Mitigation of Fusarium Head Blight in Wheat Caused by *Fusarium graminearum* Through the Use of Host Resistance Genes and Biological Controls

By

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Table of Contents

Acknowledgements i

Table of Contents ii

List of Tables v

List of Figures ix

Table of Abbreviations xii

Abstract xiii

Chapter 1.0 General Introduction 1

Chapter 2.0 Literature Review 6

2.1 Wheat and its domestication 6

2.2 Fusarium head blight and its economic importance 8

2.3 Fusarium head blight disease cycle 10

2.4 Environmental conditions needed for FHB infection 11

2.5 Fusarium head blight symptoms 12

2.6 Fusarium head blight mycotoxins 13

2.6.1 Trichothecenes: DON and its derivatives 13

2.6.2 Zearalenone and the Type A Trichothecenes 16

2.7 FHB control methods 17

2.7.1 Cultural control methods 17

2.7.2 Chemical controls 19

2.7.3 Biological controls 21

2.7.3.1 Antagonistic fungi 21

2.7.3.2 Antagonistic bacteria 23

2.7.3.3 Antagonistic earthworm species 24

2.7.4 Breeding for resistance 24

2.7.4.1 Types of FHB resistance 25

2.7.4.2 Wheat genetics 26

2.7.4.3 QTL mapping of FHB resistance 26

2.7.4.4 Sources of FHB resistance 28

2.7.4.5 Resistance and pathogen related proteins 31

2.7.4.6 Plant dwarfing genes and FHB resistance 31

Chapter 3.0 Mapping for resistant genes in a Flourish/Emerson double haploid winter wheat population 34

3.1 Abstract 34
3.2 Introduction 35
3.3 Materials and Methods 37
3.3.1 Plant material 37
3.3.2 Field trial design 38
3.3.3 *F. graminearum* inoculum 39
3.3.4 *F. graminearum* inoculation 40
3.3.5 Phenotypic disease and physical characteristic evaluation in field 40
3.3.6 Phenotypic evaluation post harvest 41
3.3.7 Plant tissue growth and collection in preparation for genotyping 42
3.3.8 Creation of genetic map using 90K SNP genotyping results 43
3.3.9 QTL mapping 44
3.3.10 Statistical analysis of phenotypic data 45
3.4.1 Field Results 46
3.4.1.1 Assessment of *Fusarium* head blight field symptoms; disease incidence, severity and FHB index 46
3.4.1.2 Assessment of physical qualities: height and thousand kernel weights 46
3.4.1.3 Presence of *Fusarium* in grain; FDK and DON analysis 47
3.4.2 QTL analysis 52
3.4 Discussion 60
Chapter 4.0 Evaluating the Effectiveness of the biological control agent, DONguard® (active ingredient *Clonostachys rosea*, ACM941) in preventing *Fusarium* Head Blight and Reducing DON mycotoxin 66
4.1 Abstract 66
4.2 Introduction 67
4.3 Materials and Methods 69
4.3.1 Treatments and their application 69
4.3.2 Disease Evaluation 75
4.3.3 Statistical analysis 77
4.3.4 Weather temperatures 78
4.4.1 Rate of DONguard® (*C. rosea* ACM941) application results 78
4.4 Results 78
4.4.1.1 ANOVA 78
4.4.1.2 Assessment of FHB disease incidence, severity, FHB Index, FDK and DON 80
4.4.1.3 Assessment of sample weights (test weights and thousand kernel weights) 85

4.4.1.4 Efficacy of DONguard® compared to fungicide and *F. graminearum* control 88
4.4.2 Time of DONguard® (*C. rosea* ACM941) application results 92
4.4.2.1 ANOVA 92
4.4.2.2 Assessment of disease severity, disease incidence, FHB Index, FDK and DON 94
4.4.2.3 Efficacy of DONguard® compared to fungicide and *F. graminearum* control 98
4.4.3 Weather results 101
4.5 Discussion 103
Chapter 5.0 General Discussion and Conclusion 110
References 114
Appendix 126

List of Tables

Table 3.1 Combined analysis of variance results for disease incidence, disease severity and FHB index from field test data from three test environments; Carman (2015), Carman (2016) and Winnipeg (2016)……**48**

Table 3.2 Flourish/Emerson double haploid (DH) population (n=178) and parental mean values for disease incidence (DI), disease severity (DS), FHB index, height, thousand kernel weight (TKW), *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) in two test locations; Carman (2016) and Winnipeg (2016)……**49**

Table 3.3 Pearson's correlation coefficients between FHB disease incidence, disease severity, FHB index, plant height, thousand kernel weight (TKW), *Fusarium* damage kernels (FDK) and deoxynivalenol (DON) values from the Flourish/Emerson double haploid population (n=178) ……**50**

Table 3.4 Combined analysis of variance results for height and thousand kernel weight from field test data from three test environments: Carman (2015), Carman (2016) and Winnipeg (2016)……**50**

Table 3.5 Combined analysis of variance results for *Fusarium* damaged kernels and deoxynivalenol from field test data from three test environments: Carman (2015), Carman (2016) and Winnipeg (2016)……**50**

Table 3.6 Summary of QTLs associated with FHB symptom reduction in the Flourish/Emerson double haploid population. Data collected from three environments; Carman, MB (2015), Carman, MB (2016) and Winnipeg, MB (2016)……**54**

Table 3.7 Summary of QTLs associated with height and thousand kernel weights in the Flourish/Emerson double haploid population. Data collected from three environments; Carman, MB (2015), Carman, MB (2016) and Winnipeg, MB (2016)……**56**

Table 4.1: List of the nine main plot treatments used in the rate experiment. The treatments include the four main classes of treatments and the six sub-treatments within the DONguard® grouping……**70**

Table 4.2: List of the eight treatments used in the time experiment. The treatments include the four main classes of treatments and the six sub-treatments within the DONguard® grouping ……**72**

Table 4.3. Analysis of variance for *Fusarium* head blight index (FHB), *Fusarium* damaged kernel (FDK), deoxynivalenol (DON), test weights and thousand kernel weights (TKW) in the 2015 Rate of DONguard® Application Test……**79**

Table 4.4 Analysis of variance for *Fusarium* head blight index (FHB), *Fusarium* damaged kernel (FDK), deoxynivalenol (DON), test weights and thousand kernel weights (TKW) in 2016 Rate of DONguard® Application Test……**79**

Table 4.5 LS means for disease incidence (%), disease severity (%), FHB index (%), *Fusarium d*amaged kernels (%) and deoxynivalenol levels (ppm) for the different treatment types tested in 2015 and 2016 site year data from the University of Manitoba field location for the DONguard ® Rate of Application Test……**84**

Table 4.6 LS means for disease incidence (%), disease severity (%), FHB index (%), *Fusarium* damaged kernels (%) and deoxynivalenol levels (ppm) for the three different spring wheat genotypes (AC Cora, CDC Teal and FHB 37) tested in 2015 and 2016 site year data from the University of Manitoba field location for the DONguard ® Rate of Application Test……...**84**

Table 4.7: LS means for test weights (Kg/hL) and thousand kernel weights (g) for the different treatment types tested in 2015 and 2016 site year data from the University of Manitoba field location for the DONguard ® Rate of Application Test……..……**87**

Table 4.8. LSD means for test weights (Kg/hL) and thousand kernel weights (g) for the three different genotypes tested (AC Cora, CDC Teal and FHB 37) in 2015 and 2016 site year data from the University of Manitoba field location for the DONguard ® Rate of Application Test.……**87**

Table 4.9. 2015 and 2016 efficacy percentage data of both the DONguard® (*C. rosea*, ACM941) and Caramba® (metconazole) treatments for disease incidence, disease severity, FHB index, Fusarium damaged kernels and Deoxynivalenol compared to the *Fusarium graminearum* inoculated control groups for the Rate of Application Test…. ……**91**

Table 4.10 Analysis of variance for disease incidence, disease severity, *Fusarium* head blight index (FHB), *Fusarium* damaged kernel (FDK), and deoxynivalenol (DON) in 2015 for the Time of Application Test ……..**93**

Table 4.11 Analysis of variance for disease incidence, disease severity, *Fusarium* head blight index (FHB), *Fusarium* damaged kernel (FDK) and deoxynivalenol (DON) in 2016 for the Time of Application Test ……...**93**

Table 4.12: Tukey-Kramer adjusted means for disease incidence, disease severity, FHB index, *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) for the different treatment types tested in 2015 and 2016 site year data for the Time of Application Test……..**96**

Table 4.13 Tukey-Kramer adjusted treatment means from 2015 and 2016 for the four different spring wheat genotypes (AC Cora, AC Morse, CDC Teal and FHB 37) tested at the University of Manitoba field location for the DONguard® Time of Application Test ……..**97**

Table 4.14: Efficacy percentage data of both the DONguard® (*C. rosea*, ACM941) and Caramba® (metconazole) treatments for the disease incidence, disease severity, FHB index, *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) compared to the *Fusarium graminearum* inoculated control groups for 2015 and 2016 Time of Application Test……..**99**

Table 4.15.: Efficacy percentage data of both the DONguard® (*C. rosea*, ACM941) and Caramba® (metconazole) treatments for the FHB index, Fusarium damaged kernels (FDK) and deoxynivalenol (DON) compared to the *Fusarium graminearum* inoculated control groups using 2015 and 2016 combined data for the DONguard ® Time of Application Test……..**100**

Table A-1 Mean values for disease incidence (DI), disease severity (DS), FHB index, height, thousand kernel weight (TKW), *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) content for the check cultivars used in the three environments; Carman (2015), Carman (2016) and Winnipeg (2016).....**126**

Table A-2 Pearson's correlation coefficients between FHB disease incidence, disease severity, FHB index, plant height, thousand kernel weight (TKW), *Fusarium* damage kernels (FDK) and deoxynivalenol (DON) values from Emerson/Flourish double haploid population (n=159) at the 2015 Carman site.....**129**

Table A-3 Pearson's correlation coefficients between FHB disease incidence, disease severity, FHB index, plant height, thousand kernel weight (TKW), *Fusarium* damage kernels (FDK) and deoxynivalenol (DON) values from Emerson/Flourish double haploid population (n=178) for combined Carman (2016) and Winnipeg (2016) data **129**

Table A-4 Chi-square results for markers in QTL regions associated with plant height on chromosome 4D in the Flourish/Emerson DH population.....**132**

Table A-5 Results of the permutation test (1000) conducted on the DH Flourish/Emerson population (Alpha=0.05) to determine if a QTL was real.....**133**

Table A-6: LS means for all variables tested for the different treatment types for each of the three spring wheat genotypes tested (AC Cora, CDC Teal and FHB 37) in 2015 from the University of Manitoba field location for the Rate of Application Test **138**

Table A-7 LSD means for all variables tested for the different treatment types tested for each of the three spring wheat genotypes tested (AC Cora, CDC Teal and FHB 37) at three different rates of application in 2016 from the University of Manitoba Fort Gary Campus field location for the Rate of Application Test.....**139**

Table A-8 2015 Efficacy values for the different Treatment*Genotype interactions between the 3 Spring Wheat genotypes (AC Cora, CDC Teal and FHB 37) ranging in resistance to FHB and the 8 different treatments given within the DONguard® Rate of Application Test **140**

Table A-9 2016 Efficacy values for the different Treatment*Genotype interactions between the 3 Spring Wheat genotypes (AC Cora, CDC Teal and FHB 37) ranging in resistance to FHB and the 8 different treatments given within the DONguard® Rate of Application Test.....**141**

Table A-10 2015 Tukey-Kramer adjusted treatment means for *Fusarium* head blight index (FHB Index), *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) for the different treatment types within each genotype tested from the University of Manitoba Fort Gary Campus field location for the DonGuard® Time of Application Test.....**142**

Table A-11 Tukey-Kramer adjusted treatment means for FHB index, *Fusarium* damaged kernels and deoxynivalenol for the different treatment types within each subgroup tested in 2016 site year data from the University of Manitoba Fort Gary Campus field location for the DONguard® Time of Application Test.....**143**

Table A-12 Minimum, maximum and average temperature of the University of Manitoba test site location over the duration of time that *C. rosea* was being applied to plants in the DONguard® Rate and Time Tests…… **144**

Table A-13 Composition of Spezieller Nährstoffarmer Agar (SNA) ……..……..…**145**

Table A-14 Composition of Carboxymethyl Cellulose (CMC) Media Culture ……..……..……**145**

List of Figures

Figure 3.1 Genetic background for development of the double haploid population crossed between winter wheat cultivars Flourish and Emerson ……**37**

Figure 3.2 Frequency distributions for FHB field symptoms; disease incidence, disease severity, FHB index (%), wheat physical characteristics; height (cm) and thousand kernel weight (g), and kernel disease; deoxynivalenol (DON) levels (ppm), *Fusarium* damage kernels (%) for a population of 178 double haploid Flourish/Emerson lines for the Carman 2016 and Winnipeg 2016 test locations ……**51**

Figure 3.3 Genetic linkage map for disease incidence QTL located on chromosome 7A for 161 DH lines developed from the Flourish/Emerson cross tested at three environments; Carman (2015), Carman (2016) and Winnipeg (2016). Marker distance is displayed on the right side of the chromosome and the names of markers present are on the right……**57**

Figure 3.4 Genetic linkage map for disease incidence QTL located on chromosome 1B in a DH population from the cross Flourish/Emerson (161 lines). Genetic linkage map for disease incidence, disease severity and kernel weight QTLs present on the Flourish/Emerson population's chromosome 2B tested at the three environments; Carman (2015), Carman (2016) and Winnipeg (2016). Marker distance is displayed on the left side of the chromosome and the names of markers present are on the right……**58**

Figure 3.5 Genetic linkage map for DON, FDK, disease severity, FHB index, height and TWK QTL located on chromosome 4D in 161DH lines developed from the cross Flourish/Emerson. Genetic linkage map for DON and FDK QTLs present on the Flourish/Emerson population's chromosome 6D tested at the three environments; Carman (2015), Carman (2016) and Winnipeg (2016), as well as QTL for pooled results. Marker distance is displayed on the left side of the chromosome and the names of markers present are on the right……**59**

Figure 4.1 Minimum temperatures recorded at the University of Manitoba Field Test Site during the *C. rosea* application (July 7 -11, 2015 and July 8-18, 2016) and *C. rosea* establishment. The dark grey area represents the time when *C. rosea* applications were given in 2015 and the lighter grey patterned area represents when *C. rosea* was applied in 2016……**102**

Figure 4.2 Average temperatures recorded at the University of Manitoba Field Test Site during the *C. rosea* application and establishment. The dark grey area represents the time when *C. rosea* applications were given in 2015 and the lighter grey patterned area represents when *C. rosea* was applied in 2016……**102**

Figure A-1 Frequency distributions for FHB field symptoms; disease incidence, disease severity, FHB index (%), wheat physical characteristics; height (cm) and thousand kernel weight (g), and kernel disease; *Fusarium* damage kernels (%), deoxynivalenol (DON) levels (ppm) for a population of 178 double haploid Flourish(F)/Emerson(E) lines for the Carman 2016 test location……..**127**

Figure A-2 Frequency distributions for FHB field symptoms; disease incidence, disease severity, FHB index (%), wheat physical characteristics; height (cm) and thousand kernel weight (g), and kernel disease; *Fusarium* damage kernels (%), deoxynivalenol (DON) levels (ppm) for a population of 178 double haploid Flourish(F)/Emerson(E) lines for the Winnipeg 2016 test location.……..**128**

Figure A-3 Correlation graph depicting the relationships between the following variables; FHB disease incidence (%), FHB disease severity (%), FHB index (%), Height (cm), Thousand Kernel Weight (TKW) (g), *Fusarium* damage kernels (FDK) and deoxynivalenol (DON). Points on the graph represent the 178 different lines in the Flourish/Emerson population. Colors of the points indicate which test location the data was taken from; Carman 2015 (blue), Carman 2016 (red) and Winnipeg 2016 (green)……**130**

Figure A-4 Correlation graph depicting the relationships between the FHB index percentage, percentage of *Fusarium* damaged kernels and the amount of deoxynivalenol (ppm) in the grain. Points on the graph represent the different lines in the Flourish/Emerson population. Colors of the points indicate which test location the data was taken from; Carman 2015 (blue), Carman 2016 (red) and Winnipeg 2016 (green) ……**131**

Figure A-5 Genetic linkage map for height QTL (checkered) located on chromosome 2D in DH lines developed from the cross Flourish/Emerson identified in Carman (2016). Marker distance is displayed on the right side of the chromosome and the names of markers present are on the right……..**134**

Figure A-6 Genetic linkage map for height (checkered) and thousand kernel weight (TKW) (dark red) QTLs present on chromosome 6A for Flourish/Emerson Population grown at three different environments; Carman (2015), Carman (2015) and Winnipeg (2016) Marker distance is displayed on the right side of the chromosome and the name of markers present are on the right.……**135**

Figure A-7 Allelic discrimination plot for Rht-B1s allele in the Flourish/Emerson population. The population included 89 lines from the DH population, the parents (Flourish and Emerson), grandparents (CDC Osprey, McClintock and CDC Falcon) and great grandparent (CDC Kestrel). Allele 1 corresponds to the line having the wildtype (*Rht-B1a*) version of the allele while Allele 2 corresponds with the dwarfing (*Rht-B1b*) allele. ……**136**

Figure A-8 Allelic discrimination plot for Rht-D1 Flourish/Emerson population. The population included 89 lines from the DH population, the parents (Flourish and Emerson), grandparents (CDC Osprey, McClintock and CDC Falcon) and great grandparent (CDC Kestrel). Allele 1 corresponds to the line having the wildtype (*Rht-D1a*) allele while Allele 2 corresponds with the dwarfing (*Rht-D1b*) allele.....**137**

Table of Abbreviations

Abbreviation	Description
3-ADON	3-acetyl-deoxynivalenol
15-ADON	15-acetyl-deoxynivalenol
ANOVA	Analysis of Variance
BLAST	Basic Local Alignment Search Tool
cM	Centimorgan
CMC	Carboxymethyl Cellulose
Crm	Carman
CTAB	Cetyl trimethalammonium bromide
DH	Double haploid
DI	Disease Incidence
DNA	Deoxyribonucleic acid
DMI	Demethylation Inhibitors
DON	Deoxynivalenol
DS	Disease Severity
ELISA	Enzyme Linked Immunosorbent Assay
FDK	*Fusarium* damaged kernels
FGSC	*Fusarium graminearum* species complex
FHB	*Fusarium* head blight
Gb	Gigabase
I	Intermediate (Resistance)
LOD	Logarithmic of odds
MAS	Marker Assisted Selection
Mb	Megabase
NIV	Nivalenol
NRC	National Research Council of Canada
PDA	Potato dextrose agar
ppm	Parts per million
PR protein	Pathogen related protein
Qol	Quinone Outside Inhibitors
QTL	Quantitative trait loci
R	Resistant
Rht	Reduced Height
RIL	Recombinant Inbred Lines
S	Susceptible
SNA	Spezieller Nährstoffarmer agar
SNP	Single nucleotide polymorphism
SSR	Single sequence repeats
TKW	Thousand Kernel Weight
USD	United States Dollar
Wpg	Winnipeg
ZEA	Zearalenone

Abstract

Nowakowski, Gabrielle, MSc., The University of Manitoba, 2018. Mitigation of Fuasarium Head Blight in Wheat Caused by *Fusarium graminearum* Through the use of Host Resistance Genes and Biological Controls. Professor; Dr. Anita Brûlé-Babel.

The fungal pathogen, *Fusarium graminearum*, is a primary causal agent of the wheat (*Triticum aestivum* L.) disease *Fusarium* head blight in North America. Heavy infection from this pathogen leads to economic losses due to a decrease in the quality and quantity of grain. Infection by *F. graminearum* also leads to accumulation of deoxynivalenol (DON), a mycotoxin that produces harmful side effects to both humans and animals when ingested. Development of resistant wheat cultivars is needed to maintain a sustainable, safe, food supply. In this study, a double haploid population derived from a cross between two winter wheat cultivars, Flourish and Emerson, was used to identify quantitative trait loci (QTL) associated with FHB symptoms and DON accumulation in the grain. So far, there are no fully resistant cultivars of wheat to protect against FHB infection. Therefore, alternative methods need to be considered. A newly developed bio-fungicide containing a fungal antagonist to *F. graminearum*, *Clonostachys rosea*, was studied and compared to the commercially available fungicide, Caramba® (active ingredient, metconazole). These QTL were present on chromosomes 1B, 2B, 4D, 6D and 7A. Results from this study showed that there was some potential in using *C. rosea* in a bio-fungicide treatment plan. However, the fungi's sensitivity to environmental conditions may have contributed to poor and inconsistent control of *F. graminearum* in this study.

Chapter 1.0 General Introduction

Approximately one third of the world's population relies on wheat (*Triticum aestivum* L.) for their daily caloric intake. As a staple crop grown around the world, wheat is used for many food items such as breads, pastas, and baker's confectionaries (Wheat Research Task Group, 2017). Maintaining a stable supply of grain is a priority, but supplies can be compromised by weather, animal activity, or disease. One of the most damaging diseases of wheat is the fungal disease; *Fusarium* head blight (FHB) or scab. This disease has been responsible for worldwide economic losses and poses a health risk to humans and some animals. Historically, FHB was not as prevalent when wide spread conventional tillage practices were utilized across North America until the early 1980's (Dill-Macky 2008). Tillage allowed for any infected plant residue to be buried underground and limited the inoculum production by the pathogen, which limited infection of the crop grown in the following year. A push towards soil conservation in the 1980s led to reduced tillage and no-till practices, which left crop residues on the soil surface, and in turn, led to an increase in FHB infection (Munkvold 2003; Dill-Macky 2008). *Fusarium* spp. also infect a variety of other cereal crops including barley (*Hordeum vulgare L.),* oat (*Avena sativa L.) and* corn (*Zea mays L.*). A combination of increased inoculum reservoirs and large acreages dedicated to cereal grains resulted in FHB epidemics. One large epidemic occurred in Ontario and other Eastern Canadian provinces in 1980 and another occurred in 1993 in Manitoba (Gilbert and Haber 2013). In North America, a variety of fungal pathogens under the *Fusarium* species complex are attributed to the disease. In North America, these include *F. culmorum* and *F. avenaceum*, however, the primary causal agent of FHB in eastern Canada and the eastern

prairies is *F. graminearum* (Schwabe (teleomorph Gibberella zeae (Schwein.) Petch)) (Gilbert and Haber 2013; Aoki *et al.* 2014).

Fusarium infection results in a combination of reduced yield, decreased end-use quality, and presence of mycotoxins in the grain leading to economic losses to producers. *Fusarium* head blight disease symptoms for wheat in the field include premature bleaching of the spike and/or pink colouration on the rachis and glumes. Once harvested, *Fusarium* damaged kernels (FDKs) will often have a pink/salmon like color and the presence of fungal mycelia (Parry *et al.* 1995; Gilbert and Fernando 2004). Infection of the kernel will result in higher nutrient and moisture loss, leading to production of a shrunken kernel with a chalky and hardened appearance. These extremely infected kernels are nicknamed “tombstone kernels” (Parry *et al.* 1995; Goswami and Kistler 2004; Gilbert and Haber 2013). Among the species that cause FHB in North America, *F. graminearum* and *F. culmorum* pose a greater threat because they produce an array of mycotoxins including deoxynivalenol (DON) and its derivatives. Two derivatives of DON are present in Manitoba; 15-acetyl-deoxynivalenol (15ADON) and 3-acetyl-deoxynivalenol (3ADON). Populations of 3ADON producing *F. graminearum* have increased in some regions and are a concern because this chemotype is the more aggressive of the two chemotypes (McMullen *et al.* 2012).

Deoxynivalenol accumulation within plant tissues results in a stunting of growth and a lowered seed germination rate (Rocha *et al.* 2005*)*. The presence of FDKs leads to a reduction of the grade of grain produced, resulting in a lower economic gain for producers. The chemical structure of DON is extremely stable at high temperatures (120-350°C) and can still be present in final products after baking (Döll and Dänicke 2011; Maresca 2013). Consumption of

contaminated wheat products results in vomiting and nausea. Higher doses of DON can result in nerve damage and even death (Rocha *et al.* 2005; Döll and Danicke 2011). Similar symptoms may be observed when DON contaminated grains are fed to some types of livestock. For example, pigs respond to DON in a similar fashion to humans, experience lethargy, reduced fertility and reduced overall growth when fed a *Fusarium* contaminated grain-based diet (Döll and Danicke 2011).

A variety of FHB prevention methods have been developed. These methods include cultural farming practices, use of chemical fungicides, biological control agents, and growing wheat cultivars that are resistant to infection. No one method can completely prevent the infection of *F. graminearum* and integrated prevention strategies are needed.

Fungicide efficacy for FHB control has not been consistent. This has been attributed to a narrow application window for the different products, which need to be applied during the few days that plants are flowering (Wegulo 2012). In addition, public health concerns of fungicide residues remaining on food products has warranted more focus on alternative prevention methods. One alternative to application of fungicides is the use of biological control agents. Application of fungi with antagonistic properties against *F. graminearum* has garnered the most research. *Clonostachys rosea* (Schroers, Samuels, Seifert and Gams [teleomorph, *Bionectria ochroleuca* (Schw.) Schroers and Samuels]) (Schroers *et al.* 1999), a saprophytic fungus is one such antagonist. This fungus works by competing for resources as well as mycoparasitizing *F. graminearum*. It is also well documented to be able to reduce disease incidence in the field as well as decreasing the amount of FDK harvested and decreasing DON levels within grain (Xue A.G. *et al.* 2009; Xue A.G. *et al.* 2014a, Xue A.G. *et al.* 2014b). However, as a living organism it

requires specific environmental conditions to grow and successfully control FHB. If such conditions are not met, it is unable to survive. *C. rosea's* sensitivity to environmental conditions including temperature (Yu and Sutton 1998; L. V. Cota *et al.* 2008), moisture (Yu and Sutton 1998; Luciano V. Cota *et al.* 2008), and ultraviolet light (Morandi *et al.* 2008, Costa *et al.* 2012) reduces its reliability. The variable reliability of this biological agent means it cannot be used as the sole prevention method against *F. graminearum*.

The use of FHB resistant cultivars is one of the most economical and reliable FHB prevention methods. Morphological characteristics such as taller heights, reduced spike number, and reduced spikelet number are often associated with reduced FHB but are not desirable features (Mesterhazy *et al.* 1999). Focus on locating genes responsible for producing pathogen related (PR) proteins and activating plant defences is required (Pritsch *et al.* 2000). Some such defenses include proteins allowing for the detoxification of DON to DON-3-O-glucoside (Lemmens *et al.* 2005) or the increase in cell wall thickness (Jia *et al.* 2009). Resistance in wheat is controlled by a combination of multiple genes, each with a small effect. These quantitative genes are difficult to breed for, so the use of genetic mapping and identification of qualitative trait loci (QTL) are needed (Brunner *et al.* 2009).

The present study consists of two projects: one of which identified resistance genes in winter wheat and another which focuses on the utilization of a new bio-fungicide control for managing FHB. The first study consisted of developing a linkage map and identifying QTLs for *Fusarium* resistance in a double haploid population. This population was developed from a cross between Flourish (FHB susceptible cultivar) and Emerson (FHB resistant cultivar). The second study examined the time and rate of application needed for a newly developed bio-

fungicide containing the active agent, *C. rosea*. Efficacy in reducing FHB symptoms in four spring wheat genotypes ranging in resistance to FHB was evaluated and compared to a commercially available chemical fungicide Caramba®.

Chapter 2.0 Literature Review

2.1 Wheat and its domestication

Our ability to take wild plant species and domesticate them allowed for humans to support larger populations as well as expand into the raising of animals for human consumption. Human domestication of cereal plants, including wheat (genus *Triticum*), began over 10,000 years ago in the Fertile Crescent (modern day southeast Turkey/ northern Syria) (Lev-Yadun *et al.* 2000; Charmet 2011; Varzakas *et al.* 2014; Uauy 2017). Through different selection pressures and unintentional hybridization, new wheat species were formed. Some phenotypic traits which distinguish a plant as being domesticated include; the presence of non-brittle rachis, naked grain and increased number of grains per spike (Charmet 2011).

The basic chromosome number of the wheat genome is seven chromosome pairs. Wheat can either be a diploid (2n=2x=14), tetraploid (2n=4x=28) or hexaploid (2n=6x=42) species (Peng *et al.* 2011; Varzakas *et al.* 2014). The development of domesticated wheat began with the cultivation of wild emmer wheat (*T. dicoccoides*). Emmer wheat is a tetraploid containing the AABB genome and was formed through hybridization of a wild diploid wheat, *Triticum urartu* (AA) and a unknown goat grass species related to *Aegilops speltoides* (BB) (Charmet 2011; Peng *et al.* 2011; Marcussen *et al.* 2014). Over time, this wild genotype developed traits beneficial for higher yields and eventually developed into the domesticated emmer species (*T. dicoccum*). This species of wheat is the ancestor to the two most widely used wheat species grown today: bread wheat (*T. aestivum L*) (AABBDD) and durum wheat (*T. turgidum spp. durum*) (AABB). The second early domesticated wheat developed in the Fertile

Crescent was the diploid einkorn wheat (*T. monococcum L.)* (AA genome). This species is still grown on a small scale in some Mediterranean countries (Charmet 2011; Peng *et al.* 2011). Ninety-five percent of the wheat grown today is *T. aestivum L.* (Varzakas *et al.* 2014)

Bread wheat (*Triticum aestivum*) is the most widely grown species in the world and makes up 20% of the world's caloric consumption (Pfeifer *et al.* 2014). This species of wheat was first appeared about 8,500 years ago, from a cross between domesticated emmer wheat and a genetic donor which carried the D genome (Charmet 2011; Peng *et al.* 2011; Marcussen *et al.* 2014). The exact donor species is unknown, however the origins of the D genome trace back to a second goat grass species, *Aegilops tauschii.* The inclusion of the D genome makes bread wheat an allohexaploid species (AABBDD genome). One special characteristic of wheat is the production of the protein gluten, which gives bread its dough making quality. The level of gluten in the grain determines the type of products that can be made. Bread wheat can be split into two classifications: hard and soft. Hard wheat varieties have protein levels higher than 11.5% (Varzakas *et al.* 2014). The higher levels of gluten give the dough strength and make it optimal for bread making. Soft wheat contains a lower gluten content and is used to make cakes and other dessert pastries.

Durum wheat (*Triticum turgidum spp. durum)* is a allotetraploid wheat species (AABB genome) and the second most grown wheat species (Thuillet *et al.* 2005). When milled, durum wheat produces semolina, a grittier flour compared to bread wheat (Varzakas *et al.* 2014). Semolina flour contains very high levels of gluten, making the dough firm and dry, as well as a yellowish color. These properties are excellent for production of Italian-style pastas.

2.2 Fusarium head blight and its economic importance

Fusarium head blight (FHB), or scab, of wheat (*Triticum aestivum L*.) is considered one of the greatest economic threats to cereal grain production. Infection occurs in many of the major cereals grown around the world including: corn (*Zea mays L.*), wheat (*Triticum aestivum L*.), rye (*Secale cereale L.)*, barley (*Hordeum vulgare L.)* and oat (*Avena sativa L.)*. *Fusarium* head blight is caused by a number of different *Fusarium* species that have been characterized as the *Fusarium graminearum* species complex (FGSC) (Aoki *et al.* 2014). Sixteen distinct species which cause the disease have been identified.

In North America, FHB is caused by a number of *Fusarium* species including *F. culmorum* and *F. avenaceum*, however, *F. graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch) (Gilbert and Haber 2013) is the primary causal agent of the disease in the Canadian prairies. Infection by *Fusarium* results in decreased yield and reduced end use quality of the grain. Some *Fusarium* species also produce mycotoxins that accumulate in the grain.

Economic losses to FHB are largely attributed to the different mycotoxins produced by certain *Fusarium* species. One of the most concerning mycotoxins is deoxynivalenol (DON) which is produced by both *F. graminearum* and *F. culmorum*. The presence of DON, also known as vomitoxin, in grain samples affects the marketability and utility of the grain. This chemical compound produces a range of hazardous effects when consumed by both humans and animals, including vomiting and diarrhea (Pestka 2010; Wegulo 2012; Maresca 2013). For samples to be considered marketable for human consumption in Canada, maximum acceptable DON levels are 1 ppm in end use products (i.e. flour). To be considered acceptable for animals,

levels of DON cannot exceed 5 ppm for feed destined for cattle or poultry and 1 ppm for pigs and young calves feed (Wu 2007). Levels of acceptable DON and other toxins vary between countries.

The first historic accounts of FHB were recorded in England in the late 1800's (Munkvold 2003; Dill-Macky 2008). Intensive tillage practices are attributed to the low incidence of the disease until recent times. In the early 1980's as no-till and conservation tillage practices were implemented, it is believed that the resulting increase of infected crop residues left on the soil surface led to an increase in *Fusarium* inoculum and higher FHB. This, along with vast acreages of monoculture farming of cereal grains, lack of resistance genes in wheat cultivars and occurrence of optimal environmental conditions for *F. graminearum* resulted in FHB epidemics across North America. One large epidemic in Canada occurred in Ontario and other Eastern provinces in 1980 and another occurred in 1993 in Manitoba (Gilbert and Haber 2013). The total economic losses to Canadian wheat producers reached around 220 million USD in Ontario and Quebec and 300 million USD in Manitoba (Windels 2000). The United States of America has also been plagued with FHB epidemics. Like Canada, problems first started appearing in US during the early to mid 1990's (McMullen *et al.* 2012). Over the course of eight years (1993-2001) it was estimated that a combined total economic loss in the Great Plains and Central United States reached 2.481 billion US dollars due to FHB infection in bread wheat, durum wheat and barley (Nganje *et al.* 2004 a). Of these areas, the Red River Valley region of North Dakota and Minnesota accounted for over half of the losses (Nganje *et al.* 2004 a; Nganje *et al.* 2004 b). Infection rates during the period of 1993-1998 were so high that the severity of FHB infection reached as high as 80% in parts of Manitoba, Minnesota and The Dakotas (Windels

2000). Ever since the 1990's, incidences of small scale *Fusarium* epidemics have become annual events.

2.3 Fusarium head blight disease cycle

Members of the *Fusarium spp.* complex are monocyclic pathogens, going through one disease cycle once per growing season (Gilbert and Fernando 2004; Wegulo 2012). The *Fusarium* disease cycle can be split into two phases. First is the saprophytic developmental stage, where *Fusarium* survives on necrotic plant tissues from plant debris over winter months and second, the pathogenic phase where the pathogen infects living plant tissues. *Fusarium graminearum* also develops both sexually and asexually (teleomorph stage: *Gibberella zeae*).

The disease cycle of *Fusarium spp.* starts with the pathogen overwintering on infected plant debris from the previous year. During the spring, spore producing structures begin to form from the fungal hyphae. Three types of asexual spores can develop, microconidia in conidiophores, macroconidia in the sporodochium and chlamydospores within the hyphae and macroconidia (Dweba *et al.* 2017). Sexual ascospores are formed in perithecia. Both sexual and asexual spores are released from the crop debris and travel to new host plant tissue by wind or water dispersal, respectively (Parry *et al.* 1995; Gilbert and Fernando 2004; Osborne and Stein 2007). Between the two, infection from ascospores is considered the primary cause of disease. Primary infection occurs during the summer months as wheat florets flower. If spores land on vulnerable portions of the host plant (i.e. the spike), infection can occur. Fungal hyphae penetrate either the stigma or the floret glume and travel to the ovary. Once inside the plant, the fungal hyphae can grow into neighboring florets and infect other kernels. Invasion into a

neighbouring spikelet is accomplished by spread through vascular bundles. Inside the floret, the pathogen takes nutrients from the plant, resulting in grain not being able to develop properly. After harvest, fungi remaining on plant residues become dormant over the winter and the cycle starts again (Gilbert and Fernando 2004).

The level of anther extrusion in the host is thought to contribute to the ability of *Fusarium spp.* to infect host plant tissues. Cultivars which retain anthers within the glume are believed to be more susceptible compared to those which have anthers fully emerged (Parry *et al.* 1995; Skinnes *et al.* 2010). It is thought that if anthers are trapped inside the spikelet they become a nutrient source for any *Fusarium* pathogen that enters (Osborne and Stein 2007; Skinnes *et al.* 2010). A study using a double haploid cross between Arina and NK93604 examined the amount that anther extrusion/retention affected FHB infection (Skinnes *et al.* 2010). Results showed that there was a strong negative correlation between anther extrusion and FHB incidence (r=-0.53 to -0.69). Anther retention resulted in an increase in FHB incidence attributed to *F. graminearum* spores germinating better on the retained dying anther tissue. The retention of anthers within the spikelet facilitated greater *Fusarium* spore germination.

2.4 Environmental conditions needed for FHB infection

Levels of *F. graminearum* infection will vary depending on specific environmental conditions for spore release and germination. The two main factors which influence infection rates are the microclimate temperature and humidity levels (Parry *et al.* 1995). The first requirement which needs to be met is temperature. The release of ascospores into the air is dependent on temperatures in the range of 5-35°C (Reis *et al.* 2016). For both spore types, the

optimum temperature required for germination is between 25-30°C. After germination *F. graminearum* can continue to grow at temperatures as high 30°C (Osborne and Stein 2007), with higher yield losses occurring at warmer temperatures (Brennan *et al.* 2005).

The second condition which needs to be met is adequate moisture levels. Higher moisture correlates to higher infection rates (Parry *et al.* 1995; Gilbert and Fernando 2004). Relative humidity around wheat spikes is important. Increasing the number of days and times of mist irrigation post inoculation in disease nurseries has been proven to help *F. graminearum* establish in wheat (Lacey *et al.* 1999) . This, in turn, increases levels of FHB severity in the field and increases the amount of FDK and DON in harvested grains. Lacey *et al.* (1999) noted that the highest levels of infection were present if irrigation covered at least a three day time period and that plots which did not receive mist irrigation failed to produce DON or NIV. Andersen *et al.* (2015) found that increasing the moisture in the environment helped with *F. graminearum* initial penetration and infection of wheat spikes post inoculation compared to control groups. Daily mist irrigation resulted in the highest disease rates and DON content in grain.

2.5 Fusarium head blight symptoms

One of the primary visual symptoms of FHB infection is the premature bleaching of wheat florets in the field (Goswami and Kistler 2004; Osborne and Stein 2007). Initial symptoms are a brownish discoloration in the area around the point of infection (Parry *et al.* 1995). Sometimes symptoms will first develop in the centre of the wheat spikes and progresses outwards. This follows the development of the spikes where florets in the middle of the spike develop first compared to the top and bottom thirds (Walter *et al.* 2010). Symptoms of primary

infection include a stunting of kernel growth. This leads to the wheat spikes being thinner compared to their healthy counterparts.

Fusarium damaged kernels (FDKs) have a distinct appearance and are nicknamed "tombstone kernels" because of their shrunken size and their hardened chalky texture (Parry *et al.* 1995; Goswami and Kistler 2004; Gilbert and Haber 2013). *Fusarium* damaged kernels are the result of the pathogen extracting nutrients and moisture from the plant. Along with the shriveled appearance, the kernels also develop a salmon color due to the presence of the pathogen (Parry *et al.* 1995; Gilbert and Fernando 2004).

2.6 Fusarium head blight mycotoxins

Fusarium contaminated grain poses a risk to consuming infected cereal products. Certain members of the *Fusarium* complex produce mycotoxins which have an adverse affect on human health. Four different classes of mycotoxins are associated with *Fusarium* infection; trichothecene, zearalenone, aurofusarin and fusarin (Trail *et al.* 2005; Osborne and Stein 2007; Lazicka 2010; Dweba *et al.* 2017). Out of these toxins, trichothecenes and zearalenone are the most regulated in North America and Europe.

2.6.1 Trichothecenes: DON and its derivatives

Deoxynivalenol (DON) is a Type B trichothecene along with 3-acetyl-deoxynivalenol, 15-acetyl-deoxynivalenol (3ADON and 15ADON), nivalenol (NIV) and 4-acetyl-nivanol (4ANIV) (Osborne and Stein 2007; Dweba *et al.* 2017). Out of all the toxins produced by *Fusarium* species, the trichothecenes are the most concerning and most studied. Production of

trichothecenes in *Fusarium* is encoded by a complex of "TRI' genes (Foroud and Eudes, 2009). The gene catalyst responsible for trichothecene production in *Fusarium* is TRI5, which codes for the production of trichodiene synthase (Foroud and Eudes, 2009). Among the different types of *Fusarium* species, *F. graminearum* and *F. culmorum* pose a greater threat because they produce these mycotoxins. In Manitoba, there are two DON chemotypes, 15-acetyl-deoxynivalenol (15ADON) and 3-acetyl-deoxynivalenol (3ADON) with the latter being more aggressive and becoming more prevalent (Guo *et al.* 2008).

Deoxynivalenol is harmful for both plants and animals. The chemical structure of DON includes an epoxide group which binds to the peptidyl transferase site in ribosomes. This blockage prevents the production of proteins within the cell (Lazicka 2010; Maresca 2013). Acute exposure in plants will lead to necrosis of tissues. Chronic exposure leads to overall growth retardation and inhibition of seed germination (Rocha *et al.* 2005*)*. Levels of DON in grain are highly associated with the amount of infection observed in the field (Dweba *et al.* 2017) as bleaching and necrotic visual symptoms are linked to DON production. Deposits of DON are concentrated in the area of initial infection, this concentration dissipates outwards as the fungus spreads (Peiris *et al.* 2011).

Higher DON levels are also highly correlated with FDKs harvested in the field (Dweba *et al.* 2017). Many of these kernels can be removed during harvesting and seed cleaning processes. However, the presence of DON can also be detected in plump healthy-looking kernels. The presence of DON in seemingly healthy grain is associated with *F. graminearum* infection taking place at the later soft dough developmental stage. The application of fungicides may not prevent DON accumulation in the grain. Simpson *et al.* (2001) found that application of

azoxystrobin resulted in the suppression of FHB symptom development, but did not prevent the fungi from continuing to produce DON. Another factor which may influence the level of DON in grain is the amount of moisture present during the growing season. Increased humidity leads to higher incidence of FHB in the field and in turn increases the level of DON in the grain (Cowger *et al.* 2009; Wegulo 2012). However, there have been some incidences of DON levels decreasing with increasing humidity and irrigation during disease nursery trials. The rationale behind this phenomenon is that DON, a water-soluble molecule, may diffuse outside the plant tissue if there are high levels of moisture in the environment (Culler *et al.* 2007).

The risks of adverse health effects from DON ingestion is high in humans and animals. Deoxynivalenol is a very stable compound at high temperatures (350°C) and can remain intact during the manufacturing processes (Döll and Dänicke 2011; Maresca 2013). The limit for acceptable DON levels in finished products in Canada is 1ppm. Other countries with a limit of 1ppm include the United States, Russia and China, while Austria, Germany and the Netherlands have a lower limit of 0.5 ppm (Foroud and Eudes 2009). Numerous historical reports have linked human illness to acute DON consumption from contaminated cereals. Outbreaks of gastroenteritis linked to DON have been reported in Japan and Korea (1946-1963) and China (1961-1981) where symptoms occurred roughly 30 minutes after consuming “scabby cereals” (Pestka 2010).

After consumption, DON easily enters the bloodstream through the intestine via passive diffusion (Maresca 2013). Once in the blood, the toxin travels throughout the body to the brain and other vital organs. Differences in the amount of DON absorbed into the bloodstream have been linked to the type, amount, and location of bacterial colonies in the intestines.

Monogastric animals (pigs and humans) are more sensitive to DON, compared to poultry and ruminant animals, and do not contain bacteria which can detoxify DON to DON-1. Pigs reared on a diet of grains are extremely vulnerable to the effects of DON, experiencing effects at 50-100 µg of DON/kg body weight (Pestka 2010). Pigs exposed to DON in the diet were found to absorb between 54-89% of the toxin and the presence of DON was detected in blood samples as soon as 30 minutes after ingestion (Maresca 2013). Acute toxic effects of DON consumption can include vomiting, nausea and diarrhea (Rocha *et al.* 2005; Lazicka 2010; Pestka 2010; Döll and Dänicke 2011). These symptoms can often be misdiagnosed as being caused by food poisoning due to *Staphylococcus aureus* or *Bacillus cereus* (Pestka 2010). Long term low dose exposure to DON can result in anorexia, fertility issues, nerve damage and even death (Rocha *et al.* 2005, Döll and Danicke 2011). Studies on pigs have also shown that FDK consumption can reduce fertility and the overall size of a pig (Döll and Danicke 2011).

2.6.2 Zearalenone and the Type A Trichothecenes

Zearalenone (ZEA) is the second most regulated toxin produced by *F. graminearum*. Zearalenone is an estrogen-like compound which can bind to oestrogen receptors in the body. Issues with high ZEA toxin levels in animal feed have been linked to reproductive issues (Warth *et al.* 2013). The daily recommended limit for ZEA is 0.5 µg/kg body mass (Lazicka 2010). Female piglets fed a diet high in zearalenone contaminated feed were shown to develop at a faster rate compared to control piglets (Döll and Danicke 2011). Zearalenone consumption in cattle has also reported faster growth rates. Human consumption of ZEA from contaminated grains and animal products (i.e. meats, eggs, cheeses and milk) has been associated with precocious

puberty in young girls (Shoental 1983, Massart *et al.* 2008, Warth *et al.* 2013). Other reproductive effects of ZEA have included reproductive issues such as failed pregnancies (Lazicka 2010).

T-2 and HT-2 are two examples of minor toxins produced in the *Fusarium* complex. These compounds are examples of Type A trichothecenes and are primarily produced by *F. sporotrichioides* and *F. poae* (Foroud and Eudes 2009). Unlike Type B trichothecenes, these do not contain a ketone at C-8 in their chemical structure (Foroud and Eudes 2009). Ingestion of T-2 produces similar acute symptoms to those caused by the Type B trichothecenes. Acute symptoms include nausea, vomiting and diarrhea (Lazicka 2010). Chronic long-term exposure to this toxin results in immune damage.

2.7 FHB control methods

Currently there are four methods which can be used to limit the levels of *F. graminearum* infection. The first is cultural farming practices, including crop rotation and tillage. The second is the use of chemical control agents, third is biological control agents and lastly breeding resistant cultivars.

2.7.1 Cultural control methods

The presence of FHB infection in the field is influenced by cultural practices implemented by farmers. Historically, the incidence of widespread FHB infection was low in the United States and Canada during the time when moldboard plowing was popular (Dill-Macky 2008; McMullen *et al.* 2012). By ploughing the fields, top soil was inverted and buried infected

plant tissue, limiting the release of *Fusarium* ascospores into the air. However, this cultural practice declined in favour of low tillage practices which limited the rate of soil erosion. Studies on the effect of tillage vs non-tillage practices on FHB have had inconsistent results. In a rotation between two *Fusarium* hosts, wheat and corn, tillage prior to sowing significantly reduced disease incidence and DON levels in wheat (Teich and Hamilton 1985; Landschoot *et al.* 2013). In a rotation between wheat, corn and soybean, Miller *et al.* (1998) found that conventional tillage reduced FDK in wheat. However, Lori *et al.* (2009) reported that the difference between tilled and non-tilled sites was not significantly different. Overall, the effects of disease pressure and environmental conditions played a larger role on the level of FHB incidence than tillage (Miller *et al.* 1998; Lori *et al.* 2009; Landschoot *et al.* 2013).

Crop rotation can influence the incidence of FHB in the field. Crop rotation strategies for FHB prevention involve rotating between host and non-host crops (Parry *et al.* 1995; Schaafsma *et al.* 2001; Gilbert and Haber 2013; Shah *et al.* 2018). *Fusarium*'s primary hosts are cereal species such as wheat, barley, oat and corn. Rotation between these crops and a non-grass species, such as canola or soybean is recommended to help lower *Fusarium* inoculum reservoirs in the field (Gilbert and Fernando 2004). Rotations between cereal crops and corn should be avoided. Corn is a sturdier plant which decomposes at a slower rate compared to wheat and houses the pathogen for a longer period (Dill-Macky 2008). Reservoirs can decrease if the stalks are mechanically chopped to allow for faster decomposition of plant debris. Crop rotation away from corn has been shown to have a large influence on the amount of FHB infection and the level of DON in wheat kernels (Munkvold 2003). Wheat from fields grown after corn/wheat

had 57% more DON contaminated grain compared to wheat harvested from fields which were previously planted to soybean (Schaafsma *et al.* 2001).

Certain harvest practices can lower the amount of FDK in collected grain. Harvesting crops at the earliest time possible helps stop continual fungal growth on wheat spikes and in turn decreases the total amount of DON in the grain (Munkvold 2003). While harvesting, increasing the fan speed on the combine can reduce FDK in harvested grain by blowing a higher proportion of FDK back into the field. This does not help to minimize inoculum sources in the field for subsequent years, but does lower the amount of FDK harvested. Ensuring that grain is dry and stored in moisture controlled facilities post harvest aids in stopping the continued growth of fungi in the grain (Munkvold 2003).

2.7.2 Chemical controls

The use of chemical control agents is one of the most effective methods in reducing the levels of FHB in the field. Two main classes of fungicides have been documented as effective against *F. graminearum:* Quinone Outside Inhibitors (QoI) and Demethylation Inhibitors (DMI).

Quinone Outside Inhibitors (QoI) work by preventing fungal respiration. The strobilurin group has been shown to reduce the incidence of FHB in the field, but they have been reported to increase DON levels in infected seeds (Gilbert and Haber 2013). For example, when azoxystrobin was sprayed after wheat was inoculated with *F. graminearum*, the levels of the DON mycotoxin increased in susceptible cultivars (Simpson *et al.* 2001). The increase in DON is attributed to the fungicide suppressing FHB symptoms, but not preventing the fungus from entering the kernel and producing mycotoxins. Inconsistencies in the claim of azoxystrobin

increasing DON levels have been reported by Pirgozliev (2002), who noted a decrease in DON levels after application. Inconsistent reports on FDK and DON reduction from replicated trials using Qol based fungicides has raised a lot of questions about its efficacy (Wegulo *et al.* 2015).

The second class of fungicides is the demethylation Inhibitor (DMI) fungicides. They are reported to be more efficient in controlling FHB than the Qol fungicides. The DMI fungicides work by disrupting the sterol biosynthesis pathway in the fungus which prevents cell membrane formation. Some examples of commonly used active ingredients include; tebuconazole, metconazole, tetraconazole and prothioconazole. Results from trials using these active ingredients have shown consistent results in FHB infection and DON accumulation reduction (Pirgozliev *et al.* 2002, Pirgozliev *et al.* 2008; Gilbert and Haber 2013). These fungicides are listed as suppressive rather than preventative.

Issues with the use of chemical fungicides are related to narrow application windows and they are only suppressive to FHB. Optimal application occurs between the time of wheat spike emergence and the beginning of flowering (Zadoks scale: 60 (Zadoks *et al*, 1974)). Fungicide application can also occur just after anthesis, however, there is a risk that FDK and DON levels will not be suppressed (Wegulo *et al.* 2015). Poor weather conditions such as high wind speeds and rain can prevent the application of fungicide all together and poses a risk to fields. Rates of application, as well as the shape and angle of applicator nozzles can also affect the overall efficacy of fungicides in reducing FHB (Mesterházy *et al.* 2011).

2.7.3 Biological controls

Unlike chemical compounds, naturally occurring antagonists to *F. graminearum* can grow on the plant and be active for longer spans of time. Unlike chemical treatments, antagonists, which are naturally present in the field, potentially pose a lower environmental threat. For a biological agent to effectively control a pest species, it must reliably establish in the environment. Studies into required environmental conditions and timing of application are crucial for the rationalization of biological control use over chemical products. Extensive studies have looked at how bacterial, fungal and animal controls can be used to reduce FHB and DON levels in grain as well as lower inoculum sources in the field. However, no biological control agent has been shown to greatly reduce FHB compared to a fungicide. This limitation does not mean that utilizing biological controls is out of the question, but that it needs to be used in combination with other control strategies.

2.7.3.1 Antagonistic fungi

Between the different types of biological controls, the application of antagonistic fungi is among the most studied. Among the vast array of fungal species, members of the *Trichoderma* species and *Clonostachys spp.* have proven to be the most aggressive. *Trichoderma* are an example of saprophytic fungal species which are naturally found in the soil. *Trichoderma spp.* are widely used for their versatility in parasitizing many pest species (Gilbert and Haber 2013). Studies of *Trichoderma's* efficacy as a seed treatment method have reported that treated seeds had better growth compared to untreated seeds in fields containing *F. graminearum*. The efficacy of *Trichoderma* spp. is attributed to its ability to produce chemical

compounds, such as chitinase, which allow them to break down fungal cell walls. The ability to produce these antagonistic compounds is attributed to two genes: ech42 and nag1 (Lutz *et al.* 2003).

Clonostachys rosea (Schroers, Samuels, Seifert and Gams [teleomorph, *Bionectria ochroleuca* (Schw.) Schroers and Samuels]) (Schroers *et al.* 1999) is another commonly found soil borne fungus which lives on necrotic tissues. Unlike other fungal species, *C. rosea* has the ability to establish in unfavorable environments, including living plant tissues located above ground. (Morandi *et al.* 2000). The mode of action of *C. rosea* against pest species includes both competition and mycoparasitism (Sutton *et al* 1997). Past field studies evaluated utilization of *C. rosea* for seed treatment programs as well as a treatment method on plants in the field. Grain treated with *C. rosea* strain CR47 produced more robust plants compared to untreated controls after exposure to *Fusarium culmorum* (Roberti *et al.* 2008). The fungal agent stimulated the plant's defense response system and the production of pathogenesis related (PR) proteins began. The application of *C. rosea* primed the plant's defense response system prior to *Fusarium* infection. One expressed protein was PR4, which encodes the production of peroxidase (POX). Another highly studied strain of *C. rosea* is ACM941. When grown in culture, a 52.3% reduction in *F. graminearum* mycelial growth was observed due to ACM941. In field trials, ACM941, reduced FDK by 49% and DON levels by 21% (Xue *et al.* 2009). ACM941 is the active ingredient in a bio-fungicide product, CLO-1. Results from field trials involving this bio-fungicide showed a reduction in the FHB index by 30-46%, FDK by 31-39% and DON by 23-33% (Allen G. Xue *et al.* 2014). The efficacy of this bio-fungicide was shown to be on par with the fungicide Folicur®, however its efficacy was greater when used on cultivars containing some

form of resistance to *Fusarium* infection. The resistant cultivar AC Nass (Nass *et al.* 2006) produced very good results, while the susceptible cultivar, AC Foremost (Thomas *et al*. 1997) , did not benefit from application of ACM941 (Allen G. Xue *et al.* 2014).

Fungal biological control agents are not as potent as current chemical controls, weakening their desirability (Wegulo *et al.* 2015). Much of the difficulty of using fungal control agents is that environmental conditions dictate if they will work. Inconsistencies with *C. rosea* efficacy has been reported. Much like *F. graminearum*, the incidence of *C. rosea* developing on the host is dependent on optimal temperature (Cota *et al.* 2008, Yu and Sutton 1998) and humidity levels. However, unlike *F. graminearum, C. rosea* is sensitive to ultraviolet light emitted by the sun (Morandi *et al.* 2008, Costa *et al.* 2012). This sensitivity to ultraviolet light limits acceptance of *C. rosea* as a reliable biological control against *Fusarium*.

2.7.3.2 Antagonistic bacteria

Bacterial agents are of interest in preventing FHB infection because of they are relatively inexpensive to produce, are broadly available, and many have antimicrobial properties. One study conducted by Franco *et al.* (2011) explored the potential of lactic acid producing bacteria against *F. graminearum* strain IAPAR 2218. A variety of *Lactobacillus* spp. were grown on agar plates with *F. graminearum*. This species limited *F. graminearum* growth through the release of antimicrobial properties. *Lactobacillus* was also tested for its ability to remove DON in liquid media. Results showed that DON decreased, but the exact mode of action was not fully understood. It was theorized that DON was removed as a result of the bacteria absorbing it (Franco *et al.* 2011). *Lasobacter enzymogenes* strain C3 has been reported to have anti-fungal

properties. The mode of action for *L. enzymogenes* is through the production of chitinase and glucases. These compounds work by breaking down the cells of *F. graminearum* (Jochum *et al.* 2006).

2.7.3.3 Antagonistic earthworm species

Earthworms (*Lumbricus* spp.) have an affinity for consuming plant debris infected with FHB. In general, earthworm species will gravitate towards plant litter infected with fungi because fungi have already partially decomposed the plant material, making it easier for the earthworm to digest. Wolfarth *et al.* (2011) conducted a study to evaluate whether earthworm activity can disrupt *Fusarium* reservoirs as well as DON content by 95% within the soil. *Lumbricus terestis*, a primary decomposer commonly found around the world, was shown to decompose *Fusarium* infected wheat straw faster than healthy straw. They discovered that *L. terestis* preferred consuming plant debris infected with *F. graminearum* and reduced *Fusarium* biomass in straw by 98%. However, earthworms have their limitations. First, access to plant debris is difficult unless some form of tillage is done. Secondly, there is no economically feasible way to produce high numbers of earthworms, nor a way to guarantee their survival in the field (Wolfarth *et al.* 2011). Lastly, like other animal species, earthworms do not have resistance to the toxic effects of DON and could experience negative effects after consuming large amounts of infected tissue (Wolfarth *et al.* 2011).

2.7.4 Breeding for resistance

The need to grow resistant cultivars to FHB infection is high. The development of new cultivars with resistance to FHB would reduce economic costs to farmers by reducing potential

yield and quality losses, and limiting the requirement for chemical or biological control inputs. Development of high yielding plant varieties with high FHB resistance combined with desired agronomic traits has proven to be difficult. Many undesirable agronomic traits are associated with increasing FHB resistance. These morphological characteristics include traits that do not promote high yield including: increased plant height, increased distance between flag leaf and spike, spikes producing fewer florets which are spread further apart, and awnless phenotypes. (Mesterházy *et al.* 1995). Additional difficulties arise over the fact that resistant gene sources are limited and there are no resistance genes that confer immunity to the disease.

2.7.4.1 Types of FHB resistance

Five types of genetic resistance to FHB have been described. The first two types of resistance are the easiest to observe and document. Type I resistance is the prevention of initial infection (Schroeder and Christensen in 1963). For this type of resistance, plants are evaluated for disease incidence under natural or artificial infection. Studies exploring Type I resistance involve spray inoculation of the plant and documenting the presence or absence of FHB related symptoms. This type of resistance study is easily applied to field-based research. Type II resistance describes the prevention of the spread of disease within the spike. Studies focusing on Type II resistance require controlled environments and inoculating a single floret on a spike and monitoring the degree of disease progression. This type of study is easily done in a greenhouse or lab-based setting. Type II resistance results from development of physical barriers around the infected tissue, which limits disease spread within the spike. Mesterházy (1995) reported that Type III resistance resulted when infection occurred, but the level of kernel damage was minimal. Type IV resistance was defined as tolerance to FHB infection,

where yield is not as severely compromised at similar levels of infection. The last form of resistance was Type V resistance which prevents the accumulation of mycotoxins in the grain. Of the five types of resistance, Types I and II are the most studied. All forms of resistance are somewhat inter-related making it difficult to separate the effects of single resistance types in the field.

2.7.4.2 Wheat genetics

The first factor attributed to the difficulty in identifying disease resistance is the massive size of the wheat genome. The total genome size of wheat is around 17 Gb (Uauy 2017) which severely dwarfs *Arabadopsis thaliana* (145 Mb), a model plant commonly used in genetic studies (Arumuganathan and Earle 1991). The allopolyploid nature and large sections of repetitive DNA in the genome contribute to the complexity of wheat genetic mapping and QTL identification (Lagudah *et al.* 2001; Uauy 2017).

For durum wheat, there is an additional problem of little genetic variation due to thousands of years of selection pressures (Charmet 2011). To reverse the bottleneck effects, researchers have begun the reintroduction of genes from wheat land races and wild relatives at the risk of lowering desirable agronomic traits.

2.7.4.3 QTL mapping of FHB resistance

Resistance to FHB in wheat is controlled by a complex combination of quantitative genes, each contributing a small effect, as opposed to major genes involved in gene-for-gene interactions. Combining resistance genes that lead to strong FHB resistance and good agronomic traits is difficult (Brunner *et al.* 2009). The use of molecular markers to locate and

map sections of DNA associated with resistance [quantitative trait loci (QTL)] is important for marker-assisted selection in plant breeding programs. Buerstmayer *et al.* (2009) reviewed and summarized the results of 52 separate mapping studies and reported that FHB resistant QTLs were present on all chromosomes excluding 7D. Since the release of that review, additional QTL have been reported on 7D (Cativelli *et al.* 2011; Eckard *et al.* 2015; McCartney *et al.* 2016; Steiner *et al.* 2017).

Typically, recombinant inbred lines (RIL), double haploid (DH) and backcrossed populations are used in QTL analysis (Buerstmayr *et al.* 2009; McMullen *et al.* 2012). These populations are homozygous or nearly homozygous at all loci to allow for the detection of QTL. The development of these populations can be expensive and time consuming. The development of near homozygous RIL needs to reach the F_7 generation (Tanksley and Nelson 1996), which can be time consuming, while the development of DH wheat populations is expensive. To construct QTL maps, you must have both phenotypic and genotypic information relating to the population of interest. In order to get adequate phenotypic information, large numbers of plants need to be evaluated (McCartney *et al.* 2007).

Phenotypic data for Types I and II resistance can either be recorded on a multiple day basis (i.e. 7, 14 and 21 days) (Cuthbert *et al.* 2006; Srinivasachary *et al.* 2008; Miedaner *et al.* 2011; Eckard *et al.* 2015) or once 18-21 days (Cuthbert *et al.* 2007; Lu *et al.* 2013; Zhang *et al.* 2014) after initial spore inoculation. The latter time of evaluation is used because that is usually the point of peak infection of the plant. After that time point, disease symptoms (i.e. bleaching) will be difficult to differentiate between spike maturation and senescence. Harvested grain can be evaluated to determine what percentage of the population developed fewer *Fusarium*

damaged kernels (FDKs) (Type III resistance) and lower levels of mycotoxins (Type V resistance). Sixteen-days after infection is when the effect of DON on the plant reaches its maximum effect (Lemmens *et al.* 2005). Finding resistance through phenotypic records alone is time and space intensive, as large numbers of plants require evaluation in differing environmental conditions (McCartney *et al.* 2007; Wang *et al.* 2012; Steiner *et al.* 2017).

Marker assisted selection (MAS) is a method to help shorten the amount of time needed and more accurately identify plants containing the trait of interest. In early generations, MAS is used to locate specific genes by looking for specific markers associated with a specific QTL region. By using multiple markers, QTL can be pyramided to increase resistance for FHB quickly (Anderson 2007; McCartney *et al.* 2007; Buerstmayr, H., Ban, T., Anderson 2009). Two main types of markers used in plant genetics are: Single Sequence Repeats (SSRs) and Single Nucleotide Polymorphism (SNPs) markers. Single Sequence Repeat markers work by detecting mutations, which are indels in repetitive DNA (Vieira *et al.* 2016). The use of SNPs is more recent in plant breeding and has surpassed SSR markers in popularity. SNP markers detect a single nucleotide base change (adenine, cytosine, guanine and thymine) in the DNA sequence. There are two SNP consensus maps of the wheat genome, on based on the 9K wheat Infinium SNP array (Cavanagh *et al.* 2013) and the other based on the 90K wheat Infinium SNP array (Wang *et al.* 2014).

2.7.4.4 Sources of FHB resistance

Due to the complexity of FHB resistance, sources of reliable FHB resistance are difficult to identify. Most of the well documented resistant cultivars have originated from Asian

countries. Among these, the Chinese spring wheat cultivar Sumai 3 and its derivatives are some of the most commonly used sources of FHB resistance genes particularly in spring wheat. Resistance in Sumai 3 is the result of a specific combination of minor QTLs, each offering a small amount of resistance. This is known because Sumai 3 was developed from two wheat cultivars which were moderately susceptible to FHB. *Fusarium* head blight resistance QTLs from Sumai 3 have been mapped on chromosomes 5A (*Qfhs.ifa-5A)*, 3BS (*Fbh1*) and 6B (Buerstmayr *et al.* 2002; Liu *et al.* 2006; Cuthbert *et al.* 2007). Resistant genes on chromosome 5A have been associated with both Type I and Type II resistance, while chromosome 3BS correlates with Type II resistance (Foroud 2012) and Type V resistance by detoxifying DON to DON-3-O-glucoside (Lemmens *et al.* 2005). Cuthbert *et al.* (2006) identified another gene from Sumai 3, *Fhb2* QTL on chromosome 6BS, which was associated with increased Type II resistance (Cuthbert *et al.* 2006). A study published by Qi *et al. (2008)*, described the QTL, *Fhb3*, which was isolated in a wild rye variety, *Leymus racemosus*, on chromosome 7Lr#1. Additional resistance QTL have been mapped in another Chinese variety; Wangshuibai, one on chromosome 4B (*Fhb4, Qfhi.nau-4B*) (Xue *et al.* 2010) and another on chromosome 5A (*Fhb5, Qfhi.nau-5A*)(Xue *et al.* 2011). Both are associated with major Type I resistance (Dweba *et al.* 2017). However, fears of over reliance on Sumai 3 resistance and the potential for *F. graminearum* to develop resistance has led to more focus in developing native resistant lines in Europe, Australia and the Americas (Gilbert and Haber 2013). In addition, linkage that reduces fitness and yield when Asian spring wheat sources of resistance are used is a concern in breeding winter wheat (Häberle *et al.* 2009, Gilbert and Haber 2013).

Multiple QTL relating to FHB resistance have been identified in European winter wheat cultivars. One QTL on chromosome 1BL (*Qfhs.lfl-1BL*) was identified in four winter wheat cultivars (Cansas, Biscay, History and Pirat) and was associated with reducing FHB index values by 42% (Häberle *et al.* 2009). Additional resistant QTL have been identified on chromosomes 6AL (*Qfhs.lfl-6A*) and 7BS (*Qfhs.lfl-7BS),* using the cross Dream (R)/ Lynx (S) (Häberle *et al.* 2009). Schmolke *et al.* (2005) had previously examined FHB resistant using the cross Dream/Lynx. Within this cross they identified four QTL relating to FHB resistance on chromosomes 1B, 2BL, 6AL and 7BS.

In the United States, two winter wheat cultivars, Freedom and Ernie (McKendry *et al.* 1995), are sources for FHB resistance (Rudd *et al.* 2001). Lui *et al.* (2007) identified the resistance QTL in Ernie on chromosomes 2B, 3B, 4BL and 5A. They also reported that the QTL conferring Type II resistance were different from those found in Sumai 3 and its derivatives. In Canada, Emerson, was the first winter wheat cultivar designated as being resistant to FHB. However, like Sumai 3, it was developed from a cross between two wheat cultivars that were moderately susceptible to FHB. So far, no published work has stated what resistance genes are present in Emerson (Graf *et al.* 2013).

Compared to bread wheat, durum wheat currently does not have as many identified FHB resistance genes (McMullen *et al.* 2012; Gilbert and Haber 2013). Unlike bread wheat, there is a lack of any allotetraploid equivalent to Sumai 3 in durum wheat. Only a few FHB resistant QTL have been identified in durum. These include one on chromosome 2A (QFhb.rwg-2A) in the cultivar Ben (Elias and Miller 1998, Zhang *et al.* 2014).

2.7.4.5 Resistance and pathogen related proteins

Pathogen related (PR) proteins are plant defense proteins released after a plant senses an attack due to fungal pathogens, insects, or herbaceous activity. Decreases in FHB symptoms and reduction in DON levels have been associated with high PR levels in resistant cultivars (Pritsch *et al.* 2000). Pathogen related proteins have antimicrobial or other defence properties and are released throughout the plant once a pathogen invasion has been detected. Genes located on chromosomes 5A (Qfhs.ifa-5A) and 3BS (Fhb1 or QFhs.ndsu-3BS) have been shown to increase plant defense components and are responsible for the production of many PR proteins. Some PR proteins are connected to the jasmonic acid biosynthesis pathway. These proteins include; PR1 and PR2 which increase β 1,3-glucase, PR3 which increases chitinase, and PRs 4, 5 and 9 which increase peroxidase (POX) level (Foroud 2012). Resistance genes on chromosomes 5A (Qfhs.ifa-5A) and 3BS increased cell wall thickness (Jia *et al* 2009, Steiner *et al.* 2009, Schweiger *et al.* 2013). Li *et al.* (2000) reported that PR related proteins produced by Sumai 3 increased chitinase and β 1,3-glucase production after inoculation with *F. graminearum*. These genes were located on the long arms of chromosomes 3B and 3D and coded for an increase in PR 1 and PR 3 production.

2.7.4.6 Plant dwarfing genes and FHB resistance

Shorter wheat cultivars are in higher demand because the plant's energy goes towards producing higher grain yield over increasing vegetative growth. Spikes of shorter plants are closer to the ground and have a higher probability of infection from spores on plant debris. In addition, short plants tend to have denser leaf growth which leads to higher moisture and humidity in the crop canopy, providing a microclimate conducive to *F. graminearum* infection

(Mesterhazy *et al.* 1999, Srinivasachary *et al.* 2008). Two semi-dwarfing genes, *Rht-B1b* and *Rht-D1b*, were introduced into breeding stocks during the "Green Revolution" (Peng *et al.* 1999; Saville *et al.* 2012; Herter *et al.* 2018). These two restricted height (*Rht*) genes work in a similar way by reducing the plant's sensitivity to the plant height hormone, gibberellin (Peng *et al.* 1999; Saville *et al.* 2012; Herter *et al.* 2018). A third major dwarfing gene is *Rht-8c* located on the short arm of chromosome 2D (Guedira *et al.* 2010). *Rht8* is less common in North American wheat varieties, only being present in about 3% of winter wheat varieties and 8% of spring wheat. An additional height gene, *Rht24,* has been described on chromosome 6S (Herter *et al.* 2018).

The interactions between these height genes and FHB resistance have been studied. The "*Rht*" dwarfing alleles correlate with increased FHB sensitivity. This is not optimal as around 90% of the wheat varieties grown in North America contain these dwarfing genes (Guedira *et al.* 2010). Srinivasachary *et al.* (2008) reported plants containing dwarfing alleles *Rht-B1b* (chromosome 4B) and *Rht-D1b* (chromosome 4D) were more susceptible to FHB compared to lines with the wild type allele. They also measured humidity levels near the spikes and found no difference between tall and shorter cultivars and concluded that the increase in FHB susceptibility was due to plant height QTLs influencing the expression of FHB resistance genes on chromosome 3A (Srinivasachary *et al.*, 2008). Draeger *et al.* (2007) found multiple FHB resistant QTL linked with the height gene *Rht-Db1* and concluded that the wild type allele confers higher resistance. Plant height itself is unlikely to be the cause of increased resistance, but rather genetic linkage or pleotropic effects with the dwarfing genes (Draeger *et al* 2007, Srinivasachary *et al.* 2008). On the other hand, Saville *et al.* (2012) examined the relationship

between the height mutants and wheat pathogen infection using a NIL population containing either the wild type (*rht-tall*), semi dwarf (*Rht-B1b*) or severe dwarf (*Rht-B1c*) alleles. These were examined for both Type I and II resistance against *F. graminearum*. Results showed that as the level of dwarfism increased, so did FHB susceptibility after spray inoculation, but the reverse was true for point inoculation. Similar results have also found that Type I resistance was higher in wild types (Yan *et al.* 2011). The reasoning being that the microclimate in taller varieties may be less humid and cooler compared to the dwarf plants. Yan *et al.* (2011) also observed that 4/10 NILs containing dwarf, *Rht-B1b* and *Rht-D1b* alleles, had better Type II resistance to *F. graminearum*, which they attributed to dwarf plants having higher cell densities which may make it more difficult for *Fusarium* to spread.

Chapter 3.0 Mapping for resistant genes in a Flourish/Emerson double haploid winter wheat population

3.1 Abstract

Fusarium head blight (FHB) of wheat (*Triticum aestivum L*.) is an economically damaging disease. It reduces both the quality and quantity of useable grain. *Fusarium* head blight infection, caused by *Fusarium graminearum*, also brings on additional risks by producing mycotoxins such as deoxynivalenol (DON). Development of FHB resistant wheat cultivars is a primary strategy to reduce losses to *F. graminearum*. In the current study, a population of 178 double haploid lines developed from a cross between two winter wheat cultivars, Flourish (susceptible to FHB) and Emerson (resistant to FHB), was phenotyped for response to *F. graminearum* in inoculated nurseries conducted at three different test sites in Manitoba; Carman (2015), Carman (2016) and Winnipeg (2016). Genotypic data were collected on the population using the Illumina 90K wheat beadchip and a linkage map was developed. Quantitative trait loci (QTL) analysis identified QTL from the resistant parent, Emerson, that reduced FHB symptoms (disease incidence and severity) and FHB damage (*Fusarium* damaged kernels, DON). The QTL were identified on five chromosomes; 1B, 2B, 4D, 6D and 7A. In addition, QTL associated with plant height were identified on chromosomes 2D, 4D and 6A and QTL on chromosomes 2B and 4D were associated with kernel weight. A QTL on chromosome 4D was associated with disease severity, FHB index, FDK, DON, plant height and kernel weight. The 4D QTL was stable and present in all three sites.

3.2 Introduction

One of the most economically important wheat (*Triticum aestivum L*.) diseases worldwide is *Fusarium* head blight (FHB), or scab, caused by several *Fusarium* species (Aoki *et al.* 2014). *Fusarium graminearum* is the primary *Fusarium* pathogen in North America. Infection by *F. graminearum* lowers yield and end-use quality of the grain and leads to accumulation of mycotoxins such as deoxynivalenol (DON), which affect food and feed safety (Gilbert and Haber 2013).

Fungicides, biological antagonists, and cultural practices are used by producers to limit FHB infection (Gilbert and Haber 2013). However, these methods do not provide sufficient or consistent control of FHB. Development and commercialization of wheat cultivars that are resistant to *F. graminearum* infection would have long term benefits to producers, including lower economic inputs and reduced environmental impacts.

The development of new resistant cultivars is hampered by the fact that FHB resistance is controlled by a combination of genes which contribute small levels of resistance (Brunner *et al.* 2009). Currently, no fully resistant wheat cultivar has been developed. Genetic pools for resistance to FHB have been identified in Europe, Asia, Australia and the Americas, but the strongest forms of FHB resistance are mostly found in Asian germplasm (Buerstmayr, H., Ban, T., Anderson 2009). Molecular markers can be used to locate and map quantitative trait loci (QTL) associated with resistance. For QTL mapping, both genotypic and phenotypic information needs to be gathered.

Prevention of initial infection (Type I) and prevention of the spread of infection (Type II) are the two most studied and well described forms of resistance (Schroeder and Christensen 1963). In the field, Type I resistance is evaluated by infecting the plants with *F. graminearum* spores, by either natural or artificial means, and evaluating how many plants get infected (disease incidence evaluation). Type II resistance evaluates how much the symptoms are expressed and spread on an individual spike. Type II resistance is usually examined in a greenhouse setting using point inoculation and recording how much the disease spreads from the point of inoculation.

FHB resistance QTL have been identified on all chromosomes of the wheat genome (Steiner *et al.* 2017). Some of the most notable ones are located on chromosomes 3BS (*Fhb1*), 5A (*Qfhs.ifa-5A)* (Liu *et al.* 2006 Buerstmayr *et al.* 2002) and 6BS (*Fhb2*) (Cuthbert *et al.* 2006). The *Fhb1* and *Fhb2* alleles were first reported in the Asian cultivar, Sumai 3, and are associated with increasing Type II resistance. The *Qfhs.ifa-5A* QTL has been associated with Type I resistance. Additional resistance genes have been mapped in another Chinese cultivar; Wangshuibai, one on chromosome 4B (*Fhb4, Qfhi.nau-4B*) (Xue *et al.* 2010) and one on chromosome 5A (*Fhb5, Qfhi.nau-5A*)(Xue *et al.* 2011) and is associated with major Type I resistance (Dweba *et al.* 2017). Concerns of over reliance on Sumai's resistance and the potential for *F. graminearum* to overcome this resistance have led to more focus in developing native resistant lines in Europe, Australia and the Americas (Gilbert and Haber 2013).

The winter wheat cultivar, Emerson, was the first cultivar to be registered as resistant to FHB in Canada. Much like Sumai 3, it was developed from a cross between two susceptible cultivars (Graf *et al.* 2013). The objective of the current study was to identify QTL associated

with FHB reaction in a double haploid (DH) population developed from the cross between Emerson and a susceptible winter wheat cultivar, Flourish.

3.3 Materials and Methods

3.3.1 Plant material

A double haploid (DH) hard red winter wheat population was developed from the cross between Flourish and Emerson (Figure 3.1). The development of the double haploid population was accomplished through wheat/corn hybridization technique (Fedak *et al.* 1997). The female parent, Flourish, is early maturing, high yielding and produces high protein content. However, this cultivar is susceptible to FHB (Graf *et al.* 2012, Graf *et al.* 2013). The male parent, Emerson, produces good yield, high protein content and is resistant to FHB (Graf *et al.* 2013) and has smaller seeds than Flourish (Graf, personal communication, August 28, 2018).

A population of 178 double haploid Flourish/Emerson lines was used in this study. Prior to this study, the lines were preselected for short-medium height and for stripe rust resistance from an original population size of 755. No pre-screening for FHB resistance occurred.

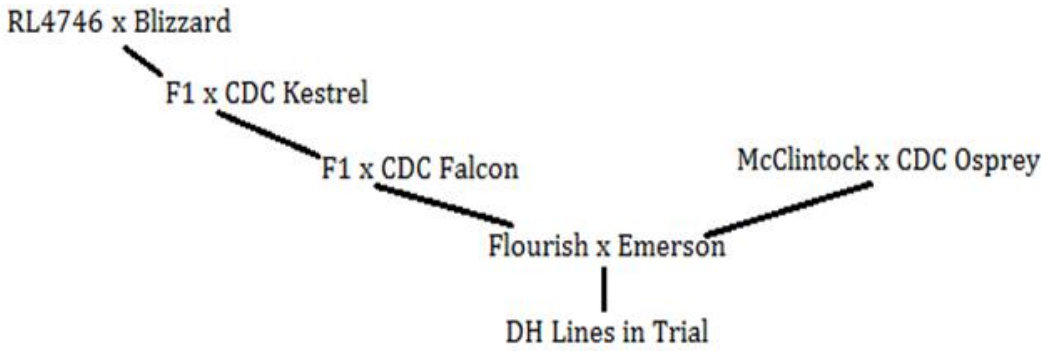

Figure 3.1 Genetic background for development of the double haploid population crossed between winter wheat cultivars Flourish and Emerson (Graf *et al.* 2012, Graf *et al.* 2013).

3.3.2 Field trial design

Field trials were conducted in the summers of 2015 and 2016. Three site years of data were collected from the following locations; Carman, MB (2015), Carman, MB (2016) and Winnipeg, MB (2016). All field trials were planted in a randomized complete block design. AGROBASE software was used to generate the randomizations. Plots in the trial were 1 meter rows planted with 70 seeds per row. Each plot represented a single genotype. Rows were spaced 30 cm apart.

The first trial was conducted at the University of Manitoba's Ian N. Morrison Research Station in Carman, Manitoba. This trial was sown on October 18th, 2014 and phenotypic data were collected over the summer of 2015. The 2015 trial consisted of two replicates and 159 double haploid lines. This site did not contain either parent (Flourish or Emerson) or check cultivars. Five cultivars grown as filler plots at the end of the block were used as FHB symptom checks for this year. These fill cultivars included; Hanover (Susceptible), Freedom (Intermediate), 43I*18 (Intermediate), 32C*17 (Resistant) and FHB148 (Resistant).

The 2016 trials included an additional 18 double haploid (DH) lines from the population (178 lines in total), three replicates as well as the parental cultivars; Flourish and Emerson. One trial was located at the Ian N. Morrison Research Station and the second was located at the University of Manitoba's Fort Gary Campus, Winnipeg (the Point). The Carman and Winnipeg trials were sown on September 30th and 31st, 2015, respectively. Phenotypic data for disease incidence, disease severity and height were collected over the summer of 2016. Check cultivars ranging in FHB resistance were used at these two sites and located randomly within each

replicate. Six check cultivars ranging in FHB resistance were used; Caledonia (Susceptible) (Sorrells *et al.* 2004), CDC Osprey (Fowler 1997a), 43I*18 (Intermediate), 32C*17 (Resistant), Hanover (Susceptible), and FHB 148 (Resistant). Plots were fertilized to provide 150 Kg/ha of N based on soil test results. In Winnipeg, 33 kg/ha 11-52-0 fertilizer was applied with the seed and 255 kg/ha 40-0-0 and 30 kg/ha 0-0-60 fertilizer was broadcast onto the plots with a Valmar fertilizer spreader. In Carman, 11-52-0 (39kh/ha in 2015 and 2016) and Urea (47 kg/ha in 2015 and 175kg/ha in2016) was broadcast on the plots.

3.3.3 *F. graminearum* inoculum

F. graminearum inoculum consisted of four isolates obtained from Dr. Jeannie Gilbert of the Cereal Research Centre of Agriculture and Agri-Food Canada; M7-07-01, M9-07-1 (3 acetyl-deoxynivalenol chemotype), M1-07-2 and M3-07-2 (15 acetyl-deoxynivalenol chemotype). The process began with single spore isolation from a stock colony and cultured on potato dextrose agar (PDA) plates. The fungus was cultured for 18-20 hours, after which the plate was examined to locate a single conidium using a high-powered dissecting microscope. A section of the PDA media containing the spore was removed with a sterile spatula and placed on a Spezieller Nährstoffarmer agar (SNA) media plate (20mL media/plate). The SNA plate was placed under a continual UV light source at room temperature for a week. Using aseptic techniques, SNA plates were sliced into 8 pieces and placed in 2 L flasks containing Carboxymethyl Cellulose (CMC) media (1.5L/flask). The flasks were placed under fluorescent shop lights and aerated for a week. Once finished the concentrated isolate was strained from the flask through sterile cheesecloth and stored in refrigerated glass bottles until preparation for inoculation.

Macroconidia concentrations for each isolate were determined using a haemocytometer. Equal proportions for each isolate were combined to produce a total concentration of 50,000 macroconidia/mL for inoculation. The calculated amount of each isolate concentrate was added to 2 liters bottles and distilled water was added to reach a total volume of 2L of inoculum per bottle. Tween 20 (20 ml) was added, to each 2L bottle of solution, in each bottle to break up the surface tension of the water and promote inoculum dispersal on the plant.

3.3.4 *F. graminearum* inoculation

Time to 50% heading (Zadoks: 50) and 50% flowering (Zadoks: 60) (Zadoks *et al*, 1974) were recorded for each plot. At 50% anthesis, plots were inoculated with 50 mL of 50,000 *F. graminearum* macro-conidia/mL spore suspension. Each plot was inoculated a second time, two days after the first inoculation. Inoculum was applied using a CO_2 backpack sprayer set at 30 psi. Inoculum was sprayed directly onto the wheat spikes using an R&D back pack sprayer with an 80.02 spray nozzle. After inoculation, plots were mist irrigated for 10 minutes/hour for 10 hours. The mist irrigation system consisted of a 4732 litre tank of water, a generator (power source), irrigation pump, Hunter system controller (controls irrigation times) and tubing carrying water to the plots. This was done to maintain high humidity levels and promotes an optimal growing environment for the pathogen.

3.3.5 Phenotypic disease and physical characteristic evaluation in field

Eighteen to twenty-one days after the initial inoculation (Cuthbert *et al.* 2007; Lu *et al.* 2013; Zhang *et al.* 2014) plots were visually assessed for the proportion of infected spikes, FHB

disease incidence (DI), and the proportion of infected spikelets on infected spikes, severity of the disease (DS). These percentage values were used to calculate the FHB index, using the following formula:

$$FHB\ Index = (Mean\ DS\ X\ Mean\ DI)/100$$

Additionally, plant heights were measured prior to harvest. Measurements were taken using a meter stick. The heights recorded were taken from an average of three height measurements recorded for each plot.

3.3.6 Phenotypic evaluation post harvest

Post-harvest measurements included: 1000 kernel weight (g), percent *Fusarium* damaged kernels (FDK) and DON (ppm). A Model U Seed Counter (Texas, USA) was used to count out 1000 kernels and the grain was weighed on a scale. If a plot produced less than 1000 seeds, then the following calculation was used to determine the thousand-kernel weight (TKW):

$$TWK = \frac{weight\ of\ sample\ \times\ 1000}{\#\ seeds\ from\ sample}$$

Composite samples were prepared for each line in the population, the two parental lines, and the check lines. Equal proportions (by weight) of each replicate were pooled together to reach a total weight of 50.0 grams. The 50.0 g samples were sent to BioVision Seed Labs (Winnipeg, Manitoba) for further FDK and DON analysis. From the 50.0 g samples, grain showing any signs of *Fusarium* infection were removed. These signs included: small shriveled kernels, kernels with a chalky appearance, kernels with a salmon/pink coloring and kernels with a fuzzy appearance. For questionable kernels, a 10X microscope was used to confirm the

presence of fungal mycelial growth. The removed kernels were weighed and a percentage value from the 50g sample was then calculated. Highly infected FDKs are smaller and lighter weight than their healthy counterparts.

DON content in the grain was calculated by first grinding the samples into powder that was fine enough to pass a 20 mesh sieve. Ten grams of ground grain was then mixed in 100mL of distilled water for three minutes. When particles had settled an aliquot was taken (min 5mL) and filtered through a Neogen filter syringe and the filtrate was collected. This was then further diluted in distilled water (1:2 ratio). The Veratox® DON 5/5 quantitative test kit (Neogen Corporation, Lansing, MI) was used to test DON concentration. This kit is an enzyme linked immunosorbent assay (ELISA) with a DON content accuracy range of 0.5- 5.0 ppm. For samples with over 5.0 ppm of DON, dilutions of the filtrate were made to keep DON concentration within the limits of the test.

3.3.7 Plant tissue growth and collection in preparation for genotyping

Five to eight seeds were taken from each of the 178 lines, the two parental cultivars (Flourish and Emerson), as well as other winter wheat cultivars related to the population (CDC Falcon (Fowler 1999), CDC Osprey (Fowler 1997a), CDC Kestrel (Fowler 1997b), McClintock, Norstar (Grant 1980)) and grown on cotton balls. Cotton balls were sterilized in an autoclave, placed in new seed trainers and moistened with distilled water. Seeds from each sample were surfaced sterilized with an alcohol solution to remove any fungal or bacterial contaminates. Trays were placed under a 32W light and grown until plants reached the 1st to 2nd leaf growth stage.

At the 1-2 leaf growth stage, approximately 4 cm of fresh whole leaf tissue were sampled from each of the lines. Leaves were cut using sterilized scissors and placed in Qiagen© collection tubes (Qiagen, Mississauga, Ontario). Hot bead sterilization on scissors was used to remove the risk of DNA contamination between the different samples. Two 96 well Qiagen© collection tube boxes were used to collect all the sample tissues. During the collection process, collection tubes were placed on ice. Each box contained tissue from the two parents and the five related cultivars. The remaining tubes in Box 1 contained tissue from 89 lines used in the DH population and Box 2 contained tissue from the remaining DH lines. Once tissue was collected, boxes were placed in a -80C freezer for storage. The samples were freeze dried for three days to remove moisture in the tissue and sent to the National Research Council of Canada (NRC) for Illumina® 90K SNP Genotyping.

DNA extraction was preformed by the NRC using an automated AutoGenprep 965CE system. A modified CTAB (Cetyl trimethylammonium bromide) extraction protocol was used with this system. Frozen plant tissue was first ground and 0.3mL CTAB Lysis solution was added and left to digest the sample for 30 minutes at 65^{O}C. These were then transferred into a 96-deep well plate and run through the AutoGenprep 965.

3.3.8 Creation of genetic map using 90K SNP genotyping results

Initial analysis of SNP data was performed at the National Research Council of Canada using GenomeStudio v2011.1 and R studio. A total of 1234 unique SNP markers were identified. Marker data across the lines was screened for poor data points. Markers were removed from the data set if they showed a high number of missing data (>10%) or showed segregation

distortion (skewed >85% to one allele) within the population. A chi square test was performed to determine if each marker fit the expected 1:1 ratio in the population. Markers with high distortion and a P value < 0.001 were removed. Data from a total of 1,185 markers and 172 lines were used to generate a preliminary linkage map using MAPDisto 1.7.7 in Excel®. The initial linkage map contained 27 linkage groups and 17 unlinked markers. The unlinked markers were removed from the data set.

The initial map was compared to the consensus map from Wang *et al.* (2014) and by analysis of the flanking DNA sequence to Chinese Spring reference sequence v 1.0.*et al.* Each linkage group was assigned to a specific chromosome. Marker orders within each linkage group were compared to their predicted order in Chinese spring based on BLAST. If markers were inverted, then the linkage group data was flipped to have information from the short arm of the chromosome listed first. Candidates with >10cM distance between the adjacent markers were changed to a missing data point (threshold error detection used was 0.01) and a final linkage map was created.

These included lines with either high amounts of missing data, or larger numbers of cross over events within a linkage group were removed from the dataset. A total of 11 lines were removed in this process. Finally, two markers were removed from the dataset because they expanded the linkage map.

3.3.9 QTL mapping

Analysis of QTLs was conducted with QGene 4.3.10 software (Joehanes and Nelson 2008) using the phenotypic data collected over the three test locations and the final linkage

map was used for this analysis. Simple interval mapping was used with a scan interval of 1cM. Logarithmic of odds (LOD) scores, R^2 (phenotypic variation explained by QTL) and additive effect values were recorded for QTLS with a LOD score greater than 3. To determine if the QTLs were significant, a permutation test of 1000x and an α of 0.05 was done (Table A-5).

Visual representations of the genetic linkage maps were made through Mapchart 2.3 software (Voorrips 2002). Confidence intervals for the QTLs were calculated by using a 1.5 LOD drop off from the peak LOD value (Visscher *et al.* 1996).

3.3.10 Statistical analysis of phenotypic data

Statistical analyses on the phenotypic data were conducted using SAS® statistical software version 9.4 (SAS institute Inc., Cary, NY, USA). Results from Levine's test of homogeneity determined that the data from the three site years could be pooled together. Analyses of variance (ANOVA) were conducted using the PROC MIXED statement. In the model, all terms were considered random effects. The model statement contained no terms. The random statement included the genotype, environment and rep(environment) for the following parameters; DI, DS, FHB Index, height and TKW. Results from FDK and DON were taken from pooled results. The model statement for these parameters had the genotype and site year as a random effect. Correlation results were generated using the PROC CORR statement on means from all three site years.

Using SAS (v. 9.4), least square mean values from the three site years were generated for the 178 DH lines in the population. These values were later used in the QTL analysis. In

Microsoft Excel® 2016, frequency distributions of the population were created and parental line means were compared to the population distribution.

3.4.1 Field Results

3.4.1.1 Assessment of *Fusarium* head blight field symptoms; disease incidence, severity and FHB index

Table 3.1 shows the ANOVA for the combined disease incidence, severity and FHB index. The effects of the genotype, environment and genotype*environment interaction were highly significant (<0.0001) for FHB disease incidence, severity and FHB index. The population means for the three field FHB variables measured in Carman and Winnipeg in 2016 were close to mid-parent values for all traits except for FHB index in Winnipeg and Carman and disease severity in Winnipeg (Table 3.2). Distributions of the DH means for DI, DS and FHB index spanned the range of the parents with few lines exhibiting transgressive segregation. Distributions for DS and FHB index appeared to be slightly skewed towards the more resistant parent, while the distribution of DI was relatively normal (Figure 3.2). Significant positive correlations were observed among the three disease parameters field site (Table 3.3). Very strong positive correlations also occurred between the three phenotypic field parameters, FDK and DON values.

3.4.1.2 Assessment of physical qualities: height and thousand kernel weights

ANOVA of the plant height data (Table 3.4) showed that the genotype, location and GXE interaction were highly significant (P= <0.0001). The overall mean height of all DH lines was between the two parental cultivars (Table 3.2). The distribution of mean height values for the

DH lines, showed transgressive segregation beyond both parents (Figure 3.2). Plant height was negatively correlated with disease severity (r=-0.31) and FHB index (r=-0.23) and a very strong positive correlation with thousand kernel weights (r=0.32). Significant negative correlations between height and FDK (r=-0.13) and height and DON (r=-0.20) content were also observed (Table 3.3.).

ANOVA of thousand kernel weight (Table 3.4) indicated that all sources of variation were highly significant (P=<0.0001). Frequency distributions of TKW show transgressive segregation beyond the means of both parents. Overall TKW mean values for 2016 were 28.5g, which was between the mean values of both parents (Table 3.2). Significant moderate negative correlations were observed between TKW and DS, FHB index, FDK and DON data (Table 3.3) of r= -0.39, -0.37, -0.38 and -0.38 respectively.

3.4.1.3 Presence of *Fusarium* in grain; FDK and DON analysis

Combined FDK and DON ANOVA results from the three site years tested are displayed in Table 3.5. Both the effects of genotype and location grown were highly significant (P= 0.0001) for both parameters. There was a strong correlation between the percent of FDK in samples and level of DON was observed (0.80) (Table 3.3). Correlations between FHB index values and FDK were 0.67, and correlations between FHB index and DON were 0.76. The DH populations showed a skewed distribution for both DON and FDK (Fig 3.2).

Table 3.1 Combined analysis of variance results for disease incidence, disease severity and FHB index from field test data from three test environments; Carman (2015), Carman (2016) and Winnipeg (2016).

Source	Disease Incidence			Disease Severity		FHB Index	
	DF	Mean Squares	P value	Mean Squares	P value	Mean Squares	P value
Genotype	177	529	<.0001	1,296	<.0001	573	<.0001
Environment	2	283,140	<.0001	121,413	0.0001	114,675	<.0001
Genotype*Environment	335	263	<.0001	324	<.0001	233	<.0001
Rep(Environment)	5	1,795	<.0001	1,489	<.0001	1,165	<.0001
Error	859	148		202		95	

Table 3.2 Flourish/Emerson double haploid (DH) population (n=178) and parental mean values for disease incidence (DI), disease severity (DS), FHB index, height, thousand kernel weight (TKW), *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) in two test locations: Carman (2016) and Winnipeg (2016).

	Carman					Winnipeg				
Trait	**Flourish Mean**	**Emerson Mean**	**Mid-Parent Mean**	**DH Population Mean**	**DH Pop Range**	**Flourish Mean**	**Emerson Mean**	**Mid-Parent Mean**	**DH Population Mean**	**DH Pop Range**
DI (%)	67.9	18.8	43.3	45.0	5-92.5	45.0	5.8	25.4	14.6	5.0-65
DS (%)	80.8	12.5	46.7	44.0	5-92.5	76.7	10	43.3	22.5	5.0-87.5
FHB Index (%)	57.8	2.5	30.1	22.0	0.3-81.0	34.9	0.6	17.8	4.1	0.3-43.9
Height (cm)	67.8	72.1	70.0	68.5	45-79.7	78.7	86.8	82.7	81.1	65.2-95.8
TKW (g)	28.1	27.7	27.9	28.2	20.2-33.9	30.3	27.4	28.9	28.7	19.3-35.7
FDK (%)	24.0	3.1	13.6	12.1	3.3-30.1	12.7	0.6	6.6	3.8	0.2-13.3
DON (ppm)	35.9	7.9	21.9	19.7	4.2-57.0	18.3	1.9	10.1	5.6	0.2-22.0

	Combined 2016 results				
Trait	**Flourish Mean**	**Emerson Mean**	**Mid Parent Mean**	**DH Population Mean**	**DH Pop Range**
DI (%)	56.6	12.3	34.4	30.1	7.9—51.3
DS (%)	78.8	11.3	45.1	33.3	6.7-72.9
FHB Index (%)	46.4	1.6	24.0	13.1	0.6-35.6
Height (cm)	73.3	78.3	75.8	74.8	63.6-84.1
TKW (g)	29.2	27.6	28.4	28.5	23.4-32.6
FDK (%)	18.4	1.9	10.1	8.0	2.0-19.9
DON (ppm)	27.1	4.9	16.0	12.7	3.9-31.9

Note: Information from Carman (2015) site was not included as there was no parental data available and the population size was smaller

Table 3.3 Pearson's correlation coefficients between FHB disease incidence, disease severity, FHB index, plant height, thousand kernel weight (TKW), *Fusarium* damage kernels (FDK) and deoxynivalenol (DON) values from the Flourish/Emerson double haploid population (n=178).

	Incidence	Severity	FHB Index	Height	TKW	FDK
Severity	0.62 <0.0001					
Fhb Index	0.82 <0.0001	0.92 <0.0001				
Height	-0.31 <0.0001	-0.08 0.2799	-0.23 <0.0001			
TKW	-0.13 0.0884	-0.39 <0.0001	-0.37 <0.0001	0.32 <0.0001		
FDK	0.60 <0.0001	0.62 <0.0001	0.67 <0.0001	-0.13 0.0908	-0.38 <0.0001	
DON	0.64 <0.0001	0.69 <0.0001	0.76 <0.0001	-0.20 0.0062	-0.38 <0.0001	0.80 <0.0001

Table 3.4 Combined analysis of variance results for height and thousand kernel weight from field test data from three test environments: Carman (2015), Carman (2016) and Winnipeg (2016).

Source	Height			Thousand Kernel Weight		
	Df	Mean Squares	P value	Df	Mean Squares	P value
Genotype	177	129.97	<.0001	177	29	<.0001
Environment	2	47,802	<.0001	2	452	<.0001
Genotype*Environment	335	15	0.0029	331	8	<.0001
Rep(Environment)	5	87	<0.001	5	7	0.0034
Error	858	12		831	2	

Table 3.5 Combined analysis of variance results for *Fusarium* damaged kernels and deoxynivalenol from field test data from three test environments: Carman (2015), Carman (2016) and Winnipeg (2016).

Source	*Fusarium* Damaged Kernels			Deoxynivalenol		
	Df	Mean Squares	P value	Df	Mean Squares	P value
Genotype	177	62	<.0001	177	120	<.0001
Environment	2	27,372	<.0001	2	21,470	<.0001
Error	331	25		331	65	

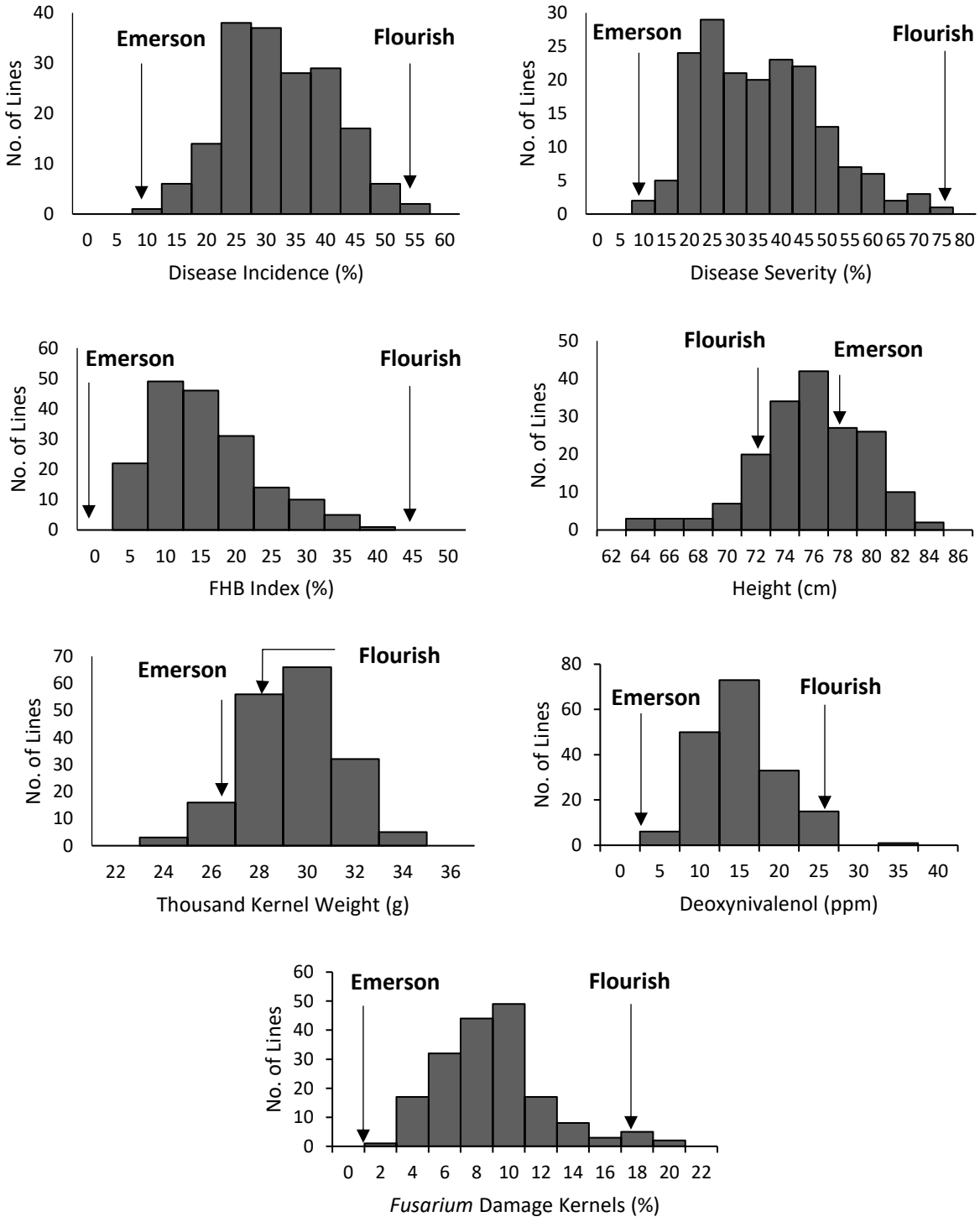

Figure 3.2 Frequency distributions for FHB field symptoms; disease incidence, disease severity, FHB index (%), wheat physical characteristics; height (cm) and thousand kernel weight (g), and kernel disease; deoxynivalenol (DON) levels (ppm), *Fusarium* damage kernels (%) for a population of 178 double haploid Flourish/Emerson lines for the Carman 2016 and Winnipeg 2016 test locations. Note: Carman 2015 was excluded because no parent information was available.

3.4.2 QTL analysis

Quantitative trait loci associated with FHB disease symptoms were located on 5 chromosomes; 7A, 1B, 2BS, 4D and 6D. Results of the FHB symptoms QTLs from each test location are presented in Table 3.6. Apart from a QTL on chromosome 2B ins Carman (2015) all QTL for FHB traits showed that the Emerson allele decreased disease and the Flourish allele increased disease. A major QTL which was strongly associated with disease severity and FHB index values was located on chromosome 4D. This QTL was stable and was detected for all three individual site years and the pooled data. The Emerson allele was identified as decreasing disease severity and FHB index values. The 4D disease severity QTL accounted for 19% of the phenotypic variability in Carman (2015), 22.7% of the phenotypic variability in Carman (2016) and 11.6% of the phenotypic variability in Winnipeg (2016). This QTL was also detected in the combined results from the three site locations (Table 3.6) and accounted for a combined phenotypic variability of 25.5%. The QTL on 4D was also associated with DON and % FDK in Carman (2015) (Table 3.6). In Carman 2015, this QTL explained 13.9% of the phenotypic variability for DON and 11.8% of the phenotypic variability for FDK. This QTL was not detected in the 2016 site years or the combined results. The position of the 4D QTL was consistently located at 13 cM (Fig 3.5). Other QTL on chromosome 7A were associated with disease incidence, disease severity, FHB index, DON and FDK at different locations. For each parameter, this QTL was only identified at one location, suggesting that environmental effects could play a part in the expression of the genes. QTL on chromosome 6D were identified in the Carman 2016 data and was associated with DON and % FDK.

QTL for the physical attributes; height and thousand kernel weights (Table 3.7) were found on chromosomes 2B, 2D, 4D and 6A. The susceptible parent, Emerson, was responsible for increasing plant height and kernel weight. QTL on 2D related to increasing height and 2B relating to kernel weight came from the resistant parent, Flourish. Two QTLs, on chromosomes 2D and 6A, were associated with plant height and accounted for 14% and 13% of phenotypic variability, respectively. The 2D QTL was identified at the Carman (2016) site and the 6A QTL was identified at the Winnipeg (2016) site. The QTL on 4D was associated with thousand kernel weights and was identified in all three site years. The phenotypic variation explained by this QTL was 9.6%, 17% and 11.3% for Carman (2015), Winnipeg (2016) and Carman (2016), respectively. This QTL was also observed in the combined TKW results and accounted 17.6% of the phenotypic variation.

Selection for plant height was done prior to this study. Chi square results for the QTL regions associated with plant height are displayed in Table A-4. Results show that the ratio between A and B alleles for over 50% of the QTL region did not significantly differ from a 1:1 ratio region for chromosomes 2D and 4D. The ratio between the two alleles on 6A was closer to 3:2 ratio between the A and B allele.

Table 3.6 Summary of QTLs associated with FHB symptom reduction in the Flourish/Emerson double haploid population. Data collected from three environments: Carman, MB (2015), Carman, MB (2016) and Winnipeg, MB (2016).

			Carman 2015			Carman 2016			Winnipeg 2016			Combined		
Chrom	Flanking Molecular Markers	Peak Position (cM)	LOD	R^2 (%)	Additive Effect	LOD	R^2 (%)	Additive Effect	LOD	R^2 (%)	Additive Effect	LOD	R^2 (%)	Additive Effect
Disease Incidence														
1B	BS00022429_51 Excalibur_c581_1220	18	3.28	9	3.646	-	-	-	-	-	-	-	-	-
2BS	BS00041585_51 Excalibur_c10376_313	4	-	-	-	5.792	15.3	5.603	-	-	-	-	-	-
7A	BS00064863_51 BS00047811_51	84	-	-	-	-	-	-	3.745	10.2	2.542	-	-	-
Disease Severity														
2BS	BS00041585_51 BobWhite_c30622_180	3	4.198	11.3	-5.426	-	-	-	-	-	-	-	-	-
4D	RAC875_REP_C105718_304 BobWhite_c20689_427	13	7.349	19	7.131	8.983	22.7	8.293	4.321	11.6	4.683	10.299	25.5	6.624
7A	BS00064863_51 wsnp_Ku_rep_c113718_96236830	113	3.002	8.2	4.776	-	-	-	-	-	-	-	-	-
FHB Index														
4D	RAC875_REP_C105718_304 BobWhite_c20689_427	13	6.993	18.1	6.149	5.81	15.3	5.1	3.17	8.7	1.31	8.574	21.7	3.78
7A	BS00064863_51 wsnp_Ku_rep_c113718_96236830	113	3.428	9.3	4.496	-	-	-	-	-	-	-	-	-
Fusarium Damaged Kernels														
4D	RAC875_rep_c105718_304 BobWhite_c20689_427	13	4.377	11.8	2.943	-	-	-	-	-	-	-	-	-
6D	IAAV8692 BS00021881_51	49	-	-	-	3.849	10.4	1.773	-	-	-	-	-	-
7A	Kukri_c38498_186 Kukri_c46218_66	126	-	-	-	4.992	13.3	2.056	-	-	-	4.483	12	1.698

Table 3.6. Cont. Summary of QTLs associated with FHB symptom reduction in the Flourish/Emerson DH population. Data collected from three environments: Carman, MB (2015), Carman, MB (2016) and Winnipeg, MB (2016).

			Carman 2015			Carman 2016			Winnipeg 2016			Combined		
Chrom	Flanking Molecular Markers	Peak Position (cM)	LOD[a]	R^2(%)[b]	Additive Effect[c]	LOD	R^2 (%)	Additive Effect	LOD	R^2 (%)	Additive Effect	LOD	R^2 (%)	Additive Effect
Deoxynivalenol														
4D	RAC875_REP_C105718_304 BobWhite_c20689_427	13	5.24	13.9	4.336	-	-	-	-	-	-	-	-	-
6D	IAAV8692 BS00012510_51	75	-	-	-	3.403	9.3	2.83	-	-	-	-	-	-
7A	Excalibur_rep_c68963_163 BobWhite_c34551_714	85	-	-	-	-	-	-	3.534	9.6	1.051	-	-	-

a: LOD is the peak LOD score detected

b: R^2 (%) is the amount of phenotypic variation

c: A positive additive effect values indicates that Flourish increased respective trait and Emerson decreases it

d: 159 lines tested in 2015 and 178 were tested in 2016 sites

Table 3.7 Summary of QTLs associated with height and thousand kernel weights in the Flourish/Emerson double haploid population. Data collected from three environments: Carman, MB (2015), Carman, MB (2016) and Winnipeg, MB (2016).

			Carman 2015			Carman 2016			Winnipeg 2016			Combined		
Chrom	Flanking Molecular Markers	Peak Position (cM)	LOD[a]	R^2 (%)[b]	Additive Effect[c]	LOD	R^2 (%)	Additive Effect	LOD	R^2 (%)	Additive Effect	LOD	R^2 (%)	Additive Effect
Height														
2D	RAC875_c66820_684 Excalibur_c14639_1632	21	-	-	-	5.26	14	1.561	-	-	-	-	-	-
4D	RAC875_c60218_63 BobWhite_c20689_427	14	-	-	-	-	-	-	-	-	-	3.134	8.6	-1.331
6A	IAAV7418 BobWhite_c14915_156	90	-	-	-	-	-	-	5.126	13.6	-1.852	4.447	11.9	-1.582
Thousand Kernel Weights														
2B	BS00041585_51 Excalibur_c10376_313	5	-	-	-	-	-	-	4.6	12.3	0.82	4.112	11.1	0.654
4D	RAC875_REP_C105718_304 BobWhite_c20689_427	12	3.544	9.6	-0.646	4.207	11.3	-0.721	6.53	17.0	-0.972	6.762	17.6	-0.852
6A	IAAV7418 BobWhite_c14915_156	97	-	-	-	-	-	-	-	-	-	3.617	9.8	-0.678

a: LOD is the peak LOD score detected

b: R^2 (%) is the amount of phenotypic variation

c: A positive additive effect values indicates that Flourish increased respective trait and Emerson decreases it

d: 159 lines tested in 2015 and 178 were tested in 2016 sites

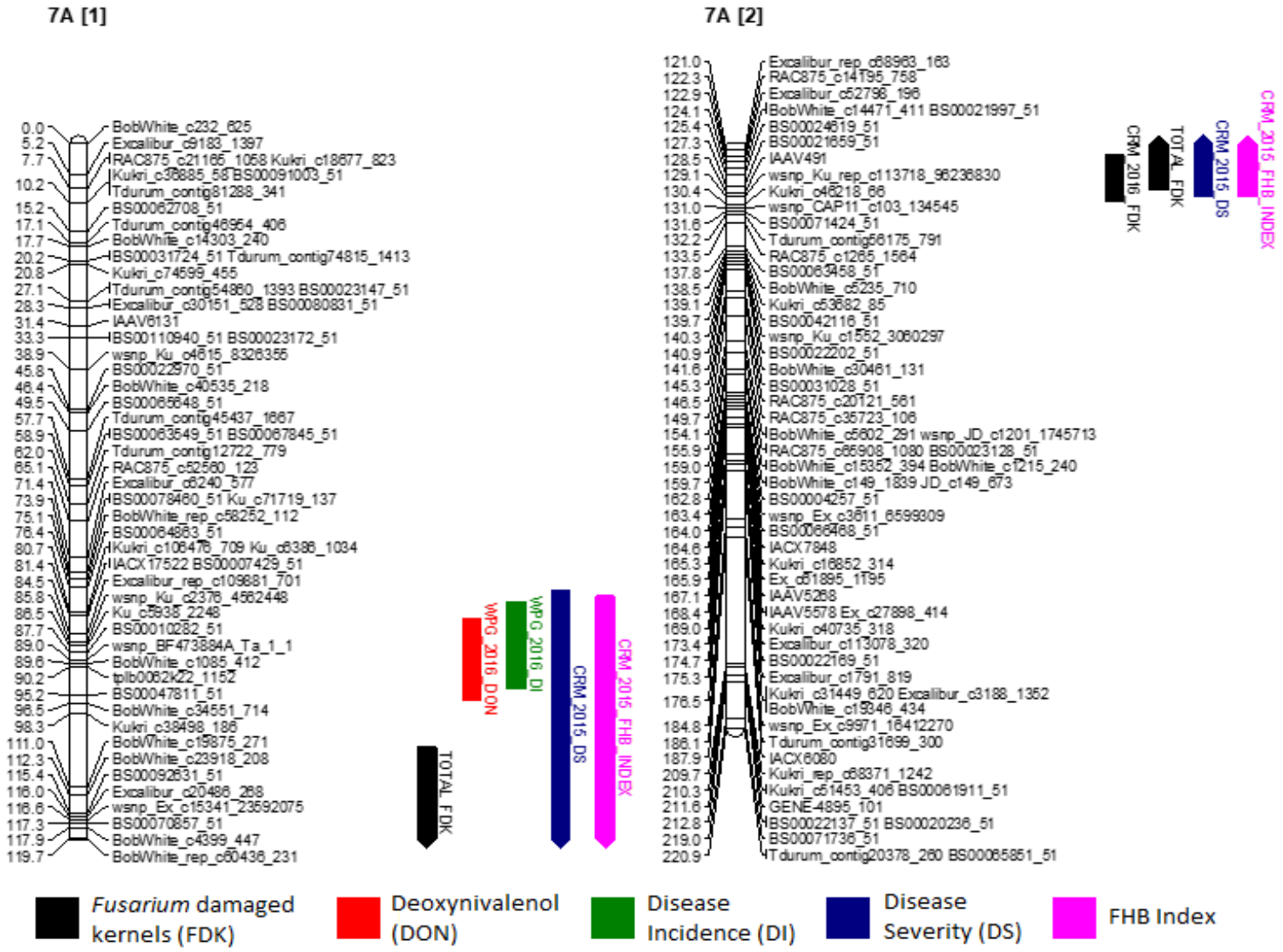

Figure 3.3 Genetic linkage map for disease incidence QTL located on chromosome 7A for 161 DH lines developed from the Flourish/Emerson cross tested at three environments; Carman (2015), Carman (2016) and Winnipeg (2016). Marker distance is displayed on the right side of the chromosome and the names of markers present are on the right. Note: Due to its length the linkage group on 7A has been split into two parts: 7A[1] and 7A[2].

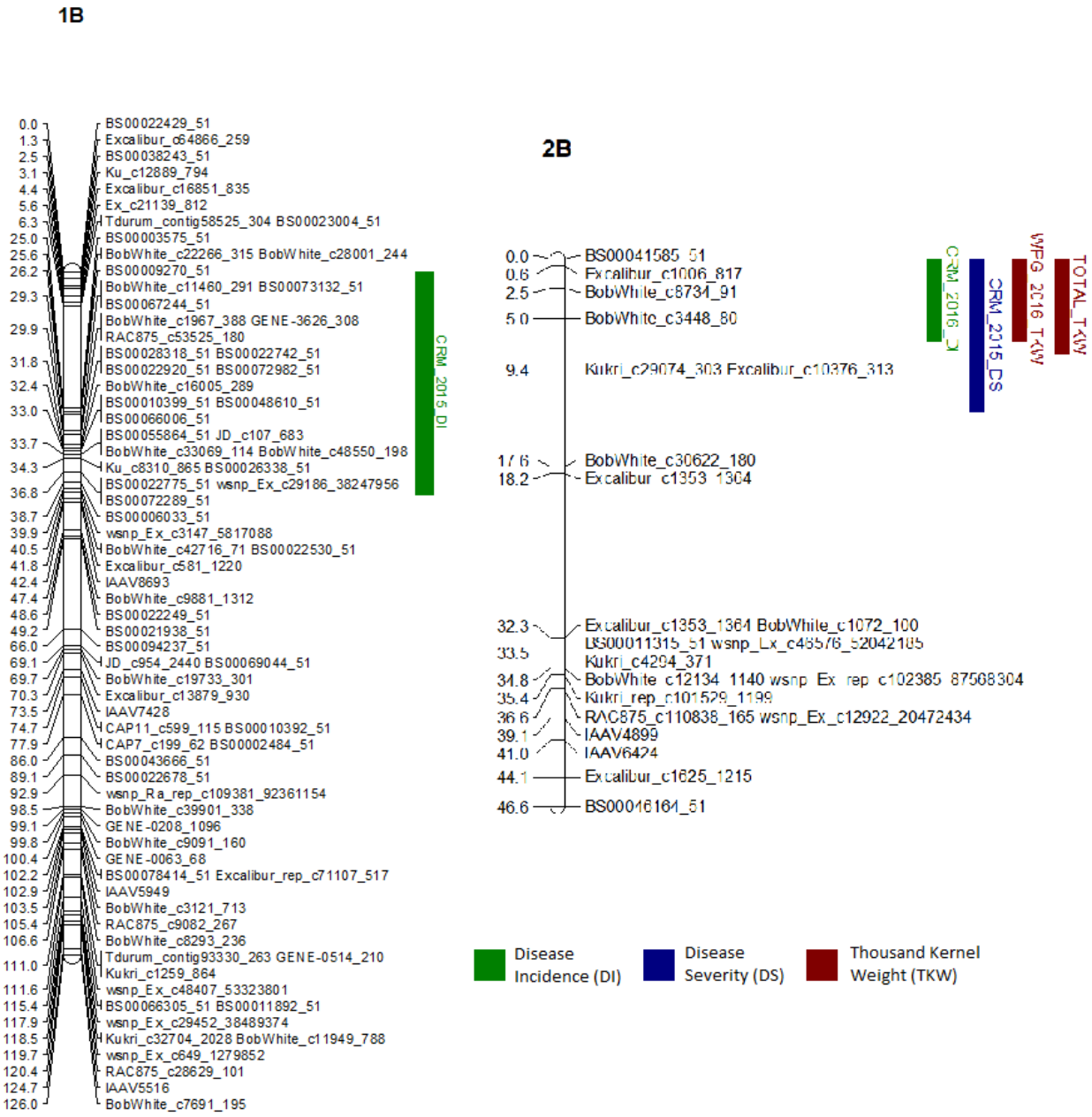

Figure 3.4 Genetic linkage map for disease incidence QTL located on chromosome 1B in a DH population from the cross Flourish/Emerson (161 lines). Genetic linkage map for disease incidence, disease severity and kernel weight QTLs present on the Flourish/Emerson population's chromosome 2B tested at the three environments; Carman (2015), Carman (2016) and Winnipeg (2016). Marker distance is displayed on the left side of the chromosome and the names of markers present are on the right.

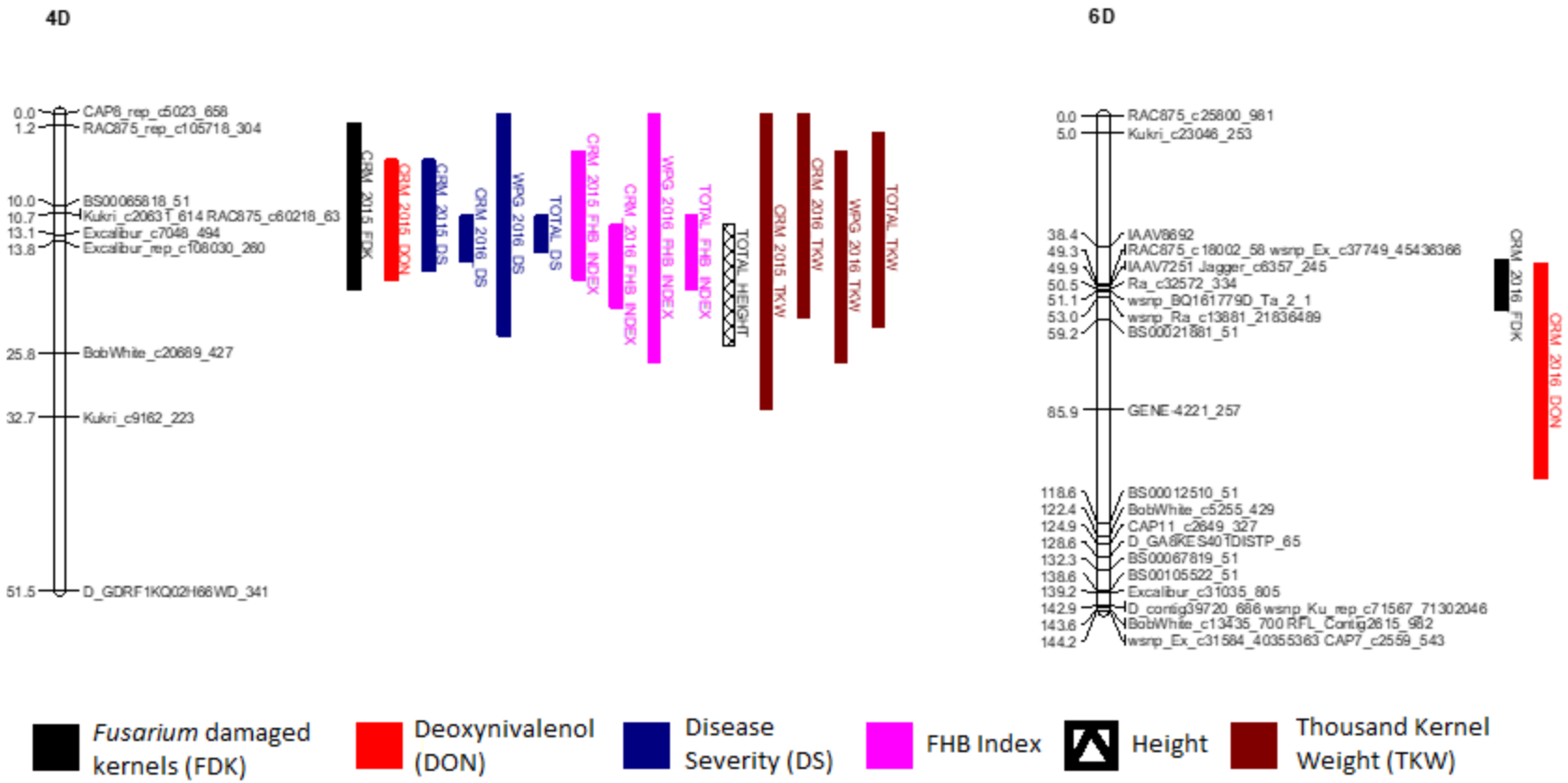

Figure 3.5 Genetic linkage map for DON, FDK, disease severity, FHB index, height and TWK QTL located on chromosome 4D in 161DH lines developed from the cross Flourish/Emerson. Genetic linkage map for DON and FDK QTLs present on the Flourish/Emerson population's chromosome 6D tested at the three environments; Carman (2015), Carman (2016) and Winnipeg (2016), as well as QTL for pooled results. Marker distance is displayed on the left side of the chromosome and the names of markers present are on the right.

3.4 Discussion

Breeding resistance to *Fusarium* head blight in wheat is a complex process. Unlike other plant diseases, FHB resistance is not the result of gene-for-gene interaction, but rather a complex of genes with small effects that can be pyramided together to form resistance (Eckard *et al.* 2015). In this study a FHB resistant hard red winter wheat cultivar, Emerson, was crossed with a susceptible cultivar Flourish. Emerson is noteworthy because, like Sumai 3, it was developed from crossing cultivars with poorer resistance to FHB infection. To date, no published data for the genetic basis of FHB resistance in Emerson are available. Results showed that QTL associated with FHB traits were present on chromosomes 1B, 2B, 4D, 6D and 7A (Table 3.6). Height related QTL were found on chromosomes 2D, 4D and 6A (Table 3.7). The source of most of the resistance in the DH population came from the paternal parent Emerson.

Results showed that QTLs located on chromosomes 7A and 4D were associated with most FHB traits measured and were consistently detected across environments. QTL for DI, DS, FHB Index, % FDK and DON were identified on chromosome 7A and QTL for DS, FHB index, % FDK and DON were identified on chromosome 4D. In addition, the QTL on chromosome 4D was associated with plant height and TKW. The 4D QTL produced stable results for disease severity and FHB index in all three environments. In 2015 the QTL on 4D was also associated with DON and FDK. Peak LOD scores for the 4D QTL occurred at 13cM position.

The 4D QTL for height co-localized with those related to FHB resistant traits. Overall increases in height had a weak negative correlation with decreased FHB index (r=-0.23), % FDK (r=-0.13) and DON content (r=-0.20), and a moderate negative correlation with disease

incidence (r=-0.31) (Table 3.3). The DH population mean value for height (74.8 cm) was slightly lower than the mid-parent mean (75.8 cm) (Table 3.2) and the population means for DS (33.3%) and FHB index (13.1%) were lower than the mid parent means of 45.1% and 24.0%. If looking at correlations for 2015 and 2016 separately, the trend was that increased height was associated with reduced FHB field symptoms (Tables A-2 and A-3). When looking at the frequency distribution of plant height, transgression segregation was present at both extremes. Of the 179 DH lines tested, 52 were shorter in height than Flourish and 33 lines were taller than Emerson. With so many lines showing lower heights yet still retaining FHB resistance does not agree with the common theory that taller plants are less susceptible to FHB (Mesterhazy 1995; Buerstmayr *et al.* 2011). Given that the population had undergone some pre-selection for excessively shorter to medium plant height it is interesting to still see that transgressive segregation still occurred (Fig 3.2). This phenomenon could be the result of a GXE interaction. With some sort of environmental conditions stimulating plant heights in Manitoba compared to where the pre-screening took place in Lethbridge, Alberta.

Alleles for two semi-dwarfing genes, *Rht-B1b* (chromosome 4B) and *Rht-D1b* (chromosome 4D), have been frequently used in wheat breeding. These genes are so popular that over 90% of modern wheat varieties carry at least one of them (Guedira *et al.* 2010). In general, the presence of these alleles leads to an increase in susceptibility to FHB compared to lines containing the wild type alleles (Srinivasachary *et al.* 2008; Mao *et al.* 2010; Yan *et al.* 2011; Saville *et al.* 2012). Screening for the height genes was done for 89 lines of the DH population and for the two parental varieties (Flourish and Emerson). Screening results (Figures A-7 and A-8) found that all the DH lines in the Flourish/Emerson population, as well as the two

parents, carried the *Rht-B1b* dwarfing allele on chromosome 4B and had the wild type allele *Rht-D1a* on chromosome 4D. Related varieties, McClintock and Norstar, contained the wildtype Rht-B1a. In the QTL analysis, for the pooled data, height localized on chromosome 4D (Table 3.7) in the same region as the disease traits and accounted for a small amount of the phenotypic variation in the population (8.6%) and LOD score of 3.134. The source of this QTL was the taller resistant parent, Emerson and is not related to segregation of Rht-*D1b*. It is interesting to note that the QTL for height is only present for the pooled data and not the individual site years (Table 3.7). One reason for the lack of QTL presence in the individual site years could be attributed to the fact that this population had undergone pre-selection for shorter plant height. Populations which are pre-selected for a certain trait give lower LOD scores than populations that did not undergo selection for that trait (Melchinger *et al.* 2012). If the LOD score for height was lower than the threshold of 3 in the individual sites could account for why they were not reported. This QTL's LOD value of 3.134 was very close to the LOD threshold of 3 and could also be a false positive due to the combination of unbalanced datasets. In the current study, Carman (2015) contained only two replicates and 18 fewer lines compared to Carman (2016) and Winnipeg (2016). The presence of a unbalanced dataset increases the chances of false-positives (Wang *et al.* 2012). Using more complex statistical models like a MIXED model does lower the risk but doesn't completely prevent it.

A third major dwarfing gene, *Rht8*, located on the short arm of chromosome 2D has been used in creating semi dwarfing wheat varieties (Korzun *et al.* 1998; Guedira *et al.* 2010; Mao *et al.* 2010). Although it is more frequently found in Asian wheat varieties, roughly 8% of winter wheat varieties grown in the United States carry this gene (Guedira *et al.* 2010). In the

current study a QTL was identified on the short arm of chromosome 2Dx at the Carman (2016) site location and was associated with the taller resistant parent, Emerson (Table 3.7). Further studies could screen this DH population to see if Emerson carries this semi dwarfing gene. The last plant height related QTL in this study was located on chromosome 6A and shorter height allele originated from the susceptible parent, Flourish. However out of the three QTL identified, the markers on 6A show the highest amount of distortion (Table 4-A). Schmolke *et al.* (2005) reported plant height QTL on chromosome 6AL in a Dream/Lynx RIL population and noted that it was related to FHB resistance.

Draeger *et al.* (2007) found multiple FHB resistant QTL linked with the height gene *Rht-D1* and concluded that the wild type allele confers higher resistance. Plant height itself is probably not the cause of increased resistance, but rather genetic linkage or pleotropic effects occur with the dwarfing genes (Draeger *et al.* 2007: Srinivasachary *et al.* 2008). The fact that the height QTL on 4D is co-localized with the FHB resistance QTL could indicate that the FHB resistance genes are linked with height in the Flourish/Emerson DH population.

QTL associated with FDK and DON accumulation were identified on chromosomes 7A, 4D and 6D. Previous studies have found similar results. Draeger *et al.* (2007) identified a QTL for DON reduction on 4DS (R^2=9.1) and QTL for FDK on chromosome 4DS (R^2=11.4) and 7AL (R^2=8.7) in a DH cross between European cultivars: Arina and Riband. The results of the current study produced results at 4D (r^2=13.9) and 7A (r^2=9.6) for reducing DON (Table 3.6). A QTL on chromosome 6D for FDK and DON was detected for the Carman 2016 site with the Emerson alleles reducing disease symptoms. This QTL explained to 9.3% of the phenotypic variation for FDK and 10.4% for DON. Overall, FHB resistance on 6D has been mapped in a RIL line using

Swiss winter wheat cultivars Arina and Forno. The effects of this QTL were major attributing to a 22% phenotypic variation in FHB reistance (Paillard *et al.* 2004). Resistance located on 6D has also been mapped in the American "Wesley" cultivar, along with QTL on 1B and 4D (Eckard *et al.* 2015).

One QTL associated with disease severity (Type II resistance) was identified on chromosome 1B. This was a minor QTL accounting for 9% of the phenotypic variation and only appearing in the Carman (2015) trial. QTL associated with FHB resistance have been reported on the long arm of chromosome 1B. *Qfhs.lfl-1BL* was reported in the winter wheat cultivar Cansas and accounted for reducing disease severity by 42% (Häberle *et al.* 2009). The same allele has been reported in other European winter wheat cultivars including Biscay, History and Pirat (Häberle *et al.* 2009). A minor QTL on chromosme 1BL has also been reported in a DH population from a cross between Arina x NK93604. This QTL co-localized with both increased FHB resistance and increased anther extrusion, linking chromosome 1B with an increase in Type I resistance (Skinnes *et al.* 2010).

Some issues with this experiment were related to poor environmental conditions at the Winnipeg field location and poor site set up for the Carman (2015) field. Winnipeg over the summer of 2016 experienced cooler temperatures, resulting in poor FHB symptom development which were half the size or lower compared to the Carmen test site in 2016 (Table 3.2). Carman (2015) had the additional problem for not having proper FHB check varieties grown within the test as well as containing 18 fewer lines and being one replicate short compared to the two 2016 site locations. Because of these issues additional environments of testing are needed to determine how stable the QTL discovered are. Additional environments

would also verify that none of the QTL detected are false positives. An additional trial and phenotypic evaluation was conducted over the summer of 2017 in Carman, MB but information from that trial was not included in this evaluation. The current study examined Type I resistance (prevention of infection), Type III resistance (prevention of DON accumulation) and Type IV resistance (prevention of kernel damage). Future work on this population could include point inoculation studies in a greenhouse setting to fully examine the presence of Type II resistance in the population. Lastly, one of the height QTL detected in this study was located on chromosome 2D. Future screening of this population could determine if the dwarfing gene *Rht8* is present.

Chapter 4.0 Evaluating the Effectiveness of the biological control agent, DONguard® (active ingredient *Clonostachys rosea*, ACM941) in preventing *Fusarium* Head Blight and Reducing DON mycotoxin

4.1 Abstract

A newly developed bio-fungicide, DONguard®, was evaluated for efficacy in controlling *Fusarium* Head Blight (FHB) infection in wheat. DONguard®, produced by Adjuvants Plus Inc., contains the ACM941 strain of *Clonostachys rosea*. *C. rosea* is a saprophytic fungus which has exhibited strong potential as a biological control agent against a variety of *Fusarium* species through competition and mycoparasitism. This study evaluated the effect of rate and time of application of DONguard® for FHB control in the field as well as compared it to a commercially available chemical fungicide. The bio-fungicide was sprayed on four different spring wheat cultivars ranging in resistance to FHB.

Overall results of the rate of application experiment showed that no matter the rate of application, DONguard® did not significantly improve ($P<0.005$) test weight, thousand kernel weight, FHB Index, FDK or DON levels compared to the *F. graminearum* inoculated control groups. Overall efficacy results were poor for all variables tested. The most concerning result was the overall increase in deoxynivalenol (DON) levels within the grain treated with the bio-fungicide product. Data from the time of DONguard® application test inferred that earlier application times resulted in lowered FHB infections.

High variation and inconsistencies between the results from the two site years is attributed to the environmental effects placed on the bio-fungicide. Unsuitable growing conditions for the *C. rosea* spores could have contributed to poor establishment on wheat spikes and poor antagonistic effects on *F. graminearum*. Within both experimental trials the overall efficacy of Caramba® (metconazole) fungicide was superior compared to DONguard® for all variables tested. DONguard® shows potential as a bio-fungicide product, however, its tendency to increase DON levels within the grain may make it unsuitable for use in the manner tested in this study. More field trials are required to understand the specific environmental conditions needed for this product to be effective.

4.2 Introduction

Fusarium head blight (FHB) is one of the most economically detrimental diseases of wheat. Infection results in the degradation of the quality and overall quantity of grain produced. This disease is the result of fungal infection by a variety of *Fusarium* species (Aoki *et al.* 2014). One of the most prominent *Fusarium* species found in North America is *Fusarium graminearum* Schwabe [teleomorph: *Gibberella zeae* Schwein (Petch)] (Gilbert and Haber 2013). Along with reducing the quality of the grain, *F. graminearum* produces a variety of mycotoxins within the seed, one of which is deoxynivalenol (DON). Deoxynivalenol is classified as a vomitoxin and consuming too much contaminated grain can result in vomiting and nausea. Prolonged consumption of contaminated wheat products can result in neurological damage, and in some cases, death (Rocha *et al.*2005*)*. Deoxynivalenol is a very stable compound and remains stable during milling and baking. Livestock that are sensitive to DON experience the

same symptom as humans when ingesting DON contaminated grain (Pestka 2010, Maresca 2013). Among livestock fed a grain-based diet, pigs are one of the most sensitive to DON's effects. Pigs reared on DON contaminated grains have reduced growth rates and develop reproductive problems (Doll and Danicke 2011, Maresca 2013).

A variety of methods have been developed to suppress FHB infection. These range from developing genetically resistant cultivars, application of fungicides, using biological controls, as well as applying appropriate crop rotation strategies. No single method can fully suppress FHB infection, so a multi-pronged approach is required to reduce losses to FHB. Studies of biological control methods have been gaining momentum as they are perceived to provide a safer alternative to use of fungicides for suppression of plant diseases. Although the use of chemical fungicides is very efficient at reducing plant diseases, public concerns over chemical residues remaining on food products have led to greater focus on alternative prevention methods.

Clonostachys rosea (Schroers, Samuels, Seifert and Gams [Telomorph, *Bionectria ochroleuca* (Schw.) Schroers and Samuels]) (Schroers *et al.* 1999) is a common saprophytic fungus which has attracted the attention of many researchers as a potential biological control agent to suppress a variety of plant diseases. The focus on use of *C. rosea* as a potential biological control agent started in the early 1990's when the pathogenic properties of the fungus were observed against *Botrytis cinerea* infection (Yu and Sutton 1998; Morandi *et al.* 2000). Although naturally occurring in the soil, this fungus can establish on above ground tissue. While it has a higher success rate on wounded, dying, or decomposing plant tissue, it can grow on living tissue (Morandi *et al.* 2000). Previous studies have evaluated *C. rosea* as a seed treatment and a treatment for wheat/corn stubble to reduce primary *Fusarium* inoculum

source levels in the field (Jensen 2000: Luongo *et al.* 2005). The next phase of research evaluates application of *C. rosea* as a foliar spray to reduce FHB infection in the field.

The objective of this study was to determine the appropriate time and rate of application needed for a newly developed foliar bio-fungicide product, DONguard®. DONguard® contains the active agent, *C. rosea* strain ACM941. The efficacy of the product on four spring wheat cultivars, varying in resistance to FHB, was also compared to the fungicide Caramba® that is registered for suppression of FHB in wheat.

4.3 Materials and Methods

4.3.1 Treatments and their application

Two field experiments were conducted in the summers of 2015 and 2016 at the University of Manitoba, Winnipeg field site. One test (hereinafter referred to as the "Rate Test") evaluated the optimal rate of DONguard® application in controlling FHB in three spring wheat genotypes. The second test studied (hereinafter referred to as the "Time Test"), examined the optimal time of application of DONguard® to reduce FHB in four different genetic lines. Both experiments were set up as a randomized split plot design, generated using AGROBASE.

A) Rate test

Nine different treatments (Table 4.1) served as the main plot and three spring wheat cultivars/lines ranging in susceptibility to *F. graminearum* were the subplots. The three cultivars were CDC Teal (susceptible) (Hughes and Hucl, 1993), AC Cora (intermediate) (Townley-Smith and Czarnecki 2008) and FHB 37 (moderately resistant). A total of four replicates were used in both experimental years.

A total of 1,200 viable seeds were planted for each subplot on May 22, 2015 for the 2015 trial and May 20, 2016 for the 2016 trial. Subplots were 1.33 x 3 meters in size and consisted of six rows spaced 17 cm apart. Each main plot was separated by a tall wheat cultivar, Amazon, to act as a buffer against contamination from neighboring plot treatments.

After maturity, subplots were harvested with a Wintersteiger Elite combine on September 1, 2015 and August 30, 2016. Grain from each subplot was collected in cotton bags, labeled and dried using forced air-drying beds for three days after harvest.

Table 4.1: List of the nine main plot treatments used in the rate experiment. The treatments include the four main classes of treatments and the six sub-treatments within the DONguard® grouping.

	Treatment Type	Rate of Application (g ha^{-1})	Amount Product in 0.9L water	Time of Application	Active Ingredient
1	DONguard® + *F. graminearum*	150	0.06 g	Heading	*C. rosea* (ACM941)
2			0.06 g	Flowering	
3		300	0.12 g	Heading	
4			0.12 g	Flowering	
5		450	0.18 g	Heading	
6			0.18 g	Flowering	
7	Caramba® + *F. graminearum*	300	0.9 mL	Flowering	Metconazole
8	*Fusarium* Inoculation Control		NA	Flowering	NA
9	Un-inoculated- Untreated Control		NA	Flowering	NA

Note: NA stands for Not Applicable

The treatments as described in Table 4.1 consisted of the biological control agent DONguard® applied at different rates, a fungicide check and inoculated and uninoculated controls.

B) Time test

For the summer of 2015, this trial was laid out in a six-replicate randomized split plot design. Treatment applications were the main plot effects and the differing genotypes were the subplot effect. For this test four genotypes ranging in FHB severity were used. These included three hexaploid bread wheat varieties; CDC Teal (S) (Hughes and Hucl, 1993), AC Cora (I) (Townley-Smith and Czarnecki 2008) and FHB 37 (MR) and one tetraploid durum wheat cultivar; AC Morse (S) (Plant Varieties Journal 2010). A total of six application treatments were tested in this year. These treatments correspond to numbers 1-4, 7, 8 in Table 4.2. Seventy seeds were planted in a single one-meter row for each subplot. These were planted on May 22, 2015. All four rows within each main plot were given one of six different treatments. A total of 144 rows were planted (6 treatments x 4 genotypes x 6 replicates). All main plots were separated by a 4 row, one-meter long border of a tall wheat cultivar, Amazon, to minimize cross contamination of treatments.

In the 2016 field trial an additional two treatment types were added resulting in a total of eight treatments being used (Table 4.2). Like the previous year, six replicates were used making a total of 192 rows being tested. The 2016 field trial was planted on May 20, 2016.

The following treatment types were used in the experiment:

Table 4.2: List of the eight treatments used in the time experiment. The treatments include the four main classes of treatments and the six sub-treatments within the DONguard® grouping.

No.	Treatment Type	Time of Application
1	*C. rosea* (ACM941) + F. *graminearum*	Flag Leaf
2		Heading
3		Anthesis
4*		Flag Leaf and Heading
5	Metconazole + *F. graminearum*	Anthesis
6*	*C. rosea* (ACM941) Control	Anthesis
7	*Fusarium* Inoculation Control	Anthesis
8	Un-inoculated Control	Anthesis

Note: Treatment numbers 4 and 6 were additions to the 2016 field trial and were not included in the 2015 year.

Treatment numbers 5 and 6 were added for the 2016 field trial. The *C. rosea* control was included to see what effect, if any, the biological control by itself would have on the wheat plants. Based on results from the previous year, the double inoculation treatment of DONguard® at the flag leaf and heading developmental stages was included. This treatment was included to see if doubling the application would increase the efficacy of the biological control treatments.

i. Bio-fungicide: DONguard®

DONguard® is a product developed by Adjuvants Plus Inc. It is comprised of the *Clonostachys rosea* strain ACM941 and a 5% C-Wet water conditioning agent (Sylvan BioProducts 2015). The desired use of this product is to prevent the development of FHB on

wheat spikes and the accumulation of DON in grain. *C. rosea* spores are sensitive to ultraviolet (UV) light and no UV protective agent was included in the DONguard® formula. Therefore, application of this product was conducted prior to sunset to limit photo-degradation. DONguard® was supplied by Adjuvant Plus as a brown powder, to be dissolved in water. Preparation of DONguard® solution was conducted one hour prior to application. This product was applied as a foliar spray.

Differing concentrations of DONguard® were made for the Rate Test experiment by adding different amounts of DONguard® powder in 0.9 Liters of water. One bottle was made for the sub plot. The different amounts of DONguard®, corresponding to the different rates of application, are displayed in Tables 4.1. For the Time Test, a concentration of 0.12g DONguard® per 0.9L of water was used. One bottle was used for applying roughly 18 rows.

ii. Fungicide

The fungicide Caramba® was used as a comparison to evaluate the efficacy of DONguard®. Caramba® is produced by BASF Chemical Company and is registered for suppression of FHB in wheat (BASF 2012). Caramba® is a Group 3 fungicide with the active ingredient, metconazole. Class 3 fungicides are classified as demethylation inhibitors (DMI) and work by disrupting the sterol biosynthesis pathway resulting in the prevention of the formation of cell membrane formation in the fungus (Gilbert and Haber 2013). For both experiments, the fungicide treatment was applied at the beginning of the flowering stage (Zadoks 60). During this experiment Caramba was applied as a foliar spray.

iii. *Fusarium graminearum* inoculum preparation

See section 3. 3 for *Fusarium graminearum* inoculum preparation.

iv. Timing and method of application

Application of treatments were performed using a CO_2 backpack sprayer calibrated at 30 psi. A six-nozzle boom attachment allowed for all six rows in the plot to be treated simultaneously with 150mL of solution (Rate Test). For the Time test no attachment was used, so each row could be given a treatment separately. Treatments were sprayed directly onto the wheat spikes.

The timing of treatment application was dependent on the growth stages of the crop. Zadok wheat growth stages were used as a reference guide to ensure that each treatment was applied at a consistent time between replicates. The number of days to heading and anthesis for each plot was recorded. Depending on the treatment (Tables 4.1 and 4.2), application of the biological control occurred when 50% of the subplot had reached either the flag leaf stage (Zadoks scale: 38) heading growth stage (Zadoks scale: 50) or the beginning of anthesis (Zadoks scale: 60) (Zadoks *et al.*, 1974). Plots were mist irrigated (10 minutes/hour for 10 hours) after application unless *F. graminearum* inoculations were occurring. To allow *C. rosea* to establish on the wheat spikes, application of *F. graminearum* occurred at least 48 hours after the DONguard® application.

Fungicide application was done in a similar fashion to the subplots receiving DONguard® treatments at the flowering stage. Subplots were sprayed when 50% of the plants reached Zadoks growth stage 60. The fungicide was prepared by diluting 0.9 mL of Caramba® fungicide

in 0.9L of water prior to application (recommended rate used). Like the biological control treatments, *Fusarium* inoculation did not occur until 48 hours had passed.

All groups, except the un-inoculated un-treated control, received two 0.9L inoculations of *F. graminearum* macroconidia spore solution. Four *F. graminearum* isolates were in the inoculum. Two 3A-DON chemotypes, M7-07-01, M9-07-1 and two 15 A-DON chemotypes, M1-07-2, M3-07-2, isolates were used. These isolates were obtained from Dr. Jeannie Gilbert at the Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg Manitoba. The solution was a 50,000 macroconidia/mL concentration and contained equal portions of the four isolates. Each bottle of inoculum contained 20mL of Tween 20 oil to break up the surface tension of the water and enhance spread of the inoculum. After inoculation the plants were irrigated using a mist irrigation system for 10 minutes/hour for 10 hours.

4.3.2 Disease Evaluation

i. Field evaluation

Eighteen to twenty-one days after the initial *F. graminearum* inoculation or water control application, disease severity (DS) and disease incidence (DI) were evaluated visually by two evaluators. The mean values from the two evaluators was used for the analysis. Disease incidence was determined by estimating the percentage of spikes within a subplot infected by *F. graminearum*. Disease severity was determined by looking at the infected spikes and averaging the severity of the infection. These two percentage values were used to calculate the *Fusarium* head blight (FHB) Index. The following formula was used in the calculation:

$$FHB\ Index = (Mean\ DS\ X\ Mean\ DI)/100$$

ii. Post harvest evaluation

a. Determining weight and moisture content

Plots were harvested with a small plot, Wintersteiger Elite combine set with a reduced wind speed to retain as many *Fusarium* damaged kernels (FDK) as possible. Harvested samples were placed on a drying bed for three days. Once drying was complete, each sample was cleaned to remove any excess chaff and straw in the sample. First, any whole spikes found in the sample were hand threshed. Each sample was then run through a blower set at a low airspeed to allow chaff to be removed without removing the FDK. Samples were sieved with a 5/64" Triangle screen to remove any broken grain and weed seeds. A Carter dockage tester was used for samples with a high proportion of weed seeds. A No.2 screen was used to separate out large debris in the sample and a 5/64 ¾ S screen was used to separate out broken kernels. A blank pan was used at the bottom to catch any broken and small FDKs. Any whole FDKs which fell through the sieve were retrieved and placed back into the sample. Moisture content was determined by using a Dicky John moisture meter (2015 only, no difference was found, data not included). Thousand kernel weights were calculated by first counting out 1000 kernels and weighing them on a LC4800P scale. Test weights were determined using a manual test weight apparatus (Canadian Grain Commission, 2017). The apparatus consisted of a Cox funnel, USA, a calibrated 0.5L measuring cup and a dish to catch excess grain. Grain was first funneled into the 0.5L cup, a hard wood striker was then used to scrape off excess grain on top of the measure's rim. The weight of the grain in the cylinder was recorded.

b. FDK and DON analysis

See section 3.3.6 for details on how FDK (%) and DON content was determined.

4.3.3 Statistical analysis

Trial data were analyzed using SAS statistical software (SAS version 9.4) (SAS institute Inc., Cary, NY, USA). Tests for homogeneity of variance using PROC MIXED were conducted to determine whether the data could be pooled. Results from the test (data not shown) determined that the variation between the two years in both experiments was too great for data combination. The following analyses were conducted separately between 2015 and 2016 site data. Variables measured in the two years underwent analysis of variance (ANOVA) testing in Proc MIXED. The model statement listed the treatments, genotypes and the treatment*genotype interaction as fixed effects. The rep(treatment) was used as the random effect. Least significance difference (LSD) mean values of the groups and subgroups were analysed to determine whether there were any significant differences between them.

Due to poor seed set from water logging in the field, results for FDK, TKW and DON were extremely poor in the 2016 field site year. Large amounts of missing data were present for AC Morse which made SAS unable to estimate values for some of the treatment groups. Because of this, AC Morse was excluded for ANOVA, LSD mean and efficacy evaluation. In the Time Test, Tukey-Kramer adjusted least squared mean values of the groups and subgroups were analysed to determine whether there were any significant differences between them. Tukey-Kramer adjustments, repeated/group function and ddmf=satterthwaite, were used to help with the heterogeneous variances and high number of missing data.

Comparisons of the efficacy of DONguard® and Caramba® treatments relative to the *F. graminearum* inoculated control groups were conducted. Both overall variable means and subgroup means were examined. To test for efficacy, the following formula was used:

$$Efficacy\ percentage = \frac{Parameter\ mean\ of\ the\ F.graminearum\ inoculated\ control - \ Parameter\ mean\ of\ group\ tested}{Parameter\ mean\ of\ the\ F.graminearum\ inoculated\ control} * 100$$

To test the efficacy percentage of the test weight and 1000 kernel count numbers, the following equation was used:

$$Efficacy\ percentage = \frac{Parameter\ mean\ of\ group\ tested - \ Parameter\ mean\ of\ the\ inoculated\ control}{Parameter\ mean\ of\ the\ inoculated\ control} * 100$$

4.3.4 Weather temperatures

Weather data were taken from temperatures recorded on the University of Manitoba's Fort Gary campus weather station over the summers of 2015 and 2016. From the data, a graph of the average temperatures was generated in Microsoft Excel® 2016.

4.4.1 Rate of DONguard® (*C. rosea* ACM941) application results

4.4 Results

4.4.1.1 ANOVA

Analyses of variance showed that there were significant differences (P<0.001) among genotypes for all variables measured in both 2015 and 2016 (Tables 4.3 and 4.4). Treatments were significantly different for all variables in both site years except for FDK in 2016 (Table 4.4). Treatment* genotype interaction was significant for all variables measured in 2015 and only for DON in 2016.

Table 4.3. Analysis of variance for *Fusarium* head blight index (FHB), *Fusarium* damaged kernel (FDK), deoxynivalenol (DON), test weights and thousand kernel weights (TKW) in the 2015 Rate of DONguard® Application Test.

Source of Variation		FHB Index (%)		FDK (%)		DON (ppm)		Test Weight (kg/hL)		TKW (g)	
	DF[a]	Mean squares	Probability	Mean squares	Probability	Mean squares	Probability	Mean squares	Probability	Mean squares	Probability
Treatment	8	4,365	<.0001	913	<.0001	482	<.0001	704	<.0001	39	0.0007
rep(Treatment)	27	124	0.0001	103	0.0061	41	0.0502	77	0.0019	8	0.0476
Genotype	2	23,323	<.0001	2,221	<.0001	2,012	<.0001	3,699	<.0001	433	<.0001
Treatment * Genotype	16	369	<.0001	76	0.0272	56	0.0105	83	0.0032	8	0.0476
Error	54	38		41		24		31		5	

[a] Degrees of Freedom

Table 4.4 Analysis of variance for *Fusarium* head blight index (FHB), *Fusarium* damaged kernel (FDK), deoxynivalenol (DON), test weights and thousand kernel weights (TKW) in 2016 Rate of DONguard® Application Test.

Source of Variation		FHB Index (%)		FDK (%)		DON (ppm)		Test Weight (kg/hL)		TKW (g)	
	DF[a]	Mean squares	Probability	Mean squares	Probability	Mean squares	Probability	Mean squares	Probability	Mean squares	Probability
Treatment	8	429	0.0281	28	0.2411	84	0.0367	81	<.0001	9	0.0372
rep(Treatment)	27	163	0.0883	20	0.0005	34	0.0045	10	0.7078	3	0.0033
Genotype	2	14,831	<.0001	383	<.0001	1,512	<.0001	340	<0.0001	122	<.0001
Treatment * Genotype	16	159	0.1303	6	0.6327	27	0.0469	16	0.2637	2	0.2932
Error	54	106		7		15		13		1	

[a] Degrees of Freedom

4.4.1.2 Assessment of FHB disease incidence, severity, FHB Index, FDK and DON

In general disease levels were higher in 2015 than in 2016. The results for both 2015 and 2016 show few differences among the treatment means for FHB index, FDK and DON for the *F. graminearum* inoculated control and the inoculated *C. rosea* treatments. In 2015 (Table 4.5) FHB disease incidence in the C. rosea treated groups and the metconazole group did not significantly differ from the *F. graminearum* control. Plants given an application of *C. rosea* at flowering showed higher disease incidence than plants treated at the heading stage with the same rate of application. There was no significant difference between disease severity symptoms and *the F. graminearum* control. The metconazole treatment was significantly lower than both the *C. rosea* treatments and the *F. graminearum* control in 2015. FHB Index results showed that all biological control treatments, as well as the fungicide treatment, did not differ significantly from the *F. graminearum* inoculated control group. All treatments had significantly higher FHB index compared to the untreated uninoculated control group. Between *C. rosea* treatments, there was no significant difference among the different rates of application used at a specific stage of application. The time of application had some effect with those treated at the later, flowering stage, showing higher FHB index values compared to those treated at the heading stage. Application at the lowest rate did show some significant differences between the time of application, with the treatment at heading being significantly lower than at flowering. All treatments of *C. rosea* applied at heading were not significantly different from the metconazole treatment in 2015. All DONguard® treatments at the flowering stage had significantly higher FHB values than metconazole in 2015.

In 2016 (Table 4.5) the results show that all treatments using *C. rosea* at any rate or stage of application did not significantly change the FHB index values from the *F. graminearum* inoculated control group. Although not significantly different, the results from plants treated at the flowering stage displayed higher FHB index values. In 2016, the fungicide treatment did not differ significantly from the un-treated and un-inoculated control. Both treatments had significantly lower disease than the *C. rosea* treatments. 2016 disease incidence results show that all *C. rosea* treatments, aside from application at 300g/ha at heading, did not significantly differ from the *Fusarium* inoculated control group. *C. rosea* applied at 300g/ha (heading stage) and the metconazole treatments could significantly lower disease incidence. The metconazole treatment was also not significantly different from the uninoculated untreated control group. Like the previous year, disease severity values for all *C. rosea* treated groups, no matter the time or rate of application did not significantly differ from both the *Fusarium* inoculated control groups. All *C. rosea* treatments (excluding Flowering @ 300g/ha) did not significantly differ from the untreated control. The metconazole group displayed the lowest disease severity of all groups but was not significantly different from the untreated control.

The amount of FDK (Table 4.5) from all *C. rosea* treated samples were not significantly different from the *F. graminearum* inoculated control group (both years). Within the *C. rosea* treated samples, there was no observable difference between the different rates of application and the timing of application in 2015. In 2015 FDK from fungicide treated samples were significantly lower than from the biological control group and the *F. graminearum* inoculated control group, but were significantly higher than the un-treated un-inoculated control. Disease levels were lower in 2016 than 2015, resulting in lower FDK values. As a result, there are not as

many clear differences between treatments in 2016. All biological control treatments as well as the fungicide treatments were not significantly different from the *F. graminearum* inoculated control.

Results of DON content in 2015 showed that all *C. rosea* treated and fungicide treated groups were not significantly different from the *F. graminearum* control. Rates of application did not affect DON accumulation in the seed. However, later application tended to lead to higher DON values compared to the *F. graminearum* inoculated control. Of these, the lowest application rate of 150 g/ha at flowering gave a mean result of 24.21 ppm of DON in the kernels. This value was 5.38 ppm higher than the *F. graminearum* inoculated control's 18.83 ppm. Application of a fungicide treatment produced the lowest DON concentration (15.53 ppm) compared to any *C. rosea* treatment group. The fungicide treatment was significantly lower than plants treated with *C. rosea* at the flowering stage.

Results for DON accumulation from 2016, agreed with the 2015 results in that *C. rosea* treatments did not significantly differ from the *F. graminearum* control group. However, unlike the previous year, the results showed that the fungicide treatment (5.14 ppm) did significantly differ from the inoculated control (8.58 ppm). Among the different *C. rosea* treatments, application at the later growth stage led to higher DON concentrations than applications at heading. The one exception was application at the flowering stage at a rate of 450 g/ha. This treatment resulted in the lowest DON values among the biological control treatments and was the only biological control treatment that resulted in a lower concentration (7.97 ppm) compared to the *F. graminearum* control group (8.58 ppm). Although not significantly different, all other applications of the biological control resulted in DON values higher than the *F.*

graminearum control, the highest of those being the application at 150 g/ha at the flowering stage. This trend was similar to the 2015 data.

Although treatment * genotype interactions were statistically significant for all variables measured in 2015 and for DON in 2016, the proportion of variance attributed to the interactions was relatively small compared to the main effects of treatment and genotype (Tables 4.3 and 4.4). For all variables, there was no significant improvement from any of the *C. rosea* treatment groups compared to the *F. graminearum* control when looking at each of the three genotypes separately (Appendix Table A-6, A-7). Mean values for each genotype are displayed in Table 4.6. The susceptible cultivar, CDC Teal, consistently gave the highest results for all parameters tested in both 2015 and 2016.

Table 4.5 LS means for disease incidence (%), disease severity (%), FHB index (%), *Fusarium d*amaged kernels (%) and deoxynivalenol levels (ppm) for the different treatment types tested in 2015 and 2016 site year data from the University of Manitoba field location for the DONguard ® Rate of Application Test.

	Treatment	Stage of App.	Rate of App. (g ha^{-1})	Disease incidence (%)		Disease severity (%)		FHB Index (%)		FDK (%)		DON (ppm)	
				2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
1	*C. rosea*	Heading	150	71.25 c	44.38 ab	74.38 a	38.75 ab	54.58 bc	22.26 a	28.29 a	7.22 abc	18.88 abc	8.87 ab
2			300	75.00 c	38.75 bc	74.50a	36.46 ab	57.45 abc	19.45 ab	28.98 a	6.64 ab	18.67 abc	9.31 a
3			450	75.13 bc	46.88 ab	75.54 a	35.00 abc	58.03 abc	20.54 ab	30.87 a	6.60 ab	18.29 bc	8.84 a
4		Flowering	150	86.92 a	52.92 a	75.08a	36.25ab	65.29 a	24.03 a	29.65 a	8.08 a	24.21a	9.58 a
5			300	83.71 ab	49.58 a	75.38 a	45.42 a	63.65 ab	27.46 a	31.01 a	7.05 ab	21.79 ab	9.37 a
6			450	84.71 a	47.50 ab	74.13 a	38.75 ab	63.58 ab	23.23 a	29.28 a	6.32 ab	20.79 ab	7.97 b
7	Metconazole	Flowering		76.50 bc	32.29 cd	61.17 b	25.00 c	49.38 c	9.53 c	20.35 b	4.98 bc	15.53 c	5.14 b
8	*F. graminearum* Control	Flowering		78.79 abc	47.08 a	72.83 a	33.96 b	58.00 abc	20.47 a	29.31 a	7.97 ab	18.83 abc	8.58 a
9	Untreated Uninoculated control	Flowering		9.04 d	23.12 d	37.7 c	29.58 bc	3.5 d	10.58 bc	4.16 c	3.23 c	2.08 d	1.61 c

Note: Values which share the same letter grouping do not significantly differ at P=0.05

Table 4.6 LS means for disease incidence (%), disease severity (%), FHB index (%), *Fusarium* damaged kernels (%) and deoxynivalenol levels (ppm) for the three different spring wheat genotypes (AC Cora, CDC Teal and FHB 37) tested in 2015 and 2016 site year data from the University of Manitoba field location for the DONguard ® Rate of Application Test.

Genotype	Resistance	Disease Incidence (%)		Disease Severity (%)		FHB Index (%)		FDK (%)		DON (ppm)	
		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
AC Cora	I[a]	63.96 c	43.33 b	77.92 b	29.93 b	51.98 b	4.78 b	17.78 c	4.78 b	12.99 b	5.85 b
CDC Teal	S[b]	81.85 a	64.93 a	91.76 a	62.57 a	78.37 a	10.21 a	33.48 a	10.21 a	26.31 a	14.90 a
FHB 37	R[c]	67.88 b	19.24 c	37.22 c	13.89 c	27.47 c	4.36 b	26.13 b	4.36 b	13.76 b	2.34 c

Note: Values which share the same letter grouping do not significantly differ at P=0.05

a. Intermediate resistance, b. Susceptible, c. Resistant

4.4.1.3 Assessment of sample weights (test weights and thousand kernel weights)

Overall, results of the different treatment groups on the test weights showed that there was no major difference in the test weight of the seeds in the treated samples compared to the *F. graminearum* inoculated control in either 2015 or 2016 (Table 4.7). One exception was that the *C. rosea* treatment applied at 450 g/ha during the flowering stage had a significantly lighter test weight than the *F. graminearum* control in 2015. This treatment was also the only one to be significantly lighter than the fungicide treatment. Out of all DONguard® treatments, the application of 150 g/ha at heading gave the largest weight in 2015. This weight was also significantly heavier than DONguard® applications given at flowering (150 g/ha and 450 g/ha). In 2016, the test weights for the fungicide treatment was significantly heavier compared to all *C. rosea* treatments, except for when it was applied at the heading stage at a rate of 150 g/ha. All treatments in both years were significantly lower than the untreated uninoculated control. Within the same growth stage, rates of application did not significantly affect test weights of the samples.

Results of the different treatment groups on the thousand kernel weight values displayed no major difference between treated samples and the *F. graminearum* inoculated control in both 2015 and 2016 (Table 4.7). In 2015, all *C. rosea* treatments and the fungicide treatment had significantly lower TKWs than the untreated uninoculated control. In 2016, the inoculated control (26.7g) was not significantly different from the uninoculated untreated control (27.83 g). All biological control groups were significantly lighter than the untreated and uninoculated control in 2015. Within the *C. rosea* treatments there were no significant differences among the different rates of application at specific growth stages. Between all the

treated groups, the fungicide treatment resulted in the highest weight (27.63g). This value was in between the two control groups and was not significantly different from the untreated uninoculated control.

Mean results for each genotype (Table 4.8) showed that the moderately resistant cultivar, FHB 37, consistently gave the heaviest test weights and thousand kernel weights. In both 2015 and 2016, the susceptible cultivar, CDC Teal gave the lightest thousand kernel weight results. In 2015 CDC Teal had the lightest test weight, while in 2016 the intermediate cultivar, AC Cora, displayed the lightest test weight.

Table 4.7 LS means for test weights (Kg/hL) and thousand kernel weights (g) for the different treatment types tested in 2015 and 2016 site year data from the University of Manitoba field location for the DONguard ® Rate of Application Test.

	Treatment	Stage of Application	Rate of Application (g ha^{-1})	Test Weights (kg/hL)		Thousand Kernel Weight (g)	
				2015	2016	2015	2016
1	*C. rosea*	Heading	150	66.08 b	75.73 bc	27.18 b	25.95 bc
2			300	64.77 bc	74.92 c	27.77 b	25.25 c
3			450	64.07 bcd	74.57 c	27.18 b	25.32 c
4		Flowering	150	62.24 cd	75.29 c	26.34 b	26.22 bc
5			300	64.25 bcd	75.54 c	27.27 b	26.36 abc
6			450	62.34 d	75.10 c	26.38 b	26.11 bc
7	Metconazole	Flowering		66.29 bc	77.07 ab	32.35 b	27.63 ab
8	*F. graminearum* Control	Flowering		65.18 bc	75.82 bc	28.48 b	26.7 abc
9	Untreated Uninoculated Control	Flowering		73.92 a	78.37 a	32.26 a	27.83 a

Note: Values which share the same letter grouping do not differ significantly (P=0.05)

Table 4.8 LSD means for test weights (Kg/hL) and thousand kernel weights (g) for the three different genotypes tested (AC Cora, CDC Teal and FHB 37) in 2015 and 2016 site year data from the University of Manitoba field location for the DONguard ® Rate of Application Test.

Genotype	Resistance	Test Weight (kg/hL)		Thousand Kernel Weight (g)	
		2015	2016	2015	2016
AC Cora	I [a]	72.26 a	80.82 b	27.36 b	24.69 c
CDC Teal	S [b]	63.86 b	78.86 c	24.53 c	25.99 b
FHB 37	R [c]	72.38 a	81.84 a	31.43 a	28.32 a

Note: Values which share the same letter grouping do not differ significantly (P=0.05)
a. Intermediate, b. Susceptible, Resistant

4.4.1.4 Efficacy of DONguard® compared to fungicide and *F. graminearum* control

No clear pattern was observed in the efficacy of different rates of DONguard® applications. Overall, the use of DONguard® compared to the *Fusarium* inoculated control group was found to increase FHB disease incidence, disease severity, FHB Index and DON when applied at the later flowering stage (Table 4.9). When applied at a rate of 150 g/ha, disease incidence values were increased by 10.3% in 2015 and 12.4% in 2016. In 2016 disease severity increased by 33.8% when *C. rosea* was applied at a rate pf 300g/ha and by 14.1% when applied at a rate of 450 g/ha.

When applied at the earlier heading growth stage, DONguard® treated groups were found to have lower disease incidence compared to the *Fusarium* control (Table 4.9). The largest improvement (17.7%) was observed in 2016 when DONguard® was applied at a rate of 300g/ha. Small improvements in FHB index values were also observed when applied DONguard® was applied during heading at a rate of 300 g/ha in both 2015 and 2016.

Aside from application at 150g/ha during flowering, DONguard's® ability to lower FDK values was high in 2016 (Table 4.9). In 2016 the efficacy of the product improved as the rate of application increased. The two highest reductions in FDK were observed when application was given at a rate of 450 g/ha. When applied at flowering FDK reduced by 20.7% and at heading it was reduced by 17.2%. However, it is concerning that although FDK was reduced, the level of DON accumulated in the grain increased. The only DONguard® treatment which gave a lower DON content value compared to the *Fusarium* control group in 2016, was the application given at a rate of 450 g/ha at the flowering stage (Table 4.9). This treatment lowered DON content by

7.1%. The highest increase in DON was observed in both years when DONguard was applied at a rate of 150 g/ha at the flowering stage. For this treatment DON increased by 28.6% in 2015 and 11.7% in 2016.

The fungicide treatment gave the largest improvements in all parameters tested when compared to the *F. graminearum* inoculated control group for disease incidence (2016 only), disease severity, FHB Index, FDK and DON. In 2015 FDK was reduced by 30.57%. In 2016 larger improvements were seen with the fungicide lowering disease incidence by 31.4%, disease severity by 26.4%, FHB Index values by 53.4%, FDK values by 37.5% and DON levels by 40.1% compared to the inoculated control.

Treatment*genotype interaction efficacies are displayed in Tables A-8 and A-9. In both years the resistant line displayed the highest reductions in FDK when treated with DONguard®. In 2015 the highest reduction was observed when FHB 37 was treated with DONguard® at a rate of 150 g/ha at heading (19.0%). In 2016 the highest FDK reduction for FHB 37 was observed when DONguard® was applied at a rate of 300 g/ha at heading (38.4%). In 2015 the intermediate cultivar, AC Cora, displayed no improvements for FDK reduction except when application was given at a rate of 450 g/ha during heading (16.2%). In 2016, AC Cora had positive improvements in FDK levels when treated with DONguard® no matter the time or rate of application. The highest FDK reduction (42.5%) for AC Cora was when DONguard® was applied at a rate of 300 g/ha at heading. Application of DONguard® at heading, no matter the rate of application, consistently improved DON content in both 2015 and 2016 for AC Cora. The largest improvement in 2015 was a DON reduction of 34.4% when applied at a rate of 150 g/ha

and in 2016 the largest reduction was 18.6% when applied at a rate of 300 g/ha. For both years the resistant cultivar, FHB 37 showed the highest increased in DON content.

Table 4.9: 2015 and 2016 efficacy percentage data of both the DONguard® (*C. rosea*, ACM941) and Caramba® (metconazole) treatments for disease incidence, disease severity, FHB index, Fusarium damaged kernels and Deoxynivalenol compared to the *Fusarium graminearum* inoculated control groups for the Rate of Application Test.

	Treatment	Stage of Application	Rate of Application (g ha^{-1})	Disease Incidence (%)		Disease Severity (%)		FHB Index (%)		*Fusarium* Damaged Kernels (%)		Deoxynivalenol (ppm)	
				2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
1	***C. rosea***	**Heading**	**150**	9.6	5.7	-2.1	-14.1	5.9	-8.7	3.5	9.4	-0.3	-3.4
2			**300**	4.8	17.7	-2.3	-7.4	1.0	5.0	1.1	16.7	0.9	-8.5
3			**450**	4.7	0.4	-3.7	-3.1	-0.1	-0.3	-5.3	17.2	2.9	-3.0
4		**Flowering**	**150**	-10.3	-12.4	-3.1	-6.7	-12.6	-17.4	-1.2	-1.4	-28.6	-11.7
5			**300**	-6.2	-5.3	-3.5	-33.8	-9.7	-34.1	-5.8	11.5	-15.7	-9.2
6			**450**	-7.5	-0.9	-1.8	-14.1	-9.6	-13.5	0.1	20.7	-10.4	7.1
7	**Metconazole**	**Flowering**		2.9	31.4	16.0	26.4	14.9	53.4	30.6	37.5	17.5	40.1

Note: Positive percentages mean that those trials had lower values compared to the inoculated control group.

4.4.2 Time of DONguard® (*C. rosea* ACM941) application results

4.4.2.1 ANOVA

Results from the analysis of variance (ANOVA) test for 2015 showed significant variation for treatment and genotype for all variables measured (Table 4.8). The treatment*genotypes interaction in 2015 was significant for FHB index (P=0.0101), FDK (P=0.0061) and for DON accumulation (P=<0.0001). No significant interaction was present for disease incidence or disease severity. ANOVA results for 2016 (Table 4.9) show that the treatments and genotypes differed significantly from one another. The interaction between genotype and treatment was also significant for Disease incidence, FHB index, FDK and DON.

Table 4.10. Analysis of variance for disease incidence, disease severity, *Fusarium* head blight index (FHB), *Fusarium* damaged kernel (FDK), and deoxynivalenol (DON) in 2015 for the Time of Application Test.

Source of Variation		Disease Incidence (%)		Disease Severity (%)		FHB Index (%)		FDK (%)		DON (ppm)	
	DF[a]	Mean squares	Probability	Mean squares	Probability	Mean squares	Probability	Mean squares	Probability	Mean squares	Probability
Treatment	5	5,370.19	<.0001	5,795.29	<.0001	4,411.23	<.0001	606.83	<.0001	363.13	<.0001
Rep(Treatment)	30	242.48	02394	194.43	0.6115	222.55	0.084	55.98	0.003	24.74	0.0522
Genotype	3	3,944.95	<.0001	18,642	<.0001	11,633	<.0001	2,129.61	<.0001	3,122.93	<.0001
Treatment * Genotype	15	212.02	0.382	290.60	0.1794	322.26	0.0101	57.01	0.0061	76.93	<.0001
Error	86	199.36		215.04		150.78		25.35		15.45	

[a] Degrees of Freedom

Table 4.11. Analysis of variance for disease incidence, disease severity, *Fusarium* head blight index (FHB), *Fusarium* damaged kernel (FDK) and deoxynivalenol (DON) in 2016 for the Time of Application Test.

Source of Variation		Disease Incidence (%)		Disease Severity (%)		FHB Index (%)			FDK (%)		DON (ppm)	
	DF[a]	Mean squares	Probability	Mean squares	Probability	Mean squares	Probability	DF[a]	Mean squares	Probability	Mean squares	Probability
Treatment	7	3,05.31	<.0001	1,371.05	0.0002	727.84	<.0001	7	12.70	0.0318	90.78	0.0031
rep(Treatment)	40	264.77	0.2603	245.65	0.7956	116.29	0.2338	29	5.46	0.0007	27.22	0.0295
Genotype	3	3,386.40	<.0001	4,756.28	<0.0001	1,496.25	<.0001	2	95.95	<.0001	485.23	<.0001
Treatment * Genotype	21	411.70	0.0244	235.28	0.8375	161.73	0.0477	14	7.19	0.0019	30.11	0.0102
Error	120	227.13		322.59		97.66		33	56.05		13.74	

Note: FDK and DON does not include AC Morse

[a] Degrees of Freedom.

4.4.2.2 Assessment of disease severity, disease incidence, FHB Index, FDK and DON

The least squared difference mean values for the different treatment types showed no significant difference between the *C. rosea + Fusarium graminearum* treatments and the *Fusarium graminearum* control group for disease incidence, disease severity, FHB Index, FDK or DON values (Table 4.12). The fungicide treatment had significantly lower disease severity, FHB index and FDK than the *F. graminearum* control in 2015. In 2016, the fungicide application was similar to the water control group for disease severity, FDK and DON. Two additional test groups were added in 2016; a double application of *C. rosea* treatment and a *C. rosea* control group. The double application of *C. rosea* did not significantly differ from any of the other *C. rosea + F. graminearum* test groups for all parameters. The *C. rosea* test group did not significantly differ from the water control group.

The differences among the genotypes tested are displayed in Table 4.13. For all parameters the moderately resistant line, FHB 37 had lower disease levels. The line with intermediate resistance, AC Cora, gave the second lowest results for all parameters. AC Cora also did not significantly differ from FHB 37 for disease incidence, disease severity (2016), FHB Index (2016) FDK or DON (2015) levels. The two susceptible genotypes, AC Morse and CDC Teal had the highest disease levels. Between the two, AC Morse had lower FHB index results in 2015 and CDC Teal had lower DON in the seed compared to AC Morse in 2015.

As with the previous experiment, the proportion of variance attributed to the treatment * genotype interactions was relatively small compared to the main treatment and genotype effects (Tables 4.10 and 4.11). Results for the different treatment*genotype means can be

found in Tables A-10 and A-11. Within genotypes, no significant differences were found between the different *C. rosea* treatment groups for FHB index, FDK and DON. *C. rosea* treatments did not significantly differ from *F. graminearum* controls. Fungicide treatments were found to improve FHB index values in 2015 for AC Morse. The fungicide application also significantly lowered FDK and DON values in CDC Teal in 2015 (Table A-10). Treatment*genotype means in 2016 show that there was very little difference between the different treatments and between the different genotypes (Table A-11).

Table 4.12 Tukey-Kramer adjusted means for disease incidence, disease severity, FHB index, *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) for the different treatment types tested in 2015 and 2016 site year data for the Time of Application Test.

	Treatment	Stage of Application	Disease Incidence (%)		Disease Severity (%)		FHB Index (%)		FDK (%)		DON (ppm)	
			2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
1	*C. rosea + F. graminearum*	Flag Leaf	56.15 b	26.56 b	74.79 a	27.08 ab	44.23 a	10.11 abc	22.01 ab	3.80 ab	15.99 ab	8.42 ab
2		Heading	58.13 ab	29.58 ab	70.83 a	31.56 a	43.11 a	11.72 ab	21.63 a	3.05 ab	17.00 a	7.52 ab
3		Flowering	72.19 a	41.98 a	65.06 a	28.44 a	44.24 a	15.00 a	22.03 a	4.88 a	16.26 a	13.81 a
4		Flag+Head	-	30.52 ab	-	32.50 a	-	11.95 ab	-	3.56 ab	-	7.70 ab
5	Metconazole + *F. graminearum*	Flowering	51.74 b	26.67 b	45.5 b	18.75 ab	25.43 b	5.72 bc	14.68 bc	1.48 b	11.82 b	3.31 b
6	*C. rosea* Control	Flowering	-	12.19 c	-	15.83 ab	-	2.91 cd	-	1.49 b	-	3.32 b
7	*F. graminearum* Control	Flowering	57.81 ab	39.06 ab	72.56 a	31.46 a	44.22 a	16.59 ab	24.48 a	3.36 ab	15.44 ab	7.27 ab
8	Untreated Uninoculated control	Flowering	21.90 c	10.21 c	33.66 b	14.06 b	10.25 c	1.58 d	8.69 c	1.72 ab	5.41 c	3.92 b

Note: Values which share the same letter grouping are not significantly different at P=0.05

Note: FDK and DON information does not include AC Morse in 2016 data

Note: Double DONguard® application at flag leaf and heading and a DONguard® control treatment were not included in the 2015 trial.

Table 4.13 Tukey-Kramer adjusted treatment means from 2015 and 2016 for the four different spring wheat genotypes (AC Cora, AC Morse, CDC Teal and FHB 37) tested at the University of Manitoba field location for the DONguard® Time of Application Test.

Genotype	Resistance	Disease Incidence (%)		Disease Severity (%)		FHB Index (%)		FDK (%)		DON (ppm)	
		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
AC Cora	**I**	45.26 b	24.74 bc	60.70 b	19.84 b	30.49 c	6.04 b	11.50 b	2.31 b	5.33 c	5.74 b
AC Morse	**S**	57.71 a	36.41 a	72.01 a	31.82 a	43.11 b	14.70 a	26.50 a	-	28.29 a	-
CDC Teal	**S**	66.06 a	30.52 ab	81.06 a	34.64 a	56.96 a	13.65 a	25.27 a	5.09 a	14.74 b	12.26 a
FHB 37	**R**	42.92 b	16.72 c	27.85 c	13.54 b	13.07 d	3.40 b	10.60 b	1.35 b	6.25 c	2.72 c

Note: Values which share the same letter grouping do not significantly differ at P=0.05
Note: AC Morse not included for 2016 FDK and DON results due to poor harvest

4.4.2.3 Efficacy of DONguard® compared to fungicide and *F. graminearum* control

The efficacy between the *C. rosea + F. graminearum* treatments and the *F. graminearum* control group changes drastically depending on the year for all parameters tested (Table 4.14). 2016 showed positive results for reducing disease incidence, disease severity and FHB index. In 2016 application at flag leaf reduced the incidence of FHB by 32.0%, disease severity by 13.9% and FHB index by 39.1%. Application at the heading growth stage reduced incidence by 24.3% and FHB index by 29.4%. Despite a reduction in visible symptoms larger negative efficacies were present in 2016 than 2015 for FDK and DON. The largest negative efficacies were present for DONguard® application at flowering with an increase in FDK by 45.2% and DON by 90.0%.

In general, fungicide applications reduced disease incidence, disease severity, FHB index, FDK and DON percent in both years (Table 4.14). Efficacy values for fungicide treatments were larger in 2016 compared to 2015. In 2015 fungicide application lowered disease severity by 37.3%, FHB index by 42.5% and FDK by 40.0 %.

Efficacies for the treatment*genotype interaction are displayed in Table 4.15. Out of the four genotypes tested, *C. rosea* decreased DON levels in AC Cora in both years when applied at the earlier flag leaf and heading developmental stages. In 2015 the application at flag leaf lowered DON levels by 21.8 %, 11.0% more compared to the metconazole treatment. A 27.7% decrease in DON was also observed in AC Cora for the double treatment group in 2016. This treatment for AC Cora paired with a large increase of FDK (70.2%) compared to the control. The largest increases of FDK were present for the resistant cultivar, FHB 37. The largest increases of FDK and DON were in 2016 when application was given at the latest, flowering

stage. FDK increased by a large 232.2% and DON by 90.7% compared to the *Fusarium* control. The susceptible cultivar, CDC Teal, generally responded positively to DONguard® application, apart from DON content in 2016. Application at earlier developmental stages gave consistent positive results (excluding DON in 2016).

Table 4.14: Efficacy percentage data of both the DONguard® (*C. rosea*, ACM941) and Caramba® (metconazole) treatments for the disease incidence, disease severity, FHB index, *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) compared to the *Fusarium graminearum* inoculated control groups for 2015 and 2016 Time of Application Test.

Treatment	Stage of Application	Disease Incidence		Disease Severity		FHB Index (%)		FDK (%)		DON (ppm)	
		2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
C. rosea	Flag Leaf	2.9	32.0	-3.1	13.9	-0.02	39.1	10.1	-13.1	-3.6	-15.8
	Heading	-0.6	24.3	2.4	-0.3	2.5	29.4	11.6	9.2	-10.1	-3.4
	Flowering	-24.9	-7.5	10.3	9.6	-0.1	9.6	10.0	-45.2	-5.3	-90.0
	Flag Leaf + Heading	-	21.9	-	-3.3	-	28.0	-	-6.0	-	-5.9
C. rosea Control	Flowering	-	68.8	-	49.7	-	82.5	-	55.7	-	54.3
Metconazole	Flowering	10.5	31.7	37.3	40.4	42.5	65.5	40.0	56.0	23.5	54.5

Note: Positive percentages mean that those trials had lower values compared to the inoculated control group.
Note: Double application of DONguard ® at flag leaf and heading and DONguard ® control treatment not included in 2015 trial.

Table 4.15.: Efficacy percentage data of both the DONguard® (*C. rosea*, ACM941) and Caramba® (metconazole) treatments for the FHB index, Fusarium damaged kernels (FDK) and deoxynivalenol (DON) compared to the *Fusarium graminearum* inoculated control groups using 2015 and 2016 combined data for the DONguard ® Time of Application Test.

	Line	Treatment	Stage of Application	FHB Index (%)		FDK (%)		DON (ppm)	
				2015	2016	2015	2016	2015	2016
1	***AC Cora***	***C. rosea***	**Flag Leaf**	-25.6	46.8	4.2	-34.6	21.8	17.6
2			**Heading**	-7.3	-16.2	4.8	-40.9	11.0	26.1
3			**Flowering**	-21.0	15.6	-20.0	-47.1	-31.1	-0.7
4			**Flag Leaf + Heading**	-	-4.9	-	-70.2	-	27.7
5		**Metconazole**	**Flowering**	17.7	20.2	24.7	10.1	10.8	36.2
6	**AC Morse**	***C. rosea***	**Flag Leaf**	10.5	-	32.6	-	-28.9	-
7			**Heading**	-7.8	-	-0.7	-	-15.0	-
8			**Flowering**	7.3	-	11.4	-	-11.6	-
9			**Flag Leaf + Heading**	-	-	-	-	-	-
10		**Metconazole**	**Flowering**	58.8	-	37.6	-	10.7	-
11	**CDC Teal**	***C. rosea***	**Flag Leaf**	1.5	60.7	22.3	7.6	27.4	-30.1
12			**Heading**	16.5	42.1	26.4	19.8	3.1	-32.6
13			**Flowering**	-7.2	-8.7	26.6	-22.6	26.0	-159.2
14			**Flag Leaf + Heading**	-	51.4	-	23.3	-	-40.8
15		**Metconazole**	**Flowering**	26.2	82.5	48.3	84.3	57.5	63.5
16	**FHB 37**	***C. rosea***	**Flag Leaf**	10.9	40.4	5.7	-125.6	-21.0	-60.2
17			**Heading**	-0.8	33.3	8.1	51.8	-62.3	20.1
18			**Flowering**	-49.8	36.4	-11.6	-232.2	-69.9	-90.7
19			**Flag Leaf + Heading**	-	-36.8	-	-77.8	-	28.3
20		**Metconazole**	**Flowering**	42.3	10.7	37.7	-17.8	-31.4	74.9

Note: Positive percentages mean that those trials had lower values compared to the inoculated control group.

Note: Double application of DONguard ® at flag leaf and heading and DONguard ® control treatment not included in 2015 trial.

Note: Not enough data was collected to perform FDK and DON testing for AC Morse in 2016

4.4.3 Weather results

Average temperature highs and lows for the *C. rosea* applications were collected by the University of Manitoba's Fort Gary Campus weather station and displayed in Figure 4.1. In 2015 DONguard® treatments were given between July 7 to July 11th. Over this period, 3 days reached average maximum temperatures over 25°C (Table A-12). The highest temperature was 30.9°C on July 10, 2015. However, three of those days also reached temperatures lower than 15°C, with the lowest temperature 10.2°C on July 7, 2015. In 2016, *C. rosea* treatments were given between July 8 and July 18. In 2016 peak temperatures did not go above 25°C in six of the eleven days that *C. rosea* was being applied (Table A-12).

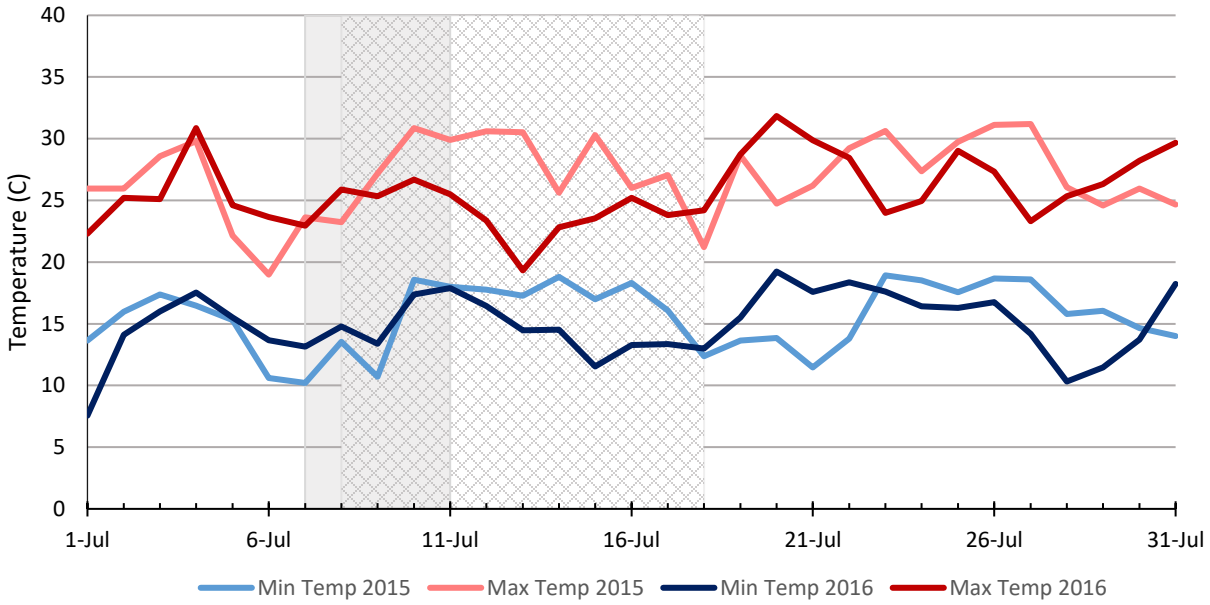

Figure 4.1 Minimum temperatures recorded at the University of Manitoba Field Test Site during the *C. rosea* application (July 7 -11, 2015 and July 8-18, 2016) and *C. rosea* establishment. The dark grey area represents the time when *C. rosea* applications were given in 2015 and the lighter grey patterned area represents when *C. rosea* was applied in 2016.

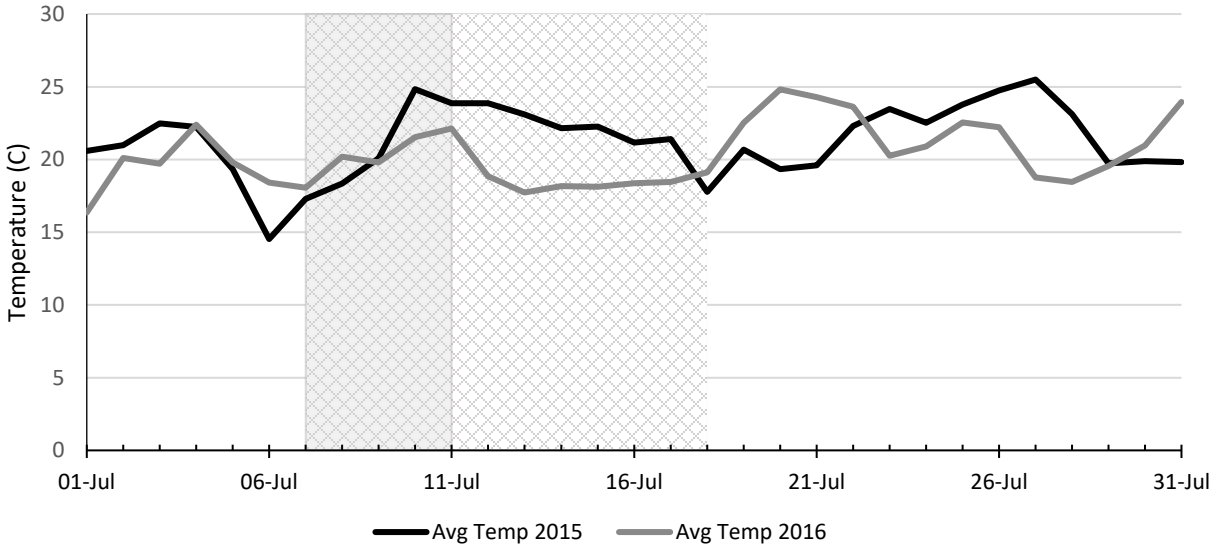

Figure 4.2 Average temperatures recorded at the University of Manitoba Field Test Site during the *C. rosea* application and establishment. The dark grey area represents the time when *C. rosea* applications were given in 2015 and the lighter grey patterned area represents when *C. rosea* was applied in 2016.

4.5 Discussion

No matter the rate of application, DONguard®, did not effectively reduce FHB Index percentages, FDK or DON levels in grain. The efficacy of the product was not consistent between the 2015 and 2016 rate of application trials (Table 4.9). Time of application appears to have a greater influence on the efficacy of the product. Within the rate of application trial, application of the bio-fungicide treatment at the earlier heading developmental stage displayed better results compared to the flowering developmental stage. No matter the rate of DONguard® application, the application of Caramba® fungicide (active ingredient, metconazole) was more efficient at lowering disease incidence (2016 only), disease severity, FHB Index, FDK and DON (Table 4.9).

The lack of consistency in the efficacy of the bio-fungicide was due to high levels of variation between the two years tested. To understand the inconsistencies and variation between the two years, the biology of the biological agent needs to be understood. The most important factor in ensuring the efficacy of a bio-fungicide is to guarantee that that the active ingredient can establish in the environment in which it is being used. *Clonostachys rosea* is a naturally occurring soil fungus which can live in many different soil environments. Primarily, the fungus lives in the soil and consumes decomposing plant tissues. Many studies of *C. rosea* as a biological control have focused on using it as a seed treatment (Jensen *et al.* 2000) or using it on plant stubble as a method to decrease the pathogen inoculum source. In this current study, the biological control product containing *C. rosea* spores was used as a foliar spray. Past studies using *C. rosea* as a foliar/spike spray have been positive in protecting against *Botrytis* in various plant species (Cota *et al.* 2008,) and lowering FHB in wheat (Xue *et al.* 2009: Xue *et al.* 2014a,

Xue *et al.* 2014b). These studies have also found that the effectiveness of using *C. rosea* as a biological control has been higher in a controlled greenhouse environment than in the field. Three environmental conditions have been studied when dealing with *C. rosea*'s survival; ultraviolet protection, moisture and temperature.

One of the key reasons why DONguard® may have been ineffective in reducing FHB infection was that the *C. rosea* spores were unable to establish due to the exposure to the sun's rays during application. UV sensitivity is cited as one of the largest limiting factors affecting *C. rosea*'s usefulness (Costa *et al.* 2012). Protein denaturation, oxidative stress and degradation of the fungi's DNA and RNA can result after exposure to UV-A (315-400 nm) and UV-B (280-315 nm) radiation (Morandi *et al.* 2008: Costa *et al.* 2012). Compared to surface fungi, *C. rosea* conidia have a light brown pigmentation. This lighter color means that it has less ability to block light radiation (Morandi *et al.* 2008: Costa *et al.* 2012). When grown on an agar medium, *C. rosea* strain LQC62 developed a negative exponential growth curve when it was exposed to natural light levels. Within a few hours, *C. rosea* germination and growth decreased to extremely slow levels and eventually stopped. When applied on leaf disks, the survival rate of *C. rosea* improved but still resulted in a negative linear growth rate (Costa *et al.* 2012). The presence of leaf tissue acted as a barrier, providing some protection against the UV rays from the test. If the strain being used can quickly penetrate the leaf tissue, survival rate will increase. Morandi *et al.* (2000), stated that *C. rosea* has a harder time penetrating into living tissue compared to diseased tissue and this could be another factor contributing to the poor results in the present study. Another study evaluated the efficacy of *C. rosea* strain LQC62 in protecting strawberries against grey mould (*Botrytis cinereal*) at different levels of UV-B radiation (Costa *et*

al. 2013). They also found that prolonged exposure to UV radiation decreased the efficacy of the biological control. By increasing the *C. rosea* conidia spore concentration, the chances of some of the spores surviving helped increase success. In the current study, the preparation of DONguard® solution was not preformed in the field. Even though application of the bio-fungicide occurred a couple of hours before sunset, some exposure to sunlight could have occurred during transportation and set up in the field. Any short amount of UV exposure could have degraded the product enough to lower its potency. To combat this issue, inclusion of using black bottles was introduced in the 2016 year. This extra layer of protection to the bio-fungicide mixture could have been one of the factors contributing to improved efficacy values in 2016 compared to 2015 in the time of application test (Table 4.13). In the Time Test, a double fungicide treatment (flag leaf + heading) was added to determine if this could boost the efficacy of the product. Unfortunately, results showed that the overall efficacy was on par or lower than that of the heading inoculation group (Table 4.13). One reason why this might not have worked is that the second round of treatment did not have enough time to fully establish before the *F. graminearum* inoculation was given.

The second factor to consider is the microclimate in which the fungus needs to establish. The temperature of the environment is a key limiting factor to the mycelial growth rate of the biological control. Lower temperatures result in a decreased mycelial growth rate in *C. rosea*, with temperatures between 10-15°C resulting in the least amount of growth and optimal growth occurring around 25°C (L. V. Cota *et al.* 2008). Maximum germination was reported to be 17% when environmental temperatures were 10°C and increased to 72% germination at temperatures of 25-30°C (Yu and Sutton 1998). When grown on tomato leaf

tissue, the highest rates of mycelial growth were found to occur at 24°C (Saraiva *et al.* 2015). In the same experiment, it was discovered that *C. rosea* could survive within the tomato tissue for a month when temperatures ranged from 18-30°C. Although higher temperatures are preferred, certain *C. rosea* strains have been reported to remain active at lower temperatures. Jensen *et al.* (2000), found that *C. rosea* strain IK726 was still capable of reducing *F. culmorum* levels when soil temperatures were in the 6 to 12°C range. In the current study, DONguard® was applied close to sunset and the fungus needed to establish within the plant during night time temperatures. Fig 4.1 shows the minimum temperatures at the University of Manitoba field location during the time of DONguard® application. Time of bio-fungicide application ran from July 7 – July 11 in 2015 and from July 8- July 18 in 2016. During the time of initial application, the temperatures regularly dropped below 15°C especially during the 2016 trial. Fig 4.2 displays the daily average temperatures recorded at the University of Manitoba during the bio-fungicide application period. In 2015, the first half of the bio-fungicide application period experienced average temperatures under 20°C. After the initial bio-fungicide application the average temperature was around the optimal temperature range for *C. rosea* growth. In 2016, lower average temperatures occurred during the total application time frame. Lower temperatures during the establishment stage could have stunted the growth of ACM941 and resulted in it not establishing itself at a high enough concentration before the *F. graminearum* inoculations. Optimal temperatures required for *F. graminearum* germination are sited to be 25-30°C (Gilbert and Fernando 2004) and would account for the lower FHB levels in 2016.

The last factor to consider is the moisture content of the environment. Field conditions between the two years differed drastically. In 2015 it was hot and dry, while in 2016 the trial

was grown in cooler, damp conditions (Table A-12). Cota *et al.* (2008) found that no matter the moisture levels, Brazilian isolates of *C. rosea* could establish on strawberry leaves, but survival rates were not as strong in lower moisture environments, only surviving for around 36 hours. Yu and Sutton (1998) found that high germination occurred when exposed to longer periods of wetness. Maximum germination was recorded when spores were exposed to 16 hours of moisture at 25-30°C. The mist irrigation system was not used after applications of bio-fungicide, unless *F. graminearum* inoculations were occurring. This was not a specified recommendation for the application of the bio-fungicide. This could mean that earlier treated groups (flag leaf and heading stages) might not have received adequate moisture for spore germination.

Published studies on the efficacy of *C. rosea* strain ACM941 in controlling FHB in wheat have been positive. A field trial found that a bio-fungicide containing ACM941 was able to reduce FHB index 30-46%, FDK 31-39% and DON 22-33% when applied to spikes (Xue *et al.* 2014a). Similar FHB Index reductions were present in the DONguard® Time Test study. Application of DONguard® at flag leaf reduced FHB index values by 39.1%, application at heading reduced it by 29.4% and a double application at flag leaf and heading resulted in a reduction of 28.0% (Table 4.14). However, these results were not consistent and were only observed in 2016. Between the different genotypes used in the *Xue et al.* study, it was found that AC Nass, the most resistant cultivar in the test, displayed the best results while AC Foremost, the least resistant in the study did not benefit from the bio-fungicide (Xue *et al.* 2014a). These results were better than the *G. zeae* controls, suggesting that the bio-fungicide acted as a supplement to reducing FHB, and was not the primary combative agent. In the current study the most resistant cultivar, FHB 37, resulted in the lowest overall levels of disease

incidence, disease severity, FHB index, FDK and DON (Table 4.13). The *C. rosea* strain ACM941 was reported to have better results when it was inoculated to reduce inoculum sources. For example, when ACM941 was applied to wheat stubble the previous year, a 58% reduction of FHB index, 49% reduction of FDK and a reduction of DON levels by 21% were recorded (Xue *et al.* 2009). The use of bio-fungicides containing ACM941 was shown to significantly reduce *G. zeae* perithecial production by up to 84% on wheat residue and 93.1% on corn (A.G. Xue *et al.* 2014b).

The biggest problem with DONguard® is the fact that it increased DON levels within the grain, especially in the resistant FHB 37 (Tables 4.14, A-6, A-7). There are no published results demonstrating that *C. rosea* application will increase the levels on DON in grain. This occurred even when FDK values were decreased in the *C. rosea* treatments. There have been reported instances where fungicide application increases DON production (Wegulo *et al.* 2015). Strobilurin, a Quinone Outside Inhibitors (QoI) classed fungicide, has been attributed to increased DON production (Gilbert and Haber 2013). For example, when azoxystrobin was sprayed after wheat was inoculated with *F. graminearum*, the levels of the DON mycotoxin increased in susceptible cultivars (Simpson *et al.* 2001). One theory behind this is that visible FHB damage, like the kernel shriveling, do not occur. With larger kernel sizes, infected grain will not be blown away during combining and cleaning processes. The production of DON and other secondary metabolites will continue to be produced resulting in higher mycotoxin levels. The abundant presence of plump grain could explain why the resistant cultivar, FHB 37, displayed positive improvements in FHB index and FDK values, but consistently produced the worst efficacy in reducing DON. Another possibility is that *F. graminearum* is increasing the

production of secondary metabolites to defend itself against the mycoparasitism. So even though the total number of FDK present may decrease, the surviving *F. graminearum* within the grain can lead to increased DON production.

A combination of unsuitable environmental conditions for the *C. rosea* spores could have contributed to poor establishment on wheat spikes and poor antagonistic effects on *F. graminearum*. An interaction between the bio-fungicide and *F. graminearum* may be the cause of increased DON content within the grain for the other cultivars. No protein analysis was done for this product so the fact that *C. rosea* penetrates the grain may mean that nutritional quality may be altered. More field trials would need to be conducted to understand the specific environmental conditions needed for this product. Optimal germination conditions can be different between different strains of *C. rosea*. The inclusion of multiple *C. rosea* strains could help decrease the risks of failure if certain environmental conditions can not be met. Lastly, this product may be more suitable as a method to lower initial inoculum sources and further study should be done by using DONguard® on stubble or as a seed treatment agent.

Overall results of the rate of application experiment showed that no matter the rate of application, DONguard® did not significantly improve ($P<0.005$) test weight, thousand kernel weight, FHB Index, FDK or DON levels compared to the *F. graminearum* inoculated control groups (Table 4.5, 4.6). Overall efficacy results (Table 4.7) were poor for all variables tested, apart from 2016's FDK results, where an increase in the rate of application helped reduce overall FDK by 17.2% when applied at the heading developmental stage and 20.7% when applied at flowering. The most concerning result is the overall increase in DON levels within the grain treated with the bio-fungicide product.

Chapter 5.0 General Discussion and Conclusion

Preventing *Fusarium* Head Blight (FHB), or scab, in wheat (*Triticum aestivum L.*) is a complicated matter. Infection from the fungal pathogen, *F. graminearum,* results in both grain yield loss, as well as, decreases in overall quality. Quality of the grain is further compromised because *F. graminearum* produces multiple types of toxic compounds, which can accumulate in the seed, including deoxynivalenol (DON). Multiple methods are used annually to try and limit the amount of infection that occurs. These include the use of biological controls, fungicide treatments, as well as developing genetically resistant wheat lines. Currently, no fully resistant wheat cultivar has been developed, so farmers apply alternative methods in combination with growing nearly resistant cultivars to suppress the disease. Two different strategies in reducing FHB infection were investigated. The first study examined the genetic resistance present in a double haploid cross between two Canadian hard red winter wheat cultivars; Flourish (S) and Emerson (R). The second study considered the efficacy of a new potential bio-fungicide product, DONguard®. The active ingredient, *Clonostachys rosea*, in past studies has shown to be antagonistic against *F. graminearum*.

The first study involved a series of field trials which evaluated the progeny from a cross between Flourish and Emerson. A total of 178 lines were grown at three different environments in Manitoba: Carman (2015), Carman (2016) and Winnipeg (2016). The objective of this study was to screen the lines for FHB symptoms in the field, test for the presence of the mycotoxin DON in the grain harvested, as well as, identify and develop a quantitative trait loci (QTL) map for FHB resistance. Results from this study found QTL responsible for FHB symptoms, FDK and

DON on five chromosomes; 7A, 1B, 2B, 4D and 6D. Similar QTL relating to reducing FHB have been previously reported in other studies using winter wheat cultivars.

To limit variation between the level of FHB infection between lines, a standardized method of *F. graminearum* inoculation was performed. To limit environmental discrepancies, a misting system was used to ensure high humidity levels needed for pathogen growth and survival. After implementing these methods, a QTL on chromosome 4D was identified as being stable and present in all three test locations. Similar QTL have previously been reported in other winter wheat studies (Draeger *et al.* 2007; Mwaniki 2017). This major QTL was associated with reducing disease severity, FHB index, FDK and DON symptoms. Other QTL identified in this study were only identified in one of the test locations. This suggests that these QTL are minor and are influenced by environmental factors (i.e. temperature). Further test locations within, or outside of, Manitoba should be performed to verify whether these QTL are consistent or if they require certain factors to activate. QTL for disease incidence, FHB index values, FDK and DON on chromosome 4D co-localized with QTL associated with plant height. Past studies have speculated that height plays a key role in dictating the resistance level of a cultivar. One of the QTL associated with plant height was found on chromosome 2D, which could potentially be the location of another dwarfing gene, *Rht8*. Previously studies have mapped this gene on the short arm of chromosome 2D (Korzun *et al.* 1998; Mao *et al.* 2010). Future screenings of this population could investigate if this QTL is *Rht8*.

The height QTL on 4D was only detected to the pooled population. More site year data containing three replicates and all 179 lines need to be repeated in a variety of environmental conditions to double check if 4D, as well as the rest of the QTL identified are not false positives.

Results from the second study showed that no matter the rate of application, DONguard® was not efficient at consistently reducing FHB field symptoms, FDK or DON levels in grain. Efficacy of the product was inconsistent between the two years tested (Table 4.7, 4.12, 4.13). Rates of application did not play a significant role in reducing FHB symptoms, however, application of the bio-fungicide treatment at the earlier developmental stages displayed better results compared to the flowering developmental stage. In the time of application study, when plants were treated with DONguard® flag leaf disease incidence reduced by 32.0% and FHB index values by 39.1%. When applied at the later heading stage, it reduced incidence by 24.3% and FHB index values by 29.4%. However, these results were only present in 2016. The reasons for inconsistent results over the two years may be a combination of unsuitable environmental conditions reducing *C. rosea* establishment and antagonistic activity. *C. rosea*'s sensitivity to ultraviolet light greatly influences its use for foliar application. Previous experiments have shown that exposing *C. rosea* to light reduces its activity and lowers its survival rates (Costa *et al.* 2012; Costa *et al.* 2013). As of yet, no 'sun blocking' agent has been included in the DONguard® formulation. Further studies with a 'sunblock' included should be done to fully determine whether *C. rosea* would be a good foliar method for reducing FHB in the field. Results from the current study also found that the use of fungicide treatment gave better overall results at reducing FHB symptoms, FDK and DON between the different cultivars used. These findings are consistent with other *C. rosea* related studies. Xue *et al.* (2014a) studied a

different bio-fungicide containing *C. rosea*, also found that the fungicide treatment, Proline 480 (active ingredient prothioconazole) resulted in significantly lower FHB symptoms in the field, FDK counts and DON levels in grain. Alternative studies considering DONguard® as a seed treatment or as an application on wheat stubble to reduce inoculum reservoirs should also be conducted. Past studies have found that bio-fungicides containing *C. rosea* were better at suppressing perithecial numbers (i.e. decreased spore development) in wheat stubble compared to the fungicide treatment, Folicur (active ingredient tebuconazole) (Xue *et al.* 2014b). Although DONguard® did show some potential as a bio-fungicide product for cultivars having at least intermediate resistance to FHB, the fact that an interaction between the bio-fungicide and *F. graminearum* may be the cause of increased DON content within the grain makes its utility questionable.

Both studies reported results showing a decrease in FHB field symptoms, FDK and DON. Progeny in the Flourish/Emerson population could out preform both parents in terms of FHB index, FDK and DON values (Table 3.2). On average DONguard® was not able to significantly reduce FHB symptoms, FDK or DON compared to the fungicide used in the experiment. However, some of the results suggest that there is a possibility that DONguard®, when paired with a moderate to resistant wheat line could reduce symptoms. Overall, both methods by themselves do not fully suppress FHB. However, in an integrated management system the combination of the two may help lower disease in the field.

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Appendix

Table A-1 Mean values for disease incidence (DI), disease severity (DS), FHB index, height, thousand kernel weight (TKW), *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) content for the check cultivars used in the three environments; Carman (2015), Carman (2016) and Winnipeg (2016).

Line	FHB Res.	Site	DI (%)	DS (%)	FHB Index (%)	Height (cm)	TKW (g)	FDK (%)	DON (%)
32C*17	R[a]	CRM 2015	18.25	15	2.99	102.3	-	11.7	12.0
		CRM 2016	15.0	14.6	2.5	77.3	33.0	2.37	9.6
		WPG 2016	5.4	10.0	0.6	105.4	34.0	0.05	1.65
		Average	13.9	13.5	2.2	96.3	33.5	3.31	6.9
43I*18	I[b]	CRM 2015	64.5	50.0	32.2	94.7	-	28.5	36.0
		CRM 2016	31.7	30.8	9.6	78.4	32.1	5.41	9.8
		WPG 2016	26.7	24.2	6.6	92.4	31.8	4.45	5.4
		Average	51.3	41.6	23.2	91.2	31.9	12.8	17.1
Caledonia	S[c]	CRM 2016	74.2	90.8	67.6	65.7	25.9	24.2	49.8
		WPG 2016	41.0	48.5	21.6	72.0	31.6	5.75	10.8
		Average	59.1	71.6	46.7	68.8	28.9	14.98	30.3
FHB148	R	CRM 2015	46.6	34.4	16.2	112.3	-	19.4	23.5
		CRM 2016	13.3	8.3	1.2	83.1	38.5	5.88	9.25
		WPG 2016	7.1	14.6	1.1	112.1	39.0	2.18	2.65
		Average	24.8	20.6	7.2	103.5	38.8	7.10	9.46
Freedom	R	CRM 2015	84	61.25	51.5	84.3	-	38.0	42.0
Hanover	S	CRM 2015	71.5	78.3	56.7	85.1	-	46.4	62.0
		CRM 2016	70.0	80.8	58.0	77.0	32.8	15.9	21.5
		WPG 2016	57.5	55.0	31.6	76.5	37.11	5.47	6.6
		Average	71.0	77.9	56.1	83.9	34.8	22.6	30.0
Osprey		CRM 2016	18.8	16.7	3.1	74.3	30.2	2.73	10
		WPG 2016	8.8	9.2	0.9	93.1	28.9	3.06	5.8
		Average	13.8	12.9	2.0	83.7	29.5	2.89	7.9

a Resistant, b Intermediate resistance, c Susceptible

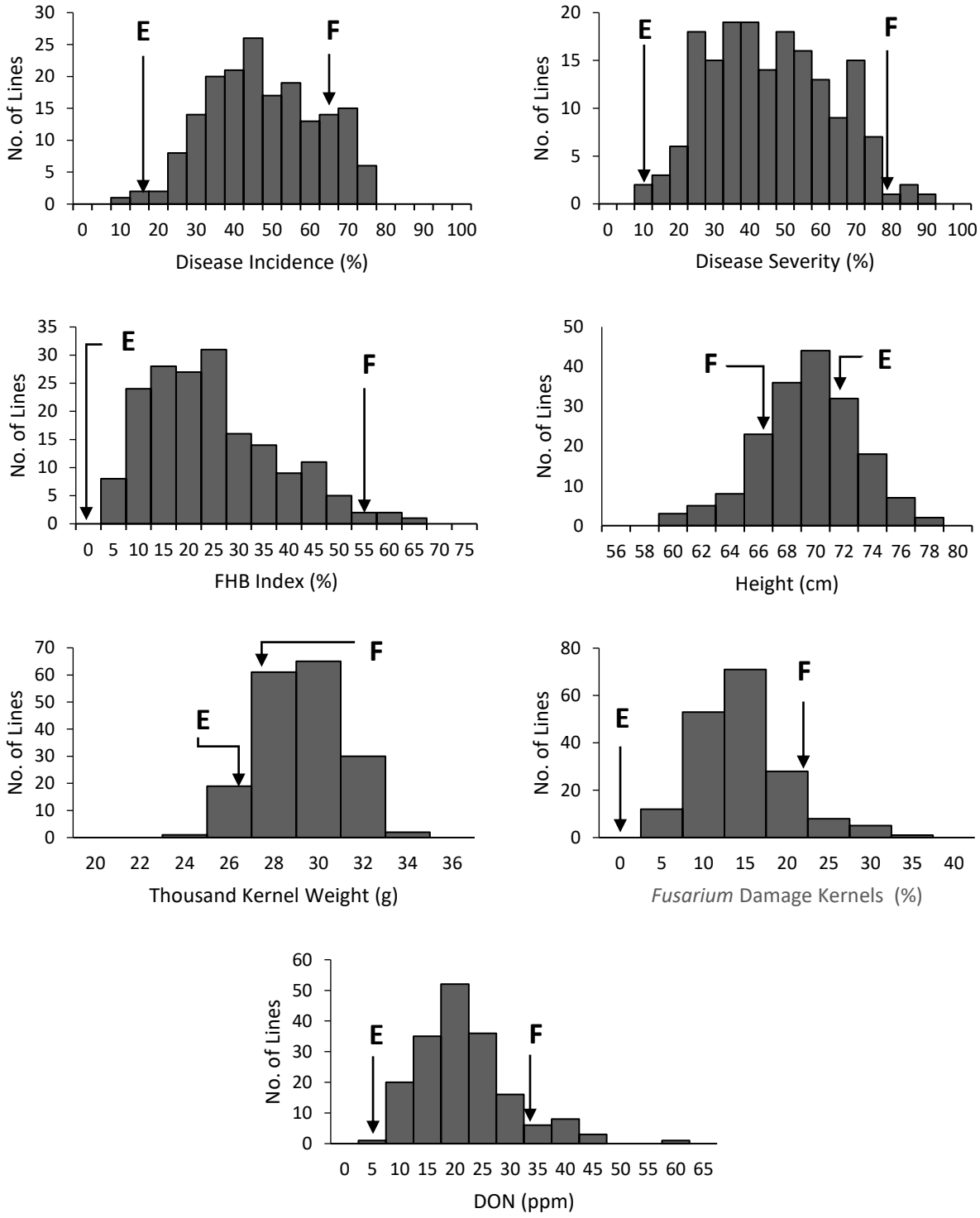

Figure A-1 Frequency distributions for FHB field symptoms; disease incidence, disease severity, FHB index (%), wheat physical characteristics; height (cm) and thousand kernel weight (g), and kernel disease; *Fusarium* damage kernels (%), deoxynivalenol (DON) levels (ppm) for a population of 178 double haploid Flourish(F)/Emerson(E) lines for the Carman 2016 test location.

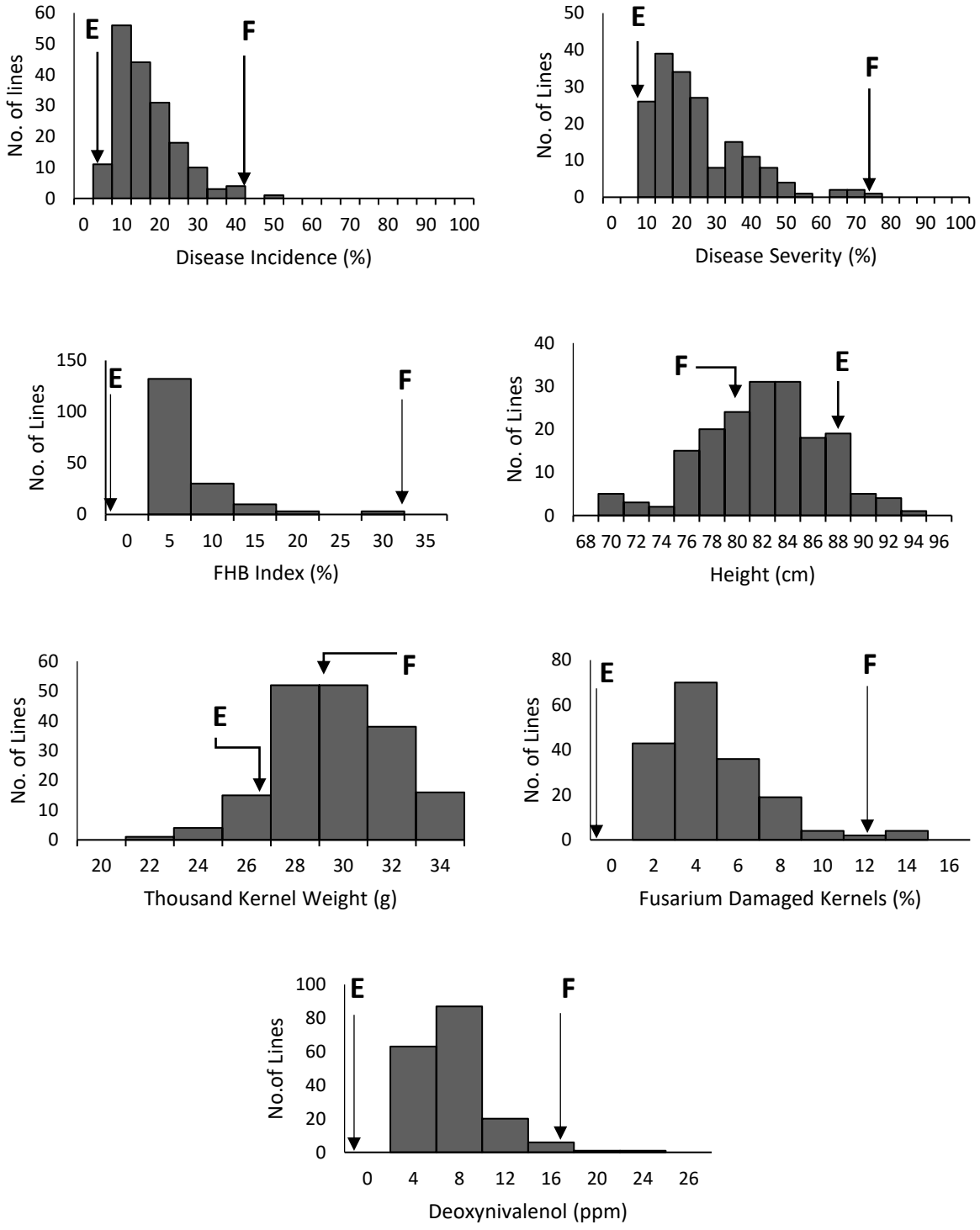

Figure A-2 Frequency distributions for FHB field symptoms; disease incidence, disease severity, FHB index (%), wheat physical characteristics; height (cm) and thousand kernel weight (g), and kernel disease; *Fusarium* damage kernels (%), deoxynivalenol (DON) levels (ppm) for a population of 178 double haploid Flourish(F)/Emerson(E) lines for the Winnipeg 2016 test location.

Table A-2 Pearson's correlation coefficients between FHB disease incidence, disease severity, FHB index, plant height, thousand kernel weight (TKW), *Fusarium* damage kernels (FDK) and deoxynivalenol (DON) values from Emerson/Flourish double haploid population (n=159) at the 2015 Carman site.

	Incidence	Severity	FHB Index	Height	TKW	FDK
Severity	0.44 <0.0001					
FHB Index	0.93 <0.0001	0.93 <0.0001				
Height	-0.35 <0.0001	-0.13 0.1083	-0.18 0.0012			
TKW	-0.25 0.0013	-0.46 <0.0001	-0.48 <0.0001	0.11 0.1805		
FDK	0.52 **<0.0001**	0.69 **<0.0001**	0.73 **<0.0001**	-0.17 0.0320	-0.47 **<0.0001**	
DON	0.46 **<0.0001**	0.65 **<0.0001**	0.70 **<0.0001**	-0.13 0.1178	-0.44 **<0.0001**	0.79 **<0.0001**

Note: n=155 for correlations involving TKW, FDK and DON

Table A-3 Pearson's correlation coefficients between FHB disease incidence, disease severity, FHB index, plant height, thousand kernel weight (TKW), *Fusarium* damage kernels (FDK) and deoxynivalenol (DON) values from Emerson/Flourish double haploid population (n=178) for combined Carman (2016) and Winnipeg (2016) data.

	Incidence	Severity	FHB Index	Height	TKW	FDK
Severity	**0.65** **<0.0001**					
FHB Index	**0.84** **<0.0001**	**0.88** **<0.0001**				
Height	**-0.22** **0.0036**	**-0.06** **0.4382**	**-0.16** **0.0337**			
TKW	**-0.02** **0.8469**	**-0.21** **0.0059**	**-0.11** **0.1361**	**0.38** **<0.0001**		
FDK	**0.51** **<0.0001**	**0.45** **<0.0001**	**0.49** **<0.0001**	**-0.09** **0.2253**	**-0.15** **0.0507**	
DON	**0.63** **<0.0001**	**0.55** **<0.0001**	**0.65** **<0.0001**	**-0.19** **0.0121**	**-0.14** **0.0656**	**0.68** **<0.0001**

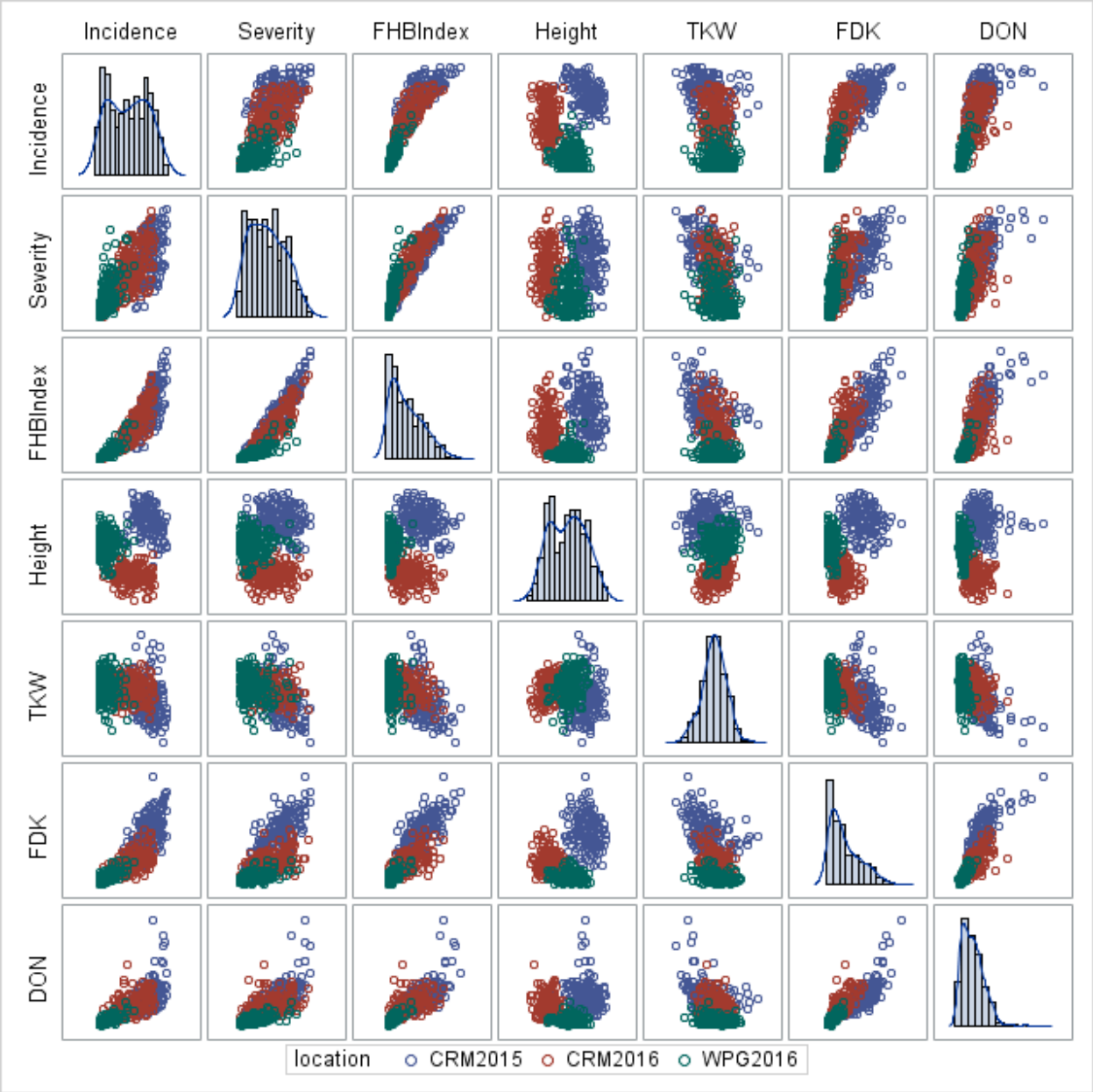

Figure A- 3: Correlation graph depicting the relationships between the following variables; FHB disease incidence (%), FHB disease severity (%), FHB index (%), height (cm), thousand kernel weight (TKW) (g), *Fusarium* damage kernels (FDK) and deoxynivalenol (DON). Points on the graph represent the 178 different lines in the Flourish/Emerson population. Colors of the points indicate which test location the data was taken from; Carman 2015 (blue), Carman 2016 (red) and Winnipeg 2016 (green).

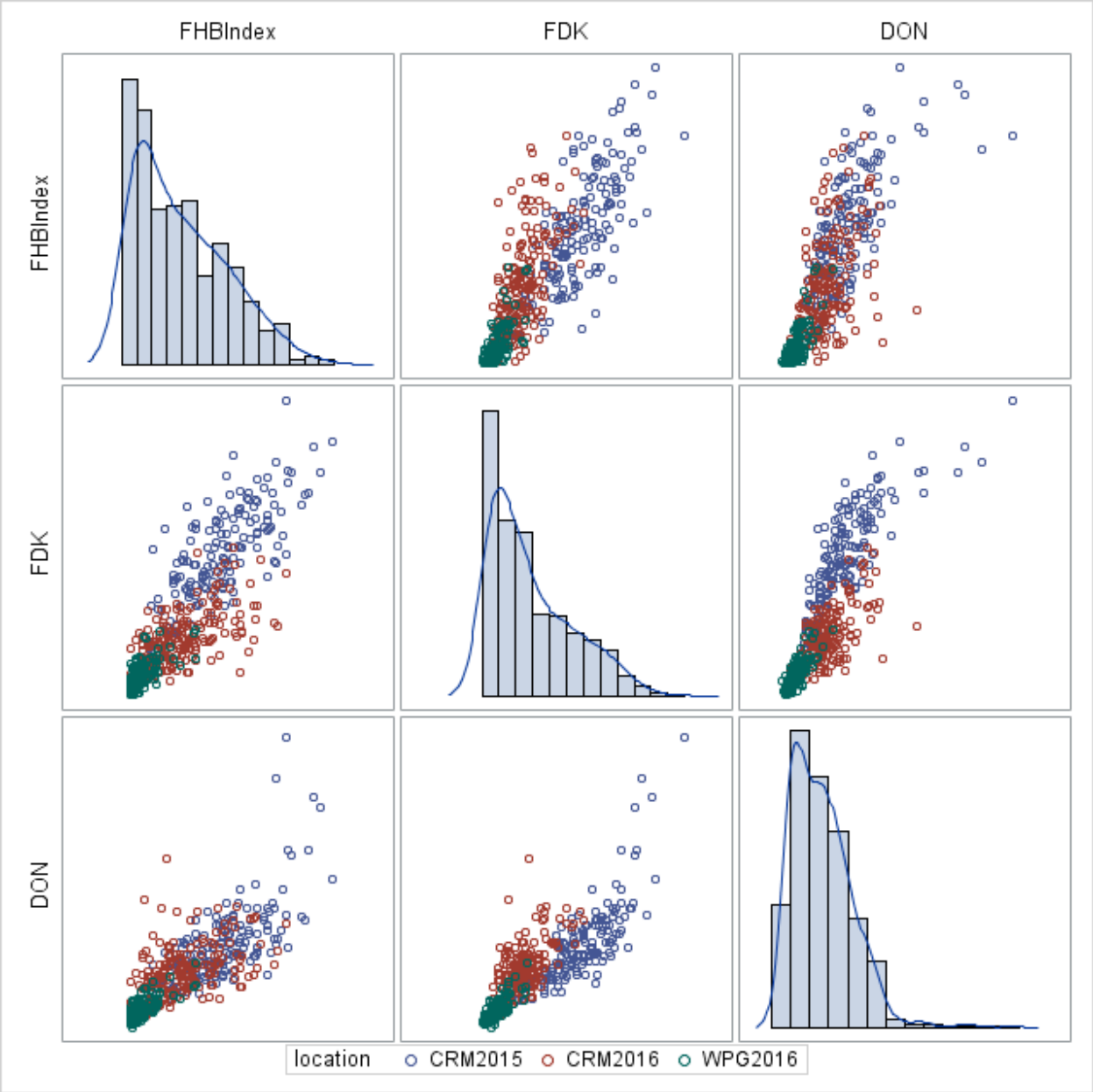

Figure A- 4: Correlation graph depicting the relationships between the FHB index percentage, percentage of *Fusarium* damaged kernels and the amount of deoxynivalenol (ppm) in the grain. Points on the graph represent the different lines in the Flourish/Emerson population. Colors of the points indicate which test location the data was taken from; Carman 2015 (blue), Carman 2016 (red) and Winnipeg 2016 (green).

Table: A-4 Chi-square results for markers in QTL regions associated with plant height on chromosome 4D in the Flourish/Emerson DH population

Chrom	Markers	No. A	No. B	No. Missing	$\chi^2_{1:1}$	*p*	
2Dx	RAC875_c66820_684	64	97	0	6.76	0.00930	**
	TA003123-0874	70	90	1	2.50	0.11385	ns
	BobWhite_c1971_839	69	92	0	3.29	0.06989	ns
	Excalibur_c77068_166	68	90	3	3.06	0.08008	ns
	Excalibur_c14639_1632	68	93	0	3.88	0.04881	*
4D	RAC875_rep_c105718_304	95	66	0	5.22	0.02228	*
	BS00065818_51	91	70	0	2.74	0.09792	ns
	Kukri_c20631_614	89	69	3	2.53	0.11158	ns
	RAC875_c60218_63	92	69	0	3.29	0.06989	ns
	Excalibur_c7048_494	96	65	0	5.97	0.01456	*
	Excalibur_rep_c108030_260	95	66	0	5.22	0.02228	*
	BobWhite_c20689_427	88	73	0	1.40	0.23714	ns
6A	IAAV7418	96	65	0	5.97	0.01456	*
	BobWhite_c5782_825	99	62	0	8.50	0.00355	**
	BobWhite_c14915_156	101	60	0	10.44	0.00123	**

Table A-5 Results of the permutation test (1000) conducted on the DH Flourish/Emerson population (Alpha=0.05) to determine if a QTL was real

Trait	Location	Chrom	Position	Add	R2	LOD	Permutation (1000) ALPHA 0.05	REAL?
FDK	CRM 2015	4D	13	2.943	0.118	4.377	3.079	YES
	CRM 2016	6D	49	1.773	0.104	3.849	3.114	YES
	CRM 2016	7A	126	2.056	0.133	4.992	2.883	YES
	CRM 2015, CRM 2016, WPG 2016	5A	38	1.378	0.082	3	3.027	NO
	CRM 2015, CRM 2016, WPG 2017	7A	114	1.698	0.12	4.483	3.115	YES
DON	CRM 2015	4D	13	4.336	0.139	5.24	2.947	YES
	CRM 2016	6D	75	2.83	0.093	3.403	3.052	YES
	WPG 2016	7A	85	1.051	0.096	3.534	2.919	YES
DI	CRM 2015	1B	18	3.646	0.09	3.28	3.19	YES
	CRM 2016	2B	4	5.603	0.153	5.792	3.265	YES
	WPG 2016	7A	84	2.542	0.102	3.745	2.983	YES
DS	CRM 2015	2B	3	-5.426	0.113	4.198	3.094	YES
	CRM 2015	4D	13	7.131	0.19	7.349	3.048	YES
	CRM 2015	7A	113	4.776	0.082	3.002	3.114	NO
	CRM 2016	4D	13	8.293	0.227	8.983	3.745	YES
	WPG 2016	4D	13	4.683	0.116	4.321	3.087	YES
	CRM 2015, CRM 2016, WPG 2017	4D	13	6.624	0.255	10.299	3.045	YES
FHB INDEX	CRM 2015	4D	13	6.149	0.181	6.993	3.102	YES
	CRM 2015	7A	113	4.496	0.093	3.428	3.059	YES
	CRM 2016	4D	13	5.1	0.153	5.81	3.13	YES
	WPG 2016	4D	13	1.318	0.087	3.17	2.725	YES
	CRM 2015, CRM 2016, WPG 2017	4D	13	3.78	0.217	8.574	3.15	YES
HEIGHT	CRM 2016	2Dx	21	1.561	0.14	5.26	3.049	YES
	WPG 2016	6A	91	-1.852	0.136	5.126	3.206	YES
	CRM 2015, CRM 2016, WPG 2017	4D	14	-1.331	0.086	3.134	3.079	YES
	CRM 2015, CRM 2016, WPG 2017	6A	89	-1.582	0.119	4.447	3.078	YES
TKW	CRM 2015	2B	5	0.93	0.086	3.137	3.185	NO
	CRM 2015	4D	13	-1.001	0.096	3.544	3.063	YES
	CRM 2016	4D	12	-0.646	0.113	4.207	3.071	YES
	CRM 2016	6A	97	-0.721	0.123	4.606	3.251	YES
	WPG 2016	2B	1	0.82	0.123	4.6	3.129	YES
	WPG 2016	4D	10	-0.972	0.17	6.53	3.171	YES
	WPG 2016	6A	97	-0.75	0.087	3.171	3.267	NO
	CRM 2015, CRM 2016, WPG 2017	2B	1	0.654	0.111	4.112	3.067	YES
	CRM 2015, CRM 2016, WPG 2017	4D	12	-0.852	0.176	6.762	3.07	YES
	CRM 2015, CRM 2016, WPG 2017	6A	97	-0.678	0.098	3.617	3.147	YES

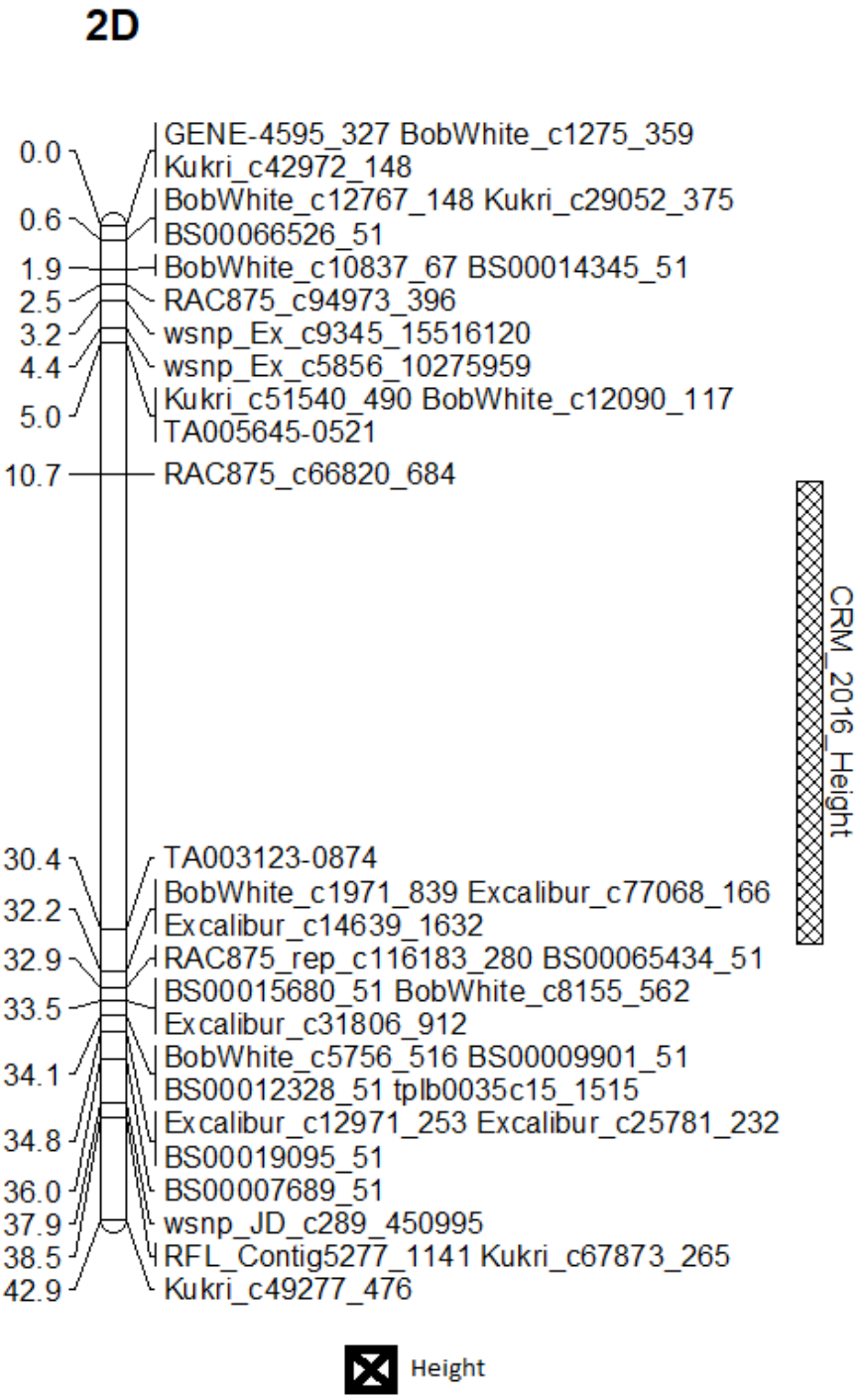

Figure A-5 Genetic linkage map for height QTL (checkered) located on chromosome 2D in DH lines developed from the cross Flourish/Emerson identified in Carman (2016). Marker distance is displayed on the right side of the chromosome and the names of markers present are on the right.

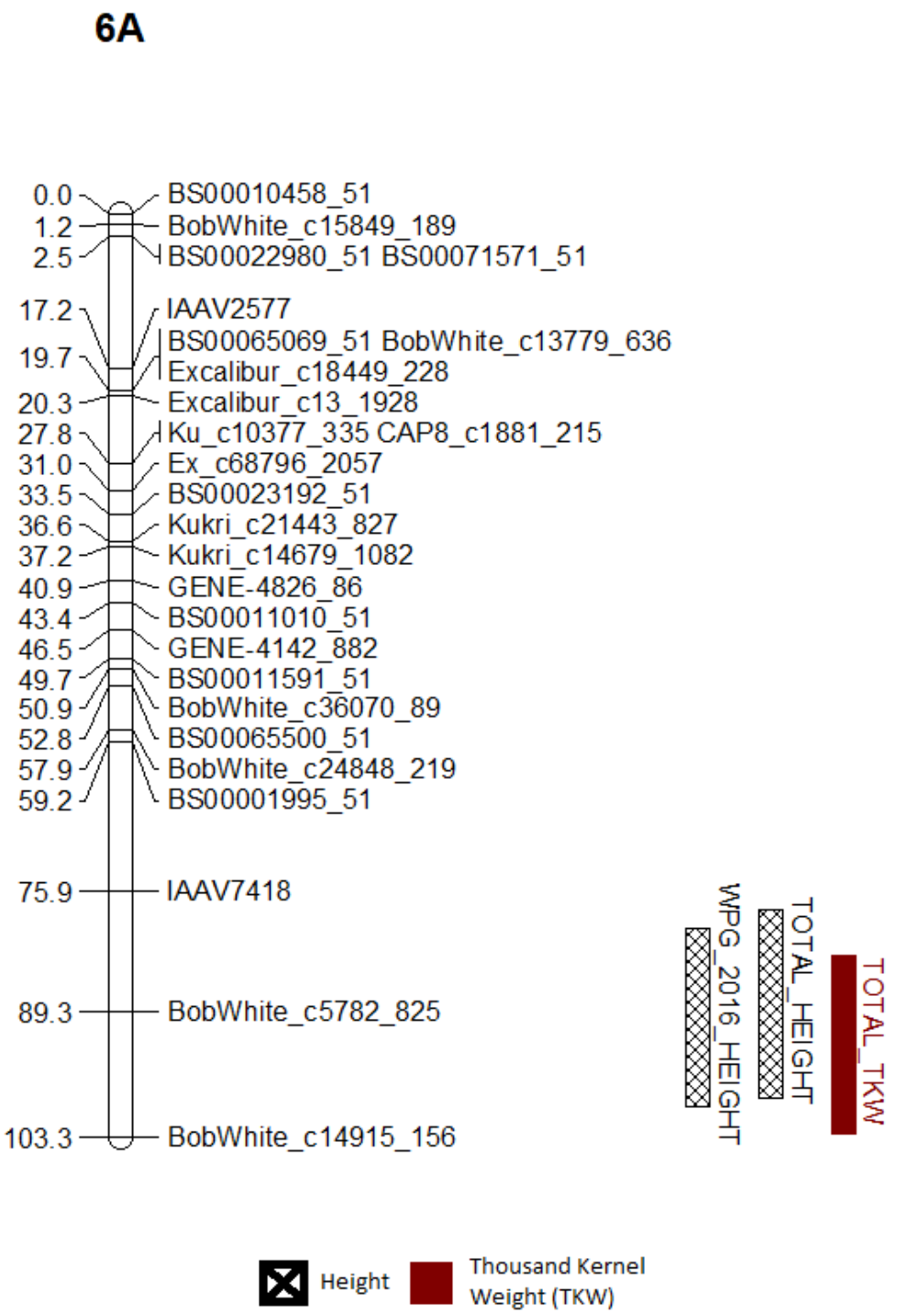

Figure A-6. Genetic linkage map for height (checkered) and thousand kernel weight (TKW) (dark red) QTLs present on chromosome 6A for Flourish/Emerson Population grown at three different environments; Carman (2015), Carman (2015) and Winnipeg (2016) Marker distance is displayed on the right side of the chromosome and the name of markers present are on the right.

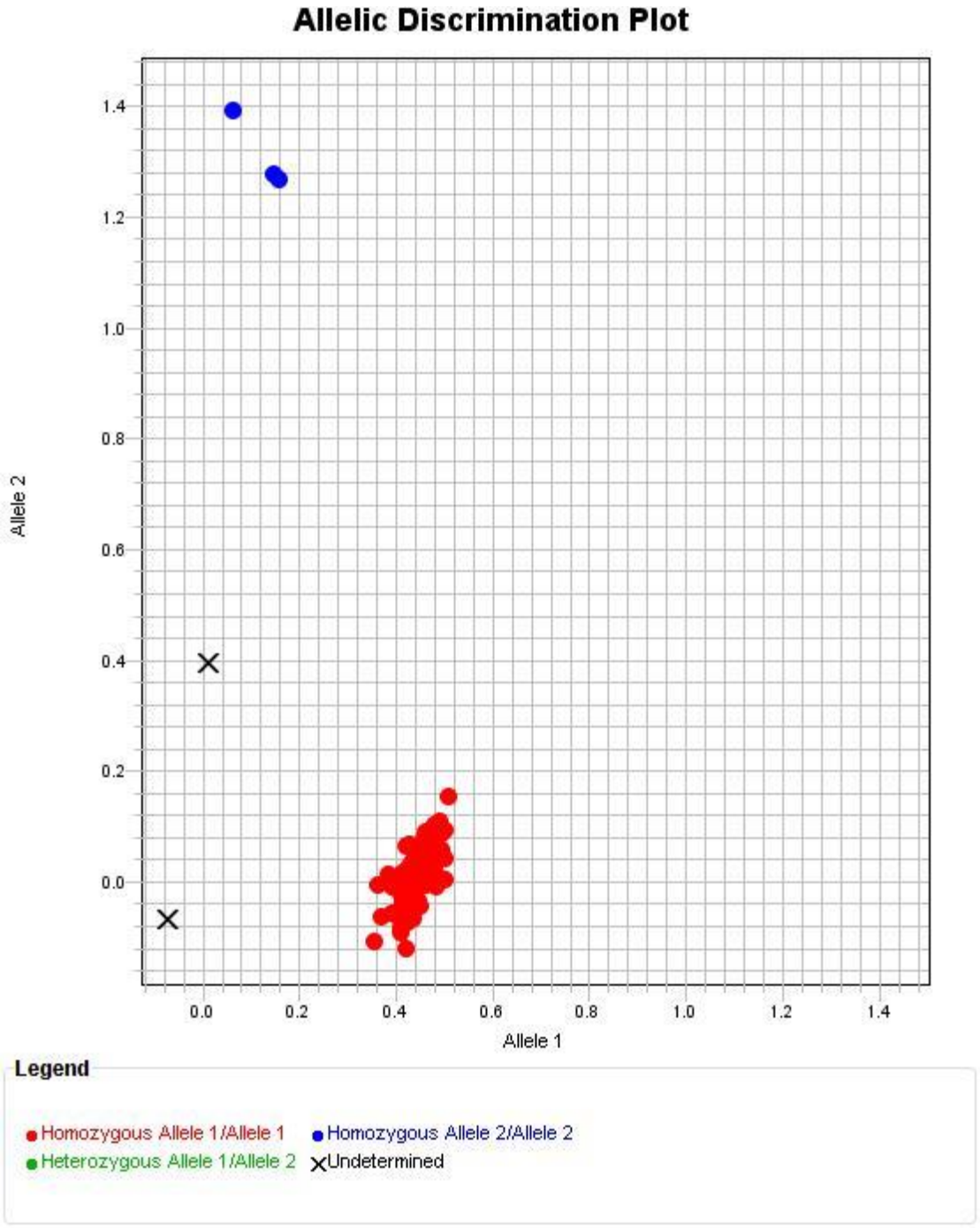

Figure A-7 Allelic discrimination plot for Rht-B1s allele in the Flourish/Emerson population. The population included 89 lines from the DH population, the parents (Flourish and Emerson), grandparents (CDC Osprey, McClintock and CDC Falcon) and great grandparent (CDC Kestrel). Allele 1 corresponds to the line having the wildtype (*Rht-B1a*) version of the allele while Allele 2 corresponds with the dwarfing (*Rht-B1b*) allele. Note: Grandparent varieties contained Allele 2.

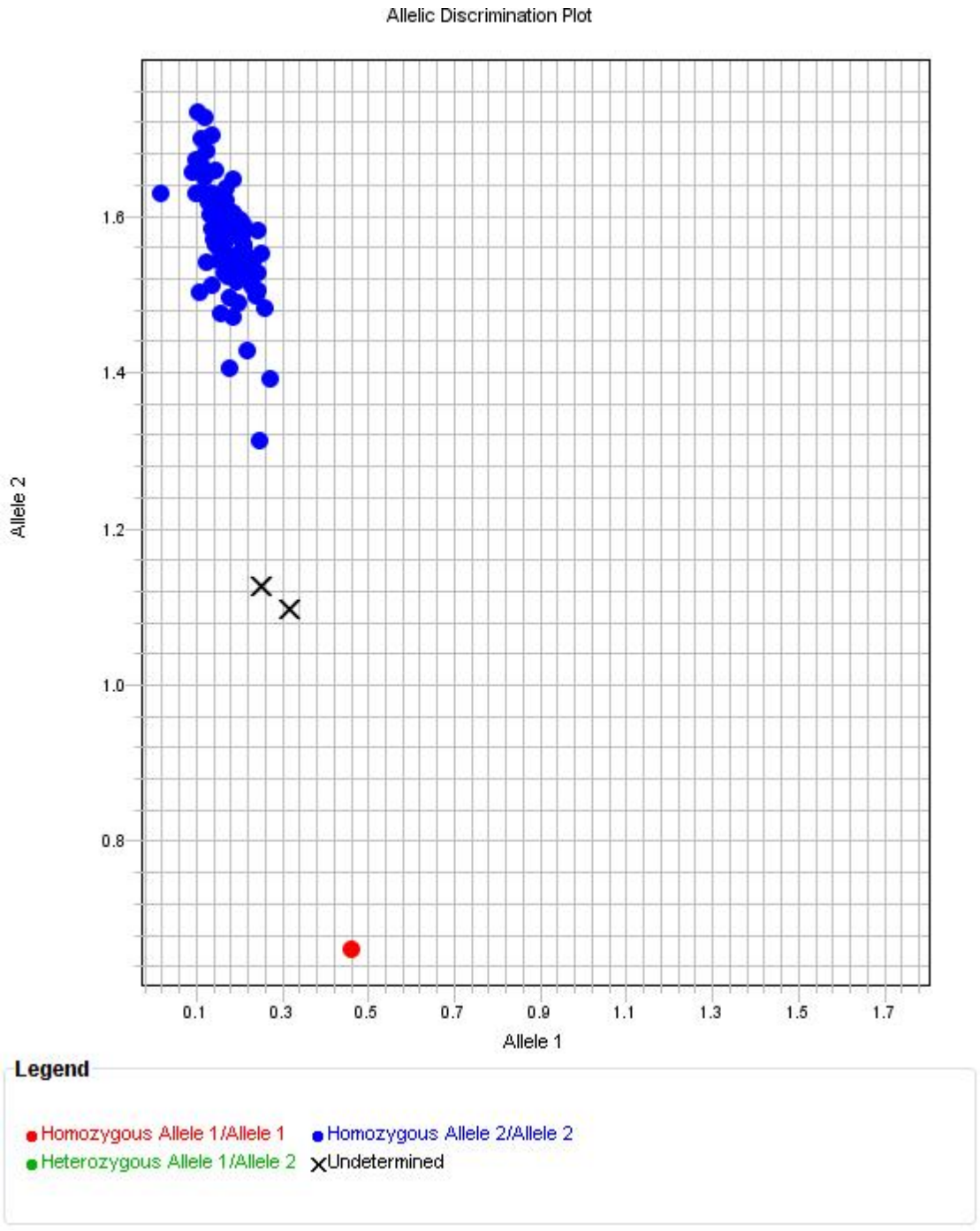

Figure A-8 Allelic discrimination plot for Rht-D1 Flourish/Emerson population. The population included 89 lines from the DH population, the parents (Flourish and Emerson), grandparents (CDC Osprey, McClintock and CDC Falcon) and great grandparent (CDC Kestrel). Allele 1 corresponds to the line having the wildtype (*Rht-D1a*) allele while Allele 2 corresponds with the dwarfing (*Rht-D1b*) allele.

Table A-6: LS means for all variables tested for the different treatment types for each of the three spring wheat genotypes tested (AC Cora, CDC Teal and FHB 37) in 2015 from the University of Manitoba field location for the Rate of Application Test.

Line	Treatment	Stage	Rate of Application (g/ha)	FHB Index [a] (%)		FDK [b] (%)		DON [c] (ppm)		Test Weight (kg/hL)		TKW [d] (g)	
AC Cora	*C. rosea*	Heading	150	48.3	GH	17.9	IJK	10.0	F	70.1	C	27.6	D
			300	52.4	FG	19.1	HIJK	13.9	EF	68.0	CDG	27.1	DE
			450	51.8	G	14.1	KLM	13.1	EF	68.2	CDE	27.0	DE
		Flowering	150	71.3	CD	25.5	FGHIL	18.3	CDEF	63.3	EFGH	25.8	DEFGH
			300	65.4	DE	27.0	BCEFGHIJK	18.3	DEF	65.5	DEF	25.6	DEFI
			450	61.6	DEF	22.4	GHIJK	14.5	EF	64.9	EF	28.2	BCDE
	Metconazole	Flowering		54.8	FG	14.5	JKLM	11.8	EF	68.4	BCDEF	27.1	CDEFG
	F. gram	Flowering		57.9	EFG	16.8	JK	15.3	EF	69.3	CD	27.2	DE
	Uninoculated	Flowering		4.4	K	2.70	N	1.90	H	74.9	A	30.8	BC
CDC Teal	*C. rosea*	Heading	150	85.3	AB	41.3	A	31.0	AB	59.9	HI	23.0	GH
			300	88.2	AB	40.4	ABC	27.1	ABCD	59.4	HI	25.6	DEFGI
			450	90.4	A	39.7	AB	27.6	BC	57.0	IJ	23.2	FGH
		Flowering	150	88.7	AB	36.5	ABCEF	37.3	AB	56.5	HIJ	22.0	H
			300	90.6	A	38.7	ABCE	30.0	AB	59.2	HIJ	24.2	EFGH
			450	92.3	A	35.9	ABCEF	32.6	A	55.3	J	21.6	HI
	Metconazole	Flowering		77.3	BC	22.0	GHIJK	18.6	DE	62.0	FHI	26.2	DEFGH
	F. gram	Flowering		87.1	AB	39.5	AD	29.0	ABC	58.6	HI	23.2	FGH
	Uninoculated	Flowering		5.5	K	7.45	MN	3.50	G	73.5	B	31.9	B
FHB 37	*C. rosea*	Heading	150	30.1	I	25.6	GHI	15.6	EF	68.3	DG	31.0	BC
			300	31.8	I	27.5	CEFGHI	15.0	EF	67.0	DEF	30.7	BC
			450	32.0	I	38.8	ABC	14.1	EF	67.0	CDEF	30.7	BC
		Flowering	150	35.8	HI	27.0	EFGHI	17.1	DEF	66.9	CDEF	31.5	ABC
			300	35.0	I	27.4	BCEFGHIJ	17.1	EF	68.1	CDEF	32.0	AB
			450	36.9	HI	30.4	BCDEFGH	15.3	E	66.9	DEF	29.4	BCD
	Metconazole	Flowering		16.0	J	24.6	EFGHIJK	16.5	EF	68.5	BCDEF	32.2	ABC
	F. gram	Flowering		29.0	I	31.7	BCEFG	12.3	EF	67.7	CDEF	31.2	BC
	Uninoculated	Flowering		0.6	L	2.33	N	0.83	I	73.3	B	34.1	A

Note: Results sharing the same letter grouping within a column do not differ significantly (P=0.05)
a. *Fusarium* head blight index, b. *Fusarium* damage kernels, c. Deoxynivalenol, d. Thousand kernel weight

Table A-7 LSD means for all variables tested for the different treatment types tested for each of the three spring wheat genotypes tested (AC Cora, CDC Teal and FHB 37) at three different rates of application in 2016 from the University of Manitoba Fort Gary Campus field location for the Rate of Application Test.

Line	Treatment	Stage	Rate of Application (g/ha)	FHB Index [a] (%)		FDK [b] (%)		DON [c] (ppm)		Test Weight (kg/hL)		TKW [d] (g)	
AC Cora	*C. rosea*	Heading	150	17.05	DEFG	5.53	ABCDE	5.95	BCDFGIJ	75.5	BCEFG	24.2	IJK
			300	8.65	EFGHJ	3.90	CDE	5.60	DEFGIJ	75.7	BCEF	24.0	IJK
			450	14.00	DEFGHJ	5.10	CD	6.53	BDH	75.2	CEF	23.0	K
		Flowering	150	21.28	DEF	5.60	CDE	7.98	BD	75.9	BCE	24.4	JK
			300	23.45	CDE	5.38	CDE	7.53	BDFG	76.4	BC	25.4	GHIJ
			450	20.78	CDEFGH	4.78	CDE	5.30	DEFG	76.0	BCE	25.0	GHIJK
	Metconazole	Flowering		9.00	FGHJ	4.08	CDE	5.90	DH	76.3	BC	25.9	GHIJ
	F. gram	Flowering		10.70	EFGHJ	6.78	BC	6.88	BDF	76.3	ABCE	24.8	GHIJK
	Uninoculated	Flowering		3.08	GHIJ	1.93	E	0.98	IJ	78.0	AB	25.5	FGHIJ
CDC Teal	*C. rosea*	Heading	150	46.95	A	10.70	ABC	17.98	AB	75.2	CDEFGH	25.9	EFGHIJ
			300	47.35	A	12.63	A	19.90	A	72.8	FGHI	24.8	GHIJK
			450	43.38	ABC	10.03	AB	17.38	AC	72.1	I	24.4	HIJK
		Flowering	150	48.58	A	12.93	A	17.63	A	73.3	FGHI	26.1	EFGHI
			300	55.05	A	11.55	AB	17.55	AC	73.3	GHI	25.5	FGHIJ
			450	46.45	AB	10.45	AB	15.75	AC	72.7	HI	25.1	GHIJK
	Metconazole	Flowering		17.68	DEI	6.28	C	7.75	BE	76.7	ABC	27.3	BDEF
	F. gram	Flowering		46.68	A	11.60	AB	16.63	AC	73.9	EFGHI	26.3	DEFGH
	Uninoculated	Flowering		27.78	BCD	5.75	C	3.58	FG	78.6	A	28.5	ABC
FHB 37	*C. rosea*	Heading	150	2.78	GHJ	5.43	ABCDE	2.68	BDFGIJ	76.5	ABCE	27.7	ABCDE
			300	2.35	HJ	3.40	CDE	2.45	DEFGIJ	76.3	ABCE	26.9	CDEFG
			450	4.25	EFGHJ	4.68	CDE	2.63	GI	76.4	ABC	28.6	ABC
		Flowering	150	2.23	GHJ	5.70	CDE	3.15	FGHIJ	76.7	ABC	28.2	ABCD
			300	3.88	GHJ	4.23	CDE	3.03	DEFGIJ	77.0	ABD	28.2	ABCD
			450	2.45	FGHIJ	3.73	CDE	2.85	FGHIJ	76.6	ABC	28.3	ABCDE
	Metconazole	Flowering		1.93	J	4.60	CDE	1.78	GIJ	78.3	A	28.6	AC
	F. gram	Flowering		4.03	GHJ	5.53	CDE	2.25	FGIJ	77.3	ABC	29.0	AB
	Uninoculated	Flowering		0.88	GHJ	2.00	DE	0.28	J	78.6	A	29.5	A

Note: Results sharing the same letter grouping within a column do not differ significantly (P=0.05)
a. *Fusarium* head blight index, b. *Fusarium* damage kernels, c. Deoxynivalenol, d. Thousand kernel weight

Table A-8: 2015 Efficacy values for the different Treatment*Genotype interactions between the 3 spring wheat genotypes (AC Cora, CDC Teal and FHB 37) ranging in resistance to FHB and the 8 different treatments given within the DONguard® Rate of Application Test.

Line	Treatment	Stage	Rate of Application (g/ha)	FHB Index [a] (%)	FDK[b] (%)	DON[d] (ppm)	Test Weight (kg/hL)	TKW[d] (g)
AC Cora	*C. rosea*	**Heading**	**150**	16.62	-6.54	34.43	1.18	1.58
			300	9.63	-13.37	8.98	-1.92	-0.52
			450	10.61	16.22	13.9	-1.62	-0.44
		Flowering	**150**	-23.13	-51.69	-19.67	-8.7	-4.86
			300	-12.86	-60.61	-19.67	5.46	-5.78
			450	-6.3	-33.27	4.92	-6.4	3.68
	Metconazole	**Flowering**		5.4	14.14	22.95	-1.25	-0.27
CDC Teal	*C. rosea*	**Heading**	**150**	2.04	-4.77	-6.9	2.2	-0.99
			300	-1.32	-2.36	6.45	1.28	9.99
			450	-3.76	-0.71	4.72	-2.73	-0.34
		Flowering	**150**	-1.89	7.6	-28.45	-3.5	-5.51
			300	-3.98	2.03	-3.45	0.97	4.3
			450	-5.94	8.92	-12.52	-5.68	-7.23
	Metconazole	**Flowering**		11.2	44.23	35.76	5.8	12.57
FHB 37	*C. rosea*	**Heading**	**150**	-3.9	19.02	-27.59	0.89	-0.58
			300	-9.59	13.18	-22.45	-0.98	-1.35
			450	-10.28	-22.53	-15.35	-0.94	-1.44
		Flowering	**150**	-23.55	14.76	-39.84	-1.11	1.22
			300	-20.79	13.59	-39.76	0.63	2.83
			450	-27.24	3.95	-24.49	-1.12	-5.52
	Metconazole	**Flowering**		44.72	22.27	-26.53	1.19	3.47

Note: A negative value indicates that the treatment performed worse than the *F. graminearum* control group in decreasing FHB symptoms group while positive results indicate that the treatment improved symptoms by that percentage

a. *Fusarium* head blight index

b. *Fusarium* damage kernels

c. Deoxynivalenol

d. Thousand kernel weight

Table A-9: 2016 Efficacy values for the different Treatment*Genotype interactions between the 3 spring wheat genotypes (AC Cora, CDC Teal and FHB 37) ranging in resistance to FHB and the 8 different treatments given within the DONguard® Rate of Application Test.

Line	Treatment	Stage	Rate of Application (g/ha)	FHB Index[a] (%)	FDK[b] (%)	DON[c] (ppm)	Test Weight (kg/hL)	TKW[d] (g)
AC Cora	*C. rosea*	Heading	150	-59.35	18.44	13.52	-1.06	-2.42
			300	19.16	42.48	18.60	-0.80	-3.23
			450	-30.84	24.78	5.09	-1.46	-7.26
		Flowering	150	-98.88	17.40	-16.00	-0.53	-1.61
			300	-119.16	20.65	-9.45	0.13	2.42
			450	-94.21	29.50	22.97	-0.40	0.81
	Metconazole	Flowering		15.89	39.82	14.24	0.00	4.44
CDC Teal	*C. rosea*	Heading	150	-0.58	7.76	-8.12	1.76	-1.52
			300	-1.44	-8.88	-19.67	-1.49	-5.70
			450	7.07	13.53	-4.51	-2.44	-7.22
		Flowering	150	-4.07	-11.47	-6.01	-0.81	-0.76
			300	-17.93	0.43	-5.53	-0.81	-3.04
			450	0.49	9.91	5.29	-1.62	-4.56
	Metconazole	Flowering		62.13	45.86	53.40	3.79	3.80
FHB 37	*C. rosea*	Heading	150	31.02	1.63	-19.11	-1.03	-4.48
			300	41.69	38.41	-8.89	-1.29	-7.24
			450	-5.46	18.84	-16.89	-1.16	-1.38
		Flowering	150	44.67	-3.26	-40	-0.78	-2.76
			300	3.72	23.37	-34.67	-0.39	-2.76
			450	39.21	32.43	-26.67	-0.91	-2.41
	Metconazole	Flowering		52.11	16.67	20.89	1.29	-1.38

Note: A negative value indicates that the treatment performed worse than the *F. graminearum* control group in decreasing FHB symptoms group while positive results indicate that the treatment improved symptoms by that percentage

a. *Fusarium* head blight index

b. *Fusarium* damage kernels

c. Deoxynivalenol

d. Thousand kernel weight

Table A-10 2015 Tukey-Kramer adjusted treatment means for FHB index, *Fusarium* damaged kernels (FDK) and deoxynivalenol (DON) for the different treatment types within each genotype tested from the University of Manitoba Fort Gary Campus field location for the DonGuard® Time of Application Test.

Line	Application Type	Time of Application	FHB Index [a] (%)		FDK [b] (%)		DON [c] (ppm)	
AC Cora	*C. rosea*	Flag leaf	43.31	ABCDEF	12.64	HIJ	4.77	IJ
		Heading	37.02	BCDEFG	12.56	GHIJ	5.43	GHIJ
		Flowering	41.73	ABCDEFG	15.83	BCDFGHIJ	8.00	FGHIJ
	Metconazole	Flowering	17.66	EFGH	9.93	GHIJ	5.44	HIJ
	F. graminearum Comtrol	Flowering	34.49	BCDEFG	13.19	DFGHIJ	6.10	FGHIJ
	Un-inoculated Control	Flowering	8.71	H	4.87	J	2.20	J
AC Morse	*C. rosea*	Flag leaf	50.83	ABCD	22.92	ABCDFG	37.16	A
		Heading	61.25	AB	34.22	A	33.13	AB
		Flowering	52.68	ABCDE	30.10	ABCD	32.17	AB
	Metconazole	Flowering	23.40	DEFGH	21.20	BCDFGH	25.75	BC
	F. graminearum Comtrol	Flowering	56.80	ABC	33.99	ABC	28.82	ABCD
	Un-inoculated Control	Flowering	13.71	GH	16.56	CDFGHI	12.75	EFGH
CDC Teal	*C. rosea*	Flag leaf	68.89	A	29.37	AB	15.67	DEF
		Heading	58.41	AB	27.83	ABE	20.92	CD
		Flowering	75.00	A	27.74	ABCDF	15.97	DEFG
	Metconazole	Flowering	51.64	ABC	19.54	BCDFGHI	9.17	FGHI
	F. graminearum Comtrol	Flowering	69.95	A	37.80	A	21.58	BCDE
	Un-inoculated Control	Flowering	17.88	EFGH	9.34	HIJ	5.16	IJ
FHB 37	*C. rosea*	Flag leaf	13.93	GH	12.22	HIJ	6.35	GHIJ
		Heading	15.76	FGH	11.91	GHIJ	8.52	FGHIJ
		Flowering	23.42	CDEFGH	14.46	CDEFGHIJ	8.92	EFGHIJ
	Metconazole	Flowering	9.02	GH	8.07	IJ	6.90	FGHIJ
	F. graminearum Comtrol	Flowering	15.63	FGH	12.95	FGHIJ	5.25	FGHIJ
	Un-inoculated Control	Flowering	0.68	H	3.97	J	1.57	J

a. *Fusarium* head blight index

b. *Fusarium* damage kernels

c. Deoxynivalenol

Table A-11 Tukey-Kramer adjusted treatment means for FHB index, *Fusarium* damaged kernels and deoxynivalenol for the different treatment types within each subgroup tested in 2016 site year data from the University of Manitoba Fort Gary Campus field location for the DONguard® Time of Application Test.

Line	Treatment	Time of Application	FHB Index [a]		FDK[b]		DON[c]	
AC Cora	*C. rosea + F. graminearum*	Flag leaf	4.52	B	2.80	ABC	6.89	BC
		Heading	9.88	AB	2.93	ABC	6.18	BC
		Flowering	7.17	AB	3.06	ABC	8.42	BC
		Flag leaf Heading	8.92	AB	3.54	ABC	6.05	BC
	C. rosea Control	Flowering	1.37	B	1.05	BC	1.58	BC
	Metconazole	Flowering	6.78	B	1.87	ABC	5.34	BC
	F. graminearum Control	Flowering	8.65	AB	2.08	ABC	8.35	ABC
	Uninoculated Untreated Control	Flowering	1.05	B	1.14	C	3.10	C
CDC Teal	*C. rosea + F. graminearum*	Flag leaf	10.53	AB	6.58	ABC	13.89	ABC
		Heading	15.52	AB	5.78	ABD	14.16	ABC
		Flowering	29.12	A	8.57	AB	27.68	A
		Flag leaf Heading	13.02	AB	5.55	A	15.04	AB
	C. rosea Control	Flowering	7.60	AB	2.47	ABC	7.02	BC
	Metconazole	Flowering	4.68	B	1.53	ABC	3.90	C
	F. graminearum Control	Flowering	26.80	AB	7.08	ABC	10.68	ABC
	Uninoculated Untreated Control	Flowering	1.93	B	3.20	ABC	5.74	BC
FHB 37	*C. rosea + F. graminearum*	Flag leaf	2.95	B	2.03	ABC	4.47	BC
		Heading	3.30	AB	0.434	ABC	2.23	BC
		Flowering	3.15	AB	2.99	ABC	5.32	BC
		Flag leaf Heading	6.77	AB	1.60	BC	2	C
	C. rosea Control	Flowering	1.03	B	0.94	CD	1.38	C
	Metconazole	Flowering	4.42	B	1.06	CD	0.7	C
	F. graminearum Control	Flowering	4.95	AB	0.90	ABC	2.79	BC
	Uninoculated Untreated Control	Flowering	0.60	B	0.83	CD	2.92	BC

Note: Values which share the same letter grouping are not significantly different at P=0.05

a. *Fusarium* head blight index, b. *Fusarium* damage kernels, c. Deoxynivalenol

Table A- 12 Minimum, maximum and average temperature of the University of Manitoba test site location over the duration of time that *C. rosea* was being applied to plants in the DONguard® Rate and Time Tests.

Julian Date	MM/DD	Min Temp 2015	Max Temp 2015	Avg Temp 2015	Precipitation 2015 (mm)	Min Temp 2016	Max Temp 2016	Avg Temp 2015	Precipitation 2016 (mm)
189	7-Jul	10.2	23.6	17.3	0	-	-	-	-
190	8-Jul	13.5	23.2	18.4	0	14.8	25.9	20.2	0.125
191	9-Jul	10.7	27.2	20.1	0	13.4	25.4	19.8	1.52
192	10-Jul	18.6	30.9	24.8	1.52	17.4	26.7	21.5	0
193	11-Jul	18.0	29.9	23.9	3.305	17.9	25.5	22.1	11.56
194	12-Jul	-	-	-	-	16.4	23.4	18.9	9.015
195	13-Jul	-	-	-	-	14.5	19.3	17.7	6.225
196	14-Jul	-	-	-	-	14.5	22.8	18.2	0.25
197	15-Jul	-	-	-	-	11.5	23.6	18.1	0
198	16-Jul	-	-	-	-	13.3	25.2	18.4	2.03
199	17-Jul	-	-	-	-	13.4	23.8	18.5	6.475
200	18-Jul	-	-	-	-	13.0	24.2	19.1	0

Table A-13 Composition of Spezieller Nährstoffarmer Agar (SNA)

Component	Amount
KH2PO4 (Potassium Phosphate)	1.0g
KNO3 (Potassium Nitrate)	1.0g
MgSO4-7H2O (Magnesium Sulphate)	0.5g
KCl (Potassium Chloride)	0.5g
Glucose	0.2g
Sucrose	0.2g
Agar	20g
Distilled Water	1L
Streptomycin Sulfate	0.25g

Table A-14 Composition of Carboxymethyl Cellulose (CMC) Media Culture

Component	Amount
KH2PO4 (Potassium Phosphate)	1.5g
NH4NO3 (Ammonium Nitrate)	1.0g
MgSO4-7H2O (Magnesium Sulphate)	0.75g
Yeast Extract	1.5g
CMC (Carboxymethyl Cellulose)	22.5g
Distilled Water	1.5L
Streptomycin Sulfate	0.375g