *Rht-D1b* increased spread within the spike (Miedaner and Voss 2008; Srinivasachary et al. 2009; Liu et al. 2013; Lu et al. 2013; Buerstmayr and Buerstmayr 2016). Conflicting results of fungal DNA accumulation from spraying Manipulator™ on AAC Penhold suggest there may be a more complex interaction between CCC and AAC Penhold in the GA biosynthesis pathway.

Altogether, it can be concluded that the role of PGRs in the accumulation of fungal DNA appears to differ, depending on the level of host resistance to the FHB and genotype of wheat. The higher fungal DNA accumulation equates to higher fungal biomass (Horevaj et al. 2011). *Fusarium* biomass can be a predictor for FHB infection. Higher *Fusarium* biomass has been associated with higher DON content in grain, FDK and severity (Horevaj et al. 2011). Therefore, it is important to consider the type of PGRs and genotype of wheat when growers use PGRs to maintain potential yield and reduce risk of FHB infection. Based on results from this study, Ethrel™ reduced plant height consistently and the response of the plant to Ethrel™ is less likely affected by environment factors. Future research under different environments could help understand how the responses of wheat from the application of Manipulator™ interact with FHB. Since the amount of fungal DNA in the spike was estimated in this study, fungal biomass in the spike could be assessed using a different standard curve. Furthermore, disease severity can be measured to evaluate types of FHB resistance of cultivar before quantifying fungal DNA accumulation.

6 Chapter 6.0 General Discussion and Conclusion

Fusarium head blight (FHB) is a devastating fungal disease mainly caused by *Fusarium graminearum* in Canada. This disease is considered as one of the most important diseases worldwide because of the impact it has on yield and grain quality, and its ability to produce high mycotoxin content in the grain. Small cereals including wheat are the main target for FHB. Canada has had substantial economic losses since early 1990s (Government of Alberta 2021). Because there is no single gene attributing complete resistance to FHB, this generates challenges for wheat breeding against FHB. Currently, available highly FHB resistant cultivars are limited, and they often come with undesirable agronomic traits such as tall height. Shorter cultivars are closer to *Fusarium* inoculum sources and have a tendency of higher anther retention which provides entrance to fungus. These make shorter cultivars vulnerable to FHB. Since shorter cultivars are a preferable option for growers, applying plant growth regulators (PGRs) to existing highly FHB resistant tall cultivars to reduce plant height and lodging may be a way to take advantage of the FHB resistance without the risk of reduced yield due to lodging. However, because PGRs target the gibberellin (GA) pathway directly or indirectly to alter plant growth, it is possible that PGRs may change FHB resistance level of highly FHB resistant cultivars. To determine the effect of PGRs on FHB infection, the first study examined the role of PGRs, Manipulator™ and Ethrel™ on FHB in five spring wheat cultivars by *F. graminearum* in field conditions. The second study investigated the effect of PGRs on anther retention in the spring wheat cultivars in controlled environments. The third study demonstrated the effect of PGRs on the accumulation of *F. graminearum* DNA in spring wheat under controlled environments.

In the first study, field trials were conducted to evaluate the effect of PGRs on five spring wheats that differ in height, semi-dwarfing alleles and level of FHB resistance at four different

environments in Manitoba; Carman (2019), Winnipeg (2019), Carman (2020), and Winnipeg (2020). There were six main effect treatments and five spring wheat cultivars. Because of hot and dry conditions in 2019 and 2020, plants were already compacted and short. However, combined results from the first study showed significant height reduction by Ethrel™. There was no change in anther retention and FHB resistance level of all tested cultivar. Cultivars with semi-dwarfing alleles had higher anther retention. This may be due to GA insensitivity which led to repression of cell elongation. Limited cell elongation in anther filaments could promote anther retention (Buerstmayr and Buerstmayr 2016). At the same FHB resistance level, AAC Brandon with *Rht-B1b* was more resistance to FHB than AAC Penhold with *Rht-D1b*. AAC Brandon with *Rht-B1b* had higher resistance for spread of infection within the spike (type II) than AAC Penhold with *Rht-D1b*. This aligns with the finding that cultivars with *Rht-B1b* have demonstrated higher resistance against disease spread within the spike (Miedaner and Voss 2008; Srinivasachary et al. 2009; Liu et al. 2013; Lu et al. 2013; Buerstmayr and Buerstmayr 2016).

The second study was conducted to determine whether PGRs affect anther retention in spring wheat cultivars under controlled environments with three treatments. Both PGRs reduced plant height. Interestingly, Manipulator™ reduced anther retention whereas Ethrel™ increased anther retention. Since Manipulator™ was applied earlier, its effect might not be still active when the plant was flowering. Early degradation of chlormequat chloride, the active ingredient of Manipulator™ was also observed in another study (Clark and Fedak 1977). Application of exogenous ethylene (ET) to wheat led to abnormal development of anthers such as smaller anther size, thereby anther retention is promoted (Bennett and Hughes 1972; Campbell et al. 2001). In addition to this, because ET inhibits cell elongation through crosstalk with the GA pathway and auxin pathway (Caldwell et al. 1988; Ramburan and Greenfield 2007; Dugardeyn et al. 2008;

Wiersma et al. 2011), application of Ethrel™ could shorten the length of filaments which encourages anther retention. The interaction between PGRs and anther retention was genotype specific. Because of GA insensitivity of the *Rht-B1b* allele, Ethrel™ was more effective in height reduction on cultivars with *Rht-B1b* allele compared to Manipulator™. Overall, there was a tendency of genotypes with higher FHB resistance to exhibit less anther retention.

The third study was conducted to demonstrate the role of PGRs on *F. graminearum* DNA accumulation in spring wheat cultivars under the controlled environment with three treatments. Ethrel™ had different effects on fungal DNA accumulation depending on cultivar. Ethrel™ significantly reduced not only fungal DNA in the spike of cultivars with *Rht-B1b,* but also reduced plant height of all tested cultivars. However, cultivars without *Rht-B1b* had higher fungal DNA accumulation when Ethrel™ was applied. Cultivars with semi-dwarfing alleles, *Rht-B1b* or *Rht-D1b,* have more DELLA proteins than cultivars without semi-dwarfing alleles. Because ethylene stabilized the DELLA proteins, DELLA proteins from semi-dwarfing cultivars could have a stronger effect on resistance against necrotrophic pathogens through jasmonic acid and ethylene signaling pathways (Navarro et al. 2008; Harberd et al. 2009). Furthermore, the role of DELLA proteins on reduction of reactive oxygen species accumulation could contribute lower accumulation of fungal DNA in cultivars with *Rht-B1b* (Achard et al. 2008). The results from the application of Manipulator™ were inconsistent for fungal DNA accumulation in spikes of cultivars, especially with *Rht-D1b*. This result could be due to variation between the two trials such as application of Manipulator™ and the batch of *F. graminearum* inoculum. Conflicting results of the AAC Penhold with *Rht-D1b* when Manipulator™ was applied indicates a complex interaction between Manipulator™ and AAC Penhold with *Rht-D1b* in the GA pathway. Overall, the effect of PGRs on the accumulation of fungal DNA in the spike is genotype specific.

All studies reported results showing Ethrel™ was effective for reduction of height in the field and under controlled environments. In the field, PGRs did not affect anther retention and FHB infection in spring wheat. Under controlled environments, PGRs had different effects on anther retention. Ethrel™ increased anther retention while Manipulator™ decreased anther retention. The effect of Ethrel™ on the accumulation of fungal DNA is genotype dependent. Manipulator™ showed inconsistent results. In this project, results regarding the effect of PGRs on anther retention and FHB infection from the field experiment and the controlled environment experiments were not consistent. Because height, anther retention and FHB resistance are quantitative traits, these traits are influenced not only by genotype of wheat, but also by environmental factors. Inconsistent results from the field and controlled environment are caused by different environment factors that affect plant growth such as photoperiod, soil type, amount of water availability, temperature, and humidity. Taken together, environment and cultivar play important roles in the performance of PGRs on height, anther retention and FHB infection. Therefore, experiments in more environments under different factors such as photoperiod, temperature, light intensity, and humidity with more cultivars will be helpful to clarify some of results that have been found in this study.

Based on field results, either of the PGRs did not significantly affect FHB and alter FHB resistance level of wheat cultivar. Therefore, growers could benefit from FHB resistance cultivar without risk of losing potential yield by choosing highly FHB resistance taller cultivar and controlling the plant height with PGRs to prevent lodging. Also, breeders could focus more on selecting for good FHB resistance and focus less on ensuring plant height is acceptable to producers. However, the results from the controlled environments indicated that anther retention and the accumulation of *F. graminearum* were affected by application of PGRs. Therefore,

growers need to consider genotype of wheat and type of PGRs when they apply PGRs depending on the environment. Altogether, it appears that plants with application of Ethrel™ were less sensitive to environmental factors in terms of reducing the height compared to plants with application of Manipulator™. Growing taller FHB resistance cultivars and controlling height with Ethrel™ could be one way to manage FHB. Further research will be required to investigate the underlying mechanism between PGRs and wheat cultivars. In addition to that, expanding the number of cultivars tested as well as the number of testing environments would be required to confirm the results from this study.

References

Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van Der Straeten, D., Peng, J., and Harberd, N.P. 2006. Integration of plant responses to environmentally activated phytohormonal signals. Science (80-.). **311**: 91–94. doi:10.1126/science.1118642.

Achard, P., Renou, J.P., Berthomé, R., Harberd, N.P., and Genschik, P. 2008. Plant DELLAs Restrain Growth and Promote Survival of Adversity by Reducing the Levels of Reactive Oxygen Species. Curr. Biol. **18**: 656–660. doi:10.1016/j.cub.2008.04.034.

Achard, P., Vriezen, W.H., Van Der Straeten, D., and Harberd, N.P. 2003. Ethylene Regulates Arabidopsis Development via the Modulation of DELLA Protein Growth Repressor Function. Plant Cell **15**: 2816–2825. doi:10.1105/tpc.015685.

Alexander, N.J., McCormick, S.P., Waalwijk, C., van der Lee, T., and Proctor, R.H. 2011. The genetic basis for 3-ADON and 15-ADON trichothecene chemotypes in Fusarium. Fungal Genet. Biol. **48**: 485–495. Elsevier Inc. doi:10.1016/j.fgb.2011.01.003.

Amarasinghe, C., Sharanowski, B., and Dilantha Fernando, W.G. 2019. Molecular phylogenetic relationships, trichothecene chemotype diversity and aggressiveness of strains in a global collection of fusarium graminearum species. Toxins (Basel). **11**: 1–17. doi:10.3390/toxins11050263.

Anukul, N., Vangnai, K., and Mahakarnchandkul, W. 2013. Significance of regulation limits in mycotoxin contamination in Asia and risk management programs at the national level. J. Food Drug Anal. **21**: 227–241. Elsevier Ltd. doi:10.1016/j.jfda.2013.07.009.

Bai, G., and Shaner, G. 2004. Management and resistance in wheat and barley to fusarium head blight. Annu. Rev. Phytopathol. **42**: 135–161. doi:10.1146/annurev.phyto.42.040803.140340.

Bakshi, A., Shemansky, J.M., Chang, C., and Binder, B.M. 2015. History of Research on the Plant Hormone Ethylene. J. Plant Growth Regul. **34**: 809–827. Springer US. doi:10.1007/s00344-015-9522-9.

Bari, R., and Jones, J.D.G. 2009. Role of plant hormones in plant defence responses. Plant Mol. Biol. **69**: 473–488. doi:10.1007/s11103-008-9435-0.

Bennett, M.D., and Hughes, W.G. 1972. Additional mitosis in wheat pollen induced by ethrel. Nature **240**: 566–568. doi:10.1038/240566a0.

Berry, P.M., Spink, J., Sterling, M., and Pickett, A.A. 2003. Methods for Rapidly Measuring the Lodging Resistance of Wheat Cultivars. J. Agron. Crop Sci. **189**: 390–401. doi:10.1046/j.0931-2250.2003.00062.x.

Bolanos-Carriel, C., Wegulo, S.N., Baenziger, P.S., Funnell-Harris, D., Hallen-Adams, H.E., and Eskridge, K.M. 2020. Effects of fungicide chemical class, fungicide application timing, and environment on Fusarium head blight in winter wheat. Eur. J. Plant Pathol. **158**: 667–679. European Journal of Plant Pathology. doi:10.1007/s10658-020-02109-3.

Brown, J.D. 2008. Effect size and eta squared. Shiken JALT Test. Eval. SIG Newsl. **12**: 38–43.

Buerstmayr, H., Ban, T., and Anderson, J.A. 2009. QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: A review. Plant Breed. **128**: 1–26. doi:10.1111/j.1439-0523.2008.01550.x.

Buerstmayr, M., and Buerstmayr, H. 2015. Comparative mapping of quantitative trait loci for Fusarium head blight resistance and anther retention in the winter wheat population Capo × Arina. Theor. Appl. Genet. **128**: 1519–1530. Springer Berlin Heidelberg. doi:10.1007/s00122-015-2527-8.

Buerstmayr, M., and Buerstmayr, H. 2016. The Semidwarfing Alleles Rht-D1b and Rht-B1b Show Marked Differences in Their Associations with Anther-Retention in Wheat Heads and with Fusarium Head Blight Susceptibility. Phytopathology **106**: 1544–1552. doi:10.1094/PHYTO-05-16-0200-R.

Buerstmayr, M., Steiner, B., and Buerstmayr, H. 2020. Breeding for Fusarium head blight resistance in wheat—Progress and challenges. Plant Breed. **139**: 429–454. doi:10.1111/pbr.12797.

Buhrow, L.M., Cram, D., Tulpan, D., Foroud, N.A., and Loewen, M.C. 2016. Exogenous Abscisic Acid and Gibberellic Acid Elicit Opposing Effects on Fusarium graminearum Infection in Wheat. Phytopathology **106**: 986–996. doi:10.1094/PHYTO-01-16-0033-R.

Bushnell, W.R., Hazen, B.E., and Pritsch, C. 2003. Histology and physiology of Fusarium Head Blight. Pages 44–83 *in* K.J. Leonard and W.R. Bushnell, eds. Fusarium Head Blight of wheat and barley. APS Press, St. Paul.

Caldwell, C.D., Norrie, J., and Mellish, D.R. 1988. a Comparison of Ethephon Alone and in Combination With Ccc or Dpc Applied To Spring Barley. Can. J. Plant Sci. **68**: 941–946. doi:10.4141/cjps88-114.

Campbell, W.F., Salisbury, F.B., Bugbee, B., Klassen, S., Naegle, E., Strickland, D.T., Bingham, G.E., Levinskikh, M., Iljina, G.M., Veselova, T.D., Sytchev, V.N., Podolsky, I., McManus, W.R., Bubenheim, D.L., Stieber, J., and Jahns, G. 2001. Comparative floral development of Mir-grown and ethylene-treated, earth-grown Super Dwarf wheat. J. Plant Physiol. **158**: 1051–1060. doi:10.1078/S0176-1617(04)70129-7.

Canadian Cereals 2019. 2019 Canadian Wheat Crop in Review.

Canadian grain commission 2018. Canadian wheat classes. [Online] Available: https://grainscanada.gc.ca/en/grain-quality/grain-grading/wheat-classes.html [2019 Oct. 30].

Canadian grain commission 2019. Wheat test weight conversion chart. [Online] Available: https://www.grainscanada.gc.ca/en/grain-quality/grain-grading/grading-factors/conversion-charts/wheat-conversion.html [2020 Oct. 30].

Canadian grain commission 2021. Frequency and severity of Fusarium damaged kernels (FDK) in Harvest Sample Program red spring wheat samples. [Online] Available: https://www.grainscanada.gc.ca/en/grain-research/export-quality/cereals/wheat/western/annual-fusarium-damage/canada-western-red-spring/ [2021 Feb. 1].

Chen, H., Moakhar, N.P., Iqbal, M., Pozniak, C., Hucl, P., and Spaner, D. 2016. Genetic variation for flowering time and height reducing genes and important traits in western Canadian spring wheat. Euphytica **208**: 377–390. Springer Netherlands. doi:10.1007/s10681-015-1615-9.

Chen, X., Steed, A., Travella, S., Keller, B., and Nicholson, P. 2009. Fusarium graminearum exploits ethylene signalling to colonize dicotyledonous and monocotyledonous plants. New Phytol. **182**: 975–983. doi:10.1111/j.1469-8137.2009.02821.x.

Chen, Y., Kistler, H.C., and Ma, Z. 2019. Fusarium graminearum Trichothecene Mycotoxins: Biosynthesis, Regulation, and Management. Annu. Rev. Phytopathol. **57**: 15–39. doi:10.1146/annurev-phyto-082718-100318.

Cheng, H., Song, S., Xiao, L., Soo, H.M., Cheng, Z., Xie, D., and Peng, J. 2009. Gibberellin acts through jasmonate to control the expression of MYB21, MYB24, and MYB57 to promote stamen filament growth in arabidopsis. PLoS Genet. **5**: 20–22. doi:10.1371/journal.pgen.1000440.

Claeys, H., De Bodt, S., and Inzé, D. 2014. Gibberellins and DELLAs: Central nodes in growth regulatory networks. Trends Plant Sci. **19**: 231–239. doi:10.1016/j.tplants.2013.10.001.

Clark, R. V, and Fedak, G. 1977. Effects of chlormequat on plant height, disease development and chemical constituents of cultivars of barley, oats, and wheat. Can. J. Plant Sci. **57**: 31–36.

Clear, R.M., and Patrick, S.K. 1990. Fusarium Species isolated from wheat samples containing tombstone (Scab) kernels from Ontario, Manitoba, and Saskatchewan. Can. J. Plant Sci. **70**: 1057–1069. doi:10.4141/cjps90-128.

Clear, R.M., and Patrick, S.K. 2000. Fusarium head blight pathogens isolated from fusarium-damaged kernels of wheat in western Canada, 1993 to 1998. Can. J. Plant Pathol. **22**: 51–60. doi:10.1080/07060660009501161.

Collard, B.C.Y., Jahufer, M.Z.Z., Brouwer, J.B., and Pang, E.C.K. 2005. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. Euphytica **142**: 169–196. doi:10.1007/s10681-005-1681-5.

Cuthbert, P.A., Somers, D.J., and Brulé-Babel, A. 2007. Mapping of Fhb2 on chromosome 6BS: A gene controlling Fusarium head blight field resistance in bread wheat (Triticum aestivum L.). Theor. Appl. Genet. **114**: 429–437. doi:10.1007/s00122-006-0439-3.

Cuthbert, R.D., DePauw, R.M., Knox, R.E., Singh, A.K., McCaig, T.N., McCallum, B., and Fetch, T. 2017a. AAC brandon hard red spring wheat. Can. J. Plant Sci. **97**: 393–401. doi:10.1139/cjps-2016-0150.

Cuthbert, R.D., DePauw, R.M., Knox, R.E., Singh, A.K., McCaig, T.N., McCallum, B., Fetch, T., and Beres, B.L. 2017b. AAC penhold Canada prairie spring red wheat. Can. J. Plant Sci. **98**: 207–214. doi:10.1139/cjps-2017-0186.

Daviere, J.M., and Achard, P. 2013. Gibberellin signaling in plants. Development **140**: 1147–1151. doi:10.1242/dev.087650.

Dill-Macky, R. 2008. Cultural control practices for fusarium head blight: Problems and solutions. Cereal Res. Commun. **36**: 653–657. doi:10.1556/CRC.36.2008.Suppl.B.55.

Dill-Macky, R., and Jones, R.K. 2000. The effect of previous crop residues and tillage on fusarium head blight of wheat. Plant Dis. **84**: 71–76. doi:10.1094/PDIS.2000.84.1.71.

Directorate, E., Meeting, J., The, O.F., Committee, C., Party, T.H.E.W., and Chemicals, O.N. 2018. Working Document on the Risk Assessment of Secondary Metabolites of Microbial Biocontrol Agents.

Draeger, R., Gosman, N., Steed, A., Chandler, E., Thomsett, M., Srinivasachary, Schondelmaier, J., Buerstmayr, H., Lemmens, M., Schmolke, M., Mesterhazy, A., and Nicholson, P. 2007. Identification of QTLs for resistance to Fusarium head blight, DON accumulation and associated traits in the winter wheat variety Arina. Theor. Appl. Genet. **115**: 617–625. doi:10.1007/s00122-007-0592-3.

Dugardeyn, J., Vandenbussche, F., and Van Der Straeten, D. 2008. To grow or not to grow: What can we learn on ethylene-gibberellin cross-talk by in silico gene expression analysis? J. Exp. Bot. **59**: 1–16. doi:10.1093/jxb/erm349.

Dweba, C.C., Figlan, S., Shimelis, H.A., Motaung, T.E., Sydenham, S., Mwadzingeni, L., and Tsilo, T.J. 2017. Fusarium head blight of wheat: Pathogenesis and control strategies. Crop Prot. **91**: 114–122. Elsevier Ltd. doi:10.1016/j.cropro.2016.10.002.

Egamberdieva, D., Wirth, S.J., Alqarawi, A.A., Abd-Allah, E.F., and Hashem, A. 2017. Phytohormones and beneficial microbes: Essential components for plants to balance stress and fitness. Frontiers Media S.A. doi:10.3389/fmicb.2017.02104.

Fabre, F., Rocher, F., Alouane, T., Langin, T., and Bonhomme, L. 2020. Searching for FHB Resistances in Bread Wheat: Susceptibility at the Crossroad. Front. Plant Sci. **11**: 1–8. doi:10.3389/fpls.2020.00731.

Fauzi, T., and Paulitz, T.C. 1994. The Effect of Plant Growth Regulators and Nitrogen on Fusarium Head Blight of the Spring Wheat Cultivar Max. Plant Dis. **78**: 289–292. doi:10.1094/PD-78-0289.

Food and Agriculture Organization of the United Nations 2020. Crops and livestock products. [Online] Available: https://www.fao.org/faostat/en/#data/QCL/visualize [2020 Aug. 31].

Foroud, N.A., Baines, D., Gagkaeva, T.Y., Thakor, N., Badea, A., Steiner, B., Bürstmayr, M., and Bürstmayr, H. 2019a. Trichothecenes in cereal grains - An update. Toxins (Basel). **11**. doi:10.3390/toxins11110634.

Foroud, N.A., McCormick, S.P., MacMillan, T., Badea, A., Kendra, D.F., Ellis, B.E., and Eudes, F. 2012. Greenhouse studies reveal increased aggressiveness of emergent Canadian Fusarium graminearum chemotypes in wheat. Plant Dis. **96**: 1271–1279. doi:10.1094/PDIS-10-11-0863-RE.

Foroud, N.A., Pordel, R., Goyal, R.K., Ryabova, D., Eranthodi, A., Chatterton, S., and Kovalchuk, I. 2019b. Chemical Activation of the Ethylene Signaling Pathway Promotes Fusarium graminearum Resistance in Detached Wheat Heads. Phytopathology **109**: 796–803.

doi:10.1094/PHYTO-08-18-0286-R.

Foroud, N.A., Yao, Y.T., Goyal, R.K., Pordel, R., Ryabova, D., Chatterton, S., and Kovalchuk, I. 2018. Exploring the role of ethylene signalling and wheat resistance to Fusarium graminearum. : 4193.

Fox, S.L., Kumar, S., Thomas, J.B., Humphreys, D.G., Mitchell Fetch, J., Green, D., Wise, I., Smith, M., Fetch, T., Gilbert, J., McCallum, B., and Menzies, J. 2016. AAC cameron Canada western red spring wheat. Can. J. Plant Sci. **96**: 1065–1072. doi:10.1139/cjps-2016-0011.

Gaikpa, D.S., Lieberherr, B., Maurer, H.P., Longin, C.F.H., and Miedaner, T. 2020. Comparison of rye, triticale, durum wheat and bread wheat genotypes for Fusarium head blight resistance and deoxynivalenol contamination. Plant Breed. **139**: 251–262. doi:10.1111/pbr.12779.

Gao, S., and Chu, C. 2020. Gibberellin metabolism and signaling: Targets for improving agronomic performance of crops. Plant Cell Physiol. **61**: 1902–1911. doi:10.1093/pcp/pcaa104.

Gilbert, J., Clear, R.M., Ward, T.J., Gaba, D., Tekauz, A., Turkington, T.K., Woods, S.M., Nowicki, T., and O'Donnell, K. 2010. Relative aggressiveness and production of 3- Or 15-acetyl deoxynivalenol and deoxynivalenol by Fusarium graminearum in spring wheat. Can. J. Plant Pathol. **32**: 146–152. doi:10.1080/07060661003740231.

Gilbert, J., and Fernando, W.G.D. 2004. Epidemiology and biological control of Gibberella zeae /Fusarium graminearum. Can. J. Plant Pathol. **26**: 464–472. doi:10.1080/07060660409507166.

Gilbert, J., and Haber, S. 2013. Overview of some recent research developments in fusarium head blight of wheat. Can. J. Plant Pathol. **35**: 149–174. doi:10.1080/07060661.2013.772921.

Government of Alberta 2021. Alberta Fusarium graminearum Management Plan. [Online] Available: https://www.alberta.ca/alberta-fusarium-graminearum-management-plan.aspx [2021 Jan. 3].

Guedira, M., Brown-Guedira, G., van Sanford, D., Sneller, C., Souza, E., and Marshall, D. 2010. Distribution of Rht genes in modern and historic winter wheat cultivars from the eastern and central USA. Crop Sci. **50**: 1811–1822. doi:10.2135/cropsci2009.10.0626.

Haidoulis, J.F., and Nicholson, P. 2020. Different effects of phytohormones on Fusarium head blight and Fusarium root rot resistance in Brachypodium distachyon. J. Plant Interact. **15**: 335–344. doi:10.1080/17429145.2020.1820592.

Harberd, N.P., Belfield, E., and Yasumura, Y. 2009. The angiosperm gibberellin-GID1-DELLA growth regulatory mechanism: How an "inhibitor of an inhibitor" enables flexible response to fluctuating environments. Plant Cell **21**: 1328–1339. doi:10.1105/tpc.109.066969.

Hayes, P. 2019. Grain and Feed Update Summer 2019 Wheat Update.

He, X., Singh, P.K., Dreisigacker, S., Singh, S., Lillemo, M., and Duveiller, E. 2016. Dwarfing genes Rht-B1b and Rht-D1b are associated with both type i FHB susceptibility and low anther extrusion in two bread wheat populations. PLoS One **11**: 1–14.

doi:10.1371/journal.pone.0162499.

Hedden, P. 2003. The genes of the Green Revolution. Trends Genet. **19**: 5–9. doi:10.1016/S0168-9525(02)00009-4.

Hedden, P., and Sponsel, V. 2015. A Century of Gibberellin Research. J. Plant Growth Regul. **34**: 740–760. Springer US. doi:10.1007/s00344-015-9546-1.

Hilton, A.J., Jenkinson, P., Hollins, T.W., and Parry, D.W. 1999. Relationship between cultivar height and severity of Fusarium ear blight in wheat. Plant Pathol. **48**: 202–208. doi:10.1046/j.1365-3059.1999.00339.x.

Horevaj, P., Milus, E.A., and Bluhm, B.H. 2011. A real-time qPCR assay to quantify Fusarium graminearum biomass in wheat kernels. J. Appl. Microbiol. **111**: 396–406. doi:10.1111/j.1365-2672.2011.05049.x.

Hou, Y.P., Mao, X.W., Wang, J.X., Zhan, S.W., and Zhou, M.G. 2017. Sensitivity of Fusarium asiaticum to a novel succinate dehydrogenase inhibitor fungicide pydiflumetofen. Crop Prot. **96**: 237–244. Elsevier Ltd. doi:10.1016/j.cropro.2017.02.011.

Igrejas, G., Ikeda, T.M., and Guzmán, C. 2020. Wheat quality for improving processing and human health. Wheat Quality For Improving Processing And Human Health. doi:10.1007/978-3-030-34163-3.

Iqbal, N., Khan, N.A., Ferrante, A., Trivellini, A., Francini, A., and Khan, M.I.R. 2017. Ethylene Role in Plant Growth, Development and Senescence: Interaction with Other Phytohormones. Front. Plant Sci. **08**: 1–19. doi:10.3389/fpls.2017.00475.

Islam, M.S., Brown-Guedira, G., Van Sanford, D., Ohm, H., Dong, Y., and McKendry, A.L. 2016. Novel QTL associated with the Fusarium head blight resistance in Truman soft red winter wheat. Euphytica **207**: 571–592. Springer Netherlands. doi:10.1007/s10681-015-1550-9.

Kamiya, Y., and García-Martínez, J.L. 1999. Regulation of gibberellin biosynthesis by light. Curr. Opin. Plant Biol. **2**: 398–403. doi:10.1016/S1369-5266(99)00012-6.

Kazan, K., and Gardiner, D.M. 2018. Transcriptomics of cereal–Fusarium graminearum interactions: what we have learned so far. Mol. Plant Pathol. **19**: 764–778. doi:10.1111/mpp.12561.

Kelly, A., Proctor, R.H., Belzile, F., Chulze, S.N., Clear, R.M., Cowger, C., Elmer, W., Lee, T., Obanor, F., Waalwijk, C., and Ward, T.J. 2016. The geographic distribution and complex evolutionary history of the NX-2 trichothecene chemotype from Fusarium graminearum. Fungal Genet. Biol. **95**: 39–48. doi:10.1016/j.fgb.2016.08.003.

Khan, N.I., Schisler, D.A., Boehm, M.J., Lipps, P.E., and Slininger, P.J. 2004. Field testing of antagonists of Fusarium head blight incited by Gibberella zeae. Biol. Control **29**: 245–255. doi:10.1016/S1049-9644(03)00157-9.

Klassen, S.P., and Bugbee, B. 2004. Ethylene synthesis and sensitivity in crop plants. HortScience **39**: 1546–1552. doi:10.21273/hortsci.39.7.1546.

Klein, A., Cameron, E.K., Heimburger, B., Eisenhauer, N., Scheu, S., and Schaefer, I. 2017. Changes in the genetic structure of an invasive earthworm species (Lumbricus terrestris, Lumbricidae) along an urban – rural gradient in North America. Appl. Soil Ecol. **120**: 265–272. Elsevier. doi:10.1016/j.apsoil.2017.08.009.

Komirenko, Z. 2018. Economic Cost of Fusarium. Farm-Level and Regional Economic Impact of Fusarium in Alberta. : 1–8. [Online] Available: https://open.alberta.ca/dataset/18054d37-911a-4feb-aac4-11866afbf535/resource/ffe92c3d-fa0d-43a1-85ee-c941af5004b2/download/economic-cost-of-fusarium-farm-level-and-regional-economic-impact-of-fusarium-in-alberta-2018.pdf.

Koning, R.E. 1983. the Roles of Auxin, Ethylene, and Acid Growth in Filament Elongation in Gaillardia Grandiflora (Asteraceae). Am. J. Bot. **70**: 602–610. doi:10.1002/j.1537-2197.1983.tb07888.x.

Lee, B.R., Zaman, R., La, V.H., Park, S.H., and Kim, T.H. 2021. Ethephon-induced ethylene enhances protein degradation in source leaves, but its high endogenous level inhibits the development of regenerative organs in brassica napus. Plants 10. MDPI. doi:10.3390/plants10101993.

Li, G., and Yen, Y. 2008. Jasmonate and ethylene signaling pathway may mediate Fusarium head blight resistance in wheat. Crop Sci. **48**: 1888–1896. doi:10.2135/cropsci2008.02.0097.

Liu, S., Griffey, C.A., Hall, M.D., McKendry, A.L., Chen, J., Brooks, W.S., Brown-Guedira, G., Van Sanford, D., and Schmale, D.G. 2013. Molecular characterization of field resistance to Fusarium head blight in two US soft red winter wheat cultivars. Theor. Appl. Genet. **126**: 2485–2498. doi:10.1007/s00122-013-2149-y.

Lori, G.A., Sisterna, M.N., Sarandón, S.J., Rizzo, I., and Chidichimo, H. 2009. Fusarium head blight in wheat: Impact of tillage and other agronomic practices under natural infection. Crop Prot. **28**: 495–502. doi:10.1016/j.cropro.2009.01.012.

Lu, Q., Lillemo, M., Skinnes, H., He, X., Shi, J., Ji, F., Dong, Y., and Bjørnstad, Å. 2013. Anther extrusion and plant height are associated with Type I resistance to Fusarium head blight in bread wheat line "Shanghai-3/Catbird." Theor. Appl. Genet. **126**: 317–334. doi:10.1007/s00122-012-1981-9.

Malihipour, A., Gilbert, J., Piercey-Normore, M., and Cloutier, S. 2012. Molecular phylogenetic analysis, trichothecene chemotype patterns, and variation in aggressiveness of fusarium isolates causing head blight in wheat. Plant Dis. **96**: 1016–1025. doi:10.1094/PDIS-10-11-0866-RE.

Mankevičiene, A., Suproniene, S., Dabkevičius, Z., and Auškalniene, O. 2008. The incidence of Fusarium fungi and mycotoxins in wheat grain as affected by growth regulators. Zemdirbyste **95**: 251–258.

Marciniak, K., and Przedniczek, K. 2019. Comprehensive Insight into Gibberellin- and Jasmonate-Mediated Stamen Development. Genes (Basel). **10**.

McCormick, S.P., Stanley, A.M., Stover, N.A., and Alexander, N.J. 2011. Trichothecenes: From

simple to complex mycotoxins. Toxins (Basel). **3**: 802–814. doi:10.3390/toxins3070802.

McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G., and Van Sanford, D. 2012a. Fusarium Head Blight Disease Cycle , Symptoms , and Impact on Grain Yield and Quality Frequency and Magnitude of Epidemics Since 1997. Plant Dis. **96**: 1712–1728.

McMullen, M., Bergstrom, G., De Wolf, E., Dill-Macky, R., Hershman, D., Shaner, G., and Van Sanford, D. 2012b. A Unified Effort to Fight an Enemy of Wheat and Barley: Fusarium Head Blight. Plant Dis. **96**: 1712–1728. The American Phytopathological Society . doi:10.1094/PDIS-03-12-0291-FE.

McMullen, M., Jones, R., and Gallenberg, D. 1997. Scab of Wheat and Barley: A re-emerging disease of devastating impact. Plant Dis. **81**.

Measures, M., Weinberger, P., and Baer, H. 1973. TEMPERATURE AND LIGHT DISTRIBUTION Description of Growth Chamber Measurements Within the Chamber. **220**: 215–220.

Mergoum, M., Frohberg, R.C., Miller, J.D., Rasmussen, J.B., and Stack, R.W. 2005. Registration of Spring Wheat Germplasm ND 744 Resistant to Fusarium Head Blight, Leaf, and Stem Rusts. Crop Sci. **45**: cropsci2005.0430. doi:10.2135/cropsci2005.0430.

Mesterhazy, A. 2003. Control of Fusarium Head Blight of wheat by fungicides. Pages 363–380 *in* K.J. Leonard and W.R. Bushnell, eds. Fusarium Head Blight of wheat and barley. APS Press, St. Paul.

Mesterházy, A. 1995. Types and components of resistance to Fusarium head blight of wheat. Plant Breed. **114**: 377–386. doi:10.1111/j.1439-0523.1995.tb00816.x.

Mesterházy, A. 2003. Breeding wheat for Fusarium Head Blight Resistance in Europe. Pages 211–240 *in* K.J. Leonard and W.R. Bushnell, eds. Fusarium Head Blight of wheat and barley. APS Press, St. Paul.

Miedaner, T., and Voss, H.H. 2008. Effect of dwarfing Rht genes on fusarium head blight resistance in two sets of near-isogenic lines of wheat and check cultivars. Crop Sci. **48**: 2115–2122. doi:10.2135/cropsci2008.02.0107.

Miller, J.D., Culley, J., Fraser, K., Hubbard, S., Meloche, F., Ouellet, T., Lloyd Seaman, W., Seifert, K.A., Turkington, K., and Voldeng, H. 1998. Effect of tillage practice on fusarium head blight of wheat. Can. J. Plant Pathol. **20**: 95–103. doi:10.1080/07060669809500450.

Mo, Y., Pearce, S., and Dubcovsky, J. 2018. Phenotypic and transcriptomic characterization of a wheat tall mutant carrying an induced mutation in the C-terminal PFYRE motif of RHT-B1b. BMC Plant Biol. **18**: 1–17. BMC Plant Biology. doi:10.1186/s12870-018-1465-4.

Muqaddasi, Q.H., Brassac, J., Börner, A., Pillen, K., and Röder, M.S. 2017a. Genetic architecture of anther extrusion in spring and winter wheat. Front. Plant Sci. **8**: 1–11. doi:10.3389/fpls.2017.00754.

Muqaddasi, Q.H., Pillen, K., Plieske, J., Ganal, M.W., and Röder, M.S. 2017b. Genetic and

physical mapping of anther extrusion in elite European winter wheat. PLoS One **12**: 1–18. doi:10.1371/journal.pone.0187744.

Mwaniki, A. 2017. Molecular mapping of QTL controlling FhB resistance and DON accumulation in two winter wheat DH population.

Navarro, L., Bari, R., Achard, P., Lisón, P., Nemri, A., Harberd, N.P., and Jones, J.D.G. 2008. DELLAs Control Plant Immune Responses by Modulating the Balance of Jasmonic Acid and Salicylic Acid Signaling. Curr. Biol. **18**: 650–655. doi:10.1016/j.cub.2008.03.060.

Nelson, S.K., and Steber, C.M. 2016. Gibberellin Hormone Signal Perception: Down-Regulating DELLA Repressors of Plant Growth and Development. Annu. Plant Rev. Gibberellins **49**: 153–188. doi:10.1002/9781119210436.ch6.

Nicholson, P., Srinivasachary, Gosman, N., Steed, A., and Chen, X. 2008. Role of phytohormone signalling in resistance of wheat to fusarium head blight. Cereal Res. Commun. **36**: 213–216. doi:10.1556/CRC.36.2008.Suppl.B.17.

Nicolaisen, M., Suproniene, S., Nielsen, L.K., Lazzaro, I., Spliid, N.H., and Justesen, A.F. 2009. Real-time PCR for quantification of eleven individual Fusarium species in cereals. J. Microbiol. Methods **76**: 234–240. doi:10.1016/j.mimet.2008.10.016.

Nirenberg, H.I. 1981. A simplified method for identifying Fusarium spp. occurring on wheat . Can. J. Bot. **59**: 1599–1609. doi:10.1139/b81-217.

Nowakowski, G. 2018. Mitigation of Fusarium Head Blight in Wheat Caused by Fusarium graminearum Through the Use of Host Resistance Genes and Biological Controls. Master's thesis, University of Manitioba, Winnipeg, MB.

Osborne, L.E., and Stein, J.M. 2007. Epidemiology of Fusarium head blight on small-grain cereals. Int. J. Food Microbiol. **119**: 103–108. doi:10.1016/j.ijfoodmicro.2007.07.032.

Palazzini, J.M., Dunlap, C.A., Bowman, M.J., and Chulze, S.N. 2016. Bacillus velezensis RC 218 as a biocontrol agent to reduce Fusarium head blight and deoxynivalenol accumulation: Genome sequencing and secondary metabolite cluster profiles. Microbiol. Res. **192**: 30–36. Elsevier GmbH. doi:10.1016/j.micres.2016.06.002.

Peng, J. 2009. Gibberellin and jasmonate crosstalk during stamen development. J. Integr. Plant Biol. **51**: 1064–1070. doi:10.1111/j.1744-7909.2009.00881.x.

Peng, J.H., Sun, D., and Nevo, E. 2011. Domestication evolution, genetics and genomics in wheat. Mol. Breed. **28**: 281–301. doi:10.1007/s11032-011-9608-4.

Petersen, S., Lyerly, J.H., McKendry, A.L., Islam, M.S., Brown-Guedira, G., Cowger, C., Dong, Y., and Murphy, J.P. 2017. Validation of Fusarium head blight resistance QTL in US winter wheat. Crop Sci. **57**: 1–12. doi:10.2135/cropsci2015.07.0415.

Pirgozliev, S.R., Edwards, S.G., Hare, M.C., and Jenkinson, P. 2003. Strategies for the control of Fusarium head blight in cereals. Eur. J. Plant Pathol. **109**: 731–742. doi:10.1023/A:1026034509247.

Plackett, A.R.G., Thomas, S.G., Wilson, Z.A., and Hedden, P. 2011. Gibberellin control of stamen development: A fertile field. Trends Plant Sci. **16**: 568–578. Elsevier Ltd. doi:10.1016/j.tplants.2011.06.007.

Porter, A.S., Evans-Fitz.Gerald, C., McElwain, J.C., Yiotis, C., and Elliott-Kingston, C. 2015. How well do you know your growth chambers? Testing for chamber effect using plant traits. Plant Methods **11**: 1–10. BioMed Central. doi:10.1186/s13007-015-0088-0.

Potvin, C., Lechowicz, M.J., Bell, G., and Schoen, D. 1990. Spatial, temporal, amd species-specific patterns of heterogeneity in growth chamber experiments. Can. J. Bot. **68**: 499–504. doi:10.1139/b90-067.

Potvin, C., and Tardif, S. 1988. Sources of Variability and Experimental Designs in Growth Chambers. Funct. Ecol. **2**: 123. doi:10.2307/2389472.

Qi, T., Huang, H., Wu, D., Yan, J., Qi, Y., Song, S., and Xie, D. 2014. Arabidopsis DELLA and JAZ proteins bind the WD-Repeat/ bHLH/MYB complex to modulate gibberellin and jasmonate signaling synergy. Plant Cell **26**: 1118–1133. doi:10.1105/tpc.113.121731.

Rademacher, W. 2000. GROWTH R ETARDANTS : Effects on Gibberellin biosynthesis and other metabolic pathways. Annu. Rev. Plant Physiol. Plant Mol. Biol. **51**: 501–531.

Rademacher, W. 2016. Chemical Regulators of Gibberellin Status and Their Application in Plant Production. Annu. Plant Rev. Gibberellins **49**: 359–404. doi:10.1002/9781119210436.ch12.

Rajala, A., Peltonen-Sainio, P., Onnela, M., and Jackson, M. 2002. Effects of applying stem-shortening plant growth regulators to leaves on root elongation by seedlings of wheat, oat and barley: Mediation by ethylene. Plant Growth Regul. **38**: 51–59. doi:10.1023/A:1020924307455.

Ramburan, S., and Greenfield, P.L. 2007. The effects of chlormequat chloride and ethephon on agronomic and quality characteristics of South African irrigated wheat. South African J. Plant Soil **24**: 106–113. doi:10.1080/02571862.2007.10634790.

Ross, J.J., Miraghazadeh, A., Beckett, A.H., Quittenden, L.J., and McAdam, E.L. 2016. Interactions Between Gibberellins and Other Hormones. Annu. Plant Rev. Gibberellins **49**: 229–252. doi:10.1002/9781119210436.ch8.

Salazar-González, C., Velásquez-Ortiz, D., and Gómez-López, E. 2020. Detection of mycotoxins produced by fusarium species in Colombia. Agron. Colomb. **38**: 208–215. doi:10.15446/agron.colomb.v38n2.77176.

Saville, R.J., Gosman, N., Burt, C.J., Makepeace, J., Steed, A., Corbitt, M., Chandler, E., Brown, J.K.M., Boulton, M.I., and Nicholson, P. 2012. The "Green Revolution" dwarfing genes play a role in disease resistance in Triticum aestivum and Hordeum vulgare. J. Exp. Bot. **63**: 1271–1283. doi:10.1093/jxb/err350.

Seed Manitoba. 2018. Variety Selection & Griwers Sourrce Guide. [Online] Available: https://www.seedmb.ca/digital-edition/seed-manitoba_2018-01-01/ [2017 Oct. 30].

Schaller, G.E. 2012. Ethylene and the regulation of plant development. BMC Biol. **10**: 9–11.

doi:10.1186/1741-7007-10-9.

Schisler, D.A., Slmmger, P.J., Boehm, M.J., and Paul, P.A. 2011. Co-culture of yeast antagonists of fusarium head blight and their effect on disease development in wheat. Plant Pathol. J. **10**: 128–137. doi:10.2923-ppj.2011.128.137.

Shaner, G. 2003. Epidemiology of Fusarium Head Blight of Small grain cereals in North America. Pages 84–119 *in* K.K. Leonard and W.R. Bushnell, eds. Fusarium Head Blight of wheat and barley. APS Press, St. Paul.

Shekhovtsov, S. V., Golovanova, E. V., and Peltek, S.E. 2016. Different dispersal histories of lineages of the earthworm Aporrectodea caliginosa (Lumbricidae, Annelida) in the Palearctic. Biol. Invasions **18**: 751–761. Springer International Publishing. doi:10.1007/s10530-015-1045-6.

Shewry, P.R., and Hey, S.J. 2015. The contribution of wheat to human diet and health. Food Energy Secur. **4**: 178–202. doi:10.1002/FES3.64.

Skelsey, P., and Newton, A.C. 2015. Future environmental and geographic risks of Fusarium head blight of wheat in Scotland. Eur. J. Plant Pathol. **142**: 133–147. doi:10.1007/s10658-015-0598-7.

Skinnes, H., Semagn, K., Tarkegne, Y., Marøy, A.G., and Bjørnstad, Å. 2010. The inheritance of anther extrusion in hexaploid wheat and its relationship to Fusarium head blight resistance and deoxynivalenol content. Plant Breed. **129**: 149–155. doi:10.1111/j.1439-0523.2009.01731.x.

Smith, W. 1884. Diseases of field and garden crops. MacMillan and Co, London.

Spolti, P., Del Ponte, E.M., Dong, Y., Cummings, J.A., and Bergstrom, G.C. 2014. Triazole sensitivity in a contemporary population of Fusarium graminearum from New York wheat and competitiveness of a tebuconazole-resistant isolate. Plant Dis. **98**: 607–613. doi:10.1094/PDIS-10-13-1051-RE.

Srinivasachary, Gosman, N., Steed, A., Hollins, T.W., Bayles, R., Jennings, P., and Nicholson, P. 2009. Semi-dwarfing Rht-B1 and Rht-D1 loci of wheat differ significantly in their influence on resistance to Fusarium head blight. Theor. Appl. Genet. **118**: 695–702. doi:10.1007/s00122-008-0930-0.

Statistis Canada 2020. Estimated areas, yield, production, average farm price and total farm value of principal field crops, in metric and imperial units. [Online] Available: https://www150.statcan.gc.ca/t1/tbl1/en/tv.action?pid=3210035901&cubeTimeFrame.startYear=2016&cubeTimeFrame.endYear=2019&referencePeriods=20160101%2C20190101 [2020 Apr. 30].

Steiner, B., Buerstmayr, M., Michel, S., Schweiger, W., Lemmens, M., and Buerstmayr, H. 2017. Breeding strategies and advances in line selection for Fusarium head blight resistance in wheat. Trop. Plant Pathol. **42**: 165–174. Tropical Plant Pathology. doi:10.1007/s40858-017-0127-7.

Steiner, B., Buerstmayr, M., Wagner, C., Danler, A., Eshonkulov, B., Ehn, M., and Buerstmayr,

H. 2019. Fine-mapping of the Fusarium head blight resistance QTL Qfhs.ifa-5A identifies two resistance QTL associated with anther extrusion. Theor. Appl. Genet. **132**: 2039–2053. Springer Berlin Heidelberg. doi:10.1007/s00122-019-03336-x.

Strange, R.N., Smith, H., and Majer, J.R. 1972. Choline, one of two fungal growth stimulants in anthers responsible for the susceptibility of wheat to fusarium graminearum. Nature **238**: 103–104. doi:10.1038/238103a0.

Strydhorst, S. 2014. Plant Growth Regulators: What Agronomists Need to Know. Page *in* Manitoba Agronomists conference 2014.

Strydhorst, S., Hall, L., and Perrott, L. 2018. Plant growth regulators: What agronomists need to know. Crop. Soils **51**: 22–26. doi:10.2134/cs2018.51.0608.

Sudakin, D.L. 2003. Trichothecenes in the environment: Relevance to human health. Toxicol. Lett. **143**: 97–107. doi:10.1016/S0378-4274(03)00116-4.

Sun, H.Y., Cui, J. he, Tian, B. hua, Cao, S. lin, Zhang, X. xiang, and Chen, H.G. 2020. Resistance risk assessment for Fusarium graminearum to pydiflumetofen, a new succinate dehydrogenase inhibitor. Pest Manag. Sci. **76**: 1549–1559. doi:10.1002/ps.5675.

Sun, T. ping 2010. Gibberellin-GID1-DELLA: A pivotal regulatory module for plant growth and development. Plant Physiol. **154**: 567–570. doi:10.1104/pp.110.161554.

Sun, Y., Xiao, J., Jia, X., Ke, P., He, L., Cao, A., Wang, H., Wu, Y., Gao, X., and Wang, X. 2016. The role of wheat jasmonic acid and ethylene pathways in response to Fusarium graminearum infection. Plant Growth Regul. **80**: 69–77. Springer Netherlands. doi:10.1007/s10725-016-0147-1.

Tamburic-Ilincic, L., Wragg, A., and Schaafsma, A. 2015. Accumulation de mycotoxines et diversité du chimiotype de Fusarium graminearum chez le blé d'hiver cultivé dans le sud-ouest de l'Ontario. Can. J. Plant Sci. **95**: 931–938. doi:10.4141/CJPS-2014-132.

Thambugala, D., Brûlé-Babel, A.L., Blackwell, B.A., Fedak, G., Foster, A.J., MacEachern, D., Gilbert, J., Henriquez, M.A., Martin, R.A., McCallum, B.D., Spaner, D., Iqbal, M., Pozniak, C.J., N'Diaye, A., and McCartney, C.A. 2020. Genetic analyses of native Fusarium head blight resistance in two spring wheat populations identifies QTL near the B1, Ppd-D1, Rht-1, Vrn-1, Fhb1, Fhb2, and Fhb5 loci. Theor. Appl. Genet. **133**: 2775–2796. Springer Berlin Heidelberg. doi:10.1007/s00122-020-03631-y.

Thomas, S.G. 2017. Novel Rht-1 dwarfing genes: Tools for wheat breeding and dissecting the function of della proteins. J. Exp. Bot. **68**: 354–358. doi:10.1093/jxb/erw509.

Triboï, E., Martre, P., and Triboï-Blondel, A.M. 2003. Environmentally-induced changes in protein composition in developing grains of wheat are related to changes in total protein content. J. Exp. Bot. **54**: 1731–1742. doi:10.1093/jxb/erg183.

Tuite, J. 1969. Plant pathological methods: fungi and bacteria. Burgess publishing Co., Minneapolis. [Online] Available: http://eprints.icrisat.ac.in/id/eprint/10287%0A.

Valverde-Bogantes, E., Bianchini, A., Herr, J.R., Rose, D.J., Wegulo, S.N., and Hallen-Adams,

H.E. 2020. Recent population changes of Fusarium head blight pathogens: drivers and implications. Can. J. Plant Pathol. **42**: 315–329. Taylor & Francis. doi:10.1080/07060661.2019.1680442.

Varga, E., Wiesenberger, G., Hametner, C., Ward, T.J., Dong, Y., Schöfbeck, D., Mccormick, S., Broz, K., Stückler, R., Schuhmacher, R., Krska, R., Kistler, H.C., Berthiller, F., and Adam, G. 2015. New tricks of an old enemy: Isolates of Fusarium graminearum produce a type A trichothecene mycotoxin. Environ. Microbiol. **17**: 2588–2600. doi:10.1111/1462-2920.12718.

Vaseva, I.I., Qudeimat, E., Potuschak, T., Du, Y., Genschik, P., Vandenbussche, F., and Van Der Straeten, D. 2018. The plant hormone ethylene restricts Arabidopsis growth via the epidermis. Proc. Natl. Acad. Sci. U. S. A. **115**: E4130–E4139. doi:10.1073/pnas.1717649115.

Wang, L., Li, Q., Liu, Z., Surendra, A., Pan, Y., Li, Y., Irina Zaharia, L., Ouellet, T., and Fobert, P.R. 2018. Integrated transcriptome and hormone profiling highlight the role of multiple phytohormone pathways in wheat resistance against fusarium head blight. PLoS One **13**: 1–24. doi:10.1371/journal.pone.0207036.

Wanguimwaniki, A. 2017. molecular mapping of quantitative trait loci controlling FHB resistance and DON accumulation in two winter wheat DH population. Doctoral dissertation, University of Manitoba, Winnipeg, MB.

Wiersma, J.J., Dai, J., and Durgan, B.R. 2011. Optimum timing and rate of trinexapac-ethyl to reduce lodging in spring wheat. Agron. J. **103**: 864–870. doi:10.2134/agronj2010.0398.

Willenborg, C.J., and Van Acker, R.C. 2008. The biology and ecology of hexaploid wheat (Triticum aestivum L.) and its implications for trait confinement. Can. J. Plant Sci. **88**: 997–1013. doi:10.4141/CJPS07144.

Wilson, Z.A., Song, J., Taylor, B., and Yang, C. 2011. The final split: The regulation of anther dehiscence. J. Exp. Bot. **62**: 1633–1649. doi:10.1093/jxb/err014.

Windels, C.E. 2000. Economic and social impacts of Fusarium head blight: Changing farms and rural communities in the Northern Great Plains. Phytopathology **90**: 17–21. doi:10.1094/PHYTO.2000.90.1.17.

Wolfarth, F., Schrader, S., Oldenburg, E., and Brunotte, J. 2016. Mycotoxin contamination and its regulation by the earthworm species Lumbricus terrestris in presence of other soil fauna in an agroecosystem. Plant Soil **402**: 331–342. Plant and Soil. doi:10.1007/s11104-015-2772-2.

Wolfarth, F., Schrader, S., Oldenburg, E., and Weinert, J. 2011. Contribution of the endogeic earthworm species Aporrectodea caliginosa to the degradation of deoxynivalenol and Fusarium biomass in wheat straw. Mycotoxin Res. **27**: 215–220. doi:10.1007/s12550-011-0098-3.

Würschum, T., Langer, S.M., Longin, C.F.H., Tucker, M.R., and Leiser, W.L. 2017. A modern Green Revolution gene for reduced height in wheat. Plant J. **92**: 892–903. doi:10.1111/tpj.13726.

Xia, R., Schaafsma, A.., Limay-Rios, V., and Hooker, D.. 2021. Effectiveness of a novel fungicide pydiflumetofen against Fusarium head blight and mycotoxin accumulation in winter wheat.

World Mycotoxin: 1–18. [Online] Available: https://doi.org/10.3920/WMJ2020.2637.

Xu, K., He, X., Dreisigacker, S., He, Z., and Singh, P.K. 2020. Anther Extrusion and Its Association with Fusarium Head blight in CIMMYT wheat germplasm. Agronomy **10**: 47.

Xue, A.G., Voldeng, H.D., Savard, M.E., Fedak, G., Tian, X., and Hsiang, T. 2009. Biological control of fusarium head blight of wheat with Clonostachys rosea strain ACM941. Can. J. Plant Pathol. **31**: 169–179. doi:10.1080/07060660909507590.

Xue, S., Li, G., Jia, H., Xu, F., Lin, F., Tang, M., Wang, Y., An, X., Xu, H., Zhang, L., Kong, Z., and Ma, Z. 2010. Fine mapping Fhb4, a major QTL conditioning resistance to Fusarium infection in bread wheat (Triticum aestivum L.). Theor. Appl. Genet. **121**: 147–156. doi:10.1007/s00122-010-1298-5.

Xue, S., Xu, F., Tang, M., Zhou, Y., Li, G., An, X., Lin, F., Xu, H., Jia, H., Zhang, L., Kong, Z., and Ma, Z. 2011. Precise mapping Fhb5, a major QTL conditioning resistance to Fusarium infection in bread wheat (Triticum aestivum L.). Theor. Appl. Genet. **123**: 1055–1063. doi:10.1007/s00122-011-1647-z.

Ye, Z. 2015. Analysis of Resistance to Fusarium Head Blight (FHB) in Winter Wheat and Evaluation of Genetics and Cultural Practices for FHB Mitigation. Master's thesis, University of Manitoba, Winnipeg, MB.

Yin, Y., Liu, X., Li, B., and Ma, Z. 2009. Characterization of sterol demethylation inhibitor-resistant isolates of Fusarium asiaticum and F. graminearum collected from wheat in china. Phytopathology **99**: 487–497. doi:10.1094/PHYTO-99-5-0487.

Zadoks, J.C., Chang, T.T., and Konzak, C.F. 1974. A decimal code for the growth stages of cereals. Weed Res. **14**: 415–421. doi:10.1111/j.1365-3180.1974.tb01084.x.

Zhang, Y.J., Fan, P.S., Zhang, X., Chen, C.J., and Zhou, M.G. 2009. Quantification of Fusarium graminearum in harvested grain by real-time polymerase chain reaction to assess efficacies of fungicides on fusarium head blight, deoxynivalenol contamination, and yield of winter wheat. Phytopathology **99**: 95–100. doi:10.1094/PHYTO-99-1-0095.

Zhao, Y., Selvaraj, J.N., Xing, F., Zhou, L., Wang, Y., Song, H., Tan, X., Sun, L., Sangare, L., Folly, Y.M.E., and Liu, Y. 2014. Antagonistic action of Bacillus subtilis strain SG6 on Fusarium graminearum. PLoS One **9**: 1–11. doi:10.1371/journal.pone.0092486.

Appendices

Table A.1. Total precipitation (mm) in each location from May to August from four test environments. The numbers in brackets indicated the average of total precipitation (mm) based on 23 and 24 years of growing seasons (1996 to 2018 and 1996 to 2019) in each location on each month.

	Carman 2019	Winnipeg 2019	Carman 2020	Winnipeg 2020
May	36.9 (75.6)	28 (72.2)	26.4 (74)	24.5 (70.3)
June	37.9 (83.2)	25 (79)	70.7 (81.3)	73.5 (76.8)
July	57.4 (61.6)	87.9 (70.3)	54 (61.5)	39.2 (71)
August	61.6 (57.1)	65.6 (66.9)	24.3 (57.3)	59.1 (66.8)
Total	193.8 (277.6)	206.5 (288.3)	175.4 (274.1)	196.3 (284.9)

Table A.2. The monthly average temperature (°C) in each location on each month from May to August from four test environments. The numbers in brackets indicated the average temperature (°C) based on 23 and 24 years of growing seasons (1996 to 2018 and 1996 to 2019) in each location on each month.

	Carman 2019	Winnipeg 2019	Carman 2020	Winnipeg 2020
May	9.6 (11)	9.8 (10.9)	10.7 (11)	10.7 (10.9)
June	17.3 (16.8)	17.8 (16.9)	18.2 (16.8)	18.7 (16.9)
July	19.5 (19.3)	20.2 (19.6)	20.2 (19.3)	20.9 (19.7)
August	18.1 (18.4)	18.1 (18.7)	18.7 (18.4)	19.4 (18.7)

Table A.3. Simple contrasts between main treatments across all tested cultivars for protein from combined experiments conducted in Carman and Winnipeg in 2019 and 2020.

Main treatments	**Probability**
Ethrel™, FHB inoculation vs FHB inoculation	0.735
Ethrel™, No FHB inoculation vs No FHB inoculation	0.5896
Manipulator™, FHB inoculation vs FHB inoculation	0.5228
Manipulator™, No FHB inoculation vs No FHB inoculation	0.3184
Ethrel™, FHB inoculation vs Manipulator™, FHB inoculation	0.3339
Ethrel™, No FHB inoculation vs Manipulator™, No FHB inoculation	0.6401
FHB inoculation vs No FHB inoculation	0.0158
All FHB inoculation vs All No FHB inoculation	0.0026

Table A.4. Simple contrasts between main treatments across all tested cultivars for test weight from combined experiments conducted in Carman and Winnipeg in 2019 and 2020.

Main treatments	Probability
Ethrel™, FHB inoculation vs FHB inoculation	0.3615
Ethrel™, No FHB inoculation vs No FHB inoculation	0.4172
Manipulator™, FHB inoculation vs FHB inoculation	0.5444
Manipulator™, No FHB inoculation vs No FHB inoculation	0.636
Ethrel™, FHB inoculation vs Manipulator™, FHB inoculation	0.1395
Ethrel™, No FHB inoculation vs Manipulator™, No FHB inoculation	0.2079
FHB inoculation vs No FHB inoculation	<.0001
All FHB inoculation vs All No FHB inoculation	<.0001

Table A.5. Simple contrasts between main treatments across all tested cultivars for thousand kernel weight from combined experiments conducted in Carman and Winnipeg in 2019 and 2020.

Main treatments	Probability
Ethrel™, FHB inoculation vs FHB inoculation	0.3088
Ethrel™, No FHB inoculation vs No FHB inoculation	0.1809
Manipulator™, FHB inoculation vs FHB inoculation	0.6325
Manipulator™, No FHB inoculation vs No FHB inoculation	0.584
Ethrel™, FHB inoculation vs Manipulator™, FHB inoculation	0.5798
Ethrel™, No FHB inoculation vs Manipulator™, No FHB inoculation	0.4114
FHB inoculation vs No FHB inoculation	<.0001
All FHB inoculation vs All No FHB inoculation	<.0001

Table A.6. Simple contrasts between main treatments across all tested cultivars for yield from combined experiments conducted in Carman and Winnipeg in 2019 and 2020.

Main treatments	Probability
Ethrel™, FHB inoculation vs FHB inoculation	0.8011
Ethrel™, No FHB inoculation vs No FHB inoculation	0.7759
Manipulator™, FHB inoculation vs FHB inoculation	0.9219
Manipulator™, No FHB inoculation vs No FHB inoculation	0.3861
Ethrel™, FHB inoculation vs Manipulator™, FHB inoculation	0.7266
Ethrel™, No FHB inoculation vs Manipulator™, No FHB inoculation	0.2561
FHB inoculation vs No FHB inoculation	<.0001
All FHB inoculation vs All No FHB inoculation	<.0001

Table A.7. Simple contrasts between main treatments across all tested cultivars for FHB disease severity from combined experiments conducted in Carman and Winnipeg in 2019 and 2020.

Main treatments	Probability
Ethrel™, FHB inoculation vs FHB inoculation	0.4319
Ethrel™, No FHB inoculation vs No FHB inoculation	0.8759
Manipulator™, FHB inoculation vs FHB inoculation	0.6947
Manipulator™, No FHB inoculation vs No FHB inoculation	0.824
Ethrel™, FHB inoculation vs Manipulator™, FHB inoculation	0.2457
Ethrel™, No FHB inoculation vs Manipulator™, No FHB inoculation	0.7059
FHB inoculation vs No FHB inoculation	<.0001
All FHB inoculation vs All No FHB inoculation	<.0001

Table A.8. Simple contrasts between main treatments across all tested cultivars for FHB disease incidence from combined experiments conducted in Carman and Winnipeg in 2019 and 2020.

Main treatments	Probability
Ethrel™, FHB inoculation vs FHB inoculation	0.306
Ethrel™, No FHB inoculation vs No FHB inoculation	0.9101
Manipulator™, FHB inoculation vs FHB inoculation	0.9227
Manipulator™, No FHB inoculation vs No FHB inoculation	0.9815
Ethrel™, FHB inoculation vs Manipulator™, FHB inoculation	0.3518
Ethrel™, No FHB inoculation vs Manipulator™, No FHB inoculation	0.8918
FHB inoculation vs No FHB inoculation	<.0001
All FHB inoculation vs All No FHB inoculation	<.0001

Table A.9. Simple contrasts between main treatments across all tested cultivars for *Fusarium* damaged kernel from combined experiments conducted in Carman and Winnipeg in 2019 and 2020.

Main treatments	Probability
Ethrel™, FHB inoculation vs FHB inoculation	0.8844
Ethrel™, No FHB inoculation vs No FHB inoculation	0.9804
Manipulator™, FHB inoculation vs FHB inoculation	0.7534
Manipulator™, No FHB inoculation vs No FHB inoculation	0.9794
Ethrel™, FHB inoculation vs Manipulator™, FHB inoculation	0.6467
Ethrel™, No FHB inoculation vs Manipulator™, No FHB inoculation	0.9598
FHB inoculation vs No FHB inoculation	0.0001
All FHB inoculation vs All No FHB inoculation	<.0001

Table A.10. Simple contrasts between main treatments across all tested cultivars for deoxynivalenol from combined experiments conducted in Carman and Winnipeg in 2019 and 2020.

Main treatments	Probability
Ethrel™, FHB inoculation vs FHB inoculation	0.7245
Ethrel™, No FHB inoculation vs No FHB inoculation	0.9736
Manipulator™, FHB inoculation vs FHB inoculation	0.9101
Manipulator™, No FHB inoculation vs No FHB inoculation	0.9963
Ethrel™, FHB inoculation vs Manipulator™, FHB inoculation	0.6423
Ethrel™, No FHB inoculation vs Manipulator™, No FHB inoculation	0.9699
FHB inoculation vs No FHB inoculation	0.0001
All FHB inoculation vs All No FHB inoculation	<.0001

Table A.11. Simple contrasts between main treatments across all tested cultivars for FHB index from combined experiments conducted in Carman and Winnipeg in 2019 and 2020.

Main treatments	Probability
Ethrel™, FHB inoculation vs FHB inoculation	0.1668
Ethrel™, No FHB inoculation vs No FHB inoculation	0.949
Manipulator™, FHB inoculation vs FHB inoculation	0.8749
Manipulator™, No FHB inoculation vs No FHB inoculation	0.9809
Ethrel™, FHB inoculation vs Manipulator™, FHB inoculation	0.1275
Ethrel™, No FHB inoculation vs Manipulator™, No FHB inoculation	0.9299
FHB inoculation vs No FHB inoculation	<.0001
All FHB inoculation vs All No FHB inoculation	<.0001

Table A.12. Analysis of variance for spike density, height, anther retention, and protein content in the Carman 2019 trial.

Source of Variation	Spike density			Height		Anther retention			Protein	
	DF[a]	MS[b]	P[c]	MS	P	DF	MS	P	MS	P
Main treatment	5	3645.64	0.4486	217.78	0.0016	5	43.51	0.4239	0.37	0.762
Cultivar	4	17214.00	<.0001	947.77	<.0001	4	3552.13	<.0001	13.88	<.0001
Main Treatment*Cultivar	20	1519.47	0.7557	11.54	0.1575	20	68.99	0.0867	0.13	0.2491
Rep	3	1119.49	0.819	241.34	0.0025	3	283.12	0.0029	3.55	0.0129
Rep* Main treatment	15	3630.41	0.0506	31.68	<.0001	15	41.51	0.5205	0.77	<.0001
Error	72	2012.40	0.4486	8.32		68	43.92		0.10	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.13. Analysis of variance for test weight, thousand kernel weight, and yield in the Carman 2019 trial.

Source of Variation	Test weight			Thousand kernel weight		Yield		
	DF[a]	MS[b]	P[c]	MS	P	DF	MS	P
Main treatment	5	40.36	<.0001	80.04	<.0001	5	4664735	<.0001
Cultivar	4	7.36	<.0001	32.81	<.0001	4	1381053	<.0001
Main treatment *Cultivar	20	0.95	0.0007	3.85	0.0016	20	160613	0.0222
Rep	3	1.39	0.0765	1.77	0.5172	3	228166	0.2016
Rep* Main treatment	15	0.53	0.1051	2.32	0.0999	15	137960	0.0774
Error	68	0.33		1.46		66	82190	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.14. Analysis of variance for Fusarium head blight (FHB) index, severity, incidence, Fusarium damaged kernel (FDK), and deoxynivalenol (DON) in the Carman 2019 trial.

Source of Variation	DF[a]	FHB Index		Incidence		Severity		FDK		DON	
		MS[b]	P[c]	MS	P	MS	P	MS	P	MS	P
Main treatment	5	3647.49	<.0001	31708.00	<.0001	5226.65	<.0001	98.02	<.0001	384.41	<.0001
Cultivar	4	540.93	<.0001	1114.38	<.0001	490.90	<.0001	30.49	<.0001	62.84	<.0001
Main Treatment*Cultivar	20	141.20	0.0001	244.62	<.0001	131.77	0.0003	6.27	<.0001	20.26	<.0001
Rep	3	425.83	0.0387	79.74	0.1346	414.32	0.0636	3.15	0.3567	6.35	0.3216
Rep* Main treatment	15	127.57	0.0011	37.05	0.7066	148.68	0.0002	2.90	0.0001	5.22	0.1106
Error	68	42.90		48.22		43.20		0.79		3.36	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.15. Analysis of variance for spike density, height, anther retention, and protein content in the Winnipeg 2019 trial.

Source of Variation	DF[a]	Spike density		Height		Anther retention		Protein	
		MS[b]	P[c]	MS	P	MS	P	MS	P
Main treatment	5	11583.00	0.2472	134.66	0.1313	65.87	0.3436	0.81	0.8938
Cultivar	4	15481.00	0.0315	3492.83	<.0001	6239.71	<.0001	31.23	<.0001
Main Treatment*Cultivar	20	8482.63	0.0938	36.61	<.0001	147.24	0.0004	0.27	0.1837
Rep	3	6919.38	0.4651	132.28	0.1572	65.10	0.3386	6.98	0.0804
Rep* Main treatment	15	7705.56	0.1708	66.12	<.0001	53.60	0.3959	2.55	<.0001
Error	72	5502.68		8.88		49.94		0.20	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.16. Analysis of variance for test weight, thousand kernel weight, and yield in the Winnipeg 2019 trial.

Source of Variation	Test weight			Thousand kernel weight			Yield		
	DF[a]	MS[b]	P[c]	DF	MS	P	DF	MS	P
Main treatment	5	74.15	<.0001	5	121.64	<.0001	5	9374597	<.0001
Cultivar	4	52.63	<.0001	4	38.11	0.0002	4	186547	0.2473
Main Treatment*Cultivar	20	7.97	<.0001	20	11.44	0.0207	20	661185	<.0001
Rep	3	0.42	0.801	3	16.02	0.2074	3	2703966	0.0467
Rep* Main treatment	15	1.26	0.0382	15	9.36	0.0956	15	806321	<.0001
Error	70	0.66		72	5.85		69	134438	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.17. Analysis of variance for Fusarium head blight (FHB) index, severity, incidence, Fusarium damaged kernel (FDK), and deoxynivalenol (DON) in the Winnipeg 2019 trial.

Source of Variation	DF[a]	FHB Index		Incidence		Severity		FDK		DON	
		MS[b]	P[c]	MS	P	MS	P	MS	P	MS	P
Main treatment	5	10189.00	<.0001	19586.00	<.0001	14271.00	<.0001	51.34	<.0001	103.45	<.0001
Cultivar	4	4311.79	<.0001	6290.15	<.0001	8338.27	<.0001	27.71	<.0001	54.13	<.0001
Main Treatment*Cultivar	20	773.82	<.0001	1124.88	<.0001	429.04	0.0076	5.41	<.0001	10.18	<.0001
Rep	3	11.55	0.9215	22.94	0.8218	150.33	0.5125	5.04	0.17	1.89	0.5163
Rep* Main treatment	15	72.18	0.0359	75.39	0.061	187.71	0.4969	2.63	<.0001	2.39	0.0053
Error	72	37.77		43.21		193.93		0.70		0.96	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.18. Analysis of variance for spike density, height, anther retention, and protein content in the Carman 2020 trial.

Source of Variation	DF[a]	Spike density		Height		Anther retention		Protein	
		MS[b]	P[c]	MS	P	MS	P	MS	P
Main treatment	5	3290.25	0.5907	92.78	0.0029	71.66	0.0605	4.95	<.0001
Cultivar	4	32734.00	<.0001	2617.06	<.0001	6855.36	<.0001	34.01	<.0001
Main Treatment*Cultivar	20	3010.87	0.2863	11.71	<.0001	145.93	<.0001	0.66	<.0001
Rep	3	1242.30	0.8335	25.85	0.2116	13.23	0.6864	0.77	0.0517
Rep* Main treatment	15	4321.70	0.0681	15.33	<.0001	26.36	0.1548	0.24	0.0559
Error	70	2523.15		3.23		18.34		0.13	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.19. Analysis of variance for lodging rating, test weight, thousand kernel weight, and yield in the Carman 2020 trial.

Source of Variation	DF[a]	Lodging rating		Test weight		Thousand kernel weight		Yield		
		MS[b]	P[c]	MS	P	MS	P	DF	MS	P
Main treatment	5	3.99	0.0002	144.73	<.0001	391.11	<.0001	5	35858523	<.0001
Cultivar	4	7.72	<.0001	78.71	<.0001	30.74	<.0001	4	6478219	<.0001
Main Treatment*Cultivar	20	1.04	0.0142	13.01	<.0001	23.25	<.0001	20	2016358	<.0001
Rep	3	0.34	0.4639	5.58	0.0959	9.35	0.0818	3	521049	0.3168
Rep* Main treatment	15	0.38	0.715	2.2	0.0204	3.45	0.0039	15	410957	0.0008
Error	70	0.50		1.05		1.33		66	132556	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.20. Analysis of variance for Fusarium head blight (FHB) index, severity, incidence, Fusarium damaged kernel (FDK), and deoxynivalenol (DON) in the Carman 2020 trial.

Source of Variation	DF[a]	FHB Index		Incidence		Severity		FDK		DON	
		MS[b]	P[c]	MS	P	MS	P	MS	P	MS	P
Main treatment	5	19055.00	<.0001	37409.00	<.0001	20725.00	<.0001	458.96	<.0001	1348.39	<.0001
Cultivar	4	5396.73	<.0001	3284.42	<.0001	6121.01	<.0001	173.23	<.0001	366.83	<.0001
Main Treatment*Cultivar	20	1114.73	<.0001	541.66	<.0001	1009.45	<.0001	36.70	<.0001	82.54	<.0001
Rep	3	123.71	0.1436	2.96	0.8907	185.59	0.1158	11.70	0.1176	3.08	0.6046
Rep* Main treatment	15	59.08	0.1515	14.34	0.6587	79.82	0.0671	5.06	0.7361	4.86	0.5006
Error	70	40.90		17.62		46.47		6.84		5.03	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.21. Analysis of variance for plant density, spike density, height, anther retention, and protein content in the Winnipeg 2020 trial.

Source of Variation	DF[a]	Plant density		Spike density		Height		Anther retention		Protein	
		MS[b]	P[c]	MS	P	MS	P	MS	P	MS	P
Main treatment	5	1138	0.9605	13271.00	0.0987	62.75	0.0221	25.26	0.8176	1.53	0.1408
Cultivar	4	8085	0.0042	61643.00	<.0001	5016.95	<.0001	6367.01	<.0001	22.63	<.0001
Main Treatment*Cultivar	20	1509	0.7193	1837.28	0.6165	15.11	<.0001	53.86	0.0014	0.65	0.0102
Rep	3	21824	0.0359	10776.00	0.1817	65.06	0.0324	297.54	0.0124	1.53	0.1635
Rep* Main treatment	15	5925	0.0008	5840.65	0.0021	17.06	<.0001	58.38	0.0015	0.78	0.0042
Error	68	1918		2097.92		4.49		20.23		0.30	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.22. Analysis of variance for test weight, thousand kernel weight, and yield in the Winnipeg 2020 trial.

Source of Variation	DF[a]	Test weight		Thousand kernel weight		Yield	
		MS[b]	P[c]	MS	P	MS	P
Main treatment	5	86.94	<.0001	424.88	<.0001	20055399	<.0001
Cultivar	4	55.32	<.0001	33.54	<.0001	515443	0.0031
Main Treatment*Cultivar	20	9.86	<.0001	34.54	<.0001	1180343	<.0001
Rep	3	10.41	0.0469	18.97	0.116	3132211	0.0038
Rep* Main treatment	15	3.10	0.0012	8.17	<.0001	454549	<.0001
Error	68	1.05		1.89		116416	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.23. Analysis of variance for Fusarium head blight (FHB) index, severity, incidence, Fusarium damaged kernel (FDK), and deoxynivalenol (DON) in the Winnipeg 2020 trial.

Source of Variation	DF[a]	FHB Index		Incidence		Severity		FDK		DON	
		MS[b]	P[c]	MS	P	MS	P	MS	P	MS	P
Main treatment	5	7010.65	<.0001	16470.00	<.0001	11978.00	<.0001	201.22	<.0001	508.82	<.0001
Cultivar	4	2222.66	<.0001	4899.83	<.0001	3552.85	<.0001	87.28	<.0001	233.02	<.0001
Main Treatment*Cultivar	20	508.34	<.0001	1060.73	<.0001	703.32	<.0001	17.60	<.0001	49.29	<.0001
Rep	3	332.62	0.0721	385.90	0.128	111.93	0.1697	25.63	0.0557	61.95	0.0636
Rep* Main treatment	15	116.65	<.0001	174.20	<.0001	58.40	0.0003	8.13	<.0001	20.67	0.0002
Error	68	24.77		35.67		17.30		2.02		5.83	

[a] DF=Degrees of Freedom, [b] MS=Mean squares, and [c] P=Probability.

Table A.24. Least square means for height and protein content for different main treatments tested across cultivars from four test environments (Carman and Winnipeg for 2019 and 2020).

Main Treatment		Height (cm)				Protein (%)			
		C19[a]	W19[b]	C20[c]	W20[d]	C19	W19	C20	W20
Ethrel™	**FHB inoculation**	64.0 C	71.7	82.8 AB	82.8 AB	18.2	15.8	17.6 A	16.8
Ethrel™	**no FHB inoculation**	64.7 BC	74.1	80.7 B	81.9 B	17.9	15.7	16.8 B	16.1
Manipulator™	**FHB inoculation**	70.8 A	74.3	82.8 AB	82.7 AB	17.8	15.8	17.7 A	16.3
Manipulator™	**no FHB inoculation**	68.5 ABC	77.1	83.7 AB	84.4 AB	17.8	16.1	16.8 B	16.1
Control	**FHB inoculation**	71.9 A	77.1	86.1 A	84.6 AB	18.0	15.9	17.8 A	16.3
Control	**no FHB inoculation**	70.2 AB	78.8	86.4 A	86.9 A	17.8	15.5	16.7 B	16.0
Experimental mean		68.4	75.5	83.8	83.9	17.9	15.8	17.2	16.3

Means followed by same letter are not significantly different at p=0.05 based on the Tukey means comparison test.

[a]Carman 2019 trial, [b]Winnipeg 2019 trial, [c]Carman 2020 trial, and [d]Winnipeg 2020 trial.

Table A.25. Least square means for spike density, height, anther retention and protein for cultivars tested across different main treatment from four test environments (Carman and Winnipeg for 2019 and 2020).

Cultivar	Spike density (spikes/m2)				Height (cm)				Anther retention (%)				Protein (%)			
	C19[a]	W19[b]	C20[c]	W20[d]	C19	W19	C20	W20	C19	W19	C20	W20	C19	W19	C20	W20
AAC Brandon	393 AB	472	553 AB	541 AB	65.5 B	67.6 D	76.7 D	75.5 D	45.0 A	49.3 B	64.1 A	59.9 A	18.7 A	17.3 A	18.5 A	17.4 A
AAC Cameron	375 BC	432	543 B	514 B	75.9 A	85.3 B	89.9 B	95.0 B	17.9 C	35.6 C	34.6 C	47.4B	18.4 B	16.1 B	17.9 B	16.6 B
AAC Penhold	352 C	413	493 C	427 C	60.7 C	61.2 E	73.6 E	65.1 E	16.7 C	28.2 D	34.6 C	45.2 B	18.1 B	16.1 B	17.7 B	16.6 B
AAC Tenacious	424 A	470	518 BC	565 A	73.7 A	90.2 A	98.6 A	102.7 A	32.6 B	17.4 E	16.1 D	14.9 C	16.7 D	14.2 D	15.4 D	14.7 D
Prosper	373 BC	454	592 A	543 AB	65.9 B	73.3 C	80.8 C	81.1 C	17.4 C	57.7 A	39.4 B	48.6 B	17.8 C	15.3 C	16.8 C	16.1 C
Mean[e]	383	448	540	518	68.3	75.5	83.9	83.9	25.9	37.6	37.8	43.2	17.9	15.8	17.2	16.3

Means followed by same letter are not significantly different at p=0.05 based on the Tukey means comparison test.

[a]Carman 2019 trial, [b]Winnipeg 2019 trial, [c]Carman 2020 trial, [d]Winnipeg 2020 trial, and [e]Experimental mean.

Table A.26. Least square means for test weight, thousand kernel weight, yield, and lodging rating for different main treatments tested across cultivars from four test environments (Carman and Winnipeg for 2019 and 2020).

Main Treatment		Test weight (Kg/hL)				TKW (g)				Yield (Kg/ha)				Lodging rating
		C19[a]	W19[b]	C20[c]	W20[d]	C19	W19	C20	W20	C19	W19	C20	W20	C20
Ethrel™	**FHB inoculation**	79.1 B	76.8 B	75.2 B	78.0 B	32.7 B	29.6 C	28.0 B	28.4 B	2663.4 B	3330.3 B	3405.9 B	3563.3 B	0.040 B
Ethrel™	**no FHB inoculation**	81.8 A	80.4 A	79.7 A	82.1 A	36.3 A	33.9 AB	35.5 A	36.8 A	3516.4 A	4519.7 A	5569.0 A	5047.7 A	0.119 B
Manipulator™	**FHB inoculation**	78.3 C	77.1 B	73.8 B	77.6 B	32.4 B	31.2 BC	28.2 B	29.3 B	2646.6 B	3501.8 B	3135.3 B	3245.8 B	0.518 B
Manipulator™	**no FHB inoculation**	81.3 A	80.3 A	79.1 A	81.5 A	36.6 A	34.8 A	36.8 A	37.8 A	3724.0 A	4921.3 A	6101.2 A	5313.6 A	0.545 B
Control	**FHB inoculation**	78.6 BC	76.7 B	74.4 B	78.0 B	32.8 B	30.7 C	29.0 B	30.6 B	2795.9 B	3461.2 B	3287.9 B	3104.0 B	1.298 A
Control	**no FHB inoculation**	81.3 A	80.6 A	79.3 A	81.7 A	36.5 A	35.7 A	37.1 A	39.1 A	3587.5 A	4584.6 A	5780.1 A	5091.4 A	0.595 B
Experimental mean		80.1	78.7	76.9	79.8	34.6	32.7	32.4	33.7	3155.6	4053.2	4546.6	4227.6	0.519

Means followed by same letter are not significantly different at p=0.05 based on the Tukey means comparison test.

[a]Carman 2019 trial, [b]Winnipeg 2019 trial, [c]Carman 2020 trial, and [d]Winnipeg 2020 trial.

Table A.27. Least square means for test weight, thousand kernel weight, and yield for cultivars tested across different main treatments from four test environments (Carman and Winnipeg for 2019 and 2020).

Cultivar	Test weight (Kg/hL)				TKW (g)				Yield (Kg/ha)				Lodging rating
	C19[a]	W19[b]	C20[c]	W20[d]	C19	W19	C20	W20	C19	W19	C20	W20	C20
AAC Brandon	80.7 A	79.6 B	77.5 B	80.2 B	33.1 C	32.1 AB	31.0 C	31.9 C	3457A	4045	3955 D	4136 B	0.230 BC
AAC Cameron	79.9 B	78.6 C	76.3 C	78.9 C	35.4 AB	34.0 A	32.5 B	33.7 B	3222 A	4153	4453 BC	4315 AB	1.456 A
AAC Penhold	79.5 B	77.5 D	74.7 D	78.9 C	34.8 B	32.9 A	31.5 C	33.7 B	2974 B	3915	4252 CD	4073 B	0.000 C
AAC Tenacious	80.6 A	80.6 A	79.6 A	82.4 A	33.6 C	33.5 A	33.9 A	35.4 A	2835 B	4096	5339 A	4167 AB	0.183 BC
Prosper	79.6 B	77.0 D	76.5 C	78.6 C	36.0 A	30.8 B	33.2 AB	33.6 B	3291 A	4057	4734 B	4446 A	0.725 B
Experimental mean	80.1	78.7	76.9	79.8	34.6	32.7	32.4	33.7	3156	4053	4547	4227	0.519

Means followed by same letter are not significantly different at p=0.05 based on the Tukey means comparison test.

[a]Carman 2019 trial, [b]Winnipeg 2019 trial, [c]Carman 2020 trial, and [d]Winnipeg 2020 trial.

Table A.28. Least square means for Fusarium head blight (FHB) index, disease severity, and disease incidence for the main treatments tested across cultivars in 2019 and 2020 data from Carman and Winnipeg.

Treatment		FHB Index (%)				Disease Incidence (%)				Disease Severity (%)			
		C19[a]	W19[b]	C20[c]	W20[d]	C19	W19	C20	W20	C19	W19	C20	W20
Ethrel™	**FHB inoculation**	20.9 A	40.0 A	50.4 B	27.0 B	74.7 A	57.4 A	80.4 A	45.2 A	25.9 A	65.0 A	56.7 B	43.7 A
Ethrel™	**no FHB inoculation**	0 B	0.8 B	0.3 C	0 C	0.08 B	1.7 B	1.3 B	0 B	0 B	12.2 B	3.8 C	0.6 B
Manipulator™	**FHB inoculation**	29.6 A	43.9 A	58.7 A	39.3 A	76.9 A	60.6 A	83.3 A	57.4 A	35.5 A	64.2 A	64.5 AB	50.0 A
Manipulator™	**no FHB inoculation**	0.005 B	1.6 B	0.3 C	0 C	0.1 B	2.6 B	2.8 B	0.06 B	0.1 B	19.2 B	3.9 C	0.1 B
Control	**FHB inoculation**	26.1 A	43.5 A	61.7 A	36.4 AB	79.5 A	60.1 A	83.1 A	57.0 A	31.4 A	64.3 A	66.7 A	43.92 A
Control	**no FHB inoculation**	0.03 B	1.4 B	0.2 C	0 C	0.4 B	2.5 B	2.5 B	0 B	0.5 B	16.3 B	2.3 C	0.03 B
Experimental mean		12.8	21.9	28.6	17.1	38.6	30.8	42.2	26.6	15.6	40.2	33.0	23.1

Means followed by same letter are not significantly different at p=0.05 based on the Tukey means comparison test.

[a]Carman 2019 trial, [b]Winnipeg 2019 trial, [c]Carman 2020 trial, and [d]Winnipeg 2020 trial.

Table A.29. Least square means for Fusarium damaged kernel (FDK) and deoxynivalenol (DON) content for the main treatment tested across cultivars in 2019 and 2020 data from Carman and Winnipeg.

Main Treatment		FDK (%)				DON (ppm)			
		C19[a]	W19[b]	C20[c]	W20[d]	C19	W19	C20	W20
Ethrel™	**FHB inoculation**	4.53 A	3.26 A	7.59 B	6.24 A	7.90 A	4.07 A	14.23 B	9.34 A
Ethrel™	**no FHB inoculation**	0.092 B	0.20 B	0.12 C	0.012 B	0.38 B	0.08 B	0.19 C	0.037 B
Manipulator™	**FHB inoculation**	3.98 A	3.24 A	10.32 A	5.98 A	8.07 A	4.11 A	17.03 A	9.81 A
Manipulator™	**no FHB inoculation**	0.10 B	0.32 B	0.14 C	0.046 B	0.025 B	0.09 B	0.22 C	0.072 B
Control	**FHB inoculation**	4.54 A	3.07 A	8.65 AB	5.78 A	9.38 A	4.53 A	14.64 B	9.51 A
Control	**no FHB inoculation**	0.09 B	0.28 B	0.14 C	0.035 B	0.10 B	0.11 B	0.24 C	0.061 B
Experimental mean		2.22	1.73	4.49	3.02	4.31	2.17	7.76	4.81

Means followed by same letter are not significantly different at p=0.05 based on the Tukey means comparison test.

[a]Carman 2019 trial, [b]Winnipeg 2019 trial, [c]Carman 2020 trial, and [d]Winnipeg 2020 trial.

Table A.30. Least square means for Fusarium head blight (FHB) index, disease incidence, disease severity for the five spring wheat cultivars (AAC Brandon, AAC Cameron, AAC Penhold, AAC Tenacious, and Prosper) tested across main effect treatments from four test environments (Carman and Winnipeg for 2019 and 2020).

Cultivar	Resistance level[a]	FHB Index (%)				Disease Incidence (%)				Disease Severity (%)			
		C19[b]	W19[c]	C20[d]	W20[e]	C19	W19	C20	W20	C19	W19	C20	W20
AAC Brandon	MR[f]	15.7 A	15.6 C	31.7 B	17.4 B	43.0 A	40.2 A	49.3 A	40.2 A	18.7 A	21.3 B	35.2 B	18.0 C
AAC Cameron	I[g]	12.6 A	26.6 B	34.2 AB	19.2 B	39.7 A	33.1 B	43.9 B	25.5 C	15.4 A	49.8 A	41.4 A	31.2 AB
AAC Penhold	MR	15.6 A	29.1 B	37.2 A	24.7 A	42.3 A	34.6 B	48.1 A	30.4 BC	17.9 A	50.0 A	42.1 A	33.7 A
AAC Tenacious	R[h]	4.4 B	1.9 D	2.1 C	0.2 C	26.3 B	2.8 C	21.5 C	2.0 D	7.6 B	19.3 B	4.8 C	3.3 D
Prosper	I	15.3 A	36.2 A	37.9 A	24.1 A	41.8 A	43.3 A	48.2 A	34.9 B	18.1 A	60.4 A	41.4 A	29.2 B
Experimental mean		12.8	21.9	28.6	17.1	38.6	30.8	42.2	26.6	15.6	40.2	33.0	23.1

Means followed by same letter are not significantly different at p=0.05 based on the Tukey means comparison test.

[a]Seed Manitoba 2018, [b]Carman 2019 trial, [c]Winnipeg 2019 trial, [d]Carman 2020 trial, [e]Winnipeg 2020 trial, [f]MR=Moderate Resistance, [g]I=Intermediate Resistance, and [h]R=Resistant.

Table A.31. Least square means for Fusarium damaged kernel and deoxynivalenol levels for the five spring wheat cultivars (AAC Brandon, AAC Cameron, AAC Penhold, AAC Tenacious, and Prosper) tested across main effect treatments from four test environments (Carman and Winnipeg for 2019 and 2020).

Cultivar	Resistance level[a]	Fusarium damaged kernel (%)				deoxynivalenol levels (ppm)			
		C19[b]	W19[c]	C20[d]	W20[e]	C19	W19	C20	W20
AAC Brandon	MR[f]	1.88 C	1.21 B	4.94 B	2.59 B	4.04 BC	1.59 C	9.38 AB	5.55 B
AAC Cameron	I[g]	2.01 C	1.83 B	4.14 B	2.82 B	3.93 C	2.50 B	8.26 B	4.96 B
AAC Penhold	MR	3.73 A	2.61 A	7.71 A	5.00 A	5.50 AB	2.51 B	9.30 AB	4.12 B
AAC Tenacious	R[h]	0.63D	0.18 C	0.32 C	0.039 C	1.83 D	0.05 D	0.96 C	0.25 C
Prosper	I	2.85 B	2.80 A	5.37 B	4.63 A	6.24 A	4.16 A	10.90 A	9.14 A
Experimental mean		2.22	1.73	4.49	3.02	4.31	2.17	7.76	4.81

Means followed by same letter are not significantly different at p=0.05 based on the Tukey means comparison test.

[a]Seed Manitoba 2018, [b]Carman 2019 trial, [c]Winnipeg 2019 trial, [d]Carman 2020 trial, [e]Winnipeg 2020 trial, [f]MR=Moderate Resistance, [g]I=Intermediate Resistance, and [h]R=Resistant

Table A.32. Pearson's correlation coefficients between fungal DNA accumulation and plant height from PGR treatments and five spring wheat cultivars (n=135) in Trial 1 and Trial 2.

	Fungal DNA (ng)	
	Trial 1	**Trial 2**
Height (cm)	**-0.21***	-0.02 ns

Note: Bold numbers show there is correlation among the parameters. * Significant at p<0.05