Improving Fusarium Head Blight Management in Durum Wheat in Saskatchewan

A dissertation submitted to the College of
Graduate and Postdoctoral Studies
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy
in the Department of Plant Sciences
University of Saskatchewan
Saskatoon, Saskatchewan, Canada

By
Gursahib Singh Sandha

PERMISSION TO USE

In presenting this dissertation in partial fulfillment of the requirements of a postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this dissertation in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised this thesis work, or in their absence, by the Head of the Department or the Dean of the College in which this thesis work was done. It is understood that any copying or publication or use of this dissertation or part thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my dissertation.

DISCLAIMER

This dissertation was exclusively created to meet the dissertation and/or exhibition requirements for the degree of Doctor of Philosophy at the University of Saskatchewan. References in this dissertation to any specific commercial products, process, or service by trade name, manufacturer or otherwise, does not constitute or imply endorsement, recommendation, or favoring by the University of Saskatchewan. The views and opinions of the author expressed herein do not reflect those of the University of Saskatchewan and shall not be used for advertising or product endorsement purposes.

Requests for permission to copy or to make other use of material in this dissertation in whole or part should be addressed to:

Head,
Department of Plant Sciences,
College of Agriculture and Bioresources
51 Campus Drive,
University of Saskatchewan, Saskatoon,
Saskatoon, Saskatchewan, S7N 5A8
Canada

OR

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatchewan S7N 5C9
Canada

ABSTRACT

Fusarium head blight (FHB) is one of the most important diseases of wheat in Canada. To manage the disease, farmers rely on an integrated pest management strategy that combines cultural practices (tillage and crop rotation), cultivar resistance and fungicide application at the current recommendation of early anthesis until 50% anthesis (BBCH61-65). Moderately resistant varieties are available in common wheat but not for durum wheat due to lack of resistance in the primary gene pool. The current study evaluated the effect of fungicide application timing and seeding rates on durum wheat affected by FHB; field trials were carried out from 2016 - 2018 at three locations in Saskatchewan. The parameters evaluated were: FHB index, Fusarium-damaged kernels (FDK), deoxynivalenol (DON) accumulation, grain protein content (GPC), and yield. Seeding rate influenced all parameters; the high seeding rate (400 seeds m^{-2}) had higher yield and FHB index, lower DON accumulation and GPC than the low seeding rate (75 seeds m^{-2}). Under extended wet conditions (high FHB risk), all anthesis applications starting at BBCH61 to BBCH69 had a similar effect on FHB index, FDK, DON accumulation, and yield, whereas in years with low disease severity, the BBCH65 application had lower disease (FHB index and FDK) and DON accumulation. The dual application (BBCH61+73) results for FHB index, FDK, and toxin content were similar to the BBCH65 application at all site-years. In North America, the triazole (demethylation inhibitors, FRAC group code - 3) fungicides (e.g., tebuconazole, metconazole, and prothioconazole) are the most effective groups against FHB. I tested 252 *Fusarium graminearum* isolates collected from western Canada for their sensitivity to tebuconazole, metconazole, and prothioconazole. Phenotyping for fungicide sensitivity of *F. graminearum* isolates revealed variation in sensitivity to each of the three fungicides. The EC_{50} of the isolates for tebuconazole, metconazole and prothioconazole was between 0.013 - 0.16 mg L^{-1}, 0.008 - 0.088 mg L^{-1}, and 0.012 - 0.058 mg L^{-1}, respectively. The EC_{50} values did not differ between trichothecene groups

3-acetyl deoxynivalenol (3ADON) and 15-acetyl deoxynivalenol (15ADON) or year of isolate collection (before/after fungicide registration). A three-year Saskatchewan wide survey of *Fusarium* spp. conducted between 2014 and 2016 revealed that *F. graminearum* was the dominant species detected and quantified in infected wheat, followed by *F. avenaceum*. Among *F. graminearum* chemotypes, the 3ADON chemotype was found more frequently than 15ADON, while the *F. graminearum*-nivalenol (NIV) chemotype was absent. This work indicated and re-confirmed that the pathogen profile in Saskatchewan is changing over time. Mycotoxin quantification revealed that 72% of the samples had toxin accumulation >1 μg kg^{-1}. Toxins present were DON, D3G, 3ADON, 15ADON, NIV, T2, and HT2. A weak correlation was detected between *F. graminearum* DNA and DON levels (R = 0.288, P = 0.008). In the final study in this thesis, I found that all four *F. graminearum* chemotypes (3ADON, 15ADON, NIV, and NX-2) were pathogenic on wheat and varied in FHB severity, with 3ADON (29.7 ± 3.6) and 15ADON (22.4 ± 2.9) isolates more aggressive than NIV (16.7 ± 2.8) and NX-2 (16.2 ± 3.1) isolates. Significant differences were observed for mycotoxin accumulation among chemotypes, which was highest for 3ADON (14.1 ± 2.26 μg kg^{-1}). All these studies will help to understand pathogen population structure and biology, which will help improve future breeding strategies and FHB management programs to reduce yield losses and mycotoxin contamination.

DEDICATION

"This thesis is dedicated to my grand father (S. Gopal Singh Sandha)"

ACKNOWLEDGEMENTS

I would like to express my gratitude and appreciation to my supervisor, Dr. Hadley R. Kutcher (Professor and Cereal Pathologist, Crop Development Centre), for accepting me as his student and provided me with the opportunity to work on such an interesting project that has all the components. I would also like to acknowledge the assistance and guidance of my advisory committee members Dr. Sabine Banniza (Professor, Crop Development Centre), Dr. Steve Shirtliffe (Professor, Crop Development Centre), Mr. Eric Johnson (Research Officer, Department of Plant Sciences, University of Saskatchewan) and Dr. Natacha Hogan (Associate Professor, Department of Animal and Poultry Science, College of Agriculture and Bioresources) for their scientific advice and insightful comments and suggestions. Special thanks to Dr. Gary Bergstrom (Cornell University, USA) for serving as the external examiner for this dissertation.

I am very grateful to Dr. Gary Peng (Research Scientist, Agriculture and Agri-Food Canada, Saskatoon Research Centre), Dr. Pierre Hucl (Professor, Crop Development Centre), Dr. Pierre Fobert (Director, Sustainable Protein Production Program at National Research Council Canada), Mr. Garry Hnatowich, (Research Director, Irrigation Research & Demonstration Program, Outlook, SK), Dr. Lipu Wang (Research Officer, University of Saskatchewan, Dr. Maria A. Henriquez (Wheat Pathologist, Agriculture and Agri-Food Canada, Morden Research and Development Center, Morden, MB), Dr. Gurcharn Brar (Assistant Professor, University of British Columbia), Ms Amy Brown (Saskatchewan Crop Insurance Corporation, SK) for their help and support in the degree program. I appreciate the technical assistance provided by Everett Boots, Mallory Dyck, Stephanie Ginter, Ehsan Sari, Magda Konopka and Mortuza Reza. Lastly, I would thank my family, especially my wife (Ravneet Kaur) for her continuous support and encouragement.

I gratefully acknowledge the financial assistance from Agriculture Development Fund of the Saskatchewan Ministry of Agriculture, Saskatchewan Wheat Development Commission, and the Western Grain Research Foundation. I am very thankful for scholarships provided by Saskatchewan Wheat Development Commission, the Henry and Erica Martynse graduate scholarship, the Canadian Phytopathological Society Graduate Scholarship, Dr. Guy Lafond Memorial Award, J. D. MacFarlane Postgraduate Scholarship, and value-added cereals award (2019 and 2020) from the Department of Plant Science, College of Agriculture & Bioresources, and the University of Saskatchewan.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS

15ADON - 15-acetyl deoxynivalenol

3ADON - 3-acetyl deoxynivalenol

BBCH - (Bayer, BASF, Ciba-Geigy, and Hoechst) - growth scale used to identify phenological development stages

CDC - Crop Development Centre

CPS - Canada prairie spring

CT - Cycle threshold

CWAD - Canada western amber durum

D3G - Deoxynivalenol-3-glucoside

DAOM- Canadian Collection of Fungal Cultures (DAOM), Ottawa, Ontario, Canada

DMI- Demethylation inhibitor

DNA - Deoxyribonucleic acid

DON - Deoxynivalenol

DPI - Days post-inoculation

EFSA - European Food Safety Authority

FDK - Fusarium damaged kernel

FGSC - *Fusarium graminearum* species complex

FHB - Fusarium head blight

FRAC - Fungicide resistance action committee

GCPSR - Genealogical concordance/discordance phylogenetic species recognition

GPC - Grain protein content

CWRS - Hard red spring wheat

JEFCA - Joint FAO/WHO Expert Committee on Food Additives

MIC - Minimum Inhibitory Concentration

NIL - Near-isogenic line

NIV - Nivalenol

NRRL - Northern Regional Research Laboratory

PBD - Potato dextrose broth

PCR - Polymerase chain reaction

PDA - Potato dextrose agar

Ppm - Part per million

QTL - Quantitative trait loci

R.M. - Rural municipality

RNA - Ribonucleic acid RNA

SAS - Statistical analysis software

SK- Saskatchewan

TDI - Tolerable daily intake

TKW - Thousand kernel weight

TW - Test weight

ZGS- Zadok growth stage

CHAPTER 1

Introduction

The province of Saskatchewan leads Canada in wheat production and produces the majority of durum wheat in the country (Saskatchewan Ministry of Agriculture 2020). The production of wheat in Canada is affected by several biotic and abiotic stresses; among biotic stresses, diseases such as Fusarium head blight (caused by *Fusarium graminearum* Schw. [Petch]), leaf rust (caused by *Puccinia triticina* Eriks.), stem rust (caused by *Puccinia graminis* Pers.: Pers f. sp. *tritici* Eriks. E. Henn), stripe rust (caused by *Puccinia striiformis* Westend f. sp. *tritici* Eriks.), and common bunt (caused by *Tilletia caries* Bjerk. and *Tilletia laevis* Bjerk.) are responsible for significant losses.

Fusarium head blight (FHB) of wheat is one of five priority diseases of wheat in Canada, caused by various toxigenic *Fusarium* spp., particularly *F. graminearum*, *F. avenaceum*, *F. culmorum*, *F. poae*, and *F. acuminatum* (Parry et al. 1995). Although more than 17 species can cause FHB in cereals, *F. graminearum* is the predominant causal agent of the disease in North America and many other parts of the world (McMullen et al. 2012). The direct losses due to FHB are reduced yield and test weight due to the presence of Fusarium damaged kernels (FDKs) (Argyis et al. 2003; Liggit et al. 1997; Wilcoxson et al. 1988). The indirect impacts of the disease are the trichothecene mycotoxins, which are accumulated in the kernels during the infection process. Mycotoxin contamination is a severe issue in the wheat industry, as consumption of mycotoxin-infected grain poses a health risk to both humans and animals (Bottalico 1998).

Generally, an integrated disease management approach is recommended to mitigate FHB, as no single control strategy is highly effective in preventing yield and quality losses in small-grain cereals (Gilbert and Haber 2013). Most researchers support the integration of cultural and chemical practices with host resistance as essential in managing FHB in wheat (Amarasinghe

et al. 2013; Scala et al. 2016; Willyerd et al. 2012). Diverse crop rotations, including four species (e.g., a four-year crop rotation in western Canada), possibly supplemented with tillage and other residue management tools is essential when trying to prevent or reduce FHB incidence. Still, this has not been common practice by growers due to economic reasons (Beres et al. 2018). Cultivar resistance is one effective management strategy in hexaploid wheat, but due to the low level of FHB resistance in durum wheat, growers rely on fungicide application as a major management tactic for in-season disease control.

Fungicide class and optimal application timing are critical for effective management of FHB. The demethylation inhibitor (DMI) triazole fungicides are one of the most important groups that have been used since the 1980s to reduce the impact of FHB (Boyacioglu et al. 1992; McMullen et al. 2012; Paul et al. 2008). Field trials on fungicide efficacy of commonly used triazole-based fungicides demonstrated that metconazole, prothioconazole, and tebuconazole + prothioconazole offer effective disease suppression and the greatest reduction in DON accumulation (Paul et al. 2008).

The timing of fungicide application is crucial for maximum FHB suppression. The most effective fungicide application timing is unclear, although application at anthesis is reported to be most effective, as the plants are most susceptible to FHB infection at this stage (Beyer et al. 2006; Haidukowski et al. 2005). Many researchers have studied the effect of pre-anthesis, anthesis, and post-anthesis application of fungicide on FHB index, FDK and DON accumulation (Chen et al. 2012; D'Angelo et al. 2014; Paul et al. 2018; Tateishi et al. 2014; Wegulo et al. 2011). Preventive fungicide treatment applied at the flag leaf or heading stages are least effective in reducing FHB index and DON accumulation (Caldwell et al. 2017; Holzapfel 2015; Hutcheon and Jordan 1992; Paul et al. 2018). Application at anthesis and post-anthesis (up to 6 days after anthesis) has proven to be effective in reducing FHB incidence and severity in spring and winter wheat; although, application at 50% anthesis is generally more

effective in moderating FHB index (Bradley et al. 2010; D'Angelo et al. 2014; Paul et al. 2018). Fungicide application later in the growing season (at the milk stage) is confirmed to be beneficial in reducing mycotoxin (DON and NIV) accumulation in grain (Tateishi et al. 2014; Yoshida et al. 2012). Despite the prevalence of FHB in western Canada and frequent epidemics of the disease in recent years, little is known about the effect of fungicide application timing on disease level and toxin accumulation in durum wheat. Thus, there was a need to test fungicide efficacy in terms of reducing disease severity and DON accumulation in durum, particularly for Saskatchewan climatic conditions.

Worldwide, the fungicides tebuconazole, metconazole, and prothioconazole have been used extensively since the 1990s in FHB management (Boyacioglu et al. 1992). Like the emergence of antibiotic resistance in human pathogens, long-term fungicides use increases the risk of insensitivity in plant pathogens. Due to the increasing use of triazole fungicides and the risk of fungicide resistance, there is an urgent need to examine field populations of *F. graminearum* for variation in sensitivity to triazole fungicides. Reports of triazole fungicide insensitivity are rare in the literature, but a few researchers have observed reduced sensitivity to triazole fungicides (Klix et al. 2007; Spolti et al. 2014; Yin et al. 2009). Fungicide insensitivity can be studied by analyzing resistance and cross-resistance levels of isolates from fields with a known history of fungicide treatment (Becher et al. 2010). Reduced sensitivity of *F. asiaticum* and *F. graminearum* isolates to benzimidazole and tebuconazole have been reported in China (Yin et al. 2009). Of 159 isolates, nine were highly insensitive to benzimidazole, and three to tebuconazole. There were no reports of cross-resistance among isolates.

Fusarium graminearum isolates collected from multiple states in the USA between 1981 and 2014 showed reduced sensitivity to tebuconazole and metconazole (Anderson et al. 2020). The mean EC_{50} values of isolates collected between 2000 and 2014 were higher than isolates collected prior to 2000. One isolate collected from Illinois had very high EC_{50} values

(metconazole - 0.1734 µg mL^{-1} and tebuconazole -1.7339 µg mL^{-1}) as compared with the means EC_{50} [mean (metconazole) EC_{50} - 0.0405 µg ml^{-1} and mean (tebuconazole) EC_{50} - 0.3311 µg ml^{-1}] of other isolates collected between 2000 and 2014. Similarly, Spolti et al. (2014) reported the first tebuconazole-resistant isolate (EC_{50} - 8.09 mg L^{-1}) in the USA collected from New York. When sensitivity to tebuconazole, metconazole, and prothioconazole was studied in a collection of *F. graminearum* isolates from Germany over a 20-year period from 1984 to 2004, a reduction in sensitivity of up to 2.59 times was detected (Klix et al. 2007). Although there are no reports of *F. graminearum* insensitivity to triazole fungicides in Canada, it is prudent to investigate the pathogen population on the prairies for insensitivity to triazole products currently used.

Another reason for the variable results obtained in breeding programs for FHB resistance and FHB control strategies across the province may be the variability among *Fusarium* spp. in terms of aggressiveness and the amount of trichothecene they produce. The aggressiveness of a pathogen is a quantitative measurement of the disease induced by a pathogenic isolate on a susceptible host (Vanderplank, 1984). In the case of *F. graminearum*, aggressiveness also depends on the trichothecene producing capacity of the isolates; non-DON producing (*Tri5* mutants) *F. graminearum* isolates cannot spread beyond the initial infection site but are still able to infect plants (Bai et al. 2001; Eudes et al. 2001; Mesterházy et al. 2002). Although multiple species can cause FHB, aggressiveness studies have revealed that *F. graminearum* and *F. culmorum* are highly pathogenic; others are considered weakly pathogenic (Wong et al. 1995; Xue et al. 2004).

Fusarium graminearum populations can be further differentiated based on the types of trichothecene chemotype they produce (Kelly et al. 2015). The existence of chemotypic groups of *F. graminearum* was first proposed by Ichinoe et al. (1983), and their distribution shows regional and seasonal differences. A chemotype is a chemical phenotype that indicates the

profile of natural compounds, including the mycotoxins an organism produces (Desjardins 2008). Trichothecenes associated with *F. graminearum* are Type A trichothecene (NX-2) and Type B (3ADON, 15ADON, and NIV) (Kelly et al. 2015; Miller et al. 1991; Varga et al. 2015). In the past, the indigenous *F. graminearum* population in North and South America was dominated by 15ADON producers; whereas 3ADON was dominant in Europe and Asia (Valverde-Bogantes et al. 2019; Ward et al. 2008). The NIV producers exist in parts of the USA, Europe, Japan, and Australia, but so far remain uncommon in Canada (Gale et al. 2011; Miller et al. 1991; Tittlemeier et al. 2013). The Asian 3ADON producing strains were first introduced to Atlantic Canada in the 1970s to 1980s on breeding material from China and Europe; the population of the 3ADON chemotype has been on the rise ever since (Ward et al. 2008). Over the years, chemo-taxonomical studies from western Canada have reported a significant increase in frequency of the 3ADON chemotype (Ahmed et al. 2020; Guo et al. 2008; Kelly et al. 2015; Ward et al. 2008). The newly emerging 3ADON population appears to be more aggressive than 15ADON, i.e., it produces more trichothecenes, is more prolific, and has higher growth rates (Ward et al. 2008).

This rapid shift from the 15ADON chemotype to the 3ADON chemotype is of increasing concern for the Canadian wheat industry. Regular monitoring of the pathogen population dynamics, the genetic basis of the chemotypic shift, and the impact of the chemotype shift can be useful for both breeding programs and current disease management strategies such as fungicide application. The overall focus of this thesis was to improve the understanding of FHB in western Canada, mainly in durum wheat, as the basis for recommendations to improve FHB management and to provide pathogen-specific information for future research investments. The specific hypotheses and the corresponding objectives included in this PhD project/ were:

1.1 Hypotheses

- The optimum timing of fungicide application to decrease FDK is BBCH61, and to reduce DON is BBCH73,
- The contemporary population of *F. graminearum* in western Canada (Saskatchewan, Manitoba, and Alberta) differs in sensitivity to tebuconazole, metconazole, and prothioconazole,
- FHB epidemics in recent years across SK were due to multiple *Fusarium* species and multiple *F. graminearum* chemotypes, and
- The aggressiveness of *F. graminearum* isolates differ among chemotypes (3ADON, 15ADON, NIV, and NX-2).

1.2 Project objectives

1. To compare the effect of various fungicide application timings on yield, FHB index, DON accumulation, FDK, TW, TKW, and grain protein content of durum wheat,
2. To determine the sensitivity of the contemporary population of *F. graminearum* in western Canada to tebuconazole, metconazole, and prothioconazole associated with symptomatic wheat spikes collected from 2014 to 2017 from durum and bread wheat,
3. To determine the population and chemotype diversity of *Fusarium* spp. among FHB infected durum and bread wheat samples from Saskatchewan, and
4. To compare the aggressiveness of 3ADON, 15ADON, NIV, and NX-2 chemotypes of *F. graminearum* and determine whether spring wheat NILs with different genes for resistance perform similarly against these four chemotypes of *F. graminearum*.

CHAPTER 2

Review of the Literature

2.1. Wheat: production and consumption

Wheat is one of the world's most important cereals in terms of production and consumption, along with corn and rice. It is also the most widely cultivated crop in Canada, with 9.95 million hectares of bread wheat and 1.98 million hectares of durum, resulting in production of 25.6 metric tonnes in the case of bread wheat and 4.9 metric tonnes of durum in 2019 (Statistics Canada 2019). The Province of Saskatchewan leads Canada in wheat production (8.5 million tonnes) and produces the majority of the durum wheat (4.5 million tonnes) (Statistic Canada 2019).

Wheat in Canada is categorized as western Canadian and eastern Canadian by the regions in which they are grown. In Western Canada, the wheat cultivars are classified into the following nine classes: Canada Northern Hard Red (CNHR), Canada Prairie Spring Red (CPSR), Canada Western Amber Durum (CWAD), Canada Western Hard White Spring (CWHWS), Canada Western Red Spring (CWRS), Canada Western Red Winter (CWRW), Canada Western Soft White Spring (CWSWS), and Canada Western Special Purpose (CWSP) (Curtis 2002). Canada Western Red Spring and Canada Western Amber Durum classes account for the greatest seeded area under cultivation.

Wheat is a good source of protein, dietary fibre, vitamins, and minerals; it is also rich in antioxidants and other healthy plant-based nutrients, making it a staple food in many countries. Wheat flour contains a high percentage of gluten (elastic protein); this makes wheat suitable for the baking industry. A considerable portion of the bread wheat produced is used in making bread; durum wheat is used mainly in making macaroni, spaghetti, couscous, and other pasta products. Additionally, wheat is also used in the brewing industry to make beer and vodka. The

residual milling products (grain and bran) and vegetative plant parts make it a valuable livestock feed and bedding component (Curtis 2002; Shewry 2009).

2.2. Fusarium head blight: the disease, causal agent and impact

Wheat is susceptible to many diseases, including rusts, bunts, powdery mildew, and Fusarium head blight (FHB). Among these, the most economically important disease of wheat, barley, and other small grain cereals worldwide is FHB (Tekauz et al. 2009; Jones and Mirocha 1999). The disease, also known as scab, is caused by various toxigenic species of Fusarium (Parry et al. 1995). The geographical distribution of these species is influenced by temperature (Mesterházy 2003). *Fusarium graminearum* is found predominantly in warmer and humid regions, whereas *F. culmorum* and *F. avenaceum* predominates primarily in cooler regions of the world. Genealogical concordance/discordance phylogenetic species recognition (GCPSR) studies revealed that the *F. graminearum* species complex (FGSC) is comprised of 16 species, including *F. graminearum sensu stricto, F. gerlachii, F. louisianens, F. asiaticum, F. ussurianum, F. nepalense, F. vorosii*, *F. acaciae-mearnsii, F. aethiopicum, F. boothii, F. mesoamericanum, F. austroamericanum, F. cortaderiae*, *F. brasilicum,* and *F. meridionale* (Aoki et al. 2012). Although more than 17 species can cause FHB in cereals, *F. graminearum* is the predominant causal agent of the disease in the USA, Canada, China, Australia, and central Europe (McMullen et al. 2012).

Fusarium graminearum is an ascomycete fungus that produces macroconidia (asexual spores) in the asexual stage and ascospores in the sexual stage (Gilbert and Fernando 2004). Extended periods of high moisture or relative humidity (>90%) and moderately warm temperatures (between 15 to 30°C) before, during, and after flowering favor inoculum production, floret infection, and colonization of developing grains (Schmale and Bergstrom 2003).

Under favourable conditions, the symptoms of FHB are often characterized by the growth of salmon pink to red coloured mycelia or sporodochia and /or purple-black perithecia on glumes at the base of the spikelet. Ultimately, the fungus spreads and diseased spikelets become prematurely bleached (Bockus et al. 2010; Parry et al. 1995). Over time, the entire head is colonized, and the developing kernels are discoloured, shrunken and chalky white in appearance with black perithecia giving the name Fusarium damaged kernels (FDK) (Parry et al. 1995). The fungus enters its host by producing cell wall degrading enzymes (hydrolytic enzymes) that vary with the associated crop (Kikot et al. 2009).

In addition to FDK, this disease also results in the production and accumulation of trichothecene toxins (mycotoxins) in the harvested grain. Mycotoxins are of major concern because they pose acute and chronic health hazards to both humans and animals (Gräfenhan et al. 2013; Miller and Richardson 2013). They not only reside in the grain during long-term storage but are also resistant to heat (Argyis et al. 2003; Liggit et al. 1997; Wilcoxson et al. 1988). Mycotoxin adulterated grain is unfit for industrial use such as milling, baking, and pasta-making due to the destruction of starch granules, cell walls, and endosperm proteins by the fungus (Nightingale et al. 1999).

2.3. Disease Epidemiology

Fusarium graminearum has a wide host range; it can survive on non-gramineous plants as a component of the root rot complex on pulses and on dead plant tissue (Xu and Chen, 1993; Chongo et al. 2001). As a saprophyte, it can survive as mycelia, ascospores, macroconidia, and chlamydospores on crop residue in the absence of the host in the field. In the subsequent crop season, primary infection starts with the production of sexual fruiting structures called perithecia at temperatures between 15-32°C. Airborne spores (ascospores and macroconidia) are the primary inoculum in the epidemiology of FHB. Warm temperatures (28-32°C) during the cropping season facilitate the release of ascospores from perithecia, which are carried by

wind or water splash to the wheat spikes (Bockus et al. 2010; Sutton 1982). During the flowering stage, warm (25-30°C) and wet weather (precipitation and relative humidity of 60-95%) promotes spore germination and causes infection of glumes, flowers, and other parts of the spikes (Dill-Macky 2010; McMullen et al. 1997). The density of the residue left from the preceding crop determines the severity of FHB as severity and mycotoxin contamination increase with an increase in the density of residues (Blandino et al. 2010). The surface crop residue then acts as a substrate for active growth of the fungus for a more extended period; therefore, deep burying of infested crop residue can effectively reduce fungal residents; however, the pathogen may survive for several years (Pereyra et al. 2004).

2.4. Trichothecenes

The members of the *Fusarium* species complex can be further differentiated based on trichothecene chemotypes (Desjardins et al. 1993). Chemotypes are sesquiterpenoid secondary metabolites, produced by *Fusarium* spp. and have a virulence role in plant disease. These are accumulated in grain after infection, and their consumption can lead to serious health problems (Proctor et al. 1995; Bottalico 1998). Trichothecenes can be broadly classified into four primary groups (Type A, Type B, Type C, and Type D); Type A and Type B are toxicologically the most important. Most *Fusarium* spp. produce Type B trichothecenes, but *F. graminearum* can also produce a newly reported Type A trichothecene (Varga et al. 2015). The most common mycotoxins associated with *F. graminearum* are Type B [deoxynivalenol (DON), its 3acetylated (3ADON) or 15acetylated derivatives (15ADON), and nivalenol (NIV)], and Type A trichothecenes (NX-2 and its derivatives) (Alexander et al. 2009; McCormick et al. 2011; Varga et al. 2015). The strain-specific trichothecene metabolites commonly found in *Fusarium* spp. are nivalenol (NIV) and DON chemotypes (O'Donnel et al. 2004). Nivalenol is more toxic than DON, and nivalenol-producing strains of *F. graminearum* are common in parts of Europe, Japan, USA, and Australia (Miller and Richardson 2013). The diversity of *Fusarium*

spp. on small grain cereals, mainly wheat in Canada, is much more comprehensive and includes less pathogenic *Fusarium* spp., principally *F. sporotrichioides, F. equiseti, F. poae*, and *F. acuminatum*. Most of these species are also potent producers of other less studied Type A (T2 and HT2 toxins) and Type B trichothecenes (NIV, beauvericin, enniatin, and moniliform) (Foroud et al. 2019). Type A trichothecenes such as T2 and HT2 toxins are generally minor contaminants in Canadian grains but are major concerns because of their higher acute toxicity than DON and other Type B trichothecenes (Miller and Richardson 2013).

Trichothecene chemotypes are encoded by *Tri* genes located in the *Tri* cluster and are synthesized through complex biosynthetic pathways. Mycotoxin production (DON or NIV) is determined by genes *Tri7* and *Tri13*. The NIV chemotype results from functional *Tri7* and *Tri13*, while nonfunctional *Tri7* and *Tri13* results in the DON chemotypes. The DON producers are further divided into 3ADON and 15ADON based on acetyl group position (C-3 and C-15), and their products depend on the *Tri8* gene from the *Tri* cluster (Alexander et al. 2011). Varga et al. (2015) identified a new trichothecene structure produced by *F. graminearum* strains (7-hydroxy, 8-deoxy) and called it a novel chemotype NX-2. The molecule structure of NX-2 is identical to 3ADON except for the absence of a keto group at the C-8. *Fusarium graminearum*-NX-2 strains never make DON but rather accumulate NX-2 and its derivative NX-3 in plants.

2.4.1. Population structure differentiation

The population of FHB pathogens can change over time at the species level, species with selective advantage can replace another species, or different populations of the same species can replace the existing population in a region. The major changes in pathogen population are mainly due to environmental conditions, resulting in displacement events if the conditions are advantageous for the invading population. In the Canadian Prairie Provinces, three species of *Fusarium* are associated with FHB in cereals: *F. graminearum*, *F. culmorum*, and *F. avenaceum* (Clear and Patrick 2010). Similarly, *Fusarium* population diversity studies from

eastern and western Canada reported *F. graminearum* as the most dominant species along with *F. avenaceum*, *F. culmorum*, *F. poae*, *F. sporotrichioides*, *F. acuminatum*, and *F. pseudograminearum* (Gräfenhan et al. 2013; Guo et al. 2008; Tittlemier et al. 2013; Xue et al. 2019). Recent surveys from Manitoba showed that the distribution of *Fusarium* spp. is changing continuously due to environmental conditions. *Fusarium graminearum* was the predominant pathogen in 2016, when excessive moisture occurred during the growing season, whereas in drier conditions of 2017, *F. poae* predominated (Banik et al. 2017; 2018). This shows that changes in climatic conditions continuously shape the species composition of FHB pathogens and therefore the mycotoxin profile in the grain.

The *Fusarium graminearum* population in Canada is composed of all Type B (3ADON, 15ADON, and NIV) and Type A (NX-2) trichothecene genotypes (Valverde-Bogantes et al. 2019). In the past, the indigenous *F. graminearum* population in Canada was dominated by 15ADON producers; whereas 3ADON was dominant in Europe and Asia (Malihipour et al. 2012; Miller and Richardson 2013; O'Donnell et al. 2004). The NIV producers exist in parts of the USA, Europe, Japan, and Australia, but so far remain rare in Canada (Gale et al. 2011; Miller et al. 1991; Tittlemeier et al. 2013; Ward et al. 2008). In North America, the first reports that the NIV chemotype was detected in *F. graminearum sensu stricto*, *F. cerealis,* and *F. asiaticum* were from wheat in southern Louisiana (Gale et al. 2011, Horevaj et al. 2011). The Asian 3ADON producing strains of *F. graminearum* were first introduced to Atlantic Canada in the 1970s to 1980s on breeding material from China and Europe; the population of the 3ADON chemotype has been on the rise since. Canadian cereal grain surveys of FHB causing species and chemotypes have shown that nearly 100% of the *F. graminearum* strains from the Maritimes are 3ADON producers, and the frequency of 3ADON producing *F. graminearum* strains is increasing in western Canadian provinces (Ahmed et al. 2020; Kelly et al. 2015; Miller and Richardson 2013; Ward et al. 2008). It was suggested that this change in chemotype

population might be due to a selective advantage of the 3ADON chemotype over the native 15ADON chemotype. In 2015, *F. graminearum* isolates capable of producing the newly discovered Type A trichothecene named NX-2 were reported in Minnesota (Varga et al. 2015). The *Tri1* gene-based diagnostic test revealed that although the NX-2 toxin was found in all *F. graminearum* isolates collected from the northern USA and southern Canada between 1999 and 2013, and the frequency was low (2.8%) (Kelly et al. 2015, 2016; Liang et al. 2014). However, a reexamination of isolates collected from northeastern New York State, previously reported as 3ADON genotypes, had a high proportion (42.1%) of NX-2 genotype strains (Lofgren et al. 2018).

2.4.2. Trichothecenes as pathogenicity factors

Trichothecene chemotypes also govern pathogenicity and aggressiveness of *Fusarium* strains. Pathogenicity is a qualitative measurement that reflects the capability of a pathogen to cause disease, while aggressiveness is a quantitative measurement of the disease induced by a pathogenic isolate on a susceptible host (Agrios 2005; Vanderplank 1984). A more aggressive pathogen will reach a given disease level faster than the less aggressive variant (Shaner et al. 1992). Aggressiveness of *Fusarium* spp. depends on their trichothecene producing capacity (Mirocha et al. 1998; Proctor et al. 1995). In FHB development on wheat, DON production by *F. graminearum* aids in the colonization of tissues, but it is not required for the infection process (Hallen-Adams et al. 2011). Desjardins et al. (1996) found that wheat spikes, when inoculated with non-trichothecene producing *Tri5* mutants of *F. graminearum,* could not colonize the wheat spike, i.e., they were restricted to the initially inoculated spikelets and caused low disease severity as compared with isolates possessing a functional *Tri5* gene. This suggested that even though trichothecenes are not required for initial infection, they are essential for pathogen spread within the spike (Bai et al. 2002).

Hallen-Adams (2011) investigated gene expression during wheat kernel colonization. The *Tri5* gene was turned on during initial infection and provided evidence that trichothecene toxins aid in pathogen spread and colonization. Sometimes *Tri5* genes are re-activated later in the growing season, contributing to toxins in the harvested grain, even in the absence of visual symptoms. Therefore, it was suggested that *F. graminearum* could rapidly resume toxin biosynthesis under favourable conditions even in infected dried grain.

The aggressiveness of *F. graminearum* is non-host specific (Gilbert et al. 2002). Many researchers have studied the role of trichothecenes as an aggressiveness factor in FHB, but the facts are still unclear (Goswami and Kistler 2005; Hestbjerg et al. 2002). When inoculated on wheat, highly aggressive isolates of *F. graminearum* and *F. culmorum* tend to produce higher DON accumulation than less aggressive isolates (Gang et al. 1998; Hestbjerg et al. 2002). In contrast, Walker et al. (2001) reported no significant difference in DON production levels between isolates with the highest and lowest aggressiveness (disease severity). There was no difference in aggressiveness between 3ADON and 15ADON producing *F. graminearum* isolates when tested on corn and carnation (Adams and Hart 1989). Goswami and Kistler (2005) reported that although the amount of the dominant trichothecene influenced the aggressiveness of *F. graminearum* on wheat, it was not affected by the type of mycotoxin produced. Ward et al. (2008) reported no difference in aggressiveness between 15ADON and 3ADON chemotypes. However, the phenotypic analysis showed that the 3ADON chemotype produces more trichothecenes, is more prolific, and has higher growth rates than 15ADON chemotypes. Foroud et al. (2012) evaluated the role of *F. graminearum* chemotypes (15ADON, 3ADON, and NIV) in disease outcomes. The DON producing isolates (3ADON and 15ADON), in some cases, were more aggressive and resulted in higher FDK and mycotoxin levels compared to NIV producers when inoculated on a moderately susceptible or resistant wheat genotype. *Fusarium graminearum* isolates collected from durum wheat in Italy did not differ

in aggressiveness among 3ADON, 15ADON, and NIV chemotypes (Purahong et al. 2014). Puri et al. (2016) reported no difference in aggressiveness between 3ADON and 15ADON chemotypes when tested for Type II resistance (point inoculation) on resistant, moderately resistant, and susceptible cultivars. Similarly, chemotype aggressiveness testing in artificially inoculated fields across Canada and Germany revealed no difference in aggressiveness of 3ADON and 15ADON chemotypes (von der Ohe et al. 2010).

In contrast, Puri and Zhong (2010) reported that the 3ADON chemotype caused a significantly higher disease level than the 15ADON chemotype on susceptible and moderately susceptible wheat genotypes. Similarly, Amarasinghe et al. (2019) and Malihipour et al. (2012) stated that NIV chemotypes were generally less aggressive than DON chemotypes (3ADON and 15ADON); however, among DON chemotypes, the 3ADON chemotype was more aggressive. Serajazari et al. (2019) reported that the 3ADON chemotype was 18% more aggressive than the 15ADON isolates in Type I resistance assays (spray inoculation), whereas no difference in the aggressiveness of the two chemotypes was observed when tested in Type II resistance.

2.4.3. Fusarium mycotoxins

Fusarium spp. can produce several chemically diverse trichothecenes mycotoxins (Desjardins 2006). There are four types of trichothecenes (Type A to D) based on substitution at the C-8 position. Trichothecenes, mostly Type A and B, are often associated with FHB in small grain cereals (Foroud et al. 2019; Miller and Richardson 2013).

The most widespread Type B trichothecene associated with *Fusarium* spp. is DON (12, 13-epoxy-3α, 7α, 15-trihydroxytrichothec-9-en-8-one) (Desjardins 2006). Deoxynivalenol, which is also known as vomitoxin, is primarily produced by *F. graminearum* and *F. culmorum*. *Fusarium graminearum* can also produce NIV, but NIV-producing strains of *F. graminearum* are uncommon in Canada. Nivalenol in Canada is mainly associated with *F. poae* and is not

currently tested in Canadian grain (Tittlemier et al. 2020). The ingestion of DON and NIV infested grain can cause alimentary hemorrhage and vomiting in humans (Bennett and Klich 2003). Animals provided mycotoxin-infested feed may exhibit feed refusal, retarded growth, reproductive disorders, immune disorders, and vomiting (Rocha et al. 2005). Health agencies around the world have established guidelines for DON levels in grain and grain-based products. The maximum tolerable level recommended for DON, and its acetylated forms (3ADON and 15ADON) is 1 μg kg^{-1} body weight per day [Joint FAO/WHO Expert Committee on Food Additives (JEFCA) 2001; JEFCA 2011]. Nivalenol is regarded as more acutely toxic to humans than DON (Minervini et al. 2004). The European Food Safety Authority (EFSA) recommended tolerable daily intake (TDI) for NIV is 1.2 μg kg^{-1} body weight per day (EFSA 2013). In Canada, the established maximum DON level in uncleaned soft wheat for human consumption is 2 μg $kg^{-1,}$ whereas the maximum DON limit in finished wheat products is 1 μg kg^{-1} (www.grainscanada.gc.ca).

Type A trichothecenes such as T2 and HT2 toxins have hydrogen, a hydroxyl group, or an ester group at C-8, and in the case of NX-2, the keto group is absent at the C-8 position (McCormick et al. 2011). The T2 and HT2 toxins are metabolites of weakly pathogenic *Fusarium* spp., principally *F. sporotrichioides,* and *F. acuminatum* (Krska et al. 2014). Although strains of *F. poae* do not usually synthesize T2 and HT2 toxins (Morcia et al. 2016; Yli-Mattila et al. 2009; Yli-Mattila et al. 2004), some authors have reported that certain *F. poae* strains can accumulate T2 and HT2 toxins (Krska et al. 2014; Edwards et al. 2012), but there are no reports of *F. poae* producing T2 or HT2 in Canada. These toxins are commonly found in parts of Europe and are even more toxic than DON. The T2 and HT2 toxins are generally present together in contaminated grain, and T2 toxin is rapidly metabolized to HT2 toxin in animals (Wanda and Kenneth 2013). The main target of the T2 and HT2 toxins is the immune system: they cause inhibition of DNA, RNA, protein synthesis, and mitochondrial function. The general symptoms

of T2 include nausea, emesis, dizziness, chills, abdominal pain, diarrhea, dermal necrosis, abortion, irreversible damage to the bone marrow, aleukia, and heavy doses can even cause the death of animals (Wanda and Kenneth 2013). The provisional maximum tolerable daily intake (PMTDI) for the sum of T2 and HT2 toxins (alone or in combination) by JECFA is 60 ng kg^{-1} body weight per day. The TDI recommended by EFSA is 100 ng kg^{-1} body weight (EFSA 2011; JEFCA 2001). *Fusarium graminearum* is the sole producer of NX-2 and NX-3. Both NX-3 and NX-2 have similar toxicity as their Type B counterparts, DON and 3ADON, respectively (Varga et al. 2015).

2.5. Disease management

The most effective management strategies for control of plant diseases are quarantine, host resistance, cultural and agronomic practices that reduce disease incidence, and chemical control. In the case of FHB, however, use of quarantine measures is unrealistic as the disease not only infects many other cereal crops, but the pathogen is endemic as well (Gale 2003). Moreover, isolation is not possible due to field-to-field spread of the disease by airborne spores. Thus, management strategies such as crop rotation (aimed at breaking the disease cycle and reducing inoculum in the field), planting resistant or tolerant cultivars, and chemical control that can reduce the severity of FHB should be practiced. The efficacy of these control strategies can vary from year to year as they depend on weather conditions during the growing season, particularly during crop anthesis. No single approach is sufficient to protect yield and quality in FHB epidemic years; therefore, a combination of two or more management strategies is more effective and recommended (Dweba et al. 2017; McMullen et al. 2012).

Environmental conditions, such as excessive rainfall and irrigation, can influence FHB index (FHB index = (% disease incidence x % disease severity)/100) (Stack and McMullen 1998). Moisture is crucial for disease development; high humidity plays an important role in fungal growth and mycotoxin production. When high moisture levels overlap with the anthesis stage

in winter wheat, it leads to a significant increase in FHB index, FDK, and DON accumulation (Cowger et al. 2009). In years with excessive rainfall, online risk assessment tools (disease forecasting models) can be handy to keep farmers informed of the risk of FHB under specific weather conditions.

2.5.1. Host resistance

Resistance to FHB varies among wheat lines and is a complex quantitative trait controlled by many genes (Mesterházy 2002; Bai and Shaner 2004). Fusarium head blight resistance mechanisms can be of different types and were first described in 1963 as being either Type I, which is resistance to initial infection, or Type II, which is resistance to spread within the spike (Schroeder and Christensen 1963). Both Type I and Type II contribute to 'field resistance' and can be evaluated in field disease nurseries (Yan et al. 2011). Type I resistance is usually assessed by visual assessment of disease following spray inoculation, which closely mimics natural infection. In contrast, Type II resistance can be evaluated by inoculating a single spikelet with the pathogen (point inoculations) and visually assessing disease spread within the spike. Miller et al. (1985) found that some cultivars have the ability to degrade toxins after they are produced in host cells and described this as Type III resistance, which is resistance to toxin accumulation, measured by analyzing DON in harvested grain (Lemmens et al. 2005; Mesterhazy 1995). Later, two additional types of resistance were described; Type IV, which is resistance to kernel infection, was visually assessed by quantifying FDK or fungal biomass, and Type V resistance was defined as tolerance or resistance to yield losses (Mesterhazy 1995, Bai and Shaner 2004). Evaluation of plant material for any particular type of resistance to FHB requires specific protocols, such as the assessment of Type I resistance, spray inoculation methods. Type II resistance is often screened by inoculating single spikelets under controlled conditions and based on the ratio or percentage of infected spikelets to the total number of spikelets over time (Wang and Miller 1988).

Despite continuous efforts to improve FHB resistance in wheat, most commercially available wheat cultivars are moderately resistant to FHB, and no registered variety is immune. Response to FHB in wheat is further complicated by the environment, which modulates infection and disease development. This complexity of the host reaction makes the disease evaluation process complicated for breeding programs (McMullen et al. 2012). Resistance breeding for commercial wheat cultivars has been challenging due to the lack of highly resistant sources (Bai and Shaner 2004). A Chinese spring wheat cultivar, Sumai 3, has been used exclusively as a resistance source in hexaploid (AABBDD) wheat breeding programs worldwide (Bai et al. 2003; Buerstmayr et al. 2002, 2003; Mesterházy 2003). In addition, the Brazilian wheat cultivars, Frontana and Encruzilhada, the USA winter wheat cultivars, Ernie and Freedom, the Japanese cultivars, Shinchunaga and Nobeokabouzu komugi, the Korean cultivar, Chokwang, and the Romanian cultivar, Fundulea 201R are used in FHB breeding programs (Bai and Shaner 2004; Van Ginkel et al. 1996; Mesterházy 2003).

Durum (tetraploid - AABB) wheat is much more susceptible than bread wheat (hexaploid), and the majority of modern durum wheat cultivars are moderately susceptible to very susceptible (Steiner et al. 2019). Despite continuous efforts and screening for FHB resistance sources in durum, scientists have identified only a few durum landraces from Tunisia and Syria with notable levels of resistance (Elias et al. 2005; Huhn et al. 2012; Talas et al. 2011).

There are two possible explanations for improved resistance in bread wheat compared to durum wheat. The first is that durum wheat lacks the D genome, which is hypothesized to play a major role in reducing disease severity of hexaploid wheat (Szabo-Hever et al. 2018). The second is that current durum wheat cultivars are mostly descendent from the germplasm that originated in the warm and dry Mediterranean basin, which had little exposure to *Fusarium* spp., and selection for improved FHB resistance was always a low priority (Buerstmayr et al. 2009).

2.5.2. Cultural practices

Some of the predisposing factors for FHB development are weather conditions, inoculum levels, and wheat growth stage. The most important is the amount of primary inoculum, which survives on the crop residue from the previous crop year. *Fusarium graminearum* is a facultative parasite pathogenic on cereal crops and grasses, so cultural practices that manage crop residue are essential to prevent FHB infection and epidemics. Field practices such as following a diverse crop rotation with non-host crops and possibly tillage after harvest on fields with a history of FHB are important when trying to mitigate the disease in future.

Conventional tillage alters soil chemical and physical properties, impacting the soil micro-environment, nutrient distribution, soil structure, and ultimately microbial population (Degrune et al. 2016; Sengupta and Dick 2015). However, no-till farm practices increase organic matter in the soil, which favours profusion of fungi as the primary decomposers (Sengupta and Dick 2015).

Conventional tillage is not a common practice of farmers on the prairies anymore, but the beneficial effect of tillage has been documented to reduce FHB incidence and DON accumulation in deep tillage vs. limited/no-till (Dill-Macky and Jones 2000; McMullen et al. 1997; Miller et al. 1998). On the other hand, the no-till production system has been reported to increase DON accumulation when climatic conditions are favourable for inoculum production and spore dispersion. Maiorano et al. (2008) studied the effect of maize debris density on DON accumulation in wheat. In dry environments, the effect was less evident, but in wet conditions (frequent rainfall during the anthesis stage), the total amount of residue in the upper 10 cm soil layer and on the surface was found to be correlated with DON accumulation (R^2 = 0.848). Krupinsky et al. (2002) reported that removing crop debris or plowing (turning the debris under) reduced infected surface debris and, ultimately, FHB incidence. It was speculated that tillage favours loss of soil moisture and rapid warming of the soil in the spring, making

conditions less suitable for FHB. Lower FHB severity was reported in fields that previously had low disease pressure and a low residue density (Champeil et al. 2004).

Fusarium graminearum can survive saprophytically on wheat, barley, and maize debris, but it also has the potential to survive on many wild grass species that act as alternate hosts (Osborne and Stein 2007; Pereyra and Dill-Macky 2008). Inch and Gilbert (2003) investigated survival of *Fusarium* spp. on wild grasses and found that inflorescences of 34 wild grasses collected from southern Manitoba harbour several *Fusarium* spp., which can cause FHB in cereal crops in Manitoba. Cultural and agronomic practices such as crop rotation, tillage, sowing date, and other integrated approaches, when used judiciously, all have a considerable impact on FHB.

Rotation with non-host crops over three or more years depending on the residue biomass, residue breakdown rate, etc., can reduce the primary inoculum. The pathogen can only survive saprophytically for approximately two years post-harvest on wheat stubble, although it may survive longer on maize stubble (Pereyra et al. 2004). Viable propagules of *Fusarium* spp. and DON accumulation in the harvested wheat were higher when wheat was grown on corn or wheat stubble than on a non-host crop stubble (Beyer et al. 2006; Schaafsma et al. 2005). A diverse crop rotation, supplemented with tillage on the field from which wheat was previously grown, has lower DON accumulation in subsequent crops (Schatzmayr and Streit 2013). Dill-Macky and Jones (2000) estimated the previous crop's effect and the tillage practice on FHB of wheat. FHB incidence and severity were low, and yields were high in fields where tillage and a wheat-soybean rotation were practiced compared with no-till plots with wheat-wheat and wheat-corn rotations. A similar effect was recorded by Wenda-Piesik et al. (2017); wheat planted after wheat or corn had higher FHB incidence as compared with wheat after sugar beet. In a four-year study from Saskatchewan, Canada, however, no consistent effects of crop rotation were reported for FHB management (Fernandez et al. 2005). FHB levels were higher in wheat following oilseed crops (mostly canola) in two years of the research and higher FDK

in wheat crops following cereals in just one year of the study. In contrast, Obst et al. (1997) found no effect of rotation on the disease in southern Germany.

Seeding rates can affect the number of emerged plants, number of tillers, number of spikes m^{-2}, length of flowering, yield, and ultimately alter FHB severity and DON accumulation. The effect of seeding rate on agronomic performance of different winter wheat cultivars was studied by Geleta et al. (2002). High seeding rate (350-450 seeds m^{-2}) treatments resulted in increased plant height, more spikes per square meter, and proportionally more main culms, which generally resulted in flowering two days earlier than the low seeding rate (<150 seeds m^{-2}) treatment. Schaafsma et al. (2005) investigated the effects of seeding rate and seed treatment on FHB. Increasing the seeding rate (350-450 to 640 seeds m^{-2}) in winter wheat reduced the flowering period by one day; however, there was an interaction between seeding rate and seed treatment on FHB index. Staggering planting dates or sowing several varieties with different heading dates can reduce the risk of FHB (McMullen and Tekauz 2002); however, because the weather is unpredictable during flowering, these strategies still do not guarantee disease control. According to Fernandez et al. (2005), seeding rate and altered planting date did not affect FHB severity in spring wheat in Saskatchewan, Canada.

Rate of nitrogen (N) fertilizer can influence FHB severity, but the results from studies in the past are inconsistent. Teich and Nelson (1984) indicated that applying N greater than recommended levels reduced FHB severity in winter wheat. In contrast, Lemmens et al. (2004) concluded that increasing N rate increased FHB under natural field conditions, although in artificially inoculated field trials, higher N benefited the crop but did not affect FHB. Subedi et al. (2007) observed no effect of N rate on FHB index but concluded that early seeded spring wheat had lower FHB severity.

In years with high FHB index, harvest management strategies can improve grain quality and reduce mycotoxin levels. Fusarium damaged kernels are lighter than healthy kernels and can

be blown back into the field by adjusting the combine fan speed and shutter opening, which improves the quality of the grain harvest. Salgado et al. (2011, 2014) reported that adjustment of fan speed and shutter opening varied the speed and volume of air flowing through the combine. When fan speed was increased and shutter opening larger than the standard configuration, it consistently resulted in lower FDK and DON. Furthermore, the grain quality resulting from these combine adjustments compensated for the loss in harvested grain quantity.

2.5.3. Chemical control

Fungicide application can be used as part of an integrated management strategy to reduce disease in the field as well as reduce DON accumulation. Ideally, farmers should follow a diverse crop rotation, choose a resistant variety, and use fungicides as a last resort if conditions are favourable for the disease (Wegulo et al. 2011). In North America, the triazole (demethylation inhibitor and FRAC group 3) fungicides (e.g., tebuconazole, metconazole, and prothioconazole) are the most effective group against FHB (Edwards et al. 2001; Mesterházy et al. 2003; Paul et al. 2008, 2010; Pirgozliev et al. 2003). These fungicides inhibit 14α demethylase, an enzyme essential for ergosterol biosynthesis (Klix et al. 2007). Ergosterol is an essential structural component in the cell membrane of fungi (ascomycetes and basidiomycetes), and its inhibition compromises fungal membrane integrity (Becher et al. 2010). When applied alone or in combination, triazole fungicides provide the best suppression of FHB and DON accumulation (Edwards et al. 2001; Zhang et al. 2011).

Group 11 fungicides, the strobilurins (quinone outside inhibitors; QoI), comprise another class of chemicals that is used to control wheat diseases. Although strobilurins are moderately effective in reducing FHB incidence, they increase DON accumulation (Magan et al. 2002; Pirgozliev et al. 2002). In several European countries, an imidazole derivate Prochloraz, with a mode of action similar to that of triazole fungicides, is widely applied to control fungal growth in cereals (Mateo et al. 2011). In Brazil, triazole and benzimidazole fungicides have been

reported to be the most effective fungicides that can be used to control FHB in wheat (Machado et al. 2017).

Just as fungicide class, the timing of fungicide application is also crucial for adequate control of both disease and mycotoxin accumulation. The timely application of fungicides has always remained a challenge for producers because of variability in crop stage (uneven flowering time) in the field, unfavourable weather conditions at the time of application, which hinder spray operation, and proper choice of spray technology (coverage of and retention) (McMullen et al. 2012; Schaafsma et al. 2005). Appropriate fungicide timing to control FHB in wheat has been studied extensively by many researchers, and application timed at the anthesis growth stage, or up to 6 days after anthesis (D'Angelo et al. 2014; Paul et al. 2018) is effective in managing both disease and mycotoxin. Despite these advances on fungicide timing, uneven flowering and unfavourable weather during the short application window have prevented timely fungicide application.

2.5.4. Disease forecasting

Forecasting models have simplified the FHB risk assessment and helped farmers in judicial and economical deployment of disease management practices. Disease forecasting models can predict disease risk based on the previous crop (inoculum) and weather variables (temperature, rainfall, and moisture), and thereby facilitate wheat growers in fungicide application to decrease FHB infection and mycotoxin risk (Giroux et al. 2016). Forecasting models are appropriate for FHB because epidemics occur regularly, and there are limited periods of pathogen sporulation, inoculum dispersal, and host infection (De Wolf et al. 2003).

Over time, weather-based forecasting models have been developed for FHB in Canada (Hooker et al. 2002; Schaafsma and Hooker 2007), the USA (De Wolf et al. 2003; De Wolf and Isard 2007; Molineros 2007; Shah et al. 2013; Shah et al. 2014), Argentina (Moschini et al. 2004),

Brazil (Del Ponte et al. 2005), Belgium (Landschoot et al. 2013), The Netherlands (Van Der Fels-Klerx et al. 2010), Switzerland (Musa et al. 2007), and Italy (Rossi et al. 2003). These models are location-specific. Researchers have tried to check their effectiveness over different climatic zones, and with a few adjustments, these models were serviceable in other regions and crops (Giroux et al. 2016).

In the USA, the model developed initially by De Wolf et al. (2003) is widely adopted to provide empirical FHB predictions. Over the years, this model has undergone several iterations to incorporate new information, and it has been used in 31 US states and in Canada (De Wolf et al. 2003; Giroux et al. 2016; Molineros et al. 2005, 2007; Shah et al. 2013). The models predict FHB risk based on weather conditions during pre-anthesis (a week before anthesis) and post-anthesis (10 days after anthesis and consider hours of relative humidity >90% and temperatures between 15 and 30°C). The risk of FHB increases with the cumulative time (hours) in which these conditions occur.

In Canada, the DONcast® model (http://www.weatherinnovations.com/doncast.cfm) developed at the University of Guelph (Ridgetown, Ontario) is a robust site-specific, commercially adopted support tool (Schaafsma and Hooker 2007). The model predicts DON accumulation at the heading stage (when 75% of the wheat spikes have emerged) based on environmental and agronomic factors (level of host resistance, crop rotation, crop residue, and tillage). The model has a prediction accuracy of 80-90%, and the threshold for recommending a fungicide application is, if predicted, DON levels ≥1 ppm.

Since 2015, FHB risk maps have been available from the beginning of winter wheat flowering until the end of the spring wheat flowering period for growers in Saskatchewan and Manitoba. The two maps are generated by different models and have their own risk rating categorization based on their respective model output. The Saskatchewan FHB risk assessment maps are based on models using relative humidity and temperature in the previous 5 days, plus the

forecast for the coming 2 days (https://saskwheat.ca/fusarium-resources). The Manitoba map is created by a model that uses the number of hours of precipitation and hours when temperatures are between 15°C and 30°C during the previous 7 days (https://www.gov.mb.ca/agriculture/crops/seasonal-reports/fusarium-head-blight-report.html).

Although all current FHB models have high prediction accuracy and can save growers time and money, they should never be taken as a stand-alone tool to make FHB management decisions.

2.6. Fungicidal management of FHB

Many researchers have studied the effect of pre-anthesis, anthesis, and post-anthesis application of fungicide on FHB and DON accumulation (Chen et al. 2012; D'Angelo et al. 2014; Paul et al. 2018; Tateishi et al. 2014; Wegulo et al. 2011). Early application of fungicide before anthesis usually results in reduced saprophytic microflora on the grain; this decreases competition from other fungi and facilitates the proliferation of toxigenic *Fusarium* spp. (Henriksen and Elen 2005). Preventive fungicide treatment applied at the flag leaf stage or 50% heading stage was the least effective in reducing FHB index and DON accumulation (Caldwell et al. 2017; Holzapfel 2015; Hutcheon and Jordan 1992; Paul et al. 2018).

The effects of post-anthesis application of triazole fungicides (tebuconazole + prothioconazole and metconazole) on FHB and DON accumulation were studied by D'Angelo et al. (2014). FHB severity, FDK, and DON were lower in post-anthesis fungicide treatments compared to the untreated check (no fungicide). The application of fungicide at 50% anthesis and up to 6 days after 50% anthesis result in the same level of disease severity, DON, and FDK. Generally, the average reduction in FHB severity was highest when fungicide was applied two days after 50% anthesis, followed by 6 days after 50% anthesis compared to applications at 50% anthesis

and 4 days after 50% anthesis. Similarly, Beyer et al. (2006) reported that application of triazole fungicides between ZGS (Zadok growth stage) 60 (start of anthesis) and ZGS69 (end of anthesis) reduced DON accumulation to approximately 53% as compared to the untreated control.

In Japan, the effect of thiophanate-methyl fungicide timing on FHB severity and mycotoxin level was examined by Yoshida et al. (2012). Four application timings (anthesis (Zadoks (Z) GS64-65), 10 (ZGS73), 20 (ZGS77), and 30 (ZGS83-85) days after anthesis), singly and in combination were applied to assess the impact on disease and mycotoxin concentration in grain (see Appendix A Table A.1 for Zadoks growth stages). The results indicated that application of fungicide at anthesis reduced FHB index (~61%) compared to the untreated check (91-100%) but did not affect mycotoxin concentration. However, the late application of fungicide reduced the level of DON and NIV without lowering the FHB index. It was concluded that fungicide application at anthesis with an additional application at 20 days after anthesis (milk stage) was useful in managing FHB severity and mycotoxin accumulation. Similarly, Tateishi et al. (2014) investigated the effect of single and double applications of metconazole at different spray timings for managing FHB and mycotoxin accumulation in wheat and barley. In wheat, a single application at 4 days after anthesis (mid-flowering stage) was the optimal application timing to control FHB symptom development, whereas application at 14 days after anthesis (milk stage) was optimal to reduce mycotoxin accumulation. In barley, a single application at 4 days after anthesis was most effective in controlling FHB development and mycotoxin accumulation. All double application combinations had similar effect on FHB development, whereas mycotoxin (DON and NIV) accumulation was lower in the dual application treatment than from a single application.

In Atlantic Canada, Caldwell et al. (2017) evaluated the effects of single and double fungicide application [flag leaf stage (ZGS39) and anthesis stage (ZGS60)] on FHB and DON

concentrations in winter wheat, spring wheat, and barley. Double fungicide application at ZGS39 + ZGS60 provided the best seed quality, highest crop yield, and decreased DON accumulation compared to single application at both stages. Similarly, Wenda-Piesik et al. (2017) suggested double application of fungicides at BBCH30-32 and BBCH65 effectively reduced the FHB incidence and mycotoxin concentration in grain.

In western Canada, MacLean et al. (2018) evaluated the effect of fungicide application timing [flag leaf stage (BBCH39), anthesis (BBCH61-65), and double application (BBCH39+61-65)] on leaf spot diseases and FHB in spring wheat. Anthesis application of prothioconazole + tebuconazole and only tebuconazole provide adequate control of FHB and leaf spots diseases. Yield of both application timings was similar, but anthesis application resulted in improved test weight and thousand kernel weight. Double application (BBCH39+61-65) provided slight benefit in disease control and yield over a single anthesis application but was not economically beneficial.

Uniform fungicide trials conducted from 1995 to 2013 in 16 USA states investigated the effects of pre-anthesis and post-anthesis fungicide applications on FHB and DON. The efficacy of post-anthesis (Feekes 10.5.1 and after) application of prothioconazole + tebuconazole or metconazole was greater than pre-anthesis (Feekes 10.5) application. Among post-anthesis treatments, application between 2 to 6 days after anthesis was as effective as anthesis (Feekes 10.5.1) application. The efficacy was similar in spring wheat and winter wheat production regions in terms of FHB control compared to the non-treated check. In contrast, in terms of percent DON reduction, efficacy was greater in spring wheat than winter wheat regions (Paul et al. 2018).

2.6.1. Integrated disease management

To minimize yield losses and manage FHB effectively, selection of fungicide and a resistant wheat cultivar is vital. Various studies have assessed the integrated effect of genetic resistance and different triazole fungicide application timings on FHB. The combined impact of crop rotation, host resistance, and fungicide application was tested by McMullen et al. (2008) in winter wheat and barley. Crop rotation alone reduced field severity of FHB by 50%; whereas, combining rotation with a tolerant cultivar reduced severity by 80%. Management practices that included crop rotation along with tolerant cultivar and fungicide provided the best results and decreased FHB severity by 92%. McMullen et al. (2008) reported that fungicide alone could reduce FHB index and DON; however, the combination of cultivar resistance and fungicide application is most effective.

Results from coordinated, integrated management trials conducted between 2007 and 2010 in 12 USA states showed that application of fungicide (prothioconazole + tebuconazole) at the early anthesis stage (Feekes 10.5.1) in combination with a moderately resistant cultivar reduced FHB index and DON by 70% compared to the non-treated susceptible check. The integration of two management strategies effectively reduced both FHB index and DON more than either moderate resistant cultivar or fungicide application alone (Willyerd et al. 2012). Multiple studies demonstrated similar results; triazole fungicide application in combination with a moderately resistant cultivar increased yield, and reduced FHB index, FDK, and DON accumulation (Amarasinghe et al. 2013; Bockus et al. 2011, 2012, 2014; Dweba et al. 2017; McMullen et al. 2008; Salgado et al. 2014; Wegulo et al. 2011).

Paul et al. (2019) evaluated the efficacy of FHB management programs, which consisted of different combinations of cultivar resistance and post-anthesis (at or after 50% anthesis) fungicide application. Selection of a moderately resistant cultivar in combination with post-anthesis (BBCH61-69) fungicide treatment of a triazole fungicide (prothioconazole +

tebuconazole) was the most effective management program in reducing FHB incidence, FHB index, FDK, and DON as compared to fungicide applied to a moderately susceptible or susceptible cultivar.

2.7. Fungicide insensitivity

Triazole fungicides (DMI - sterol demethylation inhibitor) have been used extensively since the 1990s by wheat growers for control of FHB worldwide (Boyacioglu et al. 1992. Fungicide formulation containing at least one of the three active ingredients is commercially available for use on wheat. In Canada, tebuconazole fungicides became available for protection against FHB in 1999 under emergency use registrations due to the devastating FHB epidemic in 1990's. However, metconazole and prothioconazole were not registered for use on wheat until 2011 and 2012, respectively (BASF, Canada; Bayer Crop Science, Canada).

The odds of resistance development in triazole fungicides are considered intermediate risk (Brent and Hollomon 2007; FRAC 2017) compared with other active ingredients. Although insensitivity to triazole fungicides tends to develop gradually, insensitivity has been reported in *Blumeriella jaapi*, *Erysiphe graminis*, *Monilinia fructicola,* and *Mycospharella graminicola* (Delye et al. 1998; Leroux et al. 2007; Luo et al. 2007; Ma et al. 2006; Kim Y-S et al. 2003). Strobilurin (QoI- quinone outside inhibitors) insensitive *Fusarium* spp. isolates have already been reported over large areas of Germany, France, and the United Kingdom (Parnell et al. 2006; Kim Y-S et al. 2003). However, only a few studies from the USA, China, and Germany have reported reduced sensitivity to triazole fungicides (Anderson et al. 2020; Becher et al. 2010; Spolti et al. 2014; Yin et al. 2009). Fungicide insensitivity can be studied by analyzing resistance and cross-resistance levels of isolates from fields with a known history of fungicide treatment (Becher et al. 2010).

Several assays can be used to quantify insensitive isolates, i.e., fungal radial growth measurements on fungicide amended artificial media (Anderson et al. 2020; Spolti et al. 2014), inhibition of spore germination (Avozani et al. 2014; Klix et al. 2007), germ tube elongation assays (FRAC 2006), and microtitre plate assay (FRAC 2006; Hellin et al. 2016). However, all the test assays have two standard assessment parameters: (i) MIC (Minimum Inhibitory Concentration) dose giving total control of the isolate, and (ii) EC_{50} value - the effective concentration required for 50% growth inhibition (of mycelium or spores) as compared to growth in the absence of fungicide. It is sometimes also referred to as IG_{50} (inhibition of growth) (FRAC 2006).

Fusarium graminearum isolates collected from multiple states in the USA between 1981 and 2014 showed reduced sensitivity to tebuconazole and metconazole (Anderson et al. 2020). The mean EC_{50} values of isolates collected between 2000 and 2014 were higher than isolates collected before 2000. One isolate collected from Illinois had the highest EC_{50} values (metconazole - 0.1734 µg ml^{-1} and tebuconazole - 1.7339 µg ml^{-1}) compared with the mean EC_{50} [mean (metconazole) EC_{50} - 0.0405 µg ml-1 and mean (tebuconazole) EC_{50} - 0.3311 µg ml-1] of isolates collected between 2000 and 2014 (Anderson et al. 2020). Similarly, Spolti et al. (2014) reported the first tebuconazole-resistant isolate (EC_{50} - 8.09 mg L^{-1}) in the USA, which was collected from New York. Fifty isolates (25, 3ADON and 25, 15ADON) tested showed no difference in mean sensitivity between chemotypes to tebuconazole and metconazole, although toxicity of metconazole was greater than tebuconazole. In greenhouse experiments, the resistant isolate maintained its competitiveness when it was co-inoculated with a highly aggressive *F. graminearum* isolate, suggesting that resistance had no, or an undetectable fitness penalty to the pathogen. Becher et al. (2010) reported that repeated exposure to triazole fungicides could reduce fungicide sensitivity of a pathogen population.

Insensitive nivalenol-producing *F. graminearum* isolates produced a higher level of NIV and differed in fitness-associated traits (sporulation and virulence).

In Brazil, Spolti et al. (2012) determined the sensitivity of *F. graminearum* isolates to tebuconazole and metconazole. Fifty isolates grown on media amended with increasing fungicide dosage showed large variability in sensitivity to both fungicides. For tebuconazole, the EC_{50} of 92% of the isolates was between 0.01 - 0.03 mg L^{-1}, whereas for metconazole, 85% of the isolates, the EC_{50} was between 0.03 - 0.08 mg L^{-1}. Isolates in the upper limit of EC_{50} were classified as less sensitive. The two groups of isolates (more or less sensitive) differed significantly ($P < 0.001$) for both fungicides. They also detected cross-resistance between these two triazoles ($R = 0.46$, $P < 0.0001$)

There is only one report from China that suggested reduced sensitivity of *F. asiaticum* and *F. graminearum* to benzimidazole and tebuconazole among isolates collected from wheat (Yin et al. 2009). Of 159 isolates, nine were highly insensitive to benzimidazole, and three to tebuconazole. There were no reports of cross-resistance among isolates.

In Germany, sensitivity of *F. graminearum* isolates to tebuconazole, metconazole, and prothioconazole collected between 1984 to 2004, had reduced sensitivity of up to 2.59 times within 10 years of their market introduction (Klix et al. 2007). Hellin et al. (2016) tested sensitivity of *F. culmorum* isolates collected from Europe (N =96), Russia (N =4), Turkey (N =5), Canada (N =1) and New Zealand (N =1), to tebuconazole and epoxiconazole fungicides using microtitre plate assays. Tebuconazole (mean EC_{50} - 0.32) was found to be more efficient in reducing *F. culmorum* growth than epoxiconazole (mean EC_{50} - 0.58 mg L^{-1}), and cross-resistance between both triazole fungicides were detected ($R = 0.61$). Nivalenol-producing strains of *F. culmorum* had higher EC_{50} values than DON-producing strains. *Fusarium culmorum* isolates were more sensitive to both fungicides as compared to *F. graminearum* or *F. cerealis*. Although there are no reports of reduced sensitivity of *F. graminearum* to triazole

fungicides in Canada, it is prudent to investigate the pathogen population's sensitivity on the prairies to triazole products currently used in Canada.

2.8 Research required

In Canada, the importance of triazoles in managing FHB and mycotoxin accumulation has been extensively studied in recent years. However, there are still many aspects of this disease that are not investigated, such as the fungicide timing that is most effective for FHB management in durum wheat, whether the western Canadian *F. graminearum* population is still sensitive to triazole fungicides, and how environmental conditions have shaped the pathogen population over the years on the Canadian prairies. Due to the conservation tillage movement and lack of highly resistant wheat cultivars, FHB and DON are reoccurring issues for the Canadian wheat industry. Therefore, these problems must be addressed in order to better understand this disease and provide suitable management options.

CHAPTER 3

Effect of fungicide application timing on Fusarium head blight in durum wheat.

3.1 Abstract

Fusarium head blight (FHB) is one of the most important diseases of wheat in Canada. To manage the disease, farmers rely on an integrated pest management strategy, including fungicide application, following the current recommendation at early anthesis until 50% anthesis (BBCH61-65). This study evaluated the effect of fungicide application timing and seeding rates on durum wheat affected by FHB through field trials, which were carried out from 2016 - 2018 at three locations in Saskatchewan. Eight treatments of metconazole fungicide 'Caramba®' were applied to plots with two seeding rate treatments of 75 and 400 seeds m^{-2}. The fungicide treatments consisted of an untreated check (no fungicide), a treated check (fungicide application at all stages), and applications at BBCH59 (heading), BBCH61 (early anthesis), BBCH65 (50% anthesis), BBCH69 (late anthesis), BBCH73 (soft dough) and a treatment with two applications: BBCH61 followed by BBCH73. The parameters evaluated were: FHB index, proportion of FDK, DON accumulation, grain protein content (GPC), and yield. Seeding rate influenced all parameters; the high seeding rate had higher yield and FHB index, lower DON accumulation and GPC than the low seeding rate. All fungicide application treatments led to lower FHB index, DON accumulation, and FDK than the untreated check. Under extended wet conditions (high FHB risk), all anthesis applications starting at BBCH61 to BBCH69 had a similar effect on FHB index, FDK, DON accumulation, and yield, whereas in years with low disease severity, the BBCH65 application had lower FHB index, FDK, and DON accumulation. The results of the dual application (BBCH61+73) treatment for FHB index, FDK, and DON accumulation were similar to the BBCH65 application at all site-years.

3.2 Introduction

Fusarium head blight (FHB) has significantly limited durum wheat yield and grain quality in recent years (Prat et al. 2014). Although FHB is a concern in all wheat classes and other small grain cereal crops, the economic losses in durum have been particularly high. All commercially grown durum cultivars in North America are either susceptible (S) or moderately susceptible (MS); none of the current cultivars are even moderately resistant (MR) (Clarke et al. 2010; Zhang et al. 2014). Breeders have undertaken extensive research efforts to develop resistant varieties; however, due to lack of effective sources of resistance in the cultivated gene pool, efforts to identify resistance in durum wheat have not been successful (Prat et al. 2014). Even though durum wheat is grown in drier regions of Saskatchewan, recent major outbreaks in 2012, 2014, and again in 2016 have severely affected durum wheat production (Fleury 2017).

An integrated disease management approach is recommended to mitigate FHB, as no single control strategy is highly effective in preventing yield and quality losses in small-grain cereals (Gilbert and Haber 2013). Many researchers support integrating cultural, chemical, and host resistance to manage FHB in wheat (Amarasinghe et al. 2013; Paul et al. 2019; Scala et al. 2016; Willyerd et al. 2012). Diverse crop rotations, including four species (e.g., a four-year crop rotation in western Canada) are essential to prevent or reduce FHB incidence. Still, this has not been common practice by growers due to economic reasons (Beres et al. 2018). Cultivar resistance is available as a management strategy in hexaploid wheat, but due to the low level of FHB resistance in durum wheat, growers rely on fungicides as a major management tactic for in season disease control.

Fungicide class and optimal application timing are critical for effective management of FHB. The demethylation inhibitor (DMI) triazole fungicides are one of the most important groups that have been used since the 1980s to reduce the impact of FHB (Boyacioglu et al. 1992; McMullen et al. 2012; Paul et al. 2008). Strobilurin (Quinone outside inhibitor, QoI) fungicides

are another group used to control foliar fungal diseases of wheat; however, when applied at or before anthesis, strobilurins tend to result in increased DON accumulation in the grain (Amarasinghe et al. 2013; Bissonnette et al. 2018; Paul et al. 2018; Ye et al. 2017). Field trials on fungicide efficacy of commonly used triazole-based fungicides demonstrated that metconazole, prothioconazole, and tebuconazole + prothioconazole offer the greatest reduction in DON accumulation (Paul et al. 2008).

The timing of fungicide application is crucial for maximum FHB suppression. The most effective fungicide application timing is unclear, although application at anthesis is reported to be ideal as the plants are most susceptible to FHB infection at this stage (Beyer et al. 2006; Haidukowski et al. 2005). However, frequent rainfall during crop anthesis and variability in crop growth stages (due to inconsistency among wheat spikes in terms of heading and anthesis) present challenges to growers making fungicide decisions. Current research on seeding rates and plant density has documented that variability in stand establishment, and crop growth stages can be managed by altering seeding rates (Beres et al. 2016; Isidro-Sánchez et al. 2017; Nilsen et al. 2016; Schaafsma et al. 2005). Using a higher seeding rate in cereal crops is known to increase yield and improve management of pests (Mason et al. 2007). According to Holzapfel (2015) and Schaafsma et al. (2005), increasing the seeding rate in wheat decreased the tillering and flowering window (shorter window for FHB infection), leading to earlier maturity.

Many researchers have studied the effect of pre-anthesis, anthesis, and post-anthesis application of fungicide on FHB and DON accumulation (Chen et al. 2012; D'Angelo et al. 2014; Paul et al. 2018; Tateishi et al. 2014; Wegulo et al. 2011). Application before anthesis is often more harmful to the non-pathogenic fungal population (saprophytes), which decreases competition and facilitates the proliferation of toxigenic *Fusarium* spp. (Beyer et al. 2006). Preventive fungicide treatment applied at the flag leaf or the 50% heading stages was the least

effective in reducing FHB index and DON accumulation (Caldwell et al. 2017; Holzapfel 2015; Hutcheon and Jordan 1992; Paul et al. 2018). Application at anthesis and post-anthesis (up to 6 days after anthesis) has proven to be effective in reducing FHB incidence and severity in spring and winter wheat; although, application at 50% anthesis was more effective in moderating FHB index (Bradley et al. 2010; D'Angelo et al. 2014; Paul et al. 2018). Fungicide application later in the growing season was confirmed to be beneficial in reducing mycotoxin (DON and NIV) accumulation in grain (Tateishi et al. 2014). In Japan, late application at the milk stage, approximately 20 days after anthesis, prevented mycotoxin (DON and NIV) accumulation in grain without affecting visual disease symptoms (Yoshida et al. 2012).

Despite the prevalence of FHB in Saskatchewan and frequent epidemics of FHB in recent years, there is no research on the combined effect of seeding rate and fungicide application timing to manage FHB in durum wheat in Saskatchewan. Thus, there was a need to demonstrate the feasibility and potential merits of combining seeding rate and foliar fungicide application to manage FHB and DON, particularly in durum. The objective of the present study was to compare the effect of various fungicide application timings at two seeding rates on the disease (FHB index, FDK, and DON accumulation), yield, and agronomic or end-use quality traits [test weight (TW), thousand kernel weight (TKW), and GPC].

3.3 Materials and methods

3.3.1 Study sites, experimental design, plant material, treatments, and disease inoculation

Field experiments were conducted at three locations from 2016 to 2018: Saskatoon (University of Saskatchewan), Outlook (Canada-Saskatchewan Irrigation Diversification Centre), and Melfort (Agriculture and Agri-Food Canada, Melfort Research Farm; except 2016) (Table 3.1). In total, data from six site-years were used in this study due to negligible FHB pressure at two site years [Saskatoon (2018) and Outlook (2018)]. The field plots at Saskatoon and Outlook

were irrigated with sprinkler irrigation and a low-pressure overhead system to maintain available soil moisture above 50% (available water). At both locations, irrigation was applied for four weeks beginning at early flowering to stimulate a conducive environment for disease development, whereas the experiments at Melfort were not irrigated.

The Canada Western Amber Durum wheat variety 'CDC Desire' was chosen for this study as it is susceptible to FHB, moderately resistant to stripe rust and intermediately resistant to leaf spot diseases. The triazole foliar fungicide used was 'Caramba®' [active ingredient - metconazole (8.6%) at 1 l ha^{-1}]. Each experiment was established in a randomized complete block design with four replications. In 2016, seven treatments of metconazole fungicide were applied to wheat seeded at two rates (75 and 400 seeds m^{-2}) at the following crop stages (Lancashire et al. 1991; Appendix A Table A.1):

i) BBCH59 (end of heading, spike fully emerged),

ii) BBCH61 (beginning of anthesis: first anthers visible),

iii) BBCH65 (50% of anthers mature),

iv) BBCH69 (end of anthesis: all spikelets have completed flowering, but some dehydrated anthers may remain),

v) BBCH 73 (early milk)

vi) BBCH61+73,

vii) unsprayed check, and

viii) sprayed check,

In 2017 and 2018, an additional fungicide treatment applied at BBCH73 (early milk) was included. The sprayed check was sprayed with fungicide at all timings (growth stages) in an attempt to achieve a disease and toxin-free check, whereas the unsprayed check was never

sprayed. All fungicide treatments applied were based on the developmental stage of the primary tillers. Fungicides were applied using a CO_2-pressurized backpack sprayer at Saskatoon and Melfort and a tractor-tow-behind sprayer at Outlook. All sprayers were equipped with flat fan nozzles (Agrotop Germany, Airmix® No Drift; spray angle - 110 with a 015 flowrate) sprayed at a pressure of 275.8 kPa and a carrier volume of 100 l ha^{-1}.

Certified bulk seed of CDC Desire was sourced each year commercially and was treated with Raxil® PRO [325 mL product (3 g L^{-1} tebuconazole, 15.4 g L^{-1} prothioconazole and 6.2 g L^{-1} metalaxyl) per 100 kg of seed]. Plots were direct seeded into wheat or canola stubbles using a plot drill with row spacing of 30 cm (Table 3.1). Nitrogen and P_2O_5 fertilizer were side-banded at time of seeding according to soil test recommendations. Weeds were controlled using a pre-emergent application of Roundup® (glyphosate, 900 g a.i. ha^{-1}) plus an in-crop application of Buctril® M (bromoxynil; 1 l ha^{-1}) and tank-mixed with Simplicity® (pyroxsulam; 0.5 l ha^{-1}).

Experimental sites were inoculated by spreading *F. graminearum*-colonized corn kernels approximately 12-14 days before flowering at 5 g m^{-2} (Table 3.1). Two *F. graminearum* isolates [NRRL 52068 (3ADON) and NRRL 47847 (15ADON)] were used to prepare corn spawn inoculum (Gilbert et al. 2014) following the potato dextrose agar (PDA) plate method described by Gilbert and Woods (2006). Natural rainfall and irrigation re-hydrated the corn inoculum and initiated fungal growth and sporulation.

3.3.2 Rating scale and disease assessment

Disease incidence (proportion of the plants assessed that were infected) and severity (the portion of each of the infected spikes) were evaluated at 21 to 23 days after 50% anthesis, crop stage BBCH65 using the FHB visual rating scale of Stack and McMullen (1998) (Table 3.2). Disease severity and incidence were measured from 50 spikes at five random spots in the plot. The FHB index for each plot was calculated using the formula: FHB index = (% disease

incidence x % disease severity)/100. The plots were not assessed for any other diseases (stripe rust or leaf spots) because of low incidence in 2016 and the subsequent years.

3.3.3 Yield response and seed quality

At maturity, the field plots were combine-harvested, and the grain was dried, cleaned, and weighed. In each plot, yield was recorded after data was adjusted to 14.5% moisture and then converted to tonnes ha^{-1}. Test weight (kg hl^{-1}) and TKW (g) were measured on the harvested grain sample from each plot. Test weight was measured using a 600 ml chondrometer, and TKW was determined by weighing 250 random kernels multiplied by four.

For quality evaluation, FDK, DON accumulation, and GPC were measured from the harvested grain. The FDK was visually assessed using a sub-sample of 400 seeds from each plot. For estimation of DON accumulation and GPC, a random sub-sample of 20 g of each grain sample was ground to meal using the Laboratory Mill 3100 – v3.3 EN. The LC-MS/MS based mycotoxin/deoxynivalenol (DON) platform was used for quantification of DON accumulation in the grain (Wang and Kutcher 2018). The protein extractor LECO FP-528 (3000 Lakeview Avenue, St. Joseph, MI) was used to analyze GPC. Grain protein content was determined using the crude protein-combustion method, which calculates protein based on the nitrogen content of the sample. Grain protein content was later adjusted to a 14.5% moisture basis.

3.3.4 Statistical analysis

Data were analyzed using PROC MIXED in SAS (9.4 SAS Inc, Cary, NC) to evaluate the effect of seeding rate and fungicide application timing on yield, FHB index, FDK, DON accumulation, TW, TKW, and GPC. Due to differences in climatic conditions and disease pressure over the three years of the study (Appendix A Table A.2), the analysis was separated into two groups (high disease pressure and low disease pressure). Sites were determined as high- and low-pressure sites based on the mean FHB index rating in the unsprayed check. In

2016, FHB index in the unsprayed check at Saskatoon and Outlook were 20.5 and 18.6%. In contrast, FHB

index in 2017 at Saskatoon, Outlook and Melfort were 12.9, 12.3 and 11.3%, respectively, whereas FHB rating in 2018 at Melfort was 10.8%.

Table 3.1. Description of study sites, soil type, seeding, heading, inoculation, and disease rating dates[x]

	Saskatoon, SK	**Outlook, SK**	**Melfort, SK**
Latitude and Longitude	52.24°N 106.67°W	51.28°N 107.03°W	52.51°N 104.36°W
Soil zone/series/texture	Black/Chernozemic/ loam	Dark Brown	Moist Black
Stubbles (2016/2017/2018)	wheat/canola/wheat	wheat/wheat/wheat	wheat/wheat/wheat
Plot size	2 x 10 m	1.5 x 8 m	2 x 8 m
Row spacing	30 cm	30 cm	30 cm
Sprayer[y]			
Boom length (nozzle spacing)	1.5 m (20 in)	12 m (20 in)	1.5 m (20 in)
Harvested plot size	1.5 x 8 m	1.5 x 6 m	1.5 x 6 m
Seeding date			
2016	May 20	May 17	-[z]
2017	May 19	May 8	May 25
2018	May 16	May 24	May 24
Inoculation date			
2016	June 30	June 28	-
2017	June 22	July 7	July 8
2018	June 19	June 21	July 9
Heading date (high /low seeding rate)			
2016	July 3/July 11	July 7/July 11	-
2017	July 7/ July 11	July 7/July 9	July 10/July 12
2018	July 1/July2	July 8/July 10	July 19/July 22
Disease rating (2016/2017/2018)	August 7/August 3/N.A.	July 28/August 3/ N.A.	N.A./August 12/August 15

[x] SK - Saskatchewan

[y] Sprayer - backpack sprayer at Saskatoon and Melfort and a tractor-tow-behind sprayer at Outlook

[z] Dashes indicate the trial was not conducted at Melfort in 2016

Table 3.2. Description of fungicide application dates corresponding to growth stages under high and low seeding rate treatment at each site in 2016, 2017 and 2018.

Year/ growth stage	Saskatoon		Outlook		Melfort	
	High	Low	High	Low	High	Low
2016						
BBCH59	July 9	July 10	July 10	July 13	N.A.	N.A.
BBCH61	July 14	July 15	July 12	July 15	N.A.	N.A.
BBCH65	July 15	July 18	July 14	July 18	N.A.	N.A.
BBCH69	July 18	July 20	July 17	July 20	N.A.	N.A.
BBCH73	July 31	August 3	July 31	August 3	N.A.	N.A.
2017						
BBCH59	July 13	July 15	July 10	July 11	July 22	July 24
BBCH61	July 15	July 18	July 14	July 16	July 26	July 28
BBCH65	July 18	July 20	July 16	July 18	July 28	July 31
BBCH69	July 20	July 22	July 19	July 21	July 31	August 2
BBCH73	August 1	August 2	August 4	August 5	August 8	August 8
2018						
BBCH59	July 3	July 5	July 10	July 12	July 22	July 24
BBCH61	July 5	July 8	July 13	July 15	July 24	July 26
BBCH65	July 8	July 10	July 15	July 18	July 28	July 30
BBCH69	July 11	July 12	July 17	July 21	July 31	August 1
BBCH73	July 25	July 27	July 31	August 2	August 8	August 8

N.A.- Not applicable

BBCH (Bayer, BASF, Ciba-Geigy, and Hoechst) - growth scale used to identify phenological development stages

All sites in 2016 had high disease pressure and were analysed together, whereas four sites from 2017 and 2018 with low to moderate disease pressure were analysed together. Prior to analysis, error variances of the data for all class variables were tested for normality using the Shapiro-Wilk test and homogeneity of variance using Levene's test. Heterogeneous variances were modelled with the 'repeated/group=effect' statement (Littell et al. 2006) in SAS as required. Data of all class variables were subjected to analysis of variance, where sites, years, and blocks within site-years were considered random effects, and fungicide timing (growth stage) and seeding rate as fixed effects. Mean comparisons were performed using Fisher's protected least significant difference (LSD) procedure to protect against Type-II error. Treatment differences were accepted at a significance level of $P \leq 0.05$. Due to high variation in plant densities within plots and non-significant interactions between seeding rate and fungicide treatment, the

analysis was done using plant counts as a covariate, and final modeling was based on fungicide application timing. The yield, FHB index, FDK, and DON accumulation were analyzed to determine the optimum timing of fungicide application for each factor. For all statistically significant effects, contrast and least squared means estimate statements in PROC MIXED were used to compare the unsprayed with the BBCH59, BBCH61, BBCH65, BBCH69, BBCH73, and BBCH61+73 treatments. Pearson's correlation coefficients were calculated using the correlation in SAS. Data from two groups were used to estimate the relationship between yield, disease (FHB index, FDK, and DON accumulation), and quality (TW, TKW, and GPC) parameters.

3.4. Results

The following results are from two site-years grouped as high disease pressure (2016: Saskatoon and Outlook) and four site-years grouped as low disease pressure (2017: Saskatoon, Outlook, and Melfort; 2018: Melfort). The interaction between fungicide treatments and seeding rates was not significant at any site year.

3.4.1 Plant density, spike density, and flowering window

Seeding rate (high and low) had a noticeable effect on plant and spike densities at all site years. As expected, there were more emerged plants m^{-2} in the high seeding rate treatment than the low (Fig 3.1). Spike density was also higher at the high seeding rate than the low seeding rate, but not to the extent of plant density, indicating reduced tillering of plants in response to the high seeding rate. Based on the actual plant and spike densities, a total of 2.7 - 5.4 spikes $plant^{-1}$ were observed at the 75 seeds m^{-2} seeding rate, while only 1.1 - 2.0 spikes $plant^{-1}$ were observed at the 400 seeds m^{-2} seeding rate. The duration of heading and flowering was not affected by seeding rate.

3.4.2 High disease conditions (2016 - Saskatoon and Outlook)

Under high disease conditions, the effect of seeding rate on all variables (yield, FHB index, DON accumulation, TW, and GPC) measured was significant except for FDK and TKW (Table 3.3; Table 3.4). Grain yield increased to 4.71 tonnes ha^{-1} (31.1%) in the high seeding rate compared with the low seeding rate at 3.59 tonnes ha^{-1}. Overall, the high seeding rate had high FHB index and TW, whereas GPC was higher in the low seeding rate treatment. The DON accumulation was very high in both seeding rates under high disease conditions but was comparatively lower at the high seeding rate.

The effect of fungicide treatment was significant for all variables except GPC (Table 3.3; Table 3.5). Yield in all fungicide treated plots was higher than that of the unsprayed check. Yield increase was highest in the fungicide treatment applied at the BBCH65 growth stage (4.60 tonnes ha^{-1}), which was 37.5% greater than in the untreated check. This was followed by the dual application treatment at BBCH61+73 (4.32 tonnes ha^{-1}), which was 29% greater than the untreated check (3.34 tonnes ha^{-1}). Fungicide application at BBCH59, BBCH61, and BBCH69 crop stages increased yield by 4.4, 25.1, and 19.6% over the unsprayed check.

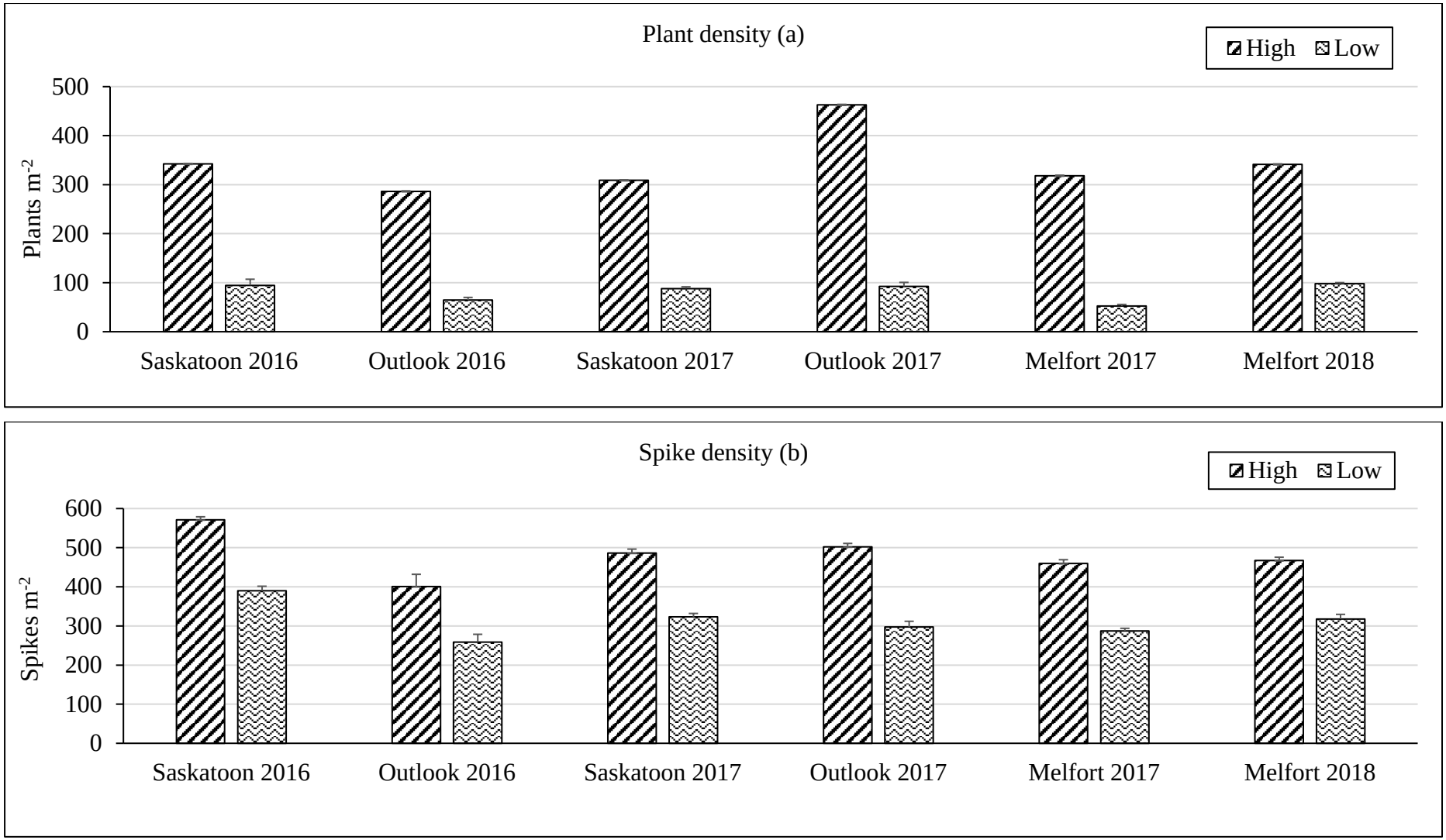

Fig. 3.1. Effect of seeding rate on (a) plant density (number of emerged plants m^{-2}) and (b) spike density (number of spikes m^{-2}) of durum wheat in Saskatchewan in 2016, 2017 and 2018.

Table 3.3. Summary statistics (*P* values) from linear mixed model analyses of fungicide timing (TRT) and seeding rate (SR) effects on yield, Fusarium head blight (FHB) index, Fusarium damaged kernels (FDK), deoxynivalenol (DON) accumulation, test weight (TW), thousand kernel weight (TKW) and grain protein content (GPC).

Factors	Yield	FHB index	FDK	DON	TW	TKW	GPC
High disease (Saskatoon 2016 + Outlook 2016)							
TRT	<.0001	<.0001	<.0001	0.0012	<.0001	<.0001	0.0738
SR	<.0001	0.0004	0.1492	0.0003	<.0001	0.1472	<.0001
SR*TRT	0.1997	0.332	0.9144	0.2798	0.6272	0.9554	0.2393
Low disease (Saskatoon 2017 + Outlook 2017 + Melfort 2017 + Melfort 2018)							
TRT	0.0324	<.0001	<.0001	0.0128	0.8012	0.0592	0.6285
SR	0.0105	0.0092	0.9942	0.0008	0.0069	<.0001	<.0001
SR*TRT	0.7516	0.3052	0.5832	0.531	0.8378	0.5062	0.5128

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

Table 3.4. Least squares mean of yield, Fusarium head blight (FHB) index, Fusarium damaged kernels (FDK), deoxynivalenol (DON) accumulation, test weight (TW), thousand kernel weight (TKW) and grain protein content (GPC) under two seeding rate treatments. Yield, TW, TKW, and grain protein estimates are based on constant moisture content.

Seeding rate treatment	Yield (tonnes ha^{-1})	FHB index (%)	FDK (%)	DON (µg kg^{-1})	TW (kg hL^{-1})	TKW (g)	GPC (%)
High disease (Saskatoon 2016 + Outlook 2016)							
High	4.71 a	9.1 a	15.6	9.8 b	74.7 a	37.0	15.9 b
Low	3.59 b	6.7 b	16.8	13.2 a	72.9 b	37.8	16.9 a
Low disease (Saskatoon 2017 + Outlook 2017 + Melfort 2017 + Melfort 2018)							
High	6.32 a	6.3 a	8.7	1.2 b	77.8 a	41.3 b	12.0 b
Low	6.09 b	5.4 b	9.8	1.8 a	76.6 b	43.0 a	12.9 a

Note: Means followed by the same letter within columns are not statistically significantly different based on Fisher's least significant differences (LSD) at $P < 0.05$.
FHB index = (percent disease incidence x percent disease severity)/100.

All fungicide treatments decreased the FHB index compared with the unsprayed check. The dual fungicide treatment had the lowest FHB index (3.5%), and the unsprayed check the highest (19.5%). Among fungicide treatments, application at BBCH59 had the highest FHB index (12.7%), whereas anthesis applications from BBCH61 to BBCH69 growth stages had similar effects on the FHB index (mean of 7.1%).

Fungicide treatments affected FDK in 2016 (Table 3.3); the lowest levels were in the dual fungicide treatment (12.1%). Among other fungicide treatments, application at BBCH59 had the highest FDK (21.1%), whereas anthesis applications at BBCH61 to BBCH69 had comparable FDK.

The DON accumulation in the grain varied among fungicide treatments (Table 3.5). It was very high in 2016 due to an unusually wet year and was highest in the untreated check (15.8 μg kg^{-1}). The DON accumulation was similar in all anthesis applications (BBCH61, BBCH65, and BBCH69) and the dual fungicide treatment.

In 2016, fungicide treatments affected TW and TKW; all fungicide treatments had greater TW and TKW than the unsprayed checks (Table 3.5). Among fungicide treatments, BBCH59 application had the lowest TW and TKW, whereas all anthesis treatments (BBCH61 to BBCH69) and dual fungicide treatment had similar TW and TKW. Fungicide effect on GPC was not significant (Table 3.3).

Table 3.5. Least squares mean of yield, Fusarium head blight (FHB) index, Fusarium damaged kernels (FDK), deoxynivalenol (DON) accumulation, test weight (TW), thousand kernel weight (TKW) and grain protein content (GPC) for fungicide treatments. Yield, TW, TKW, and GPC estimates are based on constant moisture content. Means comparison was performed after excluding sprayed check.

Fungicide treatment	Yield (tonnes ha^{-1})	FHB index (%)	FDK (%)	DON (µg kg^{-1})	TW (kg hL^{-1})	TKW (g)	GPC (%)
High disease (Saskatoon 2016 + Outlook 2016)							
BBCH59	3.50 d	12.7 b	21.1 a	14.4 ab	72.1 c	35.5 b	16.6
BBCH61	4.19 bc	7.3 c	17.0 b	12.3 bc	74.0 b	38.9 a	16.4
BBCH65	4.60 a	6.6 c	16.0 b	11.9 bc	74.7 ab	38.9 a	16.5
BBCH69	4.00 c	7.3 c	14.3 bc	10.3 c	75.1 a	38.6 a	16.1
BBCH61+73	4.32 b	3.5 d	12.1 c	9.9 c	75.5 a	39.7 a	16.2
Unsprayed check	3.35 d	19.5 a	24.0 a	15.8 a	71.7 c	32.9 c	16.7
Sprayed check	5.08	2.2	9.3	6.1	77.3	43.0	15.8
Low disease (Saskatoon 2017 + Outlook 2017 + Melfort 2017 + Melfort 2018)							
BBCH59	6.25 c	6.8 b	13.1 b	1.8 a	77.7	41.7	12.9
BBCH61	6.61 b	4.8 c	8.9 c	1.6 a	77.6	42.1	12.9
BBCH65	6.77 a	2.9 d	6.9 d	1.1 b	77.6	42.0	12.9
BBCH69	6.58 b	4.2 c	9.5 c	1.1 b	77.4	41.8	12.9
BBCH73	6.46 b	5.7 bc	8.4 c	1.2 b	77.3	42.0	12.8
BBCH61+73	6.62 b	4.6 c	7.3 d	1.0 b	77.3	41.5	12.8
Unsprayed check	6.15 c	11.8 a	15.6 a	2.0 a	77.1	41.1	12.8
Sprayed check	6.86	1.8	3.9	0.7	79.1	43.2	12.8

Note: Means followed by same letter within columns are not statistically significantly different based on Fisher's least significant differences (LSD) at $P<0.05$.

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

The BBCH73 fungicide application treatment was not included in 2016.

3.4.3 Low disease conditions [2017 (Saskatoon, Outlook, and Melfort) and 2018 (Melfort)]

Due to dry weather during crop anthesis, disease pressure was low in both 2017 and 2018. Under these conditions, seeding rate had a significant effect on yield, FHB index, DON accumulation, TW, TKW, and GPC, whereas FDK was not affected by seeding rate (Table 3.3). The high seeding rate treatment increased yield by 4.4% or 0.23 tonnes ha^{-1}, over the low seeding rate (Table 3.4). Seeding rate treatments affected FHB index (P = 0.0092); the high seeding rate treatment had higher FHB index (6.3%) compared to the low seeding rate (5.4%). There was no difference in FDK between seeding rates. Overall, DON accumulation at both seeding rates was <2 μg kg^{-1}; however, mean DON accumulation was lower in the high seeding rate treatment (1.2 μg kg^{-1}) than in the low seeding rate (1.8 μg kg^{-1}). The TW was greater in the high seeding rate treatment, whereas TKW and GPC were highest in low seeding rate treatment.

The effect of fungicide treatment was significant for yield, FHB index, FDK, and DON accumulation (Table 3.3). All fungicide treatments except BBCH59 had higher yield than the unsprayed check (Table 3.5). Fungicide treatments at BBCH65 resulted in the highest yield (6.77 tonnes ha^{-1}) with an increase of 10.1% over the unsprayed check (6.15 tonnes ha^{-1}). Fungicide applications at BBCH61, BBCH69, BBCH73, and BBCH61+73 crop stages also increased yield by 7.5, 7.1, 5.1, and 7.7% as compared to the unsprayed check. In contrast, the effect of fungicide treatment at the BBCH59 growth stage was similar to the unsprayed check.

All fungicide treatments decreased the FHB index, with the highest reduction in BBCH65 fungicide treatment, which reduced FHB index to 2.9 from 11.8% for the unsprayed check. Among fungicide treated plots, FHB index was highest when fungicide was applied at the BBCH59 growth stage (6.8%), and the levels were comparable to a single, late fungicide

application at BBCH73 (5.7%). FHB index in BBCH61, BBCH69, and dual fungicide treatments were similar.

All fungicide treatments affected FDK, and the levels were lower than the unsprayed check (Table 3.3 and 3.5). The FDK was lowest in the BBCH65 (6.9%) and dual fungicide treatments (7.3%) and was highest at BBCH59 (13.1%). The FDK in fungicide treatments at BBCH61, BBCH69, and BBCH73 were similar.

The difference in DON accumulation among fungicide treatments was very low (Table 3.5). The DON accumulation was highest in the unsprayed check and was comparable to BBCH59 and BBCH61 fungicide treatments. Fungicide treatments at BBCH65, BBCH69, BBCH73, and BBCH61+73 had lower DON accumulation than the unsprayed check but did not differ among each other (Table 3.5).

In low disease pressure site-years, fungicide treatments had no effect on TW, TKW, or GPC (Table 3.3).

3.4.4 Correlation coefficients and fungicide efficacy

Under both high and low disease severity, yield was negatively correlated with FHB index, FDK, DON accumulation and GPC, whereas yield was positively correlated with TW and TKW. FHB index was positively, but only weakly correlated with FDK and DON accumulation, and negatively and weakly correlated with TW and TKW. Fusarium damaged kernels were moderately and positively correlated with DON, but both FDK and DON accumulation were negatively correlated with TW and TKW (Table 3.6).

Table 3.6. Pearson's correlation coefficients between yield, Fusarium head blight (FHB) index, Fusarium damaged kernels (FDK), deoxynivalenol (DON) accumulation, test weight (TW), thousand kernel weight (TKW) and grain protein content (GPC).

Factors	Yield	FHB index	FDK	DON	TW	TKW	GPC
High disease pressure							
Yield	-						
FHB index	-0.58ns	-					
FDK	-0.25*	0.28*	-				
DON	-0.52**	0.22*	0.66**	-			
TW	0.58**	-0.27*	-0.73**	-0.77**	-		
TKW	0.21*	-0.34**	-0.77**	-0.65**	0.69**	-	
GPC	-0.52**	0.08ns	0.64**	0.76**	-0.77**	-0.60**	-
Low disease pressure							
Yield	-						
FHB index	-0.18*	-					
FDK	-0.29**	0.23*	-				
DON	-0.22**	0.30**	0.53**	-			
TW	0.18*	-0.32**	-0.021*	-0.38**	-		
TKW	0.10 ns	-0.21**	0.14ns	-0.27**	0.32**	-	
GPC	-0.17*	0.14*	-0.04ns	0.66**	0.60**	-0.25**	-

Note: * and **: correlation coefficients significant at $P < 0.05$ and $P < 0.01$, respectively; ns- not significant

3.5 Discussion

Integration of disease management strategies is an effective and recommended approach to FHB management in wheat and other small grain cereal crops. This research was conducted to investigate the effect of seeding rate and fungicide timing in suppressing FHB in durum wheat. Although FHB is a concern for all wheat classes, durum, in particular, is more vulnerable to this disease due to the lack of resistant varieties. In this study, I used an FHB susceptible durum wheat cultivar (CDC Desire) to evaluate the effects of seeding rate and fungicide timing to suppress this disease. The recommended seeding rate for durum wheat in Saskatchewan is 210 plants m^{-2} (Government of Saskatchewan 2019: Varieties of Grain Crops). This study tested two seeding rates to examine their effects on yield, FHB index, FDK, DON accumulation, TW, TKW, and GPC.

The results of my analyses indicated that seeding rate and fungicide timing differentially affected yield and disease parameters (FHB index, FDK, and DON accumulation) and most agronomic/end-use quality traits (TW, TKW, and GPC). The effect of seeding rate (high and low) varied among experiments but did not change with fungicide treatment as measured for the disease and quality parameters assessed. In my study, the high seeding rate treatment differed by a factor of over five-fold than the low seeding rate treatment, resulting in yield benefits due to higher plant and spike density in the high seeding rate treatment. My results agree with durum and winter wheat seeding rate responses reported by Beres et al. (2011), Holzapfel (2015), Isidro-Sánchez et al. (2017), Nilsen et al. (2016), and Schaafsma et al. (2005), who also reported that increased seeding rate increases the number of spikes m^{-2} and yield.

The most widely used fungicides to control foliar and spike diseases of wheat are the triazoles in the demethylation inhibitor (DMI) class (McMullen et al. 2012). Triazoles consist of numerous active ingredients, of which three are registered in Canada to suppress FHB:

metconazole, prothioconazole, and tebuconazole. I used metconazole fungicide, which is on the recommended list of fungicides to suppress FHB symptoms and DON accumulation in western Canada (Anonymous 2018). Metconazole was among the best of the fungicide active ingredients to reduce FHB index, FDK, and DON accumulation, according to a meta-analysis comprising >100 uniform fungicide trials conducted in the USA (Paul et al. 2008). Many other studies have also shown that triazole-based fungicides effectively suppress FHB, reduce DON accumulation in wheat kernels, and increase grain yields (D'Angelo et al. 2014; Paul et al. 2018; Wegulo et al. 2015; Wenda-Piesik et al. 2017; Yoshida et al. 2012). Despite the difference in disease pressure among site-years, yield was consistently higher in all single fungicide treatments relative to the untreated check in all field trials described here. My findings also showed that fungicides had a strong effect on yield, with increases as high as 37.5%, depending upon fungicide application timing in the high FHB group. Similar studies also reported yield increases in spring and winter wheat when a triazole fungicide was applied once (at flowering) or multiple times (before flowering + flowering or flowering + after flowering) (Caldwell et al. 2017; Machado et al. 2017; Paul et al. 2008; Sun et al. 2014).

In my study seeding rate did not affect the duration of heading and flowering. This differs from the results of Geleta et al. (2002) and Schaafsma et al. (2005), who reported that high seeding rate generally resulted in earlier flowering than the low seeding rate in winter wheat. In my study, crop staging and fungicide treatments were based on the primary tiller growth stages, which could be the reason I did not detect differences between seeding rates. Another possible explanation might be due to the fact that the maximum seeding rate in those experiments (640 seed m^{-2}) was larger than in this study (400 seed m^{-2}).

High seeding rate was associated with increased FHB index. This relationship observed in my experiments does not agree with Schaafsma et al. (2005). The most probable explanation is that the high seeding rate decreased the number of tillers per plant in my study and resulted in

greater uniformity of flowering of wheat; because the experiments were inoculated when most of the spikes were at the optimum stage for infection, higher FHB index was observed in the high seeding rate treatment than the low. In contrast, the low seeding rate treatment had a greater number of secondary, tertiary and even later tillers resulting in variable emergence of spikes and non-uniform anthesis stages. This variability in growth stages caused by the presence of additional tillers increased the flowering window, thereby lengthening the period of infection and ultimately resulting in higher variability in growth stages at the time of disease assessment. In addition, it is likely that the high seeding rate resulted in a denser crop canopy, increasing relative humidity in the canopy, which was conducive for disease development.

Several factors are responsible for the success of fungicide application in suppression of FHB and reduced DON accumulation; these include pathogen infection time, fungicide application method and time, synchrony of crop development and tillering, and the interaction of these factors with weather conditions (D'Angelo et al. 2014). Triazole fungicides have both preventive and curative effects, which has been concluded in other studies investigating the impact of fungicide application on FHB and DON accumulation. Fungicide application before anthesis (before infection) and application at or after anthesis have all reduced FHB index and DON accumulation (Chen et al. 2012; D'Angelo et al. 2014; Paul et al. 2018; Tateishi et al. 2014; Wegulo et al. 2011). Koizumi et al. (1991) reported that FHB pathogen infection in wheat occurs at anthesis. However, it is also known that "late" infection of wheat spikes occurs, affecting both FHB symptoms and DON accumulation (D'Angelo et al. 2014; Yoshida et al. 2008). Therefore, fungicide application against FHB at 50% anthesis growth stage would seem to be the optimum time to reduce disease symptoms (reviewed in McMullen et al. 2012), but to lower mycotoxin (DON and NIV) accumulation, fungicide should be applied at the milk stage (Tateishi et al. 2014; Yoshida et al. 2012).

The critical factor to consider from my results is that environmental conditions were likely responsible for differences in the FHB pressure among years. The results imply that in years with warm, wet weather during crop anthesis (high disease pressure), single fungicide applications at BBCH61, BBCH65, or BBCH69 were equally effective in reducing FHB index. Generally, there was no difference in fungicide efficacy by extending the application to the BBCH69 growth stage from that at the beginning of anthesis (BBCH61); in fact, under high FHB conditions, the trend was to lower FDK and DON accumulation at the later stage, although statistically insignificant. Under low to moderate disease pressure, a single application of a triazole at BBCH65 was better than application at BBCH61 or BBCH69 to decrease FHB index. Based on research in spring and winter wheat, D'Angelo et al. (2014) and Paul et al. (2018; 2019) showed that applications at anthesis and up to 6 days after anthesis were effective in reducing FHB index and DON accumulation. They noticed that fungicide application at 2 days post-anthesis was more effective in lowering FHB index than application at 4-6 days post-anthesis. Similarly, Bradley et al. (2010) documented comparable FHB suppression when fungicides were applied before the start of anthesis until 5 days into anthesis. Inconsistent findings of fungicide timing efficacy in previous studies and my research can be attributed to wheat type (durum vs spring vs winter wheat), cultivar resistance (FHB susceptible durum cultivar used in this study) and environment (Amarasinghe et al. 2013; Brar et al. 2019a; D'Angelo et al. 2014; Hollingsworth et al. 2008; Mesterhazy et al. 2018; Paul et al. 2008; Wegulo et al. 2011). Fungicide application at BBCH59, which is the end of the heading stage, usually reduced FHB compared with the unsprayed check, but was usually of much less benefit compared to other fungicide application timings during anthesis (BBCH61, BBCH65, and BBCH69). Preventive fungicide application, such as at the flag leaf stage or during the heading stage, was the least effective in reducing FHB index and DON accumulation (Caldwell et al. 2017; Holzapfel 2015; Hutcheon and Jordan 1992; Paul et al. 2018). My fungicide application

results at the milk stage differ from other studies (Tateishi et al. 2014; Yoshida et al. 2012); late application at BBCH73 did not reduced FHB index or DON accumulation (Table 3.5). Improved FHB suppression and reduced mycotoxin accumulation with multiple (more than one) fungicide applications at different crop growth stages are reported in some studies (Caldwell et al. 2017; Holzapfel 2015; Tateishi et al. 2014; Yoshida et al. 2012). In all cases, dual fungicide treatment always resulted in significant disease suppression, reduced DON accumulation, and improved grain quality. However, the dual application treatment (BBCH61+73), in my case, had only a slight advantage over single anthesis fungicide application (BBCH61 to BBCH69) in suppressing the FHB index under high disease pressure; it did not provide additional benefit in reducing FDK or DON accumulation. Overall, under low disease pressure, single fungicide application at BBCH65 was usually the most effective in suppressing FHB index than at BBCH61, BBCH69, or BBCH61+73.

The other important disease/quality parameter assessed in my study was FDK. Commercial grain graders have set acceptable limits for FDK at $\leq 2\%$; and their presence results in financial losses to growers due to down-grading. Levels exceeding 10% can result in total crop loss for a grower (Bianchini et al. 2015). Fusarium damaged kernels have a negative impact on durum wheat processing quality and may contain high amounts of DON (Shahin and Symons 2011). Overall, seeding rate did not affect FDK in my study, which agrees with Fernandez et al. (2005) who also reported that seeding rate did not affect FDK levels. There was no difference in FDK whether fungicide was applied at BBCH61, BBCH65, or BBCH69 growth stages. The FDK in the dual fungicide treatment did not vary from those that resulted from a single fungicide application. Yoshida et al. (2012) found no difference among fungicide treatments at anthesis, or at 10, 20, and 30 days after anthesis for FDK. However, FDK with the dual fungicide treatment at anthesis and again 20 days after anthesis was the lowest. Like my study, Holzapfel (2015) reported both a single fungicide application at ZGS60-65 and a dual application at both

ZGS57-59 and ZGS60-65 tended to have lower FDK than the GS57-59 application alone in durum wheat under Saskatchewan conditions. The FDK and DON accumulation was moderately correlated [$R = 0.66$ *(*$P < 0.0001$*)* and $R = 0.53$ *(*$P < 0.001$)] in my study, which is consistent with Paul et al. (2005), who reported a positive correlation between FDK and DON accumulation of $R = 0.73$.

Higher DON accumulation was quantified in the low seeding rate treatment relative to the high seeding rate in this study, whereas Schaafsma et al. (2005) reported no effect of seeding rate on length of the flowering period or DON accumulation. The increased DON accumulation in the low seeding rate treatment in my study may be because of the non-uniformity of crop stages due to secondary tillers, which resulted in delayed maturity. These late-maturing spikes did not exhibit FHB symptoms, but there was high DON accumulation in the grain. Del Ponte et al. (2007), Yoshida and Nakajima (2010), and Yoshida et al. (2012) also reported DON accumulation in grain without visible symptoms on spikes following *Fusarium* spp. infection at the late stage. Similarly, fungicide timing also had a significant effect on DON accumulation. The accumulation of DON in fungicide treated plots was lower relative to the unsprayed check. This is consistent with the results of Paul et al. (2018), Tateishi et al. (2014), and Yoshida et al. (2012). Under high disease pressure, fungicide application at BBCH61, BBCH65, and BBCH69 was equally effective in reducing DON accumulation; even though the levels were relatively high in these treatments, they were comparatively lower than in the unsprayed check. However, in years with low disease pressure, fungicide application at BBCH65 and BBCH69 were more effective than at BBCH61. According to a meta-analysis of more than 290 uniform fungicide trials in the United States, the percent reduction in DON accumulation was substantially higher when fungicide was applied at 50% anthesis and post-anthesis than when applied pre-anthesis and higher in spring wheat than in winter wheat (Paul et al. 2018). The late fungicide application (BBCH73 - early milk stage), which was tested only under low

disease pressure in durum wheat under Saskatchewan conditions, did not result in reduced DON accumulation. This is contrary to Tateishi et al. (2014) and Yoshida et al. (2012) as they reported that a fungicide application at later stages (milk stage) was crucial to minimize mycotoxin (DON and NIV) accumulation in Japan. Deoxynivalenol accumulation is a complex problem influenced by cultivar resistance, fungicide timing, active ingredient, application method, growing region, and weather conditions (D'Angelo et al. 2014; McMullen et al. 2012; Paul et al. 2008, 2018). The difference in efficacy of the BBCH73 application in my study compared with Yoshida et al. (2012) may be due to environmental differences, fungicide active ingredient (thiophanate-methyl vs. metconazole) or wheat class [Japanese wheat (moderately resistant) vs. durum (susceptible)]. Yoshida et al. (2012) also demonstrated the advantage of dual application; DON accumulation was lower after application at mid-flowering and milk stage than in the unsprayed check and often lower than in other single fungicide application treatments. However, the dual application tested in my experiments had almost the same DON accumulation in grain as a single application at BBCH65 or BBCH69.

In general, TW was higher at the high seeding rate. These findings are similar to those of Beavers et al. (2008), May et al. (2013), and Zecevic et al. (2014), who reported that TW increases with increased seeding rates in winter wheat, spring wheat, and durum wheat. A possible reason for this is that a low seeding rate leads to increased tillering, which results in non-uniform crop stand and, ultimately, variation in crop maturity. Late maturing spikes had smaller, less uniform kernels than those on the main stems (Beavers et al. 2008; Beres et al. 2016; Hucl and Baker 1989). Thousand kernel weight had a different response to seeding rate; overall, TKW was not affected by seeding rate when disease pressure was high. Under low disease pressure, high seeding rate resulted in lower TKW than low seeding rate, corroborated by Isidro-Sánchez et al. (2017). My results differ from those of Zecevic et al. (2014), who reported that higher seeding rates result in increased TKW, whereas Beavers et al. (2008) and

Holzapfel (2015) found no effect of seeding rate on TKW. Test weight and TKW differed among fungicide treatments only in high disease pressure conditions. The increase in TW and TKW was inversely correlated with FHB index. All the fungicide treatments resulted in a lower FHB index compared to the unsprayed check. Test weight and TKW were higher in all fungicide treated plots because FHB development is often associated with kernel damage (shrivelled kernels), directly affecting TW and TKW (Paul et al. 2018; Salgado et al. 2015). In Saskatchewan, Fernandez et al. (2012) reported increased TW and TKW when a single tebuconazole application was applied at ZGS60 as compared to applications at ZGS31–36 (stem elongation) or at ZGS37 (flag leaf emergence). My results do not agree with those of Caldwell et al. (2017) or Holzapfel (2015), where dual fungicide treatments had a significant impact on yield and seed quality (TW and TKW).

Seeding rate had a negative relationship with GPC; protein was highest for the low seeding rate treatment, which is corroborated by Bastos et al. (2020) and Beres et al. (2016) as there is less competition for available soil nitrogen with fewer plants. Fungicide treatment effect on GPC was not significant. This agrees with MacLean et al. (2018) and Ruske et al. (2003), suggesting that fungicide applications have no effect on grain protein.

This study has demonstrated the potential effects of seeding rate and fungicide application timing on FHB in durum wheat under Saskatchewan conditions. Overall, the seeding rate treatment had a more consistent impact on yield and agronomic traits than on the FHB factors measured. High seeding rate increased spike density and substantially reduced the number of tillers, which increased uniformity of crop development and made fungicide timing decisions easier. Seeding rate had a consistent influence on FHB index and DON accumulation, but less so on FDK. The more uniform flowering period in the high seeding rate treatment increased the risk of infection, which was evident from visual FHB ratings. In contrast, in the low seeding

rate treatment, more secondary tillers increased the chances of late infection and increased DON accumulation in the low seeding rate treatment.

This study confirmed the importance of the BBCH65 (50% anthesis) stage as the appropriate time to apply fungicide to manage FHB in durum wheat. However, in years when adverse field conditions due to rain prevent BBCH65 applications, farmers may still consider applying fungicide until the BBCH69 growth stage. Durum wheat growers are most likely to have the greatest reduction in DON accumulation if they choose to apply fungicide halfway through anthesis (BBCH65) up to the end of anthesis (BBCH69). It has often been argued that a single fungicide application is often not enough to provide season-long control against FHB. This study demonstrated that a dual application did not provide a yield increase, superior FHB control, or reduction in DON accumulation compared with a single, well-timed application. However, durum growers will always benefit by adopting multiple management strategies to reduce FHB index, FDK, and DON accumulation to an acceptable level in high disease pressure years.

CHAPTER 4

Sensitivity of a contemporary population of *Fusarium graminearum* from western Canada to tebuconazole, metconazole, and prothioconazole fungicides.

4.1 Abstract

Triazole fungicide application and cultural disease control strategies have been a useful tool in managing Fusarium head blight (FHB) in the absence of satisfactory FHB resistance levels in commercial wheat varieties. In this study, 252 *F. graminearum* isolates collected from western Canada were tested for sensitivity to tebuconazole, metconazole, and prothioconazole. Phenotyping for fungicide sensitivity of *F. graminearum* isolates revealed variation in sensitivity to each of the three fungicides. The EC_{50} of the isolates for tebuconazole, metconazole and prothioconazole was between 0.013 - 0.16 mg L^{-1}, 0.008 - 0.088 mg L^{-1}, and 0.012 - 0.058 mg L^{-1}, respectively. Trichothecene group (3ADON and 15ADON) and year (before/after fungicide registration) did not affect EC_{50} values. There was no evidence that the contemporary population of *F. graminearum* in western Canada has reduced sensitivity to triazole fungicides to date.

4.2 Introduction

Management of Fusarium head blight (FHB) in wheat relies on an integrated approach combing cultural practices, resistant varieties, and fungicides (Amarasinghe et al. 2013; Paul et al. 2019; Scala et al. 2016). However, in the absence of satisfactory levels of resistance in commercial wheat varieties, especially durum, crop rotation, and triazole fungicides (DMI - sterol demethylation inhibitor) together have been adopted by wheat growers for disease control. The most effective fungicide active ingredients registered for use on wheat against FHB are tebuconazole, metconazole, and prothioconazole (Paul et al. 2008; McMullen 2012). Fungicide products containing at least one of the three active ingredients are commercially available for

use on wheat. In Canada, tebuconazole-based fungicides became available for protection against FHB in 1999 under emergency use registration due to the devastating FHB epidemics in 1993 and 1997. Metconazole and prothioconazole were registered for use on wheat in 2011 and 2012, respectively (BASF, Canada; Bayer Crop Science, Canada).

Similar to the emergence of antibiotic resistance in human pathogens, long-term fungicide use increases the risk of insensitivity in plant pathogens. Although insensitivity to triazole fungicides tends to develop gradually, insensitivity has been reported in *Blumeriella jaapi*, *Erysiphe graminis*, *Monilinia fructicola*, and *Mycospharella graminicola* (Delye et al. 1998; Leroux et al. 2007; Luo et al. 2007; Ma et al. 2006; Kim Y-S et al. 2003). Nevertheless, reduced sensitivity to triazole fungicides has been reported previously in the USA, China, and Germany (Anderson et al. 2020; Becher et al. 2010; Spolti et al. 2014; Yin et al. 2009).

Fusarium graminearum isolates collected from multiple states in the USA between 1981 and 2014 showed reduced sensitivity to tebuconazole and metconazole (Anderson et al. 2020). The mean EC_{50} values of isolates collected between 2000 and 2014 were higher than isolates collected prior to 2000. An isolate collected from Illinois had the highest EC_{50} values (metconazole - 0.1734 µg mL^{-1} and tebuconazole -1.7339 µg mL^{-1}) compared with the mean EC_{50} [mean (metconazole) EC_{50} - 0.0405 µg ml^{-1} and mean (tebuconazole) EC_{50} - 0.3311 µg ml^{-1}] of other isolates collected between 2000 and 2014. Similarly, Spolti et al. (2014) reported the first tebuconazole-resistant isolate (EC_{50} = 8.09 mg L^{-1}) in the USA collected from New York. In greenhouse experiments, the resistant isolate maintained its competitiveness when co-inoculated with a highly aggressive *F. graminearum* isolate, suggesting that resistance had no, or an undetectable fitness penalty to the pathogen. The 50 isolates tested on tebuconazole and metconazole showed no difference in mean sensitivity between chemotypes. Yin et al. (2009) studied the sensitivity of *F. asiaticum* and *F. graminearum* isolates collected from wheat in China to benzimidazole and tebuconazole. Of 159 isolates, nine were highly insensitive to

benzimidazole, and three to tebuconazole. There were no reports of cross-resistance among isolates. When sensitivity to tebuconazole, metconazole, and prothioconazole was studied in a collection of *F. graminearum* isolates from Germany over 20 years from 1984 to 2004, reduction in sensitivity of up to 2.59 times was detected. Becher et al. (2010) suggested that the triazole resistant isolates produced more of the mycotoxin NIV and differed in fitness-associated traits, e.g., sporulation and virulence.

The risk of insensitivity development in fungal plant pathogens to triazole fungicides is considered intermediate (Brent and Hollomon 2007; FRAC 2017) compared with other active ingredients. Although there are no reports of *F. graminearum* insensitivity to triazole fungicides in Canada, it is prudent to investigate the pathogen population's sensitivity on the prairies to triazole products currently used in Canada. This study's objective was to determine the sensitivity of a contemporary population of *F. graminearum* in western Canada to tebuconazole, metconazole, and prothioconazole and to assess the variation in sensitivity among chemotypes.

4.2 Material and methods

4.2.1 Fungal isolates and fungicide sensitivity assay

Two hundred fifty-two single spore isolates of *F. graminearum* (Appendix A Table A.3) collected from wheat fields in Alberta, Saskatchewan, and Manitoba were tested for sensitivity to three triazole fungicides (Table 4.1). Fungicides were technical grade products of tebuconazole (CAS number: 107534-96-3), metconazole (CAS number: 125116-23-6), and prothioconazole (CAS number: 178928-70-6). All fungicides were diluted in methanol to a concentration of 1 mg ml^{-1}.

The fungicide sensitivity of the isolates was determined by microtitre plate assays (FRAC 2006). The concentrations tested for the three fungicides were 0.03, 0.1, 0.3, 1.0, 3.0, 10, 30

mg l^{-1}. Five-day-old colonies of the fungus grown on potato dextrose agar [PDA: 39 g of media (BD Difco™ Dehydrated Culture Media: Potato Dextrose Agar] in 1000 ml of distilled water, autoclaved for 20 min at 121°C) were used to prepare spore suspensions.

Table 4.1. *Fusarium graminearum* isolates, the province and year of collection and the number used to determine fungicide sensitivity

Province	Number of isolates
Saskatchewan (140)	47 (CDC, 2014) + 13 (CDC, 2015) + 31 (CDC, 2016) + 33 (NRRL) +16 (DAOM)
Manitoba (78)	49 (AAFC) + 25 (NRRL) + 4 (DAOM)
Alberta (34)	26 (NRRL) + 8 (DAOM)

NRRL culture collection – USDA/ARS Culture Collection (NRRL, Peoria, IL); DAOM-Canadian Collection of Fungal Cultures (DAOM), Ottawa, Ontario, Canada. AAFC, Agriculture and Agri-Food Canada

All spore suspensions were prepared in potato dextrose broth [PDB: 24 g of the media (BD Difco™ Dehydrated Culture Media: Potato Dextrose Broth) in 1000 ml of distilled water, autoclaved for 20 min at 121°C] with a spore concentration of approximately 850 spores ml^{-1}. Streptomycin was added at 1 ml l^{-1} of PDB to prevent bacterial contamination. Sterile, clear, and flat bottomed 96-well microtiter plates were filled with 140 µl of PDB amended with fungicide and inoculated with 60 µl of spore suspension to a final volume of 200 µl per well. The seven test concentrations were oriented vertically in the microtiter plate along with a control (no fungicide). Each plate had six isolates in two replications, and the experiment was repeated once, starting with a new conidial suspension. An initial absorbance reading was recorded using the FLUOstar Omega microtiter plate reader (BMG Labtech) at an optical density of 620 nm wavelength. The microtiter plate was sealed with sterile film, wrapped in a black plastic bag, and incubated for three days at approximately 22°C on a standard rotary shaker at 150 rpm. After three days of incubation, fungal growth was measured using the final absorbance reading to calculate EC_{50} values.

A preliminary test using one *F. graminearum* isolate was done to check the sensitivity and specificity of the protocol. The test included isolate 102-e-1 tested for all seven concentrations,

positive control (media inoculated with spores but no fungicide), and negative control [non inoculated media amended with fungicide (seven test concentrations)]. The positive control had the highest OD (optical density), whereas the negative control had lowest OD values.

4.2.2 Statistical analysis

The concentration of fungicides that reduced mycelial growth of isolates by 50% (EC_{50}) was calculated using PROC PROBIT in SAS 9.4 (SAS Institute Inc., Cary, NC, USA). A combined EC_{50} of two replicates was calculated, the means and standard errors (SE) of two repeats were calculated for each individual isolate and plotted against fungicide concentration. Before pooling the repeats to conduct ANOVA, the homogeneity of residuals for repeats was verified. After calculating the EC_{50} of each isolate and fungicide, isolates were grouped according to trichothecene group (3ADON and 15ADON). Isolates were further categorized based on year of collection (prior and after widespread use of metconazole and prothioconazole), including one group of isolates collected before registration of the fungicides (2010 and 2011) and isolates collected between 2014 and 2017. The mean of each repeat was analyzed using PROC MIXED, testing the fixed effects of isolate, trichothecene group, area of collection, and year, and the random effect of replicate nested in year on EC_{50}. To assess cross-resistance between tebuconazole, metconazole, and prothioconazole, EC_{50} values were subjected to Spearman's rank correlation analysis ($P = 0.05$).

4.3 Results

Sensitivity of 252 *F. graminearum* isolates collected from wheat fields in Alberta (N = 34), Saskatchewan (N = 140), and Manitoba (N = 78) to three triazole fungicides (tebuconazole, metconazole and prothioconazole) was tested using microtiter assay (Table 4.1). Isolate EC_{50} was significant for all three active ingredients tested (Table 4.2). A range in the sensitivity of isolates was observed for all three compounds (Fig. 4.1). The EC_{50} value of the isolates for

tebuconazole was between 0.013 and 0.16 mg $L^{-1,}$ with a mean of 0.074 mg L^{-1} (Fig. 4.1). The EC_{50} values of the isolates treated with metconazole were between 0.008 and 0.088 mg L^{-1} with a mean of 0.028 mg L^{-1} (Fig. 4.2). For prothioconazole, the EC_{50} values were between 0.012 and 0.058 mg L^{-1} with a mean of 0.027 mg L^{-1} (Fig. 4.3).

The year effect on EC_{50} values was tested only for metconazole and prothioconazole; and EC_{50} values for both did not vary between isolates collected from 1998 to 2007 and those from 2014 to 2016 (Table 4.2). The effect of trichothecene group (3ADON and 15ADON) on EC_{50} values were not significant for any of the three fungicides (Table 4.2). Similarly, for all three fungicides, the area of collection (Alberta vs Saskatchewan vs Manitoba) had no effect on EC_{50} values of isolates (Table 4.2).

No cross-resistance between these three fungicides was observed, which was inferred based on non-significant correlation coefficients: metconazole and tebuconazole (R = 0.1043, P = 0.1113), tebuconazole and prothioconazole (R = -0.0004, P = 0.9947), metconazole and prothioconazole (R = -0.0897, P = 0.1714).

Table 4.2. Analysis of variance [*F*-values and corresponding *P*-values] of EC_{50} derived from *Fusarium graminearum* spore suspensions incubated in tebuconazole, metconazole and prothioconazole for isolate, trichothecene group, year and province of collection effects

Fixed effects	**tebuconazole**		**metconazole**		**prothioconazole**	
	F value	***P* value**	**F value**	***P* value**	**F value**	***P* value**
Isolate	23.39	<0.0001	23.83	< 0.0001	24.87	<0.0001
Trichothecene group	0.38	0.8179	1.08	0.3852	1.01	0.4182
Year		NM	1.65	0.1901	0.80	0.5344
Province	1.21	0.332	1.3	0.2798	0.7	0.6272

NM: Not measured.

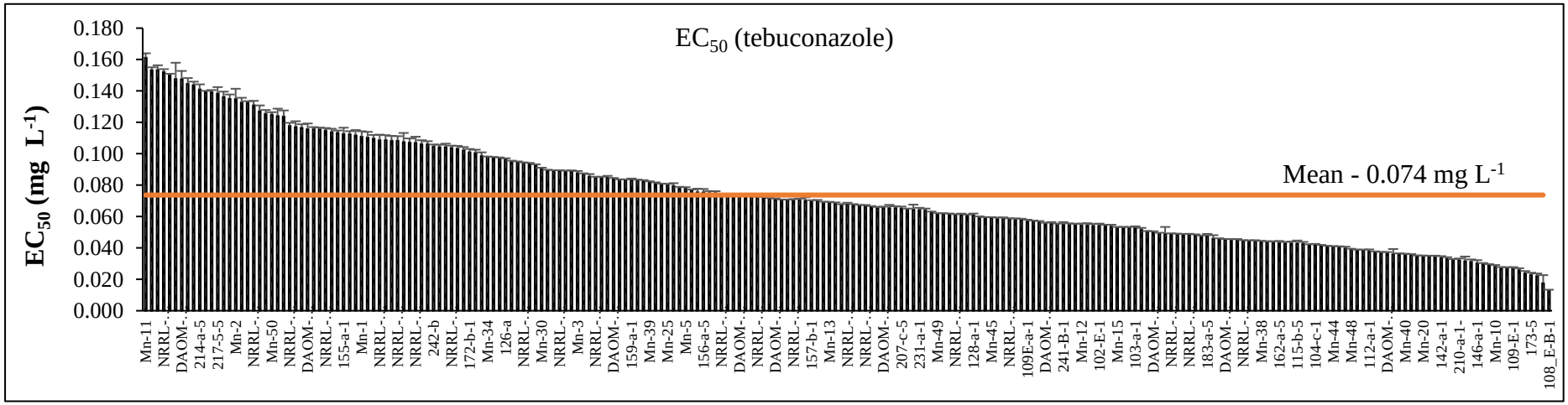

Fig. 4.1. Effective concentration of tebuconazole required to reduce mycelial growth by 50% (EC_{50}) for isolates of *Fusarium graminearum*; red line indicates the mean EC_{50} value.

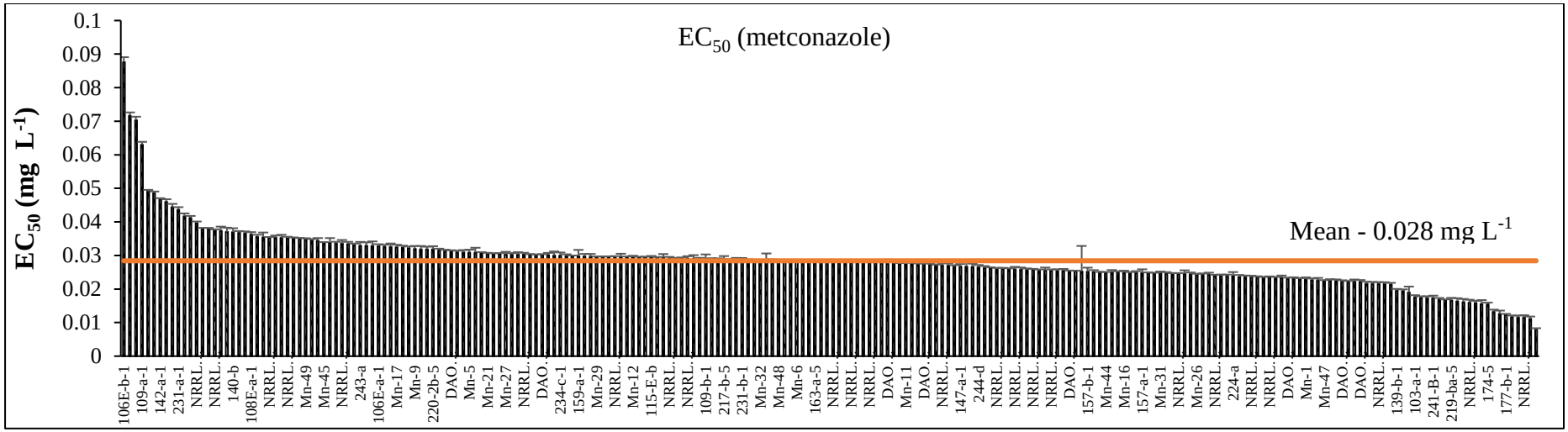

Fig. 4.2. Effective concentration of metconazole required to reduce mycelial growth by 50% (EC_{50}) for isolates of *Fusarium graminearum*; red line indicates the mean EC_{50} value.

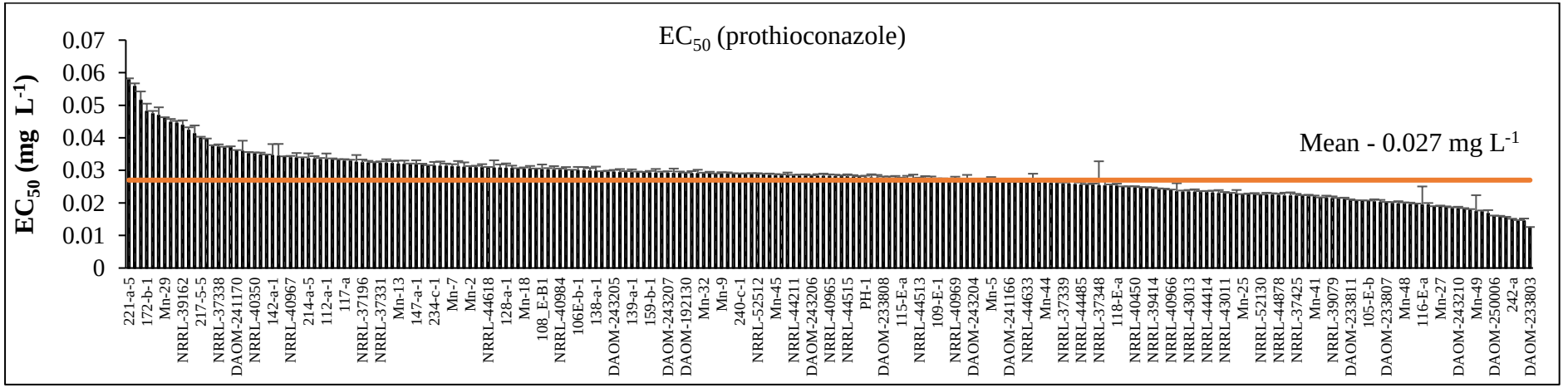

Fig. 4.3. Effective concentration of prothioconazole required to reduce mycelial growth by 50% (EC_{50}) for isolates of *Fusarium graminearum*; red line indicates the mean EC_{50} value.

4.4 Discussion

Fungicide insensitivity in plant pathogens is a global problem that threatens agricultural productivity. As new chemicals become available and are used by farmers, tracking the decline in fungicide sensitivity is crucial for long-term agricultural sustainability. The development of fungicide resistance in plant pathogenic fungi is an emerging issue due to selection pressure and absence of anti-resistance strategies (Pirgozliev 2002; Spolti et al. 2014). Various studies from around the world have shown a wide range in the sensitivity of isolates to the three fungicides tested in my study. Only a few of these have specified that triazole effectiveness has been compromised (Anderson et al. 2020; Avozani et al. 2014; Klix et al. 2007; Spolti et al. 2012, 2014; Yin et al. 2009;).

This study is the first in Canada to screen *F. graminearum* isolates for sensitivity to the three triazole fungicides tebuconazole, metconazole, and prothioconazole. The range of EC_{50} values of *F. graminearum* isolates varied among the three fungicides. I found a low EC_{50} range for the three active ingredients. No EC_{50} values above 0.1 mg L^{-1} were observed for either metconazole or prothioconazole, and 76% of the isolates tested had an EC_{50} of less than 0.1 mg L^{-1} for tebuconazole. A similar range of EC_{50} was observed in previous studies from Brazil and Japan (Spolti et al. 2012; Tateshi et al. 2010). In contrast, researchers from the USA and China reported a much higher range than those found in my study (Anderson et al. 2020; Spolti et al. 2014; Yin et al. 2009). The difference in the range of EC_{50} values among studies may be due to differences in test procedures (radial growth vs. ascospore germination vs. microtiter), test fungicide (technical grade vs. formulated product), and length of time isolates were exposed to particular fungicide.

Metconazole and prothioconazole were more fungitoxic to the isolates tested than tebuconazole, which is consistent with Anderson et al. (2020), Klix et al. (2007), and Spolti et al. (2014). In contrast, Spolti et al. (2012) reported higher EC_{50} values for isolates exposed to

metconazole than tebuconazole. Previous studies from the USA and China identified isolates resistant to tebuconazole from the natural population of *F. graminearum* (Anderson et al. 2020; Spolti et al. 2014; Yin et al. 2009). The EC_{50} values of these resistant isolates were 28.8, 57.8, and 300-fold higher than isolates with the lowest EC_{50} values. The difference between isolates with the highest and lowest EC_{50} values in this study were 9.1 (tebuconazole), 11 (metconazole), and 4.6-fold (prothioconazole), which was not strong evidence to categorize them as resistant or susceptible as this difference in sensitivity is likely a reflection of the natural variation in the population.

Studies have shown that repeated exposure to triazole fungicides can reduce fungicide sensitivity of a pathogen population (Anderson et al. 2020; Becher et al. 2010; Klix et al. 2007). This study indicated no such decline in sensitivity of metconazole and prothioconazole to isolates tested over years. The possible explanation behind increased tolerance of the *F. graminearum* population to triazole fungicides in studies from the USA and Germany may be that these fungicide products were registered for use on wheat earlier in these countries compared to Canada.

No significant difference in EC_{50} values between trichothecene groups was detected suggesting that triazoles are equally effective against both 3ADON and 15ADON chemotypes of *F. graminearum*. Similarly, Spolti et al. (2014) reported no difference in EC_{50} values between isolates of these two chemotypes for tebuconazole or metconazole. Cross-resistance between triazole fungicides were shown previously by Becher et al. (2010) for *in-vitro* mutated *F. graminearum* isolates. The penalty for cross-resistance is that an isolate may show reduced sensitivity to several fungicides with the same mode of action even though that isolate was never exposed to other active ingredients in the group. No cross-resistance in isolates was detected in my study, further evidence of no fungicide insensitivity to the three active ingredients.

In conclusion, the contemporary population of *F. graminearum* in western Canada is sensitive to tebuconazole, metconazole, and prothioconazole. However, with increasing reports of triazole insensitive isolates, there remains a risk of resistance development in the Canadian *F. graminearum* population. In the future, studies with larger sets of isolates representing multiple species and trichothecene groups must be repeated from time to time. This information will facilitate the monitoring of pathogen populations for shifts in fungicide sensitivity well before it becomes widespread. This knowledge will also give researchers/industry time to forewarn farmers about the problem and recommend alternate disease management strategies. Growers should adopt sound crop production practices and follow necessary anti-resistance guidelines and protocols to manage fungicide resistance effectively to promote healthy crops and fungicide longevity.

CHAPTER 5

Population structure of *Fusarium* spp., chemotype diversity, and mycotoxin contamination in wheat samples collected in 2014, 2015 and 2016 from Saskatchewan, Canada.

5.1 Abstract

This study determined the *Fusarium* spp., the chemotype diversity, and the mycotoxins levels in wheat samples collected from Saskatchewan from 2014 to 2016. Five *Fusarium* spp. (*F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, and *F. sporotrichioides*) were identified and quantified from 132 wheat samples using morphological identification and quantitative real-time PCR assays. *Fusarium graminearum* was the dominant species detected and quantified, followed by *F. avenaceum*. Among *F. graminearum* chemotypes, the 3ADON chemotype was found more frequently than the 15ADON, while the *F. graminearum*-NIV chemotype was absent. Mycotoxin quantification revealed that 72% of the samples had toxin accumulation above 1 μg kg^{-1}. Toxins present were DON, D3G, 3ADON, 15ADON, NIV, T2, and HT2. A weak correlation was detected between the amount of *F. graminearum* DNA and DON accumulation (R = 0.288, P = 0.008).

5.2 Introduction

Fusarium head blight (FHB), also known as scab, is caused by several *Fusarium* species, which infects a wide range of crops, including cereals such as wheat, corn, barley, and oat (Parry et al. 1995; Shen et al. 2012). The common contributors and economically important FHB pathogens are members of the *F. graminearum* species complex (FGSC) (Aoki et al. 2012). The FGSC comprises more than 17 *Fusarium* spp.; in North America and other warm regions of the world, it is *F. graminearum* that is most widespread and the leading cause of FHB (McMullen et al. 2012; Mesterházy 2003; Somma et al. 2014; Wang et al. 2011). In addition

to *F. graminearum*, other species like *F. culmorum, F. avenaceum,* and *F. poae* are also frequently found to cause FHB infection (Klix et al. 2008). The disease not only results in yield losses accounted for by shriveled lightweight, chalky colored kernels, regarded as Fusarium damaged kernels (FDK), but more importantly, many species in the genus produce mycotoxins responsible for severe health issues in humans and farm animals (McMullen et al. 2012).

Members of FGSC can be differentiated based on the types of trichothecene they produce (Kelly et al. 2015). Trichothecenes are sesquiterpenoid secondary metabolites (SMs) produced by *Fusarium* spp. and have a role in disease development (Bottalico 1998; Proctor et al. 1995). Trichothecenes are classified into four types (Types A to D) among which Types A and B are toxicologically the most important. *Fusarium graminearum* populations can be differentiated into three chemotypes of Type B trichothecenes: 3ADON, 15ADON, and NIV (Miller et al. 1991). Most *Fusarium* spp. produce Type B trichothecenes, but *F. graminearum* can also produce the recently reported Type A trichothecene called NX-2 (Varga et al. 2015). In the past, the indigenous *F. graminearum* population in North and South America was dominated by 15ADON producers, whereas 3ADON was dominant in Europe and Asia (Valverde-Bogantes et al. 2019; Ward et al. 2008). The NIV producers exist in parts of the USA, Europe, Japan, and Australia, but so far remain uncommon in Canada (Gale et al. 2011; Miller et al. 1991; Tittlemeier et al. 2013). The Asian 3ADON-producing strains were first introduced to Atlantic Canada in the 1970s to 1980s on breeding material from China and Europe, and the population of the 3ADON chemotype has been on the rise ever since (Ward et al. 2008). Canadian cereal grain surveys of FHB-causing species and *F. graminearum* chemotypes have shown that nearly 100% of the *F. graminearum* strains from the Maritimes are 3ADON producers (Kelly et al. 2015). The frequency of 3ADON-producing *F. graminearum* strains is also on the rise in western Canada (Ahmed et al. 2020; Kelly et al. 2015; Miller and Richardson 2013; Ward et al. 2008).

The common mycotoxins associated with the most important *Fusarium* species causing FHB, such as *F. graminearum* and *F. culmorum,* are zearalenone (ZEA) and the Type B trichothecenes (DON, 3ADON, 15ADON, and NIV). *Fusarium avenaceum* is a species well known for the biosynthesis of moniliform (MON), whereas NIV is mainly associated with *F. poae,* which is acutely more toxic than DON (Miller and Richardson 2013). Apart from NIV, *F. poae* can also produce the Type A trichothecenes, T2 and HT2 toxins, the most acutely toxic simple trichothecenes. These are also produced by other weakly pathogenic *Fusarium* species (*F. sporotrichioides* and *F. acuminatum*), which are more common in parts of Europe than western Canada (Alexander et al. 2009; McCormick et al. 2011). Many surveys have indicated that DON is the most frequently detected mycotoxin in Canadian cereals and other wheat-growing areas of the world (Covarelli et al. 2015; Cowger et al. 2020; Desjardins 2006; Gräfenhan et al. 2013; Tittlemier et al. 2013). It has been determined that mycotoxins have diverse and numerous mycotoxicosis effects on human and animal health (Miller and Richardson 2013). Many countries have standardized the maximum tolerated toxin levels, especially for DON, in different foodstuffs and animal feed (JECFA 2011; EFSA 2013; Charmley and Trenholm 2000). The recommended level for DON or the provisional maximum tolerable daily intake (PMTDI) is 1 μg/kg body weight/per day, and for NIV is 1.2 μg/kg body weight per day (EFSA 2013; JECFA 2001; 2011). Due to the high acute toxicity of T2 and HT2, the established PMTDI for T2 and HT2 toxins alone or in combination are lower than that of DON (60 ng/kg body weight per day: JECFA 2001).

Several researchers have proposed that climate change and minimum or no-till have increased the severity of FHB epidemics, the concentrations of mycotoxins in the harvested grain, and changed the population patterns of FHB pathogens (Kelly et al. 2015; Pereyra and Dill-Macky 2008; Valverde-Bogantes et al. 2019; Zhang et al. 2014). No recent information on these factors is available for the FHB pathogen population in Saskatchewan, which can help understand the

cause and development of an FHB epidemic and improve the existing management strategies. Therefore, the present study aimed to determine the population and chemotype diversity of *Fusarium* spp. among FHB infected spring and durum wheat grain samples from Saskatchewan.

5.3 Materials and methods

5.3.1 Plant Material

Samples of wheat (n = 132; Appendix A Table A.4) were obtained from the Saskatchewan Crop Insurance Corporation (SCIC) that had been collected in 2014 (n= 69), 2015 (n= 18), and 2016 (n=45) from Saskatchewan, Canada (Fig. 5.1). The wheat samples represented three classes: Canada Western Amber Durum (CWAD; n = 92), Canada Prairie Spring wheat (CPS; n= 13), and Canada Western Red Spring wheat (CWRS; n = 27).

Each of the 132 samples was divided into two subsamples of 50 g each, one for seed mycological analyses and one finely ground by a Laboratory Mill 3100-v3.3 EN, for *Fusarium* spp. quantification and mycotoxin analysis.

5.3.2 Morphological examination

A sub-sample of approximately 100 wheat kernels was selected at random for plating. Before plating, the seeds were surface sterilized with a 5% NaOCl (sodium hypochlorite) water solution and rinsed three times with sterile water. One hundred sterilized kernels were placed on potato dextrose agar (PDA) in plastic Petri dishes, each containing ten kernels. After five days of incubation at 20 to 22°C, *Fusarium* spp. were identified visually based on mycelial growth. The number of kernels infected in each sample was expressed as the percentage of Fusarium damaged kernels (FDKs). Seed with a wrinkled seed coat and chalky white appearance showed abundant mycelial growth. The mycelium growth around the seeds was observed under the microscope to determine the *Fusarium* spp. based on macro and micro-

morphological characteristics (Gerlach and Nirenberg 1982). Developing colonies from each seed were transferred to a new Petri dish and were left to grow for seven days at room temperature. Numerous pure cultures of *Fusarium* spp. were isolated from the incubated kernels.

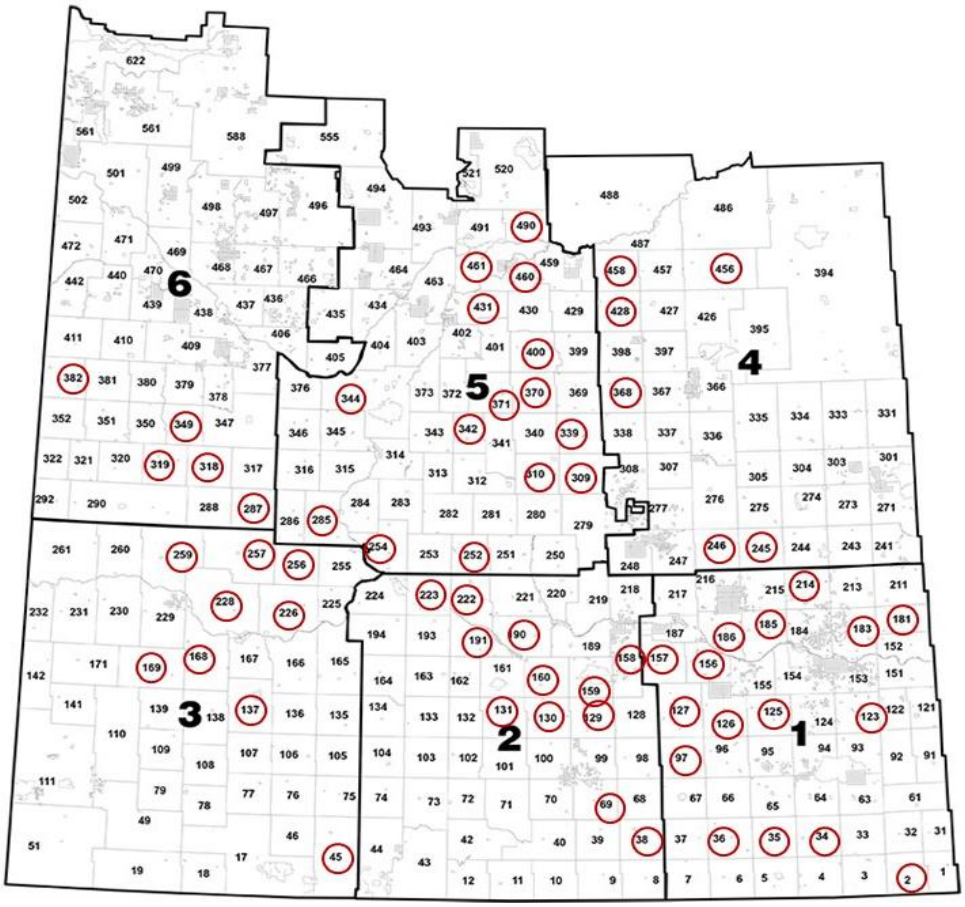

Fig. 5.1. Crop districts of Saskatchewan from which grain samples were obtained for this study.

5.3.3 DNA extraction, *Fusarium* spp. quantification and chemotyping

Homogenized wheat kernels (10 g) were ground using a Laboratory Mill 3100-v3.3 EN, and 50 mg of sample was used for DNA extraction using the QIAGEN DNeasy Blood & Tissue Kit (50) following the instruction manual provided by the manufacturer. The concentration and quality of the extracted DNA were determined by a Nanodrop ND-1000 spectrometer (Thermo Scientific, Wilmington, DE, USA).

Pure isolates of five *Fusarium* spp. (*F. graminearum, F. culmorum, F. avenaceum, F. poae,* and *F. sporotrichioides*) were grown in PDB (liquid media) at 20 to 22°C for five days. Mycelium growth on the surface was collected in 15 ml centrifuge tubes. After centrifugation at 8300 g for 20 min, the medium was removed, and the mycelia were ground to a powder in a mortar using liquid nitrogen. DNA was extracted from ground fungal mycelium using the DNA extraction kits [QIAGEN DNeasy Blood & Tissue Kit (50)]. Extracted DNA from the five *Fusarium* spp. were used in real-time PCR for specificity tests, standard curves, and positive controls.

Specific primers and probes (Table 5.1) were used to detect each of the five species that were identified from the infected wheat kernels. The 10 µl reaction mixture for *F. graminearum* (*FgMito* assay) detection by real-time PCR consisted of 1 µl of template DNA, 0.5 µl of 20X assay mix (primer + probe; Table 5.1), 5 µl TaqMan Fast Advanced Master Mix, and 3.5 µl of H_2O, while the reaction mixture for the other four *Fusarium* spp. (*TMTRI* assay) consisted of 5 µl 2× TaqMan Fast Advanced Master Mix, 0.2 µl (250 nM) of each primer (Table 5.1), and 0.2 µl of probe and 1 µl template DNA. The reaction conditions for the FgMito assay were 95°C for 20 s (95°C for 3 s, 60°C for 30 s) × 40 and 50°C for 2 min; 95°C for 10 min; (95°C for 15s, 60°C for 60s) × 40 for the *TMTRI* assay. All reactions were performed on a 7900HT Sequence Detection System (Applied Biosystems) in triplicate; the sensitivity and specificity of each assay were determined by tenfold dilutions (10, 1, 0.1, 0.01, and 0.001 ng) of DNA extracted from a pure culture of each of the five *Fusarium* spp. The serial dilution of pure fungal DNA was used to generate standard curves for each assay. The amount of fungal DNA was calculated from cycle threshold (CT) values using the standard curve, and fungal biomass was presented as a picogram (pg) of fungal DNA per nanogram (ng) of total extracted DNA. The threshold level in most of the cases was 0.01 (CT<32) to quantify the smaller amount of

DNA. The sensitivity of each primer used in the assay was high, as there was a strong correlation between the known DNA quantities and CT values (Fig. 5.2).

Isolates of *F. graminearum* identified by morphological examination were tested for the presence of chemotypes 3ADON, 15ADON, and NIV. All *F. graminearum* isolates were revalidated by the species-specific PCR marker (Demeke et al. 2005). The *Tri3* gene-specific primers were used in multiplex PCR to determine the chemotype of *F. graminearum* isolated from the wheat samples (Ward et al. 2008). The multiplex PCR reaction mixture was set as described in Ward et al. (2008). The resulting PCR product was resolved on a 2% agarose gel and scored relative to a 100-bp DNA ladder. The bands were visualized under UV light. The reaction produced amplicons for each chemotype: approximately 243 bp for 3ADON, 610 bp for 15ADON, and 840 bp for NIV.

Table 5.1. List of primers and probes used for qPCR quantification of five *Fusarium* spp. DNA in wheat samples collected from Saskatchewan between 2014 and 2016.

Species	Primer/probe	Primer/probe sequence	Reference
F. graminearum	COB1 primer	TGGCCTGAATGAAGGATTTCTAG	Kulik et al. 2015
	COB2 primer	CATCGTTGTTAACTTATTGGAGATG	
	COB probe	FAM-TTAAACACTCAAACACTACA-MGB	
F. culmorum	TMFcf	CACCGTCATTGGTATGTTGTCACT	Nicolaisen et al. 2009
	TMFcr	CGGGAGCGTCTGATAGTGG	
	TMFcp	TGCTGTCATCACATTCTCATACTAAC	
F. avenaceum	TMAVf	AGATCGGACAATGGTGCATTATAA	Halstensen et al. 2006
	TMAVr	GCCCTACTATTTACTCTTGCTTTTG	
	TMAVp	6FAM-CTCCTGAGAGGTCCCAGAGATGAACATAACTTC	
F. poae	TMpoaef	GCTGAGGGTAAGCCGTCCTT	Yli-Mattila et al. 2008
	TMpoaer	TCTGTCCCCCCTACCAAGCT	
	probe	ATTTCCCCAACTTC GACTCTCCGAGGA	
F. Sporotrichioides	TMLANf	GAGCGTCATTTCAACCCTCAA	Halstensen et al. 2006
	TMLANr	GACCGCCAATCAATTTGGG	
	TMLANp	6FAM-AGCTTGGTGTTGGGATCTGTCCTTACCG	

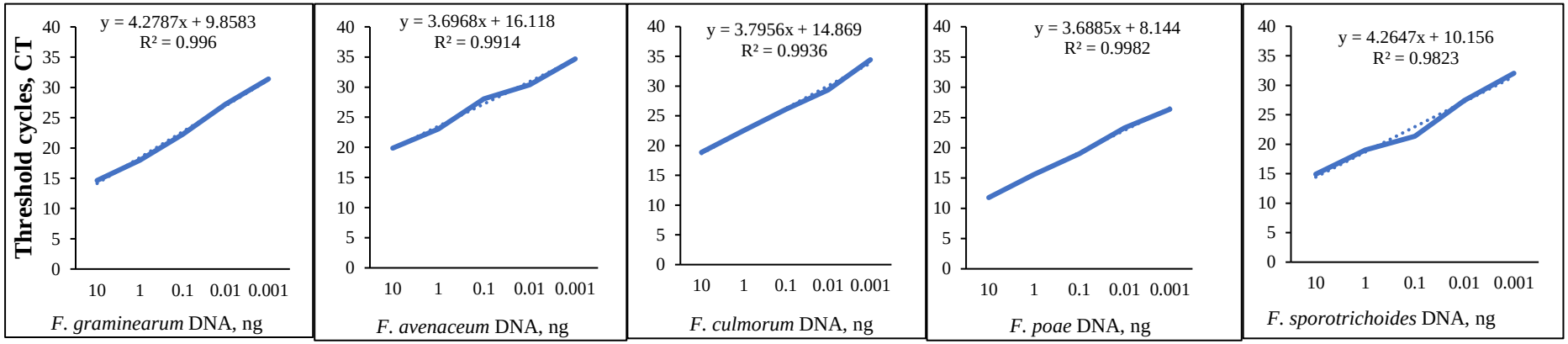

Fig. 5.2. Standard curves of the qPCR assay for five primers, used for quantification of *F. graminearum*, *F. avenaceum*, *F. culmorum*, *F. poae*, and *F. sporotrichioides*. Correlation between the relative amount of *Fusarium* spp. DNA and Ct values in a wheat sample.

5.3.4 Mycotoxin and fusarium damaged kernels (FDK)

The ground samples were tested for the presence of seven mycotoxins (DON, 3ADON, 15ADON, NIV, D3G (deoxynivalenol-3-glucoside), T2, and HT2 toxin) using LC-MS/MS based mycotoxin/deoxynivalenol (DON) platform (established by Dr. Lipu Wang).

5.4 Results

5.4.1 Species and chemotype identification

All 132 samples from three wheat classes grown in western Canada were infected by *Fusarium* spp. Fusarium damaged kernel levels were high in the CWAD wheat class (mean = 16.7%; range = 5 - 83%) followed by CWRS (mean = 13.6%; range = 4 - 65%) and CPS (mean = 13.3%; range = 6 - 31%). Comparison of FDK levels among years showed higher levels in samples from 2014 (mean - 17.5%), followed by 2016 (mean - 13.4%), and lowest in 2015 (mean - 12.6%) (Fig 5.3A).

From 132 wheat samples, five *Fusarium* spp. were recovered, and plating of a single sample usually resulted in a recovery of a mixture of *Fusarium* spp. The *Fusarium* spp. identified were *F. graminearum, F. culmorum, F. avenaceum,* and *F. poae*. *Alternaria alternata* and *Epicoccum nigrum* were also observed. *Fusarium graminearum* was detected in all 132 samples and was the most prevalent species present in more than 60% of the FDK (Fig. 5.3B). The second highest among the five species identified was *F. avenaceum,* which was present in 117 wheat samples; *F. poae* and *F. culmorum* were identified in 113 and 107 samples, respectively. *Fusarium sporotrichioides* was detected in only six samples, and all were CWAD.

The assays *FgMito* and *TMTRI* allowed for the detection and quantification of *Fusarium* spp. in all the wheat samples. All standard concentrations confirmed the presence of individual *Fusarium* spp. (Fig 5.2) with a fluorescent signal, although the concentration of each *Fusarium* spp. varied greatly among samples due to differences in the amount of template DNA extracted

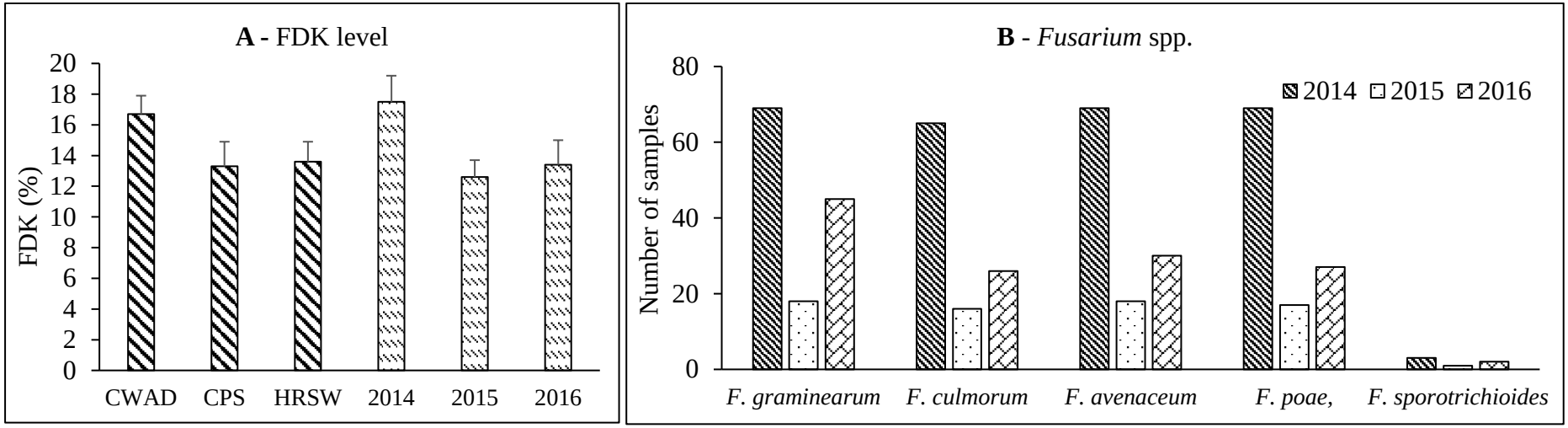

Fig. 5.3. (A) Average percentage of kernels (FDK) infected by *Fusarium* spp. for each wheat class and year of collection. **(B)** Presence of *Fusarium* spp. in the wheat kernels.

(2.1 - 742.0 ng). *Fusarium graminearum* was detected and quantified in all wheat samples, and the concentration of DNA ranged from 0.5 to 139.4 pg ng^{-1}. The species with the second-highest DNA concentration was *F. avenaceum,* which ranged between 0.02 to 19.8 pg ng^{-1}, followed by *F. poae* (0.01 - 1.56 pg ng^{-1}) and *F. culmorum* (0.01 to 3.2 pg ng^{-1}). *Fusarium sporotrichioides* DNA content was limited; it ranged between 0.3 to 0.71 pg ng^{-1} and was detected in only six samples, all CWAD. There was a difference in the average DNA concentrations of *Fusarium* spp. over years (Table 5.2). The detection rate and level of all *Fusarium* spp. were highest in samples collected in 2014, followed by 2016 and lowest in 2015 (Table 5.2).

All 263 isolates of *F. graminearum* were validated with the Fg16F/ Fg16R primer and were confirmed as *Fusarium graminearum* sensu *stricto*. Genetic chemotyping of 263 *F. graminearum* isolates collected in 2014, 2015, and 2016 showed that a substantial fraction (63.5%) of these isolates were of the 3ADON chemotype, and the remaining were the 15ADON chemotype (36.5), with a total absence of the NIV chemotype (Fig 5.4A). When compared further based on wheat class, the number of 3ADON isolates (71.3%) collected from CWAD was higher than 15ADON (28.7%; Fig. 5.4B). Eighty-five *F. graminearum* isolates were collected from CPS and CWRS, and 45 of them were the 15ADON chemotype (Fig. 5.4C).

Table 5.2. Summary of *Fusarium* spp. DNA detected (pg/ng) among FHB symptomatic durum and spring wheat samples collected from Saskatchewan in 2014, 2015, and 2016.

	2014				2015				2016			
***Fusarium* spp.**	**N**	**Min**	**Max**	**Mean**	**N**	**Min**	**Max**	**Mean**	**N**	**Min**	**Max**	**Mean**
F. graminearum	69	5.31	139.40	20.04	18	0.53	4.82	2.30	32	0.19	49.81	7.78
F. avenaceum	65	0.09	19.80	3.03	16	0.02	8.07	1.60	28	0.08	14.44	4.65
F. poae	69	0.01	1.56	0.28	17	0.01	0.75	0.12	29	0.04	1.48	0.37
F. culmorum	69	0.01	3.20	0.49	18	0.01	0.95	0.17	44	0.01	1.97	0.23
F. sporotrichioides	2	0.34	0.38	0.36	1	0.70	0.70	0.70	3	0.08	0.26	0.15

N – sample number; Min – minimum DNA quantity of *Fusarium* spp. (pg) in total DNA (ng); Max – maximum DNA quantity of *Fusarium* spp. (pg) in total DNA (ng); Mean – mean DNA concentration among samples.

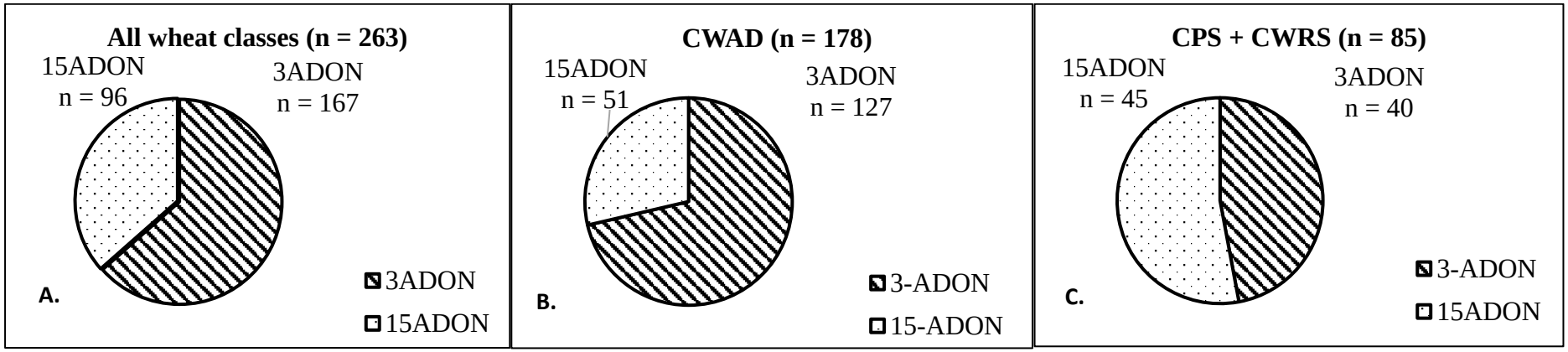

Fig. 5.4. (A) Trichothecene chemotype frequencies in Saskatchewan based on analyses of 263 *F. graminearum* isolates collected from wheat between 2014 and 2016, **(B)** chemotype frequency in CWAD, and **(C)** chemotype frequency in CPS + CWRS.

5.4.2 Mycotoxin

Data on the seven trichothecene mycotoxins quantified from the 132 wheat samples are presented in Table 5.3. The highest accumulation was of DON, followed by D3G. Other mycotoxins detected were 3ADON, 15ADON, NIV, T2, and HT2. Deoxynivalenol content was highest in the 2014 wheat samples (0.180 - 31.074 μg kg^{-1}), followed by the 2016 samples (0.007 - 20.645 μg kg^{-1}), and lowest in the 2015 samples (0.142 - 10.370 μg kg^{-1}). Nivalenol was present in all years, but the detection range was less than 1 μg kg^{-1}. The detection rate and concentration level of HT2 were higher than T2 in all the samples.

5.4.3 Correlation between qPCR and mycotoxin results

The Pearson correlation statistics in SAS was used to determine the correlation between the concentration of *Fusarium* spp. DNA, FDK, and the mycotoxins (DON, NIV, D3G, 3ADON, 15ADON, T2, and HT2) (Table 5.4). There was a weak correlation between the *F. graminearum* and DON (R = 0.288, P = 0.008), and between *F. poae* and NIV (R = 0.315, P = 0.002). A positive and moderate correlation between *F. sporotrichioides* and T2 (R = 0.671, P = 0.009) and HT2 (R = 0.533, P = 0.002) was also detected. There was no correlation between *Fusarium* spp. DNA and FDK levels in the samples.

Table 5.3. Summary of *Fusarium*-produced mycotoxins (μg kg^{-1}) in FHB symptomatic durum and spring wheat samples collected from Saskatchewan in 2014, 2015, and 2016.

	2014				2015				2016			
Mycotoxins	**N**	**Min**	**Max**	**Mean**	**N**	**Min**	**Max**	**Mean**	**N**	**Min**	**Max**	**Mean**
DON	66	0.180	31.074	6.012	18	0.142	10.370	4.137	44	0.007	20.645	6.935
NIV	53	nd	0.107	0.018	14	nd	0.039	0.019	39	nd	0.284	0.042
D3G	66	nd	4.222	0.282	18	0.003	0.456	0.200	43	nd	0.648	0.264
3ADON	63	nd	0.340	0.056	14	nd	0.109	0.066	41	nd	0.089	0.029
15ADON	48	nd	0.327	0.066	14	nd	0.079	0.047	25	nd	0.059	0.026
T2	45	nd	0.046	0.011	17	nd	0.014	0.012	8	nd	0.048	0.018
HT2	58	nd	0.615	0.036	18	0.004	0.044	0.020	37	nd	2.016	0.065

N – sample number; Min – minimum mycotoxin quantified (μg kg^{-1}); Max – maximum mycotoxin quantified (μg kg^{-1}); Mean – mean mycotoxin concentration among samples; DON - deoxynivalenol; NIV- nivalenol; D3G - deoxynivalenol-3-glucoside; 3ADON - 3acetyldeoxynivalenol, 15ADON - 15acetyldeoxynivalenol

Table 5.4. Correlation between *Fusarium* spp., Fusarium damaged kernel (FDK) and mycotoxin (DON, NIV, D3G, 3-ADON, 15-ADON, T2, and HT2) content (μg kg^{-1}).

	F. graminearum	*F. avenaceum*	*F. culmorum*	*F. poae*	*F. sporotrichioides*
FDK	-0.021 ns	0.097 ns	0.005 ns	-0.053 ns	-0.078 ns
DON	0.288 **	-0.037 ns	-0.123 ns	-0.062 ns	0.328 ns
NIV	-0.118 ns	0.073 ns	0.043 ns	0.315 **	0.774 ns
D3G	0.082 ns	-0.100 ns	-0.116 ns	-0.037 ns	0.467 ns
3ADON	0.135 ns	0.205 ns	-0.086 ns	-0.121 ns	0.875 ns
15ADON	0.197 ns	-0.132 ns	-0.138 ns	0.008 ns	0.848 ns
T2	-0.064 ns	0.149 ns	-0.144 ns	0.325 **	0.671 **
HT2	0.032 ns	0.201 ns	-0.020 ns	0.341 **	0.533 **

Note: **: Correlation coefficients significant at $P < 0.001$; ns - not significant DON - deoxynivalenol; NIV- nivalenol; D3G - deoxynivalenol-3-glucoside; 3ADON - 3acetyldeoxynivalenol, 15ADON - 15acetyldeoxynivalenol

5.5 Discussion

In this study, I report the presence of different toxigenic *Fusarium* species responsible for FHB in durum and bread wheat samples collected from Saskatchewan in 2014, 2015, and 2016. This study also reports the chemotype diversity of *F. graminearum* and the mycotoxins present in naturally infected wheat samples. All five *Fusarium* spp. tested were present in the wheat samples confirming the findings of earlier *Fusarium* diversity studies on wheat in western and eastern Canadian provinces (Gräfenhan et al. 2013; Guo et al. 2008; Tittlemier et al. 2013; Xue et al. 2019). As expected, the dominant FHB causing species in the wheat samples investigated was *F. graminearum*. It was isolated and quantified in all wheat samples in all three years regardless of wheat class and environmental conditions. These findings are in line with the worldwide occurrence of *F. graminearum*, which is best known for its impact on yield, grain quality, and its ability to produce several different toxins (Gale et al. 2003; Gräfenhan et al. 2013; McMullen et al. 2012; Vogelgsang et al. 2019; Xue et al. 2019).

Fusarium avenaceum, F. culmorum, and *F. poae* were also isolated and quantified in all years at an alarming rate. These *Fusarium* spp. are frequently associated with small grain cereals, confirming the results obtained in the same area in 2010 (Gräfenhan et al. 2013; Tittlemier et al. 2013). *Fusarium sporotrichioides* was detected at very low frequency and only in the CWAD samples plated and by qPCR quantification, which was also consistent with previous research (Gräfenhan et al. 2013, Guo et al. 2008 and Xue et al. 2019), in which *F. poae* and *F. sporotrichioides* were associated with FHB on oat, barley, and wheat. The year of collection of the isolates has a considerable effect on the *Fusarium* species recovered from the wheat samples. In years when environmental conditions were favourable for FHB epidemics (2014 and 2016), the isolation frequency and the quantity of *F. graminearum* and other resident *Fusarium* spp. recovered increased dramatically. This is most probably due to diverse weather

conditions that favoured some FHB-causing strains over the others (Covarelli et al. 2015; Vogelgsang et al. 2019).

The toxigenic potential of *Fusarium* spp. of a particular geographical area can be determined by chemotype characterization. The population structure of *F. graminearum* in Canada by Kelly et al. (2015) and Ward et al. (2008) reported significantly higher 3ADON isolates from the Maritime Provinces and Manitoba, whereas the 15ADON chemotype was more frequently isolated in Ontario, Saskatchewan, and Alberta. Analysis of trichothecene chemotypes of 263 isolates of *F. graminearum* collected from Saskatchewan wheat fields revealed the presence of 3ADON and 15ADON chemotypes, but a complete absence of NIV. The existence of chemotype patterns of western Canadian *F. graminearum* populations is in accordance with Ahmed et al. (2020) and Valverede-Bogantes et al. (2019). The results obtained in my study showed that the 3ADON (63.5%) chemotype was the most prevalent in the *F. graminearum* population isolated from durum and spring wheat in Saskatchewan. My results agree with population diversity studies of *F. graminearum* isolates collected between 1998 and 2004 (Ward et al. 2008) and 2005 - 2007 (Kelly et al. 2015) from wheat in western Canada. These two studies reported increased frequency of the 3ADON chemotype in the *F. graminearum* population on the Canadian prairies over years. My results also agree with those of Ahmed et al. (2020), who reported an increased proportion of the 3ADON chemotype over time in the *F. graminearum* population of isolates collected from wheat in Alberta (3ADON:15ADON = 1:1). The trichothecene chemotype composition also varied among years and wheat classes, but the number of 3ADON isolates was always higher than the number of 15ADON isolates.

In recent years, environmental conditions have favored FHB, and Canadian producers have seen an increased occurrence of mycotoxin contamination in small grain cereals due to frequent epidemics of FHB. *Fusarium* spp. produce a wide range of mycotoxins, among DON, 3ADON, 15ADON, ZEN, MON, NIV, T2, and HT2 toxins are of major concern in temperate climates

like Canada (Miller and Richardson 2013). My mycotoxin analyses demonstrated a mixture of mycotoxins in Saskatchewan wheat samples. The main toxin detected at the highest frequency and concentration was DON, which is generally the main toxin of economic concern in Canada and the USA in small grain cereals (Cowger et al. 2020). The range of DON concentrations in my study over three years was 0.007 to 31.07 μg kg^{-1}, with a mean of 5.7 μg kg^{-1}, which is much higher than the maximum limit for DON (1 μg kg^{-1}) in specific foods sold in Canada and those reported earlier from the Canadian prairies (Government of Canada 2020). Gräfenhan et al. (2013) and Tittlemier et al. (2013) reported much lower levels of mycotoxin in spring wheat (up to 1.1 μg kg^{-1}) and durum wheat (up to 4.7 μg kg^{-1}) samples collected from the three western Canadian provinces in 2010.

Similarly, samples collected between 2000 and 2002 from Manitoba (mean - 1.4 μg kg^{-1}), Saskatchewan (mean - 0.3 μg kg^{-1}), and Alberta (absent) had low concentrations of DON (Clear et al. 2005). The occurrence of relatively high DON concentrations observed in the samples in my study was likely due to above-average rainfall in 2014 and 2016, which encouraged FHB and DON accumulation. Also, these samples were specifically provided by SK Crop Insurance because these samples were submitted by the farmers making insurance claims because the FHB was very severe. The acetylated forms of DON (3ADON and 15ADON) were also detected, and the 3ADON toxin concentration was higher than 15ADON. The D3G detection rate was similar to that of DON, but the concentrations were much lower (approximately 0.03 μg kg^{-1}). Overall, NIV was detected in 80% of the samples, and the concentration was lower (approximately 0.03 μg kg^{-1} or 0.3 μg kg^{-1}) than EFSA established tolerable daily intake (1.2 μg kg^{-1} body weight per day) (EFSA 2013). My results contradict those of Tillemier et al. (2013), who reported the absence of NIV in durum samples collected in 2010 from Alberta and Saskatchewan. Few studies have reported T2 and HT2 in small grain cereal samples in Canada (Gräfenhan et al. 2013; Tamburic-Ilincic et al. 2010; Tittlemier et al. 2019; Van der Fels-Klerx

et al. 2010). The frequency of T2 and HT2 in my study was high (86% and 53%), but concentrations measured were low (approximately 0.040 and 0.014 μg kg^{-1}). Clear et al. (2005) and Tittlemier et al. (2019) did not report these two mycotoxins in composite wheat samples collected from the Canadian prairies. In contrast, Gräfenhan et al. (2013) reported the presence of T2 and HT2 in approximately 10% of the fields sampled at concentrations of ≤0.5 mg/kg.

The correlation between DON and the incidence of the potential DON producer, *F. graminearum* was weak (R = 0.29, P = 0.008). Such relationships have been reported previously in wheat surveys from western Canada and Europe (Eckard et al. 2011; Gräfenhan et al. 2013). Western Canadian isolates of *F. poae* have been shown to produce NIV in inoculated wheat (Tittlemier et al. 2019). A significant but weak correlation between *F. poae* and NIV toxin was detected. Like Miller and Richardson (2013), a moderate correlation between *F. sporotrichioides* and its associated mycotoxins T2 and HT2 was detected in my study.

In conclusion, this 3-year study has demonstrated that the population structure of FHB pathogens in Saskatchewan is changing continuously. It is evident from my results that multiple *Fusarium* spp. were responsible for FHB epidemics in commercial wheat fields in Saskatchewan between 2014 and 2016, resulting in mycotoxin contamination of grain. Two chemotypes of *F. graminearum* (3ADON and 15ADON) were present in the population; 3ADON was predominant, and the proportion was much higher than reported by earlier studies. My results also demonstrated that DON was present in all samples at an alarming rate, and the value exceeded the tolerable daily intake for both humans and animals. Furthermore, NIV, T2, and HT2 toxins and acetylated forms of DON were also detected in higher proportions than reported previously from western Canada, which was due to the fact that these samples were from highly FHB infected crops. In the future, additional and extensive sampling will be necessary to monitor the effect of various agronomic practices and climate change on the

pathogen population and on subsequent mycotoxin production, which will help in the improvement of current FHB management tools. The Canadian grain grading system must also monitor the concentrations of emerging mycotoxins (NIV, T2, and HT2) from the perspective of safety because of their higher toxicity compared to DON.

CHAPTER 6

The chemotypes 3ADON, 15ADON, NIV, and NX-2 of *Fusarium graminearum* vary in aggressiveness on wheat.

6.1 Abstract

The aggressiveness of *Fusarium graminearum* chemotypes was assessed in a greenhouse experiment on spring wheat genotypes that varied in resistance to Fusarium head blight (FHB). Fifteen isolates (five 3ADON, five 15ADON, three NIV, and two NX-2) of *F. graminearum* isolated from Saskatchewan wheat fields were evaluated using point inoculation (Type II resistance assay). Evaluation was on three wheat genotypes assessed as resistant, moderately resistant, and susceptible to FHB and near-isogenic lines (NILs) in the CDC Go (moderately susceptible) background, carrying *Fhb* quantitative trait loci (QTL) in four combinations: *Fhb1*, *Fhb2*, *Fhb5*, and *Fhb1+Fhb2+Fhb5*. All four *F. graminearum* chemotypes were pathogenic on wheat and varied in aggressiveness. Disease severity caused by 3ADON, 15ADON, NIV, and NX-2 isolates differed, with 3ADON (29.7 ± 3.6) and 15ADON (22.4 ± 2.9) isolates more aggressive than NIV (16.7 ± 2.8), and NX-2 (16.2 ± 3.1) isolates. Significant differences were observed for mycotoxin accumulation among chemotypes, which was highest for 3ADON (14.1 ± 2.26 μg kg^{-1}). There was no interaction between chemotype and wheat line for disease severity or mycotoxin accumulation.

6.2 Introduction

Fusarium head blight (FHB) or head scab is a devastating disease of small grain cereals such as wheat, barley, oat, and canaryseed in Canada and other parts of the world (Brar et al. 2019b; McMullen et al. 2012; Valverde-Bogantes et al. 2019). The disease causes significant yield loss and reduces the quality of harvested grain due to contamination with mycotoxins produced by *Fusarium* spp., which pose acute and chronic health hazards to humans and animals

(Gräfenhan et al. 2013; Miller and Richardson 2013). Various *Fusarium* spp. in the *F. graminearum* species complex (FGSC) can cause FHB in wheat, but *F. graminearum* is the prime causal agent of the disease in North America (Valverde-Bogantes et al. 2019).

The most common mycotoxins associated with FGSC are trichothecene metabolites, which are eukaryotic protein synthesis inhibitors, but some species can also produce the estrogenic mycotoxin zearalenone (Miller and Richardson 2013; O'Donnell et al. 2000). Members of the FGSC usually produce Type B trichothecenes, but *F. graminearum* can also produce Type A trichothecenes. Based on the toxigenic profile, *F. graminearum* isolates can be grouped into four chemotypes: (i) 3ADON (3-acetyldeoxynivalenol), (ii) 15ADON, (15-acetyldeoxynivalenol) (iii) NIV (nivalenol), and (iv) NX-2 (Miller et al. 1991; Varga et al. 2015; Ward et al. 2002). Chemotype 3ADON primarily produces 3ADON along with deoxynivalenol (DON), while the 15ADON chemotype produces DON and primarily 15ADON (Miller and Richardson 2013). Isolates of the NIV chemotype produce NIV and its acetylated derivatives, whereas the recently reported NX-2 chemotype never produces DON, but rather accumulates NX-2 and the deacetylated form called NX-3 in plants (Varga et al. 2015). Population structure studies of *F. graminearum* have indicated that the 15ADON chemotype predominates in Canada, followed by 3ADON, whereas both NIV and NX-2 chemotypes are present but at lower frequencies (Abramson et al. 2001; Kelly et al. 2016). In recent chemotype diversity studies, a significant increase in the 3ADON chemotype frequency was reported in Canada (Ahmed et al. 2020; Guo et al. 2008; Kelly et al. 2015; Ward et al. 2008). The newly emerging 3ADON population appears to be more aggressive than 15ADON, i.e., it produces more trichothecenes, is more prolific, and has higher growth rates (Ward et al. 2008).

Breeding for resistance to FHB is complicated due to differences in the types of resistance to the pathogen. Studies on FHB resistance have determined five types of *Fusarium* resistance

mechanisms in wheat: Type I - resistance to initial infection, Type II - resistance to spread of the disease from the point of infection, Type III - resistance to mycotoxin accumulation, Type IV - resistance to kernel infection, and Type V - general tolerance to the disease or resistance to yield loss (Bai and Shaner 2004; He et al. 2016; Lemmens et al. 2005; Mesterházy 1995, 2003; Miller et al. 1985; Schroeder and Christensen1963). The evaluation of plant material for any particular type of resistance to FHB is based on specific protocols. In the Type I resistance assessment, spray inoculation methods are used, whereas, for Type II resistance, single floret point inoculation is used under controlled conditions (Wang and Miller 1988). Breeding for FHB resistance is a challenging task as resistance to FHB is a quantitative trait highly influenced by environment (Malihipour et al. 2012; McMullen et al. 2012; Serajazari et al. 2019). Genetic studies have identified many 'minor' QTLs and some 'major' QTLs, which, when combined, have improved resistance to FHB (Buerstmayr et al. 2003; Salameh et al. 2011).

Previous studies attempting to examine *F. graminearum* isolate aggressiveness, host resistance reaction (the ability of host plant to reduce pathogen colonization), and interaction of the pathogen with host genotypes have revealed contradictory results. A pathogens aggressiveness is a quantitative measurement of the disease induced by a pathogenic isolate on a susceptible host (Vanderplank, 1984). In the case of *F. graminearum*, aggressiveness also depends on the trichothecene producing capacity of the isolates as non-DON producing *F. graminearum* isolates cannot spread beyond the initial infection site but are still able to infect plants (Bai et al. 2001; Eudes et al. 2001; Mesterházy et al. 2002). Multiple studies have reported high variation in aggressiveness among *F. graminearum* isolates and chemotypes from different geographical regions. Puri et al. (2016) reported no difference in aggressiveness between 3ADON and 15ADON chemotypes when tested for Type II resistance on resistant, moderately resistant, and susceptible cultivars. Similarly, aggressiveness associated with chemotype was

tested in artificially inoculated fields across Canada and Germany; no difference in aggressiveness between 3ADON and 15ADON chemotypes was reported (von der Ohe et al. 2010). In contrast, Serajazari et al. (2019) stated that the 3ADON chemotype was 18% more aggressive than the 15ADON isolates in Type I resistance assays, although no difference in the aggressiveness of the two chemotypes was observed when tested for Type II resistance. Foroud et al. (2012) compared aggressiveness of 3ADON, 15ADON and NIV-producing isolates and found DON producers were more aggressive than NIV producers.

With the inconsistency among studies and continuous changes in chemotype diversity of *F. graminearum*, I hypothesized that the four chemotypes of *F. graminearum* would vary in aggressiveness, and spring wheat genotypes with varying levels of FHB-resistance would respond differently when inoculated with isolates of 3ADON, 15ADON, NIV and NX-2 chemotypes. Therefore, the objective of this study was to compare the aggressiveness of 3ADON, 15ADON, NIV, and NX-2 producing *F. graminearum* isolates by evaluating FHB disease severity and quantifying mycotoxin accumulation under controlled conditions. An additional objective was to evaluate whether the spring wheat near-isogenic lines (NILs) with different *Fhb* QTLs perform similarly against the four chemotypes of *F. graminearum*.

6.3 Materials and methods

6.3.1 Fungal isolates

Fusarium graminearum isolates corresponding to four chemotypes were tested for Type II resistance in the greenhouse (Table 6.1). Ten *F. graminearum* isolates were collected from Saskatchewan from 2014 to 2016 (Chapter 4) and five isolates were obtained from the USDA/ARS Culture Collection (NRRL, Peoria, IL).

Table 6.1. *Fusarium graminearum* isolates, their corresponding chemotypes, and the number of isolates of each chemotype used to determine aggressiveness in wheat.

***Fusarium* spp.**	**Chemotype**	**Number**	**Isolates ID.**
F. graminearum	3ADON	5	103-a-1, 109-b-1, 161-d-2, 205-a-1, 210-a-1
F. graminearum	15ADON	5	108-b-1, 150-b-3, 156-b-1, 202-a-4, 218-a
F. graminearum	NIV	3	NRRL- 45226, NRRL-39206, NRRL-52332
F. graminearum	NX-2	2	NRRL-39173, NRRL-44211

3ADON - 3-acetyl-deoxynivalenol; 15-ADON -15-acetyl-deoxynivalenol; and NIV - nivalenol

6.3.2 Plant material

Seven wheat genotypes were included in the study: CDC Go, AAC Carberry, AAC Tenacious, and four near-isogenic lines (NILs) carrying *Fhb1*, *Fhb2*, *Fhb5*, and *Fhb1+Fhb2+Fhb5* in the CDC Go background (Table 6.2; Brar et al. 2019).

Table 6.2. Commercial wheat varieties and near-isogenic lines (NILs) in the CDC Go background with corresponding FHB resistance QTLs.

Genotype	**Disease reaction/ resistant QTLs**
CDC Go	MS check
AAC Carberry	*Fhb1* + *Fhb5* + unknown QTL; MR check
AAC Tenacious	R check
CDC Go (NIL-38)	*Fhb1*
CDC Go (NIL- 20)	*Fhb2*
CDC Go (NIL-21)	*Fhb5*
CDC Go (NIL-28)	*Fhb1* + *Fhb2* + *Fhb5*

R – resistant; MS - moderately susceptible and S - susceptible

6.3.3 Greenhouse FHB evaluations

Fusarium graminearum isolates were individually inoculated on seven wheat genotypes (Table 6.2) to assess disease spread within the spike. Three plants were grown in one-gallon pots (~13 cm diameter) in the greenhouse equipped with incandescent lamps, 16 h photoperiod, and 22/16°C day/night temperatures. Plants were watered regularly and fertilized with 10 g of slow-release fertilizer N-P-K (14-14-14). Fifteen isolates (four chemotypes) of *F. graminearum* (Table 6.1) were cultured individually on potato dextrose agar (PDA), and the spores were harvested and diluted in distilled water, attaining the working concentration of 10^5

macroconidia/ml. Spikes were inoculated at 50% anthesis crop stage (BBCH65, Lancashire et al. 1991). Two spikelets positioned two-thirds of the way from the base of the spike on the main stem were point inoculated with 10 µl of macroconidia spore suspension (5 x 10^4 spores/ml) containing 0.02% Tween 20. The inoculations were performed as described by Cuthbert et al. (2006). Inoculated spikes were covered with a clear plastic bag for 48 hours to provide high humidity for spore germination. A total of 8-12 spikes per pot were inoculated, and there were two replications for each treatment, and the experiment was repeated twice. Disease severity was measured as the number of infected spikelets per spike (Type II resistance) at 21 days post-inoculation (DPI) (Schroeder and Christensen 1963) using the scale: 0 = no spikelets infected, 100 = all spikelets infected (% affected spikelets).

6.3.4 Mycotoxin production

To evaluate mycotoxin accumulation in grain, the inoculated spikes were harvested from the three replicates at the fully ripe stage (BBCH 92, Lancashire et al. 1991) and dried to minimal water content. All spikes were hand threshed and later ground to a fine powder with a Laboratory Mill 3100 – v3.3 EN. The amount of DON, its derivatives (3ADON and 15ADON), NIV, and NX-2 was quantified using the LC-MS/MS based mycotoxin platform (Wang and Kutcher 2018).

6.3.5 Mycelial growth and conidial count

Mycelial growth and sporulation of isolates were evaluated *in vitro* on PDA. Small agar plugs (5 mm in diameter) of five-day-old cultures of each isolate were placed in the center of fresh PDA plates and incubated at 25°C. The PDA colony diameter was measured on the third and seventh day after placement. For sporulation, the conidia were harvested by adding 1 ml of distilled water and scraping the surface of the Petri plate with a plastic loop (SL10S, Thermo Fisher, Canada). The spores were counted under a compound microscope using a

hemocytometer. This experiment had two replication and was repeated once, and the average values were generated.

6.3.6 Statistical analysis

The disease severity data collected in the greenhouse and the mycotoxin values quantified were subjected to analysis of variance (ANOVA) and correlation analysis. Before analysis, normality (Shapiro-Wilk's test) and homogeneity of error variance (Levene's tests) for all class variables were estimated in procedure UNIVARIATE and heterogeneous variances were fixed using the 'repeated/group=effect' statement in the mixed model procedure of SAS (Littell et al. 2006). Replication and repeats were considered a random factor, and genotypes and chemotypes fixed factors. The effect of chemotype, genotype, and the chemotype by genotype interaction was analyzed by ANOVA. Least-squared means were estimated using the LSMEANS statement in PROC MIXED. A second analysis was performed to determine the effect of chemotype on mycelial growth and sporulation. The chemotype was considered a fixed effect and replication and repeats as random effects. Pearson's correlation coefficients among various parameters were calculated using procedure CORR. All analyses were conducted in the Statistical Analysis Software program (9.4 SAS Inc, Cary, NC).

6.4 Results

6.4.1 Greenhouse FHB evaluations

The FHB inoculations were successful in all replications, and there was enough disease pressure to discriminate chemotype effects. There were substantial differences in FHB severity among the four chemotypes: 3ADON, 15ADON, NIV, and NX-2 (Table 6.3 and 6.4). The ANOVA indicated that the interaction between chemotypes and wheat genotypes was not significant, but disease severity differed among chemotypes and wheat genotypes (Table 6.3). The average disease severity of four chemotypes averaged across seven wheat genotypes

ranged from 3.7 ± 1.2 to 65.2 ± 4.7%. Chemotype 3ADON was highly aggressive, with the highest disease severity rating, followed by chemotype 15ADON. Disease severity of the NIV chemotype was not significantly different from NX-2 (Table 6.4).

Table 6.3. *F* values and statistical significance among *Fusarium graminearum* chemotypes, wheat genotypes and their interaction for disease severity, mycotoxin content, radial growth (colony diameter) of isolates on solid agar media, and macroconidia production.

Source of variation	*F* value
Disease severity	
Chemotype	4.28 *
Genotype (wheat)	17.52 **
Chemotype × Genotype (wheat)	0.44 ns
Mycotoxin	
Chemotype	15.19 **
Genotype (wheat lines)	0.95 ns
Chemotype × Genotype (wheat)	0.79 ns
Radial growth (mm)	
Chemotype	7.31 **
Macroconidia	
Chemotype	9.04 **

*, **, ns represent significant at *P* = 0.05, 0.001, and not significant respectively

Table 6.4. Chemotype aggressiveness based on disease severity, and mycotoxin accumulation of the *Fusarium graminearum* isolates used in the study (N = 6).

Chemotype	Disease severity (%)	Mycotoxin (µg kg^{-1})				
		DON	3ADON	15ADON	NIV	NX-2
3ADON	29.7 ± 3.6 a	12.5 ± 2.7	1.2 ± 0.3	n.d.	n.d.	n.d.
15ADON	22.4 ± 2.9 b	2.9 ± 0.8	n.d.	0.7 ± 0.4	n.d.	n.d.
NIV	16.7 ± 2.8 c	n.d.	n.d.	n.d.	2.7 ± 2.6	n.d.
NX-2	16.2 ± 3.1 c	n.d.	n.d.	n.d.	n.d.	1.2 ± 0.1

DON = deoxynivalenol, 3ADON = 3-acetyl-deoxynivalenol, 15ADON =15-acetyl-deoxynivalenol, and NIV = nivalenol, n.d. = not determined, ± = standard error of mean, disease severity values indicated with the same lowercase letters were not significantly different at *P* = 0.05.

Among the seven wheat genotypes tested, AAC Tenacious had the lowest disease severity (3.7 ± 1.2%), while CDC Go exhibited the highest (65.2 ± 4.7%). Disease severity observed on CDC Carberry (14.9 ± 1.8%) and NIL-28 (*Fhb1* + *Fhb2* + *Fhb5*; 13.6 ± 2.1%) was similar.

However, no significant difference in disease severity was detected among NIL-38 (*Fhb1*), NIL- 20 (*Fhb2*), and NIL- 21 (*Fhb5*) (Fig. 6.1).

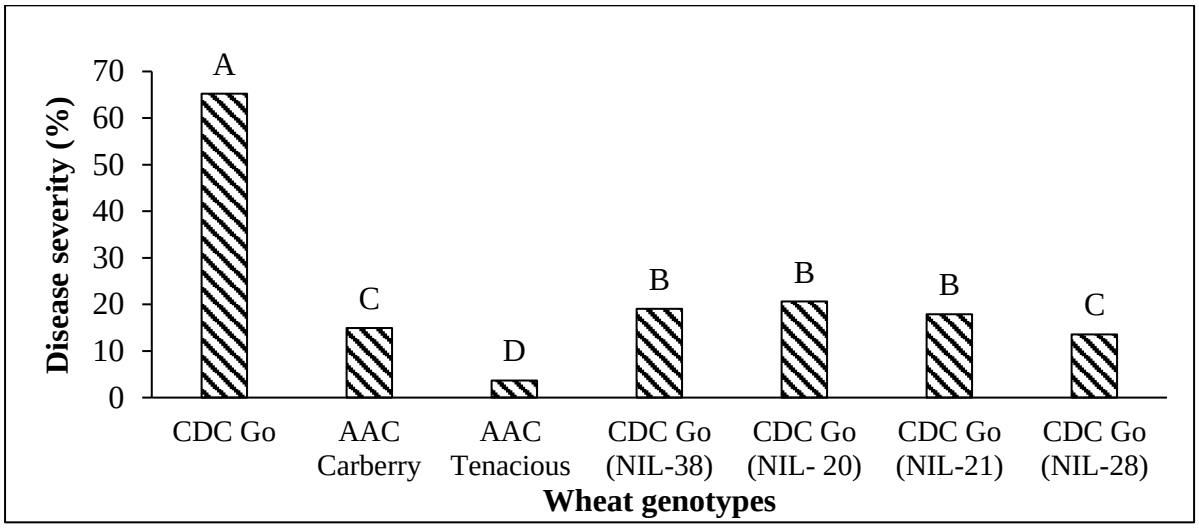

Fig. 6.1. Disease severity on each of the seven wheat genotypes [CDC Go, AAC Carberry, AAC Tenacious, NIL-38 (*Fhb1*), NIL- 20 (*Fhb2*), NIL-21 (*Fhb5*), and NIL-28 (*Fhb1 + Fhb2 + Fhb5*)] averaged over chemotypes [3-acetyl-deoxynivalenol (3ADON; N = 30), 15-acetyl-deoxynivalenol (15ADON; N = 30), nivalenol (NIV; N = 18), and NX-2 (N = 12)].

6.4.2 Mycotoxin production

Mycotoxin accumulation (DON, 3ADON, 15ADON, NIV, and NX-2) was measured in the kernels of all inoculated spikes. The chemotypes differed in the total amount of mycotoxin produced. The amount of mycotoxin measured in samples inoculated with the 3ADON chemotype was highest, followed by the 15ADON chemotype. The NIV levels produced by the *F. graminearum*–NIV chemotype was 2.7 ± 2.6 μg kg^{-1}; whereas the NX-2 chemotype accumulated the least mycotoxin among the chemotypes tested (Table 6.4). The DON produced by *F. graminearum*-3ADON was four-fold greater than that produced by the 15ADON chemotype (Table 6.4). Different levels of FHB resistance in wheat genotypes did not affect the level of mycotoxin accumulation, and the interaction between *F. graminearum* chemotype x wheat genotype was not significant (Table 6.3).

6.4.3 Mycelial growth and conidia count

The mycelial growth rate of *F. graminearum*-3ADON was the highest of the four chemotypes, followed by the 15ADON chemotype (Table 6.5). No significant differences in growth rate were observed between the NIV and NX-2 chemotypes. *In vitro* tests also showed that *F. graminearum*-3ADON produced significantly more (P = 0.0004) conidia than either *F. graminearum*-15ADON, NIV, or NX-2 (Table 6.5).

Table 6. 5. Mycelial growth (colony diameter) and conidial production of *F. graminearum* chemotypes.

Chemotype	**Mycelial growth (mm)**		**Conidia (10^4/ml)**
	Day 3	Day 7	
3ADON	46.1 ± 9.4 a	80.2 ± 7.3 a	77.0 ± 16.1 a
15ADON	35.0 ± 5.3 b	69.4 ± 6.0 b	42.7 ± 12.4 b
NIV	28.5 ± 6.7 c	73.7 ± 4.8 b	24.8 ± 9.3 c
NX-2	27.2 ± 4.0 c	72.0 ± 4.6 b	22.1 ± 2.7 c

3ADON - 3-acetyl-deoxynivalenol, 15ADON - 15-acetyl-deoxynivalenol, and NIV - nivalenol, means with the same lowercase letters were not significantly different at P = 0.05.

6.4.4 Correlation between disease severity and mycotoxin

The correlation coefficient between disease severity and DON was positive and significant, although weak (R = 0.33; P = 0.0001). Similarly, a positive but weak correlation existed between disease severity and NIV accumulation in the harvested grain (R = 0.21; P = 0.001). However, no significant correlation was found between disease severity and 3ADON, 15ADON, or NX-2.

6.5 Discussion

This research was conducted to investigate the aggressiveness of four well-known chemotypes of *F. graminearum*. My results indicated that the *F. graminearum*-3ADON chemotype caused greater disease severity than the other three chemotypes (15ADON, NIV, and NX-2) on all seven wheat genotypes evaluated. The FHB symptoms caused by 3ADON isolates developed faster on all seven wheat genotypes than the less aggressive chemotypes (15ADON, NIV, and NX-2) in the greenhouse. Spikes of CDC Go (MS check) point inoculated with *F.*

graminearum-3ADON isolates had higher disease severity (>50%) at 14 DPI, while other chemotypes, which were considered less aggressive, reached this level later (data not shown). Previous studies attempting to examine aggressiveness of *F. graminearum* chemotypes on wheat genotypes with different FHB QTL have shown inconsistent results. Puri and Zhong (2010) reported that the 3ADON chemotype caused significantly greater disease severity than the 15ADON chemotype on susceptible and moderately susceptible wheat genotypes. Similarly, Amarasinghe et al. (2019), Foroud et al. (2012), and Malihipour and Gilbert (2012) reported that NIV chemotypes were generally less aggressive than DON chemotypes (3ADON and 15ADON); however, among DON chemotypes, the 3ADON chemotype was the most aggressive. In contrast, Gilbert et al. (2010), Puri et al. (2016), Purahong et al. (2014), von der Ohe et al. (2010), and Ward et al. (2008) reported no significant difference in aggressiveness among *F. graminearum* chemotypes.

Despite the differences in aggressiveness of the *F. graminearum* chemotypes observed in my study, no significant interaction between wheat genotypes and chemotype was detected, but disease severities differed among wheat genotypes. Similar results are described by Puri and Zhong (2010) and Serajazari et al. (2019). In my experiments, disease severity was highest on CDC Go (MS check) and lowest on AAC Tenacious (R check). There was no difference in disease severity of NILs carrying QTLs *Fhb1*, *Fhb2,* and *Fhb5* in the CDC Go background. In contrast, NIL 28 carrying QTLs *Fhb1+ Fhb2+Fhb5* had lower disease severity comparable to AAC Carberry (MR check). The disease severity reactions on CDC Go NILs were similar to those of Brar et al. (2019), who also reported that there was improvement in field FHB resistance when *Fhb1*, *Fhb2,* and *Fhb5* were introgressed together into elite Canadian hard red spring wheat genotypes (CDC Alsask and CDC Go).

This study indicated that mycotoxin accumulation, mostly DON, was higher in wheat kernels inoculated with the *F. graminearum*-3ADON chemotype than with the 15ADON chemotype.

This is consistent with Puri and Zhong (2010) and Ward et al. (2008) who reported that DON accumulation was higher in wheat kernels inoculated with isolates of the 3ADON chemotype than the 15ADON chemotype. The NIV accumulation quantified in this study was lower than the DON accumulation, which agrees with Gale et al. (2011) and Jang et al. (2019), indicating that the NIV chemotype of *F. graminearum* produced less trichothecenes than the DON chemotypes. A possible reason for this may be that the synthesis of NIV requires two additional enzymatic steps catalyzed by *Tri13* and *Tri7*, respectively, compared to that of DON (Lee et al. 2002). For *F. graminearum*-NX-2, only the NX-2 mycotoxin was quantified, and the amount was the lowest among the four chemotypes. Mycotoxin accumulation in different wheat genotypes and the interaction of *F. graminearum* chemotype x wheat genotype was not significant in this study. This contrasts with the results of Gilbert et al. (2010), Puri and Zhong (2010) and Ward et al. (2008) who suggested that *F. graminearum* chemotypes may interact in different ways with wheat genotypes that differ in resistance to FHB and that the level of mycotoxins is generally higher in susceptible cultivars. The inconsistency between my results and earlier studies suggests that differences in mycotoxin accumulation in grain caused by *F. graminearum* chemotypes vary with differences in experimental conditions and inoculation methods (spray vs. point inoculation), isolates (location), and the genetic background of the wheat genotypes.

Fusarium graminearum-3ADON isolates had higher radial growth rate and produced more conidia as compared to isolates of other chemotypes. This is consistent with the results of previous studies from the USA and China, in which *F. graminearum*-3ADON and *F. asiaticum*-3ADON were able to grow faster and produce more trichothecene and had more and larger conidia than isolates of the *F. graminearum*-15ADON and *F. asiaticum*-NIV chemotypes, respectively (Puri and Zhong 2010; Ward et al. 2008; Zhang et al. 2012). Although the growth rate and spore count were not statistically different in *F. graminearum*-

NIV and NX-2, the small sample size limits the confidence that these two chemotypes were similar in growth characters based on my study. Studies with greater numbers of isolates would be necessary to differentiate between these two trichothecene-producing genotypes.

According to my findings, a weak correlation was detected between disease severity and mycotoxin (either DON or NIV), which does not agree with Ma et al. (2009) or Puri and Zhong (2010), who reported a strong positive correlation between FHB severity and DON accumulation. However, Alvarez et al. (2010) and Pasquali et al. (2010) found negative or no correlation between FHB severity and DON accumulation in grain.

In conclusion, this study indicated that *F. graminearum*-3ADON isolates collected from Saskatchewan, Canada, are more aggressive based on disease severity on wheat and mycotoxin accumulation when tested for type II resistance. The novel chemotype NX-2 was less aggressive than DON derivatives, but the disease severity was similar to NIV. It is hard to draw conclusions on the mycotoxin-producing ability of the four chemotypes of *F. graminearum* as there are qualitative and quantitative differences in the synthesis and toxicity of the mycotoxins produced. With an increase in the proportion of *F. graminearum*-3ADON in Saskatchewan (Chapter 4), this information will be useful to wheat breeding programs to develop FHB-resistant wheat cultivars for disease management. Further experiments with more isolates of the lesser-studied chemotype (NX-2) is required to compare differences in aggressiveness between the four chemotypes at the molecular level.

CHAPTER 7

General discussion and conclusion

Fungicide application is one of the main control strategies used to manage FHB in durum wheat. In a three-year field study, I assessed the efficacy of fungicide (metconazole) sprayed at different crop growth stages (BBCH59, BBCH61, BBCH65, BBCH69, BBCH73 and BBCH61+73) in durum wheat under Saskatchewan conditions. In general, all fungicide treatments in this study significantly reduced the FHB index, FDK and DON accumulation and increased the yield compared to the unsprayed check. In years with warm and wet summers, fungicide application at BBCH61, BBCH65, or BBCH69 effectively reduced FHB index, FDK, and DON accumulation, but the yield was higher with application at BBCH65. This wider window of fungicide application (BBCH61 to BBCH69) will benefit Saskatchewan farmers in years when wet field conditions restrict fungicide application at the BBCH65 growth stage. Similar results have also been reported in fungicide efficacy studies in spring and winter wheat, indicating that application at anthesis and up to 6 days after anthesis effectively reduced FHB index and DON accumulation (D'Angelo et al. 2014; Paul et al. 2018). The late fungicide application (BBCH73) did not prove efficient in reducing DON accumulation, which contradicts findings of Tateishi et al. (2014) and Yoshida et al. (2012) as they reported that a fungicide application at later stages (milk stage) effectively reduced mycotoxin (DON and NIV) accumulation in Japan. I also documented that dual fungicide treatment (BBCH61+BBCH73) is unnecessary for managing FHB. My results indicated that even though dual application had a slight advantage over single anthesis fungicide application (BBCH61 to BBCH69) in suppressing the FHB index, it still did not provide benefits in reducing DON accumulation and FDK.

This study also demonstrated that fungicides alone cannot completely control the development of FHB. Therefore, growers must adopt integrated disease management strategies, i.e., combining a diverse crop rotation with FHB resistant wheat cultivars (if available) plus fungicide application to improve management of FHB. From my experiments and recent research [Chapter 2 (section 2.6)], a wider window of fungicide application may be appropriate for spring wheat in Saskatchewan because of subtle differences in crop physiology within wheat classes. However, further research and field trials are still needed to provide recommendations for this.

Fungicide resistance in plant pathogens is a global problem that threatens agricultural productivity. As new chemicals become available and are used by farmers, tracking the decline in fungicide sensitivity is crucial for long-term agricultural sustainability. The development of fungicide resistance in plant pathogenic fungi is an emerging issue due to selection pressure and the absence of anti-resistance strategies (Pirgozliev 2002; Spolti et al. 2014). I tested the sensitivity of 252 *F. graminearum* isolates collected from Alberta, Saskatchewan, and Manitoba to three triazole active ingredients (tebuconazole, metconazole, and prothioconazole) currently recommended for FHB management. The risk of resistance development in triazole fungicides is considered to be intermediate compared with other fungicide groups (Brent and Hollomon 2017; FRAC 2017), but few studies from the USA, China, or Germany have indicated that effectiveness of triazoles has been compromised (Anderson et al. 2020; Becher et al. 2010; Spolti et al. 2014; Yin et al. 2009). Fungicide sensitivity phenotyping revealed that the *F. graminearum* population of western Canada is still sensitive to the three triazole fungicides tested. I did not detect any decline in effectiveness of fungicide products over the years. The two chemotypes (3ADON and 15ADON) of *F. graminearum* were equally sensitive to the three active ingredients tested. Overall, this project has provided a baseline for future analyses of *F. graminearum* fungicide sensitivity in western Canada. It is now imperative to

continue surveillance efforts in the future to detect any change in the sensitivity of pathogen populations over time before it becomes a widespread problem.

Like every living being, plant-pathogen populations are also evolving due to natural selection, competition, and mutation. In the past decade, FHB epidemics occurred during 2012, 2014 and 2016 (www.phytopath.ca/publication/cpds), and the frequency and intensity of the outbreaks is not likely to decrease in the future due to three reasons. First, the amount of inoculum within fields is increasing due to no-till practices, intensive crop rotations that include wheat frequently and increasing production of corn on the prairies (Statistics Canada 2020). Secondly, due to climate change, future years are predicted to have more frequent and intense rainfall events (CCCR 2019). Lastly, there has been a change in the pathogen profile across the continent in the past decade from native *F. graminearum*-15ADON to 3ADON, which has higher toxigenicity, pathogenicity and reproductive ability (Ward et al. 2008; Kelly et al. 2015). This 3-year study demonstrated that the population structure of FHB pathogens in Saskatchewan is changing continuously. Inspection of FHB-infected wheat samples collected from Saskatchewan showed that multiple *Fusarium* spp. were responsible for FHB epidemics in 2014 and 2016, but *F. graminearum* was the most common species. Further examination of *F. graminearum* isolates revealed that the proportion of the *F. graminearum*-3ADON chemotype has increased in Saskatchewan. These findings are important and add crucial information to identify the chemotype cline in Canada. Mycotoxin quantification of infected wheat samples revealed that DON accounted for the highest proportion of mycotoxin. The toxins NIV, T2 and HT2 were also detected in the samples collected at an alarming rate, and the value exceeds the tolerable daily intake for both humans and animals. Therefore, I recommend that the present grading system monitor the concentrations of emerging mycotoxins (NIV, T2, and HT2), as well as DON, because of safety concerns associated with their higher toxicity compared to DON.

Over the years, researchers have postulated that environmental factors and the differential sensitivity of chemotypes to fungicides may be reasons for the adaptive advantage of one chemotype over another (Albuquerque et al. 2019; McMullen et al. 2012). However, results from my fungicide sensitivity study (Chapter 4) showed no difference in triazole sensitivity between 3ADON and 15ADON chemotypes. At this point, the only possible reasons for differences in the distribution patterns of 3ADON and 15ADON chemotypes in Canada might be weather conditions, especially temperature, as biosynthesis of the acetyl derivatives is regulated by temperature (Albuquerque et al. 2019). Under the new climate change scenario, significant chemotype shifts could complicate current control strategies. Therefore, further research on the factors that cause these changes is a must to correctly evaluate the risk and generate knowledge to develop adequate disease control measures.

Results from the greenhouse experiments (Chapter 6) indicated that *F. graminearum*-3ADON isolates collected from Saskatchewan, Canada, are more aggressive than other chemotypes with respect to disease severity on wheat, and there was a greater accumulation of mycotoxin in grain of the wheat genotypes tested for type II resistance. The novel chemotype (NX-2) was less aggressive than DON derivatives, but the disease severity was similar to NIV. With an increase in the proportion of *F. graminearum*-3ADON in Saskatchewan (Chapter 5), future FHB epidemics may result in higher disease severity and DON accumulation, making FHB management more complicated.

In conclusion, the studies described in this thesis provide essential information regarding the importance of fungicide application timing and how this can benefit durum growers in reducing the economic losses caused by FHB. Currently, registered fungicides for managing FHB remain effective in western Canada; there is no evidence of insensitivity to the triazole fungicides in common use. The FHB pathogen profile (chemotypes) in western Canada is

changing with time, which will need to be taken into account in breeding programs and management strategies.

REFERENCES

Abramson, D., Clear, R. M., Gaba, D., Smith, D. M., Patrick, S. K., and Saydak, D. 2001. Trichothecene and moniliformin production by *Fusarium* species from western Canadian wheat. J Food Prot. 64(8):1220–1225.

Adams, G. C., and Hart, L. P. 1989. The role of deoxynivalenol and 15-acetyldeoxynivalenol in pathogenesis by *Gibberella zeae,* as elucidated through protoplast fusions between toxigenic and nontoxigenic strains. Phytopathology 79:404-408.

Agrios, G. 2005. Plant Pathology. Amsterdam: Elsevier Academic Press. US.

Ahmed, H., Zhou, Q., Zahr, K. et al. 2020. Diversity in the population of *Fusarium graminearum* isolated from wheat and corn in Alberta, Canada. J Plant Dis Prot. 127:583–590.

Albuquerque, D. R., Patriarca, A., and Pinto, V. F. 2019. Can discrepancies between *Fusarium graminearum* trichothecene genotype and chemotype be explained by the influence of temperature in the relative production of 3-ADON and 15-ADON? Fungal Biol. 125(2):153-159.

Alexander, N. J., McCormick, S. P., Waalwijk, C., Van der Lee, T., and Proctor, R. H. 2011. The genetic basis for 3-ADON and 15-ADON trichothecene chemotypes in *Fusarium*. Fungal Genet. Biol. 48:485-495.

Alexander, N. J., Proctor, R. H., and McCormick, S. P. 2009. Genes, gene clusters, and biosynthesis of trichothecenes and fumonisins in *Fusarium*. Toxin Rev. 28:198-215.

Alvarez, C. L., Somma, S., Moretti, A., and Pinto, V. F. 2010. Aggressiveness of *Fusarium graminearum sensu stricto* isolates in wheat kernels in Argentina. J. Phytopathol. 158:173-181.

Amarasinghe, C., Tamburic-Ilincic, L., Gilbert, J., Brûlé- Babel, L. A., and Fernando, W. G. D. 2013. Evaluation of different fungicides for control of Fusarium head blight in wheat

inoculated with 3ADON and 15ADON chemotypes of *Fusarium graminearum* in Canada. Can. J. Plant Pathol. 35:2.

Amarasinghe, C., Sharanowski, B., and Fernando, W. G. D. 2019. Molecular phylogenetic relationships, trichothecene chemotype diversity and aggressiveness of strains in a global collection of *Fusarium graminearum* species. Toxins 11(5):263.

Anderson, N. R., Freije, A. N., Bergstrom, G. C., Bradley, C. A., Cowger, C. et al. 2020. Sensitivity of *Fusarium graminearum* to metconazole and tebuconazole fungicides before and after widespread use in wheat in the United States. Plant Health Prog. 21:85-90.

Anonymous. 2018. Canadian wheat. 2017 Crop Review (accessed on November 20, 2020). https://canadianwheat.ca/review/Canadian%20Wheat%202017%20Crop%20in%20Review_1

Aoki, T., Ward, T. J., Kistler, H. C., and O'Donnel, K. 2012. Systematics, phylogeny and trichothecene mycotoxin potential of Fusarium head blight cereal pathogens. Mycotoxins 62 (2):91-102.

Argyris, J., Sanford, D. V., and TeKrony, D. 2003. *Fusarium graminearum* infection during wheat seed development and its effect on seed quality. Crop Sci. 43:1782-1788.

Avozani, A., Tonin, R. B., Reis, E. M., Camera, J., and Ranzi, C. 2014. In vitro sensitivity of *Fusarium graminearum* isolates to fungicides. Summa Phytopathol. 40:231–247.

Bai, G. H., and Shaner, G. 2004. Management and resistance in wheat and barley to Fusarium head blight. Annu. Rev. Phytopathol. 42:135-161.

Bai, G. H., Desjardins, A. E., and Plattner, R. D. 2002. Deoxynivalenol nonproducing *Fusarium graminearum* causes initial infection but does not cause disease spread in wheat spikes. Mycopathologia 15:91-98.

Bai, G. H., Guo, P. G., and Kolb, F. L. 2003. Genetic relationships among head blight resistant cultivars of wheat assessed on the basis of molecular markers. Crop Sci. 43:498-507.

Bai, G. H., Plattner, R., Desjardins, A., and Kolb, F. L. 2001. Resistance to Fusarium head blight and deoxynivalenol accumulation in wheat. Plant Breed. 120:1-6.

Banik, M., Beyene, M., and Wang, X. 2017. Fusarium head blight of barley in Manitoba - 2016. Can Plant Dis Surv. 97:100–101.

Banik, M., Beyene, M., and Wang, X. 2018. Fusarium head blight of barley in Manitoba - 2017. Can Plant Dis Surv. 98:95–96.

Bastos, L. M., Carciochi, W., Lollato, R. P., Jaenisch, B. R., Rezende, C. R., Schwalbert, R. et al. 2020. Winter wheat yield response to plant density as a function of yield environment and tillering potential: a review and field studies. Front. Plant Sci. 11:54.

Beavers, R. L., Hammermeister, A. M., Frick, B., Astatkie, T., and Martin, R. C. 2008. Spring wheat yield response to variable seeding rates in organic farming systems at different fertility regimes. Can. J. Plant. Sci. 88:43-52.

Becher, R., Hettwer, U., Karlovsky, P., Deising, H. B., and Wirsel, S. G. R. 2010. Adaptation of *Fusarium graminearum* to tebuconazole yielded descendants diverging for levels of fitness, fungicide resistance, virulence, and mycotoxin production. Phytopathology 100:444-453.

Bennett, J. W., and Klich, M. 2003. Mycotoxins. Clin. Microbiol. Rev. 16:497-516.

Beres, B. L., Cárcamo, H. A., Yang, R. C., and Spaner, D. M. 2011. Integrating spring wheat sowing density with variety selection to manage wheat stem sawfly. Agron. J. 103(6):1755–1764.

Beres, B. L., Brûlé-Babel, A. L., Ye, Z., Graf, R. J., Turkington, T. K., Harding, M. W., Kutcher, H. R., and Hooker, D. C. 2018. Exploring genotype x environment x management synergies to manage Fusarium head blight in wheat. Can. J. Plant. Pathol. 40(2):179–188.

Beres, B. L., Turkington, T. K., Kutcher, H. R., Irvine, B., Johnson, E. N., O'Donovan, J. T., Harker, K. N., Holzapfel, C. B., Mohr, R., Peng, G., and Spaner, D. M. 2016. Winter wheat cropping system response to seed treatments, seed size, and sowing density. Agron. J. 108(3):1101–1111.

Beyer, M., Klix, M. B., Klink, H., and Verreet, J. A. 2006. Quantifying the effects of previous crop, tillage, cultivar and triazole fungicides on the deoxynivalenol content of wheat grain. J. Plant Dis. Prot. 113:241-246.

Bianchini, A., Horsley, R., Jack, M. M., Kobielush, B., Ryu, D., Tittlemier, S., Wilson, W., et al. 2015. "DON occurrence in grains: A North American perspective." Cereal Foods World 60 (1):32-56.

Bissonnette, K. M., Kolb, F. L., Ames, K. A., and Bradley, C. A. 2018. Effect of Fusarium head blight management practices on mycotoxin contamination of wheat straw. Crop Sci. 102(6):1141-1147.

Blandino, M., Pilati, A., Reyneri, A., and Scudellari, D. 2010. Effect of maize crop residue density on Fusarium head blight and on deoxynivalenol contamination of common wheat grains. Cereal Res. Commun. 38:550–559.

Bockus, W. W. E., Bowden, R., Hunger, R., Morrill, W., Murray, T., and Smiley, R. 2010. Compendium of Wheat Diseases and Pests. Third Edition. ed. APS Press, St. Paul.

Bockus, W. W., DeWolf, E. D., and Wegulo, S. N. 2011. Effect of foliar fungicide application on Fusarium head blight in eight winter wheat cultivars, 2010. Plant Dis. Manag. Rep. 5, CF009.

Bockus, W. W., DeWolf, E. D., and Wegulo, S. N. 2012. Effect of foliar fungicide application on Fusarium head blight in eight winter wheat cultivars, 2011. Plant Dis. Manag. Rep. 6, CF004.

Bockus, W. W., DeWolf, E. D., and Wegulo, S. N. 2014. Effect of foliar fungicide application on Fusarium head blight in eight winter wheat cultivars, 2013. Plant Dis. Manag. Rep. 8, CF005.

Bottalico, A. 1998. *Fusarium* diseases of cereals: species complex and related mycotoxin profiles in Europe. J. Plant Pathol. 80:85-103.

Boyacioglu, D., Hettiarachchy, N. S., and Stack, R. W. 1992. Effect of 3 Systemic Fungicides on Deoxynivalenol (Vomitoxin) Production by *Fusarium graminearum* in Wheat. Can. J. Plant Sci. 72:93-101.

Bradley, C. A., Adee, E. A., Ebelhar, S. A., Grybauskas, A. P., Dill-Macky, R., Wiersma, J. J., Grybauskas, A. P., et al. 2010. Multi-state uniform fungicide evaluations for control of Fusarium head blight and associated mycotoxins. Page 74 in Proc. 2010 National Fusarium Head Blight Forum. S. Canty, A. Clark, A. Anderson-Scully, D. Ellis, and D. Van Sanford, eds. Milwaukee, WI:74.

Brar, G. S., Brûlé-Babel, A. L., Ruan, Y., Henriquez, M. A., Pozniak, C. J., Kutcher, H. R., and Hucl, P. J. 2019b Genetic factors affecting Fusarium head blight resistance improvement from introgression of exotic Sumai 3 alleles (including Fhb1, Fhb2, and Fhb5) in hard red spring wheat. BMC Plant Biol. 19:179.

Brar, G. S., Hnatowich, G., Peng, G., Hucl, P. J., and Kutcher, H. R. 2019a. The effect of Fhb1 and Fhb5 QTL in hard red spring wheat does not depend on fungicide use for managing Fusarium head blight in wheat. Plant Dis. 103:1850-1857.

Brent, K. J., and Hollomon, D. W. 2007. Fungicide Resistance in Crop Pathogens: How Can it be Managed? FRAC Monograph 1. 2nd Ed. Brussels, CropLife International, Brussels:55.

Buerstmayr, H., Ban, T., and Anderson, J. 2009. Review QTL mapping and marker-assisted selection for Fusarium head blight resistance in wheat: a review. Plant Breed. 128:1-26.

Buerstmayr, H., Lemmens, M., Hartl, L., Doldi, L., Steiner, B., Stierschneider, M., and Ruckenbauer, P. 2002. Molecular mapping of QTLs for Fusarium head blight resistance in spring wheat. I. Resistance to fungal spread (type II resistance). Theor. Appl. Genet. 104:84–91

Buerstmayr, H., Stierschneider, M., Steiner, B., Lemmens, M., Griesser, M., Nevo, E., and Fahima, T. 2003. Variation for resistance to head blight caused by *Fusarium graminearum* in wild emmer (*Triticum dicoccoides*) originating from Israel. Euphytica. 130:17-23.

Caldwell, C. D., MacDonald, D., Jiang, Y., Cheema, M. A., and Li, J. 2017. Effect of fungicide combinations for Fusarium head blight control on disease incidence, grain yield, and quality of winter wheat, spring wheat, and barley. Can. J. Plant Sci. 97:1036–1045.

CCCR. 2019. Canada's Changing Climate Report (accessed on November 20, 2020). https://changingclimate.ca/CCCR2019/.

Champeil, A., Doré, T., and Fourbet, J. T. 2004. Fusarium head blight: Epidemiological origin of the effects of cultural practices on head blight attacks and the production of mycotoxins by Fusarium in wheat grains. Plant Sci. 166:1389-1415.

Charmley, L. L., and Trenholm, H. L. 2000. A Review of Current Literature on Mycotoxins and Their Regulations. (Unpublished review for Canadian Food Inspection Agency, Government of Canada).

Chen, Y., Zhang, A-Z., Gao, T-Z., Zhang, Y., Wang, W-X., Ding, K-J., Chen, L., et al. 2012. Integrated Use of pyraclostrobin and epoxiconazole for the Control of Fusarium head blight of wheat in Anhui Province of China. Plant Dis. 96(10):1495-1500.

Chongo, G., Gossen, B. D., Kutcher, H. R., Gilbert, J., Turkington, T. K., Fernandez, M. R., and Mclaren, D. 2001. Reaction of seedling roots of 14 crop species to *Fusarium graminearum* from wheat heads. Can. J. Plant Pathol. 23:132-137.

Clarke, J. M., Clarke, F. R., and Pozniak, C. J. 2010. Forty-six years of genetic improvement in Canadian durum wheat cultivars. Can. J. Plant Sci. 90:791–801.

Clear, R. M., and Patrick, S. K. 2010. Fusarium head blight in western Canada. Grain Research Labortory. Canadain Grain Commission. https://grainscanada.gc.ca/en/grain-research/scientific-reports/fhb-western/fhb-1.html.

Clear, R. M., Patrick, S. K., Gaba, D., Abramson, D., and Smith, D. M. 2005. Prevalence of fungi and fusariotoxins on hard red spring and amber durum wheat seed from western Canada, 2000 to 2002. Can. J. Plant Pathol. 27:528–540.

Covarelli, L., Beccari, G., Prodi, A., Generotti, S., Etruschi, F., Juan, C. et al. 2015. Fusarium species, chemotype characterisation and trichothecene contamination of durum and soft wheat in an area of central Italy. J. Sci. Food Agric. 95:541–551.

Cowger, C., Patton-Ozkurt, J., Brown-Guedira, G., and Perugini, L. 2009. Post-anthesis moisture increased Fusarium head blight and deoxynivalenol levels in North Carolina winter wheat. Phytopathology 99:320-327.

Cowger, C., Ward, T. J., Nilssonb, K., Arellanod, C., McCormick, S. P., and Busman, M. 2020. Mark Busmanc Regional and field-specific differences in Fusarium species and mycotoxins associated with blighted North Carolina wheat. Int. J. Food Microbiol. 323:108594.

Curtis, B. C. 2002. Wheat in the world. In Bread Wheat Improvement and Production. Eds by B. C. Curtis, S. Rajaram and H. G. Macpherson. ISBN 92-5-104809-6. Food and Agriculture Organization of the United Nations, Rome.

Cuthbert, P. A., Somers, D. J., Thomas, J., Cloutier, S., and Brûlé-Babel, A. 2006. Fine mapping Fhb1, a major gene controlling fusarium head blight resistance in bread wheat (Triticum aestivum L.). Theor Appl Genet. 112:1465-1472.

D'Angelo, D. L., Bradley, C. A., Ames, K. A., Willyerd, K. T., Madden, L. V., and Paul, P. A. 2014. Efficacy of fungicide applications during and after anthesis against Fusarium head blight and deoxynivalenol in soft red winter wheat. Plant Dis. 98:1387-1397.

De Wolf, E. D., and Isard, S. A. 2007. Disease cycle approach to plant disease prediction. Annu. Rev. Phytopathol. 45:203-220.

De Wolf, E. D., Madden, L. V., and Lipps, P. E. 2003. Risk assessment models for wheat Fusarium head blight epidemics based on within-season weather data. Phytopathology 93:428-435.

Degrune, F., Theodorakopoulos, N., Dufrene, M. et al. 2016. No favorable effect of reduced tillage on microbial community diversity in a silty loam soil (Belgium). Agric Ecosyst Environ. 224:12-21.

Del Ponte, E. M., Fernandes, J. M. C., and Bergstrom, G. C. 2007. Influence of growth stage on Fusarium head blight and deoxynivalenol production in wheat. J. Phytopathol. 155:577–581.

Del Ponte, E. M., Fernandes, J. M. C., and Pavan, W. 2005. A risk infection simulation model for Fusarium head blight of wheat. Fitopatol Bras. 30:634-642.

Delye, C., Bousset, L., and Corio-Costet, M. F. 1998. PCR cloning and detection of point mutations in the eburicol 14a-demethylase (CYP51) gene from *Erysiphe graminis f. sp. hordei*, a "recalcitrant" fungus. Curr. Genet. 34:399-403.

Demeke, T., Clear, R. M., Patrick, S. K., and Gaba, D. 2005. Species-specific PCR-based assays for the detection of Fusarium species and a comparison with the whole seed agar plate method and trichothecene analysis. Int. J. Food Microbiol. 103:271-284.

Desjardins, A. E. 2006. Fusarium Mycotoixns Chemistry, Genetics and Biology. APS Press, St. Paul.

Desjardins, A. E. 2008. Natural product chemistry meets genetics: when is a genotype a chemotype? J. Agric. Food Chem. 56:7587–7592.

Desjardins, A. E., and Proctor, R. H. 2007. Molecular biology of Fusarium mycotoxins. Int. J. Food Microbiol. 119:47-50.

Desjardins, A. E., Hohn, T. M., and McCormick, S. P. 1993. Trichothecene biosynthesis in Fusarium: Chemistry, genetics, and significance. Microbiol Rev. 57:595-604.

Desjardins, A. E., Proctor, R. H., Bai, G. H., McCormick, S. P., Shaner, G., Buechley, G., and Hohn, T. M. 1996. Reduced virulence of trichothecene nonproducing mutants of *Gibberella zeae* in wheat field tests. Mol. Plant Microbe Interact. 9 (9):775-781.

Dill-Macky, R., and Jones, R. K. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis. 84:654-660.

Dill-Macky, R., Bockus, W., Bowden, R., Hunger, R., Morrill, W., Murray, T., and Smiley, R. 2010. Fusarium head blight (scab). Compendium of wheat diseases and pests.WW Bockus, RL

Bowden, RM Hunger, WL Morrill, TD Murray, and RW Smiley, eds. American Phytopathological Society, St.Paul, MN:34-36.

Dweba, C. C., Figlan, S., Shimelis, H. A., Motaung, T. E., Sydenham, S., Mwadzingeni, L., and Tsilo, T. J. 2017. Fusarium head blight of wheat: pathogenesis and control strategies. Crop Prot. 91:114-122.

Edwards, S.G., Imathiu, S.M., Ray, R.V., Back, M., and Hare, M.C. 2012. Molecular studies to identify the Fusarium species responsible for HT-2 and T-2 mycotoxins in UK oats. Int. J. Food Microbiol. 156:168–175.

Eckard, S., Wettstein, F. E., Forrer, H. R., and Vogelgsang, S. 2011. Incidence of Fusarium species and mycotoxins in silage maize. Toxins (Basel) 3:949-967.

Edwards, S., Pirgozliev, S., Hare, M., and Jenkinson, P. 2001. Quantification of trichothecene-producing fusarium species in harvested grain by competitive PCR to determine efficacies of fungicides against fusarium head blight of winter wheat. Appl. Environ. Microbiol. 67(4):1575-1580.

EFSA. 2011. Panel on Contaminants in the Food Chain (CONTAM); Scientific Opinion on the risks for animal and public health related to the presence of T-2 and HT-2 toxin in food and feed. EFSA J 9:2481.

EFSA. 2013. Panel on Contaminants in the Food Chain) (CONTAM); Scientific Opinion on risks for animal and public health related to the presence of nivalenol in food and feed. EFSA J 11:3262.

Eudes, F., Comeau, A., Rioux, S., and Collin, J. 2001. Impact of trichothecenes on Fusarium head blight (*Fusarium graminearum*) development in spring wheat (*Triticum aestivum*). Can. J. Plant Pathol. 23:318-322.

Fernandez, M. R., May, W. E., Chalmers, S., Savard, M. E., and Singh, A. K. 2012. The seventh Canadian workshop on fusarium head blight/7e Colloque canadien sur la fusariose de l'épi. Can. J. Plant Pathol. 34:141–163.

Fernandez, M. R., Selles, F., Gehl, D., DePauw, R. M., and Zentner, R. P. 2005. Crop production factors associated with Fusarium head blight in spring wheat in eastern Saskatchewan. Crop Sci. 45:1908-1916.

Fernando, W. G. D., Paulitz, T. C., Seaman, W. L., and Martin, R. A. 1997. Fusarium head blight susceptibility of wheat inoculated at different growth stages. Phytopathology 87:S30.

Fleury, D. 2017. Top Crop Manager: Improving FHB management in durum (accessed on November 20, 2020). https://www.topcropmanager.com/improving-fhb-management-in-durum-20068/

Foroud, N. A., Baines, D., Gagkaeva, T. Y., Thakor, N., Badea, A., Steiner, B., et al. 2019. Trichothecenes in cereal grains - an update. Toxins 11:634.

Foroud, N. A., McCormick, S. P., MacMillan, T., Badea, A., Kendra, D. F., Ellis, B. E., and Eudes, F. 2012. Greenhouse studies reveal increased aggressiveness of emergent Canadian *Fusarium graminearum* chemotypes in wheat. Plant Dis. 96(9):1271–1279.

FRAC. 2006. Fungicide Resistance Action Committee (accessed on November 20, 2020). https://www.frac.info/docs/default-source/publications/monographs/monograph-3.pdf

FRAC. 2017. Fungicide Resistance Action Committee (accessed on November 20, 2020). https://www.frac.info/

Gale, L. R. 2003. Population biology of Fusarium species causing head blight of grain crops. Pages 120-143 in: Fusarium head blight of wheat and barley. K. J. Leonard and W. R. Bushnell, eds. American Phytopathological Society, St. Paul, MN.

Gale, L. R., Harrison, S. A., Ward, T. J., O'Donnell, K., Milus, E. A., Gale, S. W. and Kistler, H. C. 2011. Nivalenol-type populations of *Fusarium graminearum* and *F. asiaticum* are prevalent on wheat in Southern Louisiana. Phytopathology 101:124-134.

Gang, G., Miedaner, T., Schuhmacher, U., Schollenberger, M., and Geiger, H. H. 1998. Deoxynivalenol and nivalenol production by *Fusarium culmorum* isolates differing in aggressiveness toward winter rye. Phytopathology 88:879–84.

Geleta, B., Atak, M., Baenziger, P. S., Nelson, L. A., Baltenesperger, D. D., Eskridge, K. M., et al. 2002. Seeding rate and genotype effect on agronomic performance and end-use quality of winter wheat. Crop Sci. 42:827–832.

Gerlach, W., and Nirenberg, H. I. 1982. The Genus Fusarium: a pictorial atlas. Berlin, Germany: Mitteilungen und der Biologichen Bundesanstalt für Land-und Forstwirtschaft.

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

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

Gilbert, J., and Tekauz, A. 2000. Review: Recent developments in research on Fusarium head blight of wheat in Canada. Can J. Plant Pathol. 22:1-8.

Gilbert, J., and Woods, S. M. 2006. Strategies and considerations for multi-location FHB screening nurseries. In: T. Ban, J. M. Lewis, and E. E. Phipps (eds.), The Global Fusarium Initiative for International Collaboration: A Strategic Planning Workshop, CIMMYT, El Batan, Mexico. 14-17 March, 2006. pp. 93-102.

Gilbert, J., Abramson, D., McCallum, B., and Clear, R. 2002. Comparison of Canadian *Fusarium graminearum* isolates for aggressiveness, vegetative compatibility, and production of ergosterol and mycotoxins. Mycopathologia. 153:209–215.

Gilbert, J., Brûlé-Babel, A., Guerrieri, A. T., Clear, R. M., Patrick, S., Slusarenko, K., and Wolfe, C. 2014. Ratio of 3-ADON and 15-ADON isolates of *Fusarium graminearum* recovered from wheat kernels in Manitoba from 2008 to 2012. Can J Plant Pathol. 36:54-63.

Gilbert, J., Clear, R. M., Ward, T. J., Gaba, D., Tekauz, A., Turkington, T. K., Woods, S. M., Nowicki, T., and O'Donnell, K. 2010. Relative aggressiveness and production of 3- or 15-acetyl deoxynivalenol and deoxynivalenol by *Fusarium graminearum* in spring wheat. Can J Plant Pathol. 32(2):146-152.

Giroux, M. E., Bourgeois, G., Dion, Y., Rioux, S., Pageau, D., Zoghlami, S., et al. 2016. Evaluation of forecasting models for Fusarium head blight of wheat under growing conditions of Quebec, Canada. Plant Dis. 100(6):1192-1201.

Goswami, R. S., and Kistler, H. C. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol. 5:515–525.

Goswami, R. S., and Kistler, H. C. 2005. Pathogenicity and in planta mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. Phytopathology 95: 1397–1404.

Government of Canada. 2020: Health Canada's Maximum Levels for Chemical Contaminants in Foods (accessed on November 20, 2020). https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/chemical-contaminants/maximum-levels-chemical-contaminants-foods.html

Government of Saskatchewan. 2019: Varieties of Grain Crops 2019 (accessed on November 20, 2020). file:///C:/Users/gursa/Downloads/96889varieties_of_Grain_Crops_2018.pdf.

Gräfenhan, T., Patrick, S. K., Roscoe, M., Trelka, R., Gaba, D., Chan, J. M., et al. 2013. Fusarium damage in cereal grains from Western Canada. 1. Phylogenetic analysis of moniliformin-producing Fusarium species and their natural occurrence in mycotoxin-contaminated wheat, oats, and rye. J Agric Food Chem. 61:5425–5437.

Guo, X. W., Fernando, W. G. D., and Seow-Brock, H. Y. 2008. Population structure, chemotype diversity, and potential chemotype shifting of *Fusarium graminearum* in wheat fields of Manitoba. Plant Dis. 92:756– 62.

Haidukowski, M., Pascale, M., Perrone, G., Pancaldi, D., Campagna, C., and Visconti, A. 2005. Effect of fungicides on the development of Fusarium head blight, yield and deoxynivalenol accumulation in wheat inoculated under field conditions with *Fusarium graminearum* and *Fusarium culmorum*. J. Sci. Food Agric. 85:191-198.

Hallen-Adams, H. E., Cavinder, B. L., and Trail, F. 2011. *Fusarium graminearum* from expression analysis to functional assays. in fungal genomics: methods and protocols, eds. Jin-Rong Xu and Burton H Bluhm. New York: Humana Press:79–101.

Halstensen, A. S., Nordby, K. C., Eduardand, W., and Klemsdal, S. S. 2006. Real-time PCR detection of toxigenic Fusarium airborne and settled grain dust and associations with trichothecene mycotoxins. J Environ Monit. 8:1235-1241.

He, X. Y., Lillemo, M., Shi, J. R., Wu, J. R., Bjørnstad, Å., Belova, T., et al. 2016. QTL characterization of Fusarium head blight resistance in CIMMYT bread wheat line Soru#1. PLoS One 11:e0158052.

Hellin, P., Scauflaire, J., VanHese, V., Munaut, F., and Legrève, A. 2016. Sensitivity of *Fusarium culmorum* to triazoles: impact of trichothecene chemotypes, oxidative stress response and genetic diversity. Pest Manag. Sci. 73:1244–1252.

Henriksen, B., and Elen, O. 2005. Natural Fusarium grain infection level in wheat, barley and oat after early application of fungicides and herbicides. J Phytopathol. 153:214-220.

Hestbjerg, H., Felding, G., and Elmholt, S. (2002). *Fusarium culmorum* infection of barley seedlings: correlation between aggressiveness and deoxynivalenol content. J. Phytopathol. 150:308–312.

Hollingsworth, C. R., Motteberg, C. D., Wiersma, J. V., and Atkinson, L. M. 2008. Agronomic and economic responses of spring wheat to management of Fusarium head blight. Plant Dis. 92:1339-1349.

Holzapfel, C. 2015. Managing Fusarium Head Blight in Durum with Higher Seeding Rates and Fungicides. Annual report 2015 for Agricultural Demonstration of Practices and Technologies (ADOPT) Program. https://iharf.ca/wp-content/uploads/2016/04/Managing-FHB-in-Durum-with-Higher-Seeding-Rates-and-Fungicides.pdf.

Hooker, D. C., Schaafsma, A. W., and Tamburic-Ilincic, L. 2002. Using weather variables pre- and post-heading to predict deoxynivalenol content in winter wheat. Plant Dis. 86:611-619.

Horevaj, P., Gale, L. R., and Milus, E. A. 2011. Resistance in winter wheat lines to initial infection and spread within spikes by deoxynivalenol and nivalenol chemotypes of *Fusarium graminearum*. Plant Dis. 95:31-37.

Hucl, P., and Baker, R. J. 1989. LOTIL high-yielding spring wheat. Can. J. Plant Sci. 75:209-210.

Huhn, M., Elias, E., Ghavami, F., Kianian, S., Chao, S., Zhong, S., Alamri, M., Yahyaoui, A., and Mergoum, M. 2012. Tetraploid Tunisian wheat germplasm as a new source of Fusarium head blight resistance. Crop Sci. 52:136-145.

Hutcheon, J. A., and Jordan, V. W. L. 1992. Fungicide timing and performance for Fusarium control in wheat. In: Proceedings of the brighton crop protection conference-pests and disease 1992, Vol 2 (pp 633–638) British Crop Protection Council, Farnham, UK.

Ichinoe, M., Kurata, H., Sugiura, Y., and Ueno, Y. 1983. Chemotaxonomy of *Gibberella zeae* with special reference to production of trichothecenes and zearalenone. Appl. Environ. Microbiol. 46:1364–1369.

Inch, S., and Gilbert, J. 2003. The incidence of Fusarium species recovered from inflorescences of wild grasses in southern Manitoba. Can. J. Plant Pathol. 25:379-383.

Isidro-Sánchez, J., Akdemir, D., and Montilla-Bascón, G. 2017. Genome-wide association analysis using R. In S. Gasparis (Ed.), Oat methods. New York, NY, USA: Springer Nature.

Jang, J. Y., Baek, S. G., Choi, J-H., Kim, S., Kim, J., Kim, D-W., Yun, S-H., and Lee, T. 2019. characterization of nivalenol-producing *Fusarium asiaticum* that causes cereal head blight in Korea. Plant Pathol. J. 35(6):543-552.

JECFA. 2001. Safety Evaluation of Certain Contaminants in Food 56th Meeting of the Joint FAO/WHO Expert Committee on Food Additives, WHO, Geneva.

JECFA. 2011. Safety Evaluation of Certain Contaminants in Food.72nd Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), WHO, Geneva.

Jones, R. K., and Mirocha, C. J. 1999. Quality parameters in small grains from Minnesota affected by Fusarium head blight. Plant Dis. 83:506–511.

Kelly, A. C., Clear, R. M., O'Donnell, K., McCormick, S. P., Turkington, T. K., Tekauz, A., Gilbert, J., Kistler, H. C., Busman, M., and Ward, T. J. 2015. Diversity of Fusarium head blight populations and trichothecene toxin types reveals regional differences in pathogen composition and temporal dynamics. Fungal Genet. Biol. 82:22-31.

Kelly, A., Proctor, R. H., Belzile, F., Chulze, S. N., Clear, R. M., Cowger, C., et al 2016. The geographic distribution and complex evolutionary history of the NX-2 trichothecene chemotype from *Fusarium graminearum*. Fungal Genet. Biol. 95:39–48.

Kikot, G. E., Hours, R. A., and Alconada, T. M. 2009 Contribution of cell wall degrading enzymes to pathogenesis of *Fusarium graminearum*: a review. J. Basic Microbiol. 49:231-241.

Kim, Y-S., Dixon, E. D., Vincelli, P., and Farman, M. L. 2003. Field resistance to strobilurin (QoI) fungicides in *Pyricularia grisea* caused by mutations in the mitochondrial cytochrome b gene. Phytopathology 93(7):891-900.

Klix, M. B., Beyer, M., and Verreet, J. A. 2008. Effects of cultivar, agronomic practices, geographic location, and meteorological conditions on the composition of selected Fusarium species on wheat heads. Can. J. Plant Pathol. 30:46-57.

Klix, M. B., Verreet, J. A., and Beyer, M. 2007. Comparison of the declining triazole sensitivity of *Gibberella zeae* and increased sensitivity achieved by advances in triazole fungicide development. Crop Prot. 26:683-690.

Koizumi, S., Kato, H., Yoshino, R., Hayashi, N., and Ichinoe, M. 1991. Distribution of causal Fusaria of wheat and barley scab in Japan. Annales of the Phytopathological Society of Japan 57:165-173.

Kotowicz, N. K., Frąc, M., and Lipiec, J. 2014. The importance of Fusarium fungi in wheat cultivation – pathogenicity and mycotoxins production: a review. J. Anim. Plant Sci. 21:3326-43.

Krupinsky, J. M., Bailey, K. L., McMullen, M. P., Gossen, B. D., and Turkington, T. K. 2002. Managing plant disease risk in diversified cropping systems. Agron. J. 94:198-209.

Kulik, T., Ostrowska, A., Buśko, M., Pasquali, M., Beyer, M., Stenglein, S., Załuski, D., et al. 2015. Development of an FgMito assay: A highly sensitivemitochondrial based qPCR assay for quantification of *Fusarium graminearum sensu stricto*. Int. J. Food Microbiol. 210:16–23.

Krska, R., Malachova, A., Berthiller, F., and van Egmond, H. P. Determination of T-2 and HT-2 toxins in food and feed: an update. World Mycotoxin J. 7:131−142.

Lancashire, P. D., Bleiholder, H., Langeluddecke, P., Stauss, R., van den Boom, T., Weber, E., Witzenberger, A. 199.1 A uniform decimal code for growth stages of crops and weeds. Ann Appl Biol 119:561-601.

Landschoot, S., Waegeman, W., Audenaert, K., Van Damme, P., Vandepitte, J., De Baets, B., and Haesaert, G. 2013. A field-specific web tool for the prediction of Fusarium head blight and deoxynivalenol content in Belgium. Comput. Electron. Agric. 93:140-148.

Lee, T., Han, Y-K., Kim, K-H., Yun, S-H., and Lee, Y-W. 2002. Tri13 and Tri7 determine deoxynivalenol- and nivalenol producing chemotypes of *Gibberella zeae*. Appl. Environ. Microbiol. 68:2148-2154.

Lemmens, M., Haim, K., Lew, H., and Ruckenbauer, P. 2004. The effect of nitrogen fertilization on Fusarium head blight contamination in wheat. J Phytopathol.152:1-8.

Lemmens, M., Scholz, U., Berthiller, F., Dall'asta, C., Koutnik, A., Schuhmacher, R., et al. 2005. The ability to detoxify the mycotoxin deoxynivalenol colocalizes with a major

quantitative trait locus for Fusarium head blight resistance in wheat. Mol. Plant Microb. Int. 18:1318–1324.

Leroux, P., Albertini, C., Gautier, A., Gredt, M., and Walker, A. S. 2007. Mutations in the CYP51 gene correlated with changes in sensitivity to sterol 14α-demethylation inhibitors in field isolates of *Mycosphaerella graminicola*. Pest. Manage. Sci. 63:688-698.

Liang, J. M., Xayamongkhon, H., Broz, K., Dong, Y., McCormick, S. P., Abramova, S., Ward, T. J., Ma, Z. H., and Kistler, H. C. 2014. Temporal dynamics and population genetic structure of *Fusarium graminearum* in the upper Midwestern United States. Fungal Genet. Biol. 73:83-92.

Liggitt, J., Jenkinson, P., and Parry, D. W. 1997. The role of saprophytic microflora in the development of Fusarium ear blight of winter wheat caused by *Fusarium culmorum*. Crop Prot. 16:679-685.

Littell, R. C., Milliken, G. A., Stroup, W. W., Wolfinger, R., and Schabenberger, O. 2006. SAS for mixed models (2nd Edition). SAS Institute Inc., Cary, NC, USA.

Lofgren, L., Riddle, J., Dong, Y., Kuhnem, P., Cummings, J., Del Ponte, E., Bergstrom, G., and Kistler, H. C. 2018. A high proportion of NX-2 genotype strains are found among *Fusarium graminearum* isolates from northeastern New York State. Eur J Plant Pathol. 150(3):791–796.

Luo, C. X., and Schnabel, G. 2007. The cytochrome P450 lanosterol 14 alpha-demethylase gene is a demethylation inhibitor fungicide resistance determinant in *Monilinia fructicola* field isolates from Georgia. Appl. Environ. Microbiol. 74:359-366.

Ma, H., Ge, H., Zhang, X., Lu, W., Yu, D., Chen, H., and Chen, J. 2009. Resistance to Fusarium head blight and deoxynivalenol accumulation in Chinese barley. J. Phytopathol. 157:166-171.

Ma, Z., Proffer, T. J., Jacobs, J. L., and Sundin, G. W. 2006. Overexpression of the 14alpha-demethylase target gene (CYP51) mediates fungicide resistance in *Blumeriella jaapii*. Appl. Environ. Microbiol. 72:2581–2585.

Machado, F. J., Santana, F. M., Lau, D., and Del Ponte, E. M. 2017. Quantitative review of the effects of triazole and benzimidazole fungicides on fusarium head blight and wheat yield in Brazil. Plant Dis. 101:633-41.

MacLeana, D. E., Loboa, J. M., Colesb, K., Harding, M. W., Mayd, W. E., Penge, G., Turkington, T. K., and Kutcher, H. R. 2018. Fungicide application at anthesis of wheat provides effective control of leaf spotting diseases in western Canada. Crop Prot. 112:343–349.

Magan, N., Hope, R., Colleate, A., and Baxter, E. S. 2002. Relationship between growth and mycotoxins production by Fusarium species, biocides and environment. J. Plant Pathol. 108:611-619.

Maiorano, A., Blandino, M., Reyneri, A., and Vanara, F. 2008. Effects of maize residues on the *Fusarium* spp. infection and deoxynivalenol (DON) contamination of wheat grain. Crop Prot. 27:182-188.

Malihipour, A., Gilbert, J., Piercey-Normore, M., and Cloutier, S. 2012. Molecular phylogenetic analysis, trichothecene chemotype patterns, and variation in aggressiveness of Fusarium isolates causing head blight in wheat. Plant Dis. 96(7):1016-1025.

Mason, H. E., Navabi, A., Frick, B. L., O'donovan, J. T., and Spaner, D. M. 2007. The weed-competitive ability of Canada western red spring wheat cultivars grown under organic management. Crop Sci. 47:1167-1176.

Mateo, E. M., Valle-Algarra, F. M., Mateo, R., Jiménez, M., and Magan, N. 2011. Effect of fenpropimorph, prochloraz and tebuconazole on growth and production of T-2 and HT-2 toxins by Fusarium langsethiae in oat-based medium. Int. J. Food Microbiol. 151:289-98.

May, W. E., Fernandez, M. R., Selles, F., and Lafond, G. P. 2013. Agronomic practices to reduce leaf spotting and Fusarium kernel infections in durum wheat on the Canadian prairies. Can. J. Plant Sci. 94:141-152.

McCallum, B. D., and Tekauz, A. 2002. Influence of inoculation method and growth stage on fusarium head blight in barley. Can. J. Plant Pathol. 24:77-80.

McCormick, S. P., Stanley, A. M., Stover, N. A., and Alexander, N. J. 2011 Trichothecenes: from simple to complex mycotoxins. Toxins 3:802–814.

McMullen, M., Bergstrom, G., de Wolf, E, Dill-Macky, R., Hershman, D., Shaner, G., et al. 2012. A unified effort to fight an enemy of wheat and barley: *Fusarium* head blight. Plant Dis. 96 (12):712-1728.

McMullen, M., Halley, S., Schatz, B., Meyer, S., Jordahl, J., and Ransom, J. 2008. Integrated strategies for Fusarium head blight management in the United States. Cereal Res. Commun. 36:563-568.

McMullen, M., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Dis. 81(12):1340-1348.

Mesterhazy, A. 1995. Types and components of resistance to Fusarium head blight of wheat. Plant Breed. 114:377-386.

Mesterházy, Á. 2002. Role of deoxynivalenol in aggressiveness of Fusarium graminearum and F. culmorum and in resistance to Fusarium head blight. Eur. J. Plant Pathol. 108:675–684

Mesterházy, Á. 2003. Control of Fusarium head blight of wheat by fungicides. Pages 363-380 in: Fusarium Head Blight of Wheat and Barley, K. J. Leonard and W. R. Bushnell, eds. The American Phytopathological Society, St. Paul, MN.

Mesterhazy, A., Varga, M., Toth, B., Kotai, C., Bartok, T., Veha, A., Acs, K., Vagvolgyi, C., and lehoczki-Krsjak, S. 2018. Reduction of deoxynivalenol (DON) contamination by improved fungicide use in wheat. Part 1. Dependence on epidemic severity and resistance level in small plot tests with artificial inoculation. Eur J Plant Pathol 151:39-55.

Miller, D. J., and Richardson, S. N. 2013. Mycotoxins in Canada: a perspective for 2013. Department of Chemistry, Carleton University. Canada.

Miller, J. D., Culley, J., Fraser, K., Hubbard, S., Meloche, F., Ouellet, T., Seaman, W. L., Turkington, K., and Voldeng, H. 1998. Effect of tillage practice on fusarium head blight of wheat. Can J. Plant Pathol. 20:95-103.

Miller, J. D., Greenhalgh, R., Wang, Y., and Lu, M. 1991. Trichothecene Chemotypes of Three Fusarium Species. Mycologia. 83:121-130.

Miller, J. D., Young, J. C., and Sampson, R. D. 1985. Deoxynivalenol and Fusarium head blight resistance in spring cereals. Phytopathology 113:359-367.

Minervini, F., Fornelli, F., and Flynn, K. M. 2004. Toxicity and apoptosis induced by the mycotoxins nivalenol, deoxynivalenol and fumonisin B1 in a human erythroleukemia cell line. Toxicol. In Vitro.18(1):21-8.

Mirocha, C. J., Kolaczkowski, E., Xie, W., Yu, H., and Jelen, H. 1998. Analysis of deoxynivalenol and its derivatives (batch and single kernel) using gas chromatography/mass spectrometry. J. Agric. Food Chem. 46:1414-1418.

Molineros, J. 2007. Understanding the challenge of Fusarium head blight forecasting. Thesis (Ph.D.), The Pennsylvania State University, University Park, PA.

Molineros, J., De Wolf, E., Madden, L., Paul, P., and Lipps, P. 2005. Incorporation of host reaction and crop residue level into prediction models for Fusarium head blight. Pages 119-122 in: National Fusarium Head Blight Forum, Milwaukee, WI. S. M. Canty, T. Boring, J. Wardwell, L. Siler, and R. W. Ward, eds. Michigan State University, East Lansing.

Morcia, C., Tumino, G., Ghizzoni, R., Badeck, F. W., Lattanzio, V. M., Pascale, M., and Terzi, V. 2016. Occurrence of Fusarium langsethiae and T-2 and HT-2 Toxins in Italian Malting Barley. Toxins 8(8):247.

Moschini, R. C., Carranza, M. R., and Carmona, M. A. 2004. Meteorological-based predictions of wheat head blight epidemic in the southern Argentinean Pampas region. Cereal Res. Commun. 32: 45-52.

Musa, T., Hecker, A., Vogelgsang, S., and Forrer, H. R. 2007. Forecasting of Fusarium head blight and deoxynivalenol content in winter wheat with FusaProg. Bulletin OEPP/EPPO Bulletin 37, 283-289.

Nicolaisen, M., Suproniene, S., Neilson, L. K., Lazzaro, I., Spliid, N. H., and Justesen, A. F. 2009. Real-time PCR for quantification of eleven individual Fusarium species in cereals. J. Microbiol. Methods. 76(3): 234-240.

Nightingale, M. J., Marchylo, B. A., Clear, R. M., Dexter, J. E., and Preston, K. R. 1999. Fusarium head blight: effect of fungal proteases on wheat storage proteins. Cereal Chem. 76: 150-158.

Nilsen, K. T., Clarke, J. M., Beres, B. L., and Pozniak, C. J. 2016. Sowing density and cultivar effects on pith expression in solid-stemmed durum wheat. Agron. J. 108(1):219–228.

O'Donnell, K., Ward, T. J., Geiser, D. M., Kistler, H. C., and Aoki, T. 2004. Genealogical concordance between the mating type locus and seven other nuclear genes supports formal recognition of nine phylogenetically distinct species within the *Fusarium graminearum* clade. Fungal Genet Biol. 41(6):600–623.

Obst, A., Lepschy-von, Gleissenthall, J., and Beck, R. 1997. On the etiology of Fusarium head blight of wheat in south Germany - preceding crops, weather conditions for inoculum production and the head infection, proneness of the crop to infection and mycotoxin production. Cereal Res. Commun. 25:699-703.

O'Donnell, K., Kistler, H. C., Tacke, B. K., and Casper, H. H. 2000. Gene genealogies reveal global phylogeographic structure and reproductive isolation among lineages of *Fusarium graminearum*, the fungus causing wheat scab. Proc National Academy of Sci 97:7905-7910.

Osborne L E and Stein J M 2007. Epidemiology of Fusarium head blight on small-grain cereals. Int. J. Food Microbiol. 119:103-108.

Parnell, S., van der Bosch, F., and Gilligan, C. A. 2006. Large-scale fungicide spray heterogeneity and the regional spread of resistant pathogen strains. Phytopathology 96:549-555.

Parry, D. W., Jenkinson, P., and McLeod, L. 1995. Fusarium ear blight (scab) in small grain cereals - a review. Plant Pathol. 44:207-238.

Pasquali, M., Giraud, F., Brochot, C., Cocco, E., Hoffmann, L., and Bohn, T. 2010. Genetic Fusarium chemotyping as a useful tool for predicting nivalenol contamination in winter wheat. Int J Food Microbiol. 137:246–253.

Paul, P. A., Bradley, C. A., Madden, L. V., Dalla Lana, F., Bergstrom, G. C., Dill-Macky, R., et al. 2018. Meta-analysis of the effects of QoI and DMI fungicide combinations on Fusarium head blight and deoxynivalenol in wheat. Plant Dis. 102:2602-2615.

Paul, P. A., Lipps, P. E., and Madden, L. V. 2005. Relationship between visual estimates of Fusarium head blight intensity and deoxynivalenol accumulation in harvested wheat grain: a meta-analysis. Phytopathology 95:1225-36.

Paul, P. A., Lipps, P. E., Hershman, D. E., McMullen, M. P., Draper, M. A., and Madden, L. V. 2008. Efficacy of triazole-based fungicides for Fusarium head blight and deoxynivalenol control in wheat: a multivariate meta-analysis. Phytopathology 98:999-1011.

Paul, P. A., McMullen, M. P., Hershman, D. E., and Madden, L. V. 2010. Meta-analysis of the effects of triazole-based fungicides on wheat yield and test weight as influenced by Fusarium head blight intensity. Phytopathology 100:160-171.

Paul, P. A., Salgado, J. D., Bergstrom, G., Bradley, C. A., Byamukama, E., Byrne, A. M., et al. 2019. Integrated effects of genetic resistance and prothioconazole + tebuconazole application timing on fusarium head blight in wheat. Plant Dis. 103:223–237.

Pereyra, S. A., and Dill-Macky, R. 2008. Colonization of the residues of diverse plant species by *Gibberella zeae* and their contribution to fusarium head blight inoculum. Plant Dis. 92, 800–807.

Pereyra, S. A., Dill-Macky, R., and Sims, A. L. 2004. Survival and inoculum production of *Gibberella zeae* in wheat residue. Plant Dis. 88(7):724-73.

Pirgozliev, S. R., Edwards, S. G., Hare, M. C., and Jenkinson, P. 2002. Effect of dose rate of azoxystrobin and metconazole on the development of Fusarium head blight and the accumulation of deoxynivalenol (DON) in wheat grain Eur. J. Plant Pathol. 108:469-478.

Pirgozliev, S. R., Edwards, S. G., Hare, M. C., and Jenkinson, P. 2003. Strategies for the control of Fusarium head blight in cereals. Eur. J. Plant Pathol. 109(7):731-742.

Prat, N., Buerstmayr, M., Steiner, B., Robert, O., and Buerstmayr, H. 2014. Current knowledge on resistance to Fusarium head blight in tetraploid wheat. Mol Breed 34:1689–1699.

Proctor, R. H., Hohn, T. M., and McCormick, S. P. 1995. Reduced virulence of *Gibberella zeae* caused by disruption of a trichothecene toxin biosynthetic gene. Mol Plant Microbe-Interact. 8:593-601.

Purahong, W., Nipoti, P., Pisi, A., Lemmens, M., and Prodi, A. 2014. Aggressiveness of different *Fusarium graminearum* chemotypes within a population from Northern-Central Italy. Mycoscience 55(1):63–69.

Puri, K. D., and Zhong, S. 2010. The 3ADON population of *Fusarium graminearum* found in North Dakota is more aggressive and produces a higher level of DON than the prevalent 15ADON population in spring wheat. Phytopathology 100(10):1007–1014.

Puri, K. D., Yan, C., Leng, Y., and Zhong, S. 2016. RNA-Seq revealed differences in transcriptomes between 3ADON and 15ADON populations of *Fusarium graminearum* in vitro and in planta. PLoS One 11(10):e0163803.

Quarta, A., Mita, G., Haidukowski, M., Logrieco, A., Mule, G., and Visconti, A. 2006. Multiplex PCR assay for the identification of nivalenol, 3- and 15-acetyl-deoxynivalenol chemotypes in Fusarium. FEMS Microbiol. Lett. 259:7-13.

Rocha, O., Ansari, K., and Doohan, F. M. 2005. Effects of trichothecene mycotoxins on eukaryotic cells: a review. Food Addit Contam. 22:369-378.

Rossi, V., Giosuè, S., Pattori, E., Spanna, F., and Del Vecchio, A. 2003. A model estimating the risk of Fusarium head blight on wheat. EPPO Bulletin 33:421-425.

Ruske, R. E., Gooding, M. J., and Jones, S. A. 2003. The effects of adding picoxystrobin, azoxystrobin and nitrogen to a triazole programme on disease control, flag leaf senescence, yield and grain quality winter wheat. Crop Protect. 22:975–987.

Salameh, A., Buerstmayr, M., Steiner, B., Neumayer, A., Lemmens, M., and Buerstmayr, H. 2011. Effects of introgression of two QTL for fusarium head blight resistance from Asian spring wheat by marker-assisted backcrossing into European winter wheat on fusarium head blight resistance, yield and quality traits. Mol Breed. 28:485-494.

Salgado, J. D., Madden, L. V., and Paul, P. A. 2014. Efficacy and economics of integrating infield and harvesting strategies to manage Fusarium head blight of wheat. Plant Dis. 98:1407-1421.

Salgado, J. D., Madden, L. V., and Paul, P. A. 2015. Quantifying the effects of Fusarium head blight on grain yield and test weight in soft red winter wheat. Phytopathology 105, 295–306. 10.1094/PHYTO-08-14-0215-R

Salgado, J. D., Wallhead, M., Madden, L. V., and Paul, P. A. 2011. Grain harvesting strategies to minimize grain quality losses due to Fusarium head blight in wheat. Plant Dis. 95:1448-1457.

Saskatchewan Ministry of agriculture. 2020: Final Crop Report - For the Period October 13 to 19, 2020 (accessed on November 20, 2020). https://www.saskatchewan.ca/crop-report.

Scala, V., Aureli, G., Cesarano, G., Incerti, G., Fanelli, C., Scala, F., and Bonanomi, G. 2016. Climate, soil management, and cultivar affect Fusarium head blight incidence and deoxynivalenol accumulation in durum wheat of southern Italy. Front Microbiol. 7:1014.

Schaafsma, A. W., and Hooker, D. C. 2007. Climatic models to predict occurrence of Fusarium toxins in wheat and maize. Int. J. Food Microbiol. 119:116-125.

Schaafsma, A., Tamburic-Ilincic, L., and Hooker, D. 2005. Effect of previous crop, tillage, field size, adjacent crop, and sampling direction on airborne propagules of *Gibberella zeae/Fusarium graminearum,* Fusarium head blight severity, and deoxynivalenol accumulation in winter wheat. Can. J. Plant Pathol. 27:217-224.

Schatzmayr, G., and Streit, E. 2013. Global occurrence of mycotoxins in the food and feed chain: facts and figures. World Mycotoxin J. 6(3):213-222.

Schmale, D. G., and Bergstrom, G. C. 2003. Fusarium head blight in wheat. The Plant Health Instr. updated 2010.

Schroeder, H., and Christensen, J. 1963. Factors affecting resistance of wheat to scab caused by Gibberella zeae. Phytopathology 53:831-838.

Sengupta, A., and Dick, W. A. 2015. Bacterial community diversity in soil under two tillage practices as determined by pyrosequencing. Microb. Ecol.70:853–9.

Serajazari, M., Hudson, K., Kaviani, M., and Navabi, A. 2019. *Fusarium graminearum* chemotype-spring wheat genotype interaction effects in Type I and II resistance response assays. Phytopathology 109(4):643–9.

Shah, D. A., De Wolf, E. D., Paul, P. A., and Madden, L. V. 2014. Predicting Fusarium head blight epidemics with boosted regression trees. Phytopathology 104:702-714.

Shah, D. A., Molineros, J. E., Paul, P. A., Willyerd, K. T., Madden, L. V., and De Wolf, E. D. 2013. Predicting Fusarium head blight epidemics with weathe3r-driven pre- and post-anthesis logistic regression models. Phytopathology 103:906-919.

Shahin, M. A., and Symons, S. 2011. Detection of Fusarium damaged kernels in canada western red spring wheat using visible/near-infrared hyperspectral imaging and principal component analysis. Comput. Electron. Agric. 75(1):107-112.

Shaner, G., Stromberg, E. L., Lacy, G, H., Barker, K. R., and Pirone, T. P. 1992. Nomenclature and concepts of aggressiveness and virulence. Annu. Rev. Phytopathol. 30:47–66.

Shen, C., Hu, Y., Sun, H., Li, W., Guo, J., and Chen, H. 2012. geographic distribution of trichothecene chemotypes of the *Fusarium graminearum* species complex in major winter wheat production areas of China. Plant Dis. 96(8):1172-1178.

Shewry, P. R. Wheat. 2009. Darwin Review. Journal of Experimental Botany. 60:1537- 1553.

Somma, S., Petruzzella, A. L., Logrieco, A. F., Meca, G., Cacciola, O. S., Moretti, A. 2014. Phylogenetic analyses of *Fusarium graminearum* strains from cereals in Italy, and characterisation of their molecular and chemical chemotypes. Crop Pasture Sci. 65(1):52–60.

Spolti, P., De Jorge, B. C., and Del Ponte, E. M. 2012. Sensitivity of *Fusarium graminearum* causing head blight of wheat in Brazil to tebuconazole and metconazole fungicides. Trop. Plant Pathol. 37(6):419-423.

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

Stack, R. W., and McMullen, M. P. 1998. A visual scale to estimate severity of Fusarium head blight in wheat. North Dakota State University Extension Service: Small Grains Publications. Online Publication/PP-1095. https://library.ndsu.edu/ir/handle/10365/9187.

Starkey, D. E., Ward, T. J., Aoki, T., Gale, L. R., Kistler, H. C., Geiser, D. M., Suga, H., et al. 2007. Global molecular surveillance reveals novel Fusarium head blight species and trichothecene toxin diversity. Fungal Genet. Biol. 44:1191-1204.

Statistic Canada. 2019: Principal field crop areas, June 2020 (accessed on November 20, 2020). https://www150.statcan.gc.ca/n1/daily-quotidien/200629/dq200629c-eng.htm

Steiner, B., Buerstmayr, M., Wagner, C., et al. 2019. Fine-mapping of the Fusarium head blight resistance QTL Qfhs.ifa-5A identifies two resistance QTL associated with anther extrusion. Theor. Appl. Genet. 132:2039–2053.

Subedi, K. D., Ma, B. L., and Xue, G. A. 2007. Planting date and nitrogen effects on Fusarium head blight and leaf spotting diseases in spring wheat. Agron. J. 99:113-121.

Suga, H., Karugia, G. W., Ward, T., Gale, L. R., Tomimura, K., Nakajima, T., Miyasaka, A., et al. 2008. Molecular characterization of the *Fusarium graminearum* species complex in Japan. Phytopathology 98:159-166.

Sun, H. Y., Zhu, Y. F., Liu, Y. Y., Deng, Y. Y., Li, W., Zhang, A. X., and Chen, H. G. 2014. Evaluation of tebuconazole for the management of Fusarium head blight in China. Australas. Plant Pathol. 43(6):631-638.

Sutton, J. C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum.* Can. J. Plant Pathol. 4:195-209.

Szabo-Hever, A., Zhang, Q., Friesen, T. L., Zhong, S., Elias, E. M., Cai, X., et al. 2018. Genetic diversity and resistance to Fusarium head blight in synthetic hexaploid wheat derived from *Aegilops tauschii* and diverse *Triticum turgidum* Subspecies. Front. Plant Sci. 9:1829.

Talas, F., Longin, F., and Miedaner, T. 2011. Sources of resistance to Fusarium head blight within Syrian durum wheat landraces. Plant Breed. 130:398-400.

Tamburic-Ilincic, L. 2010. Fusarium species and mycotoxins associated with oat in southwestern Ontario, Canada. Can J Plant Sci 90:211–216.

Tateishi, H., Miyake, T., Mori, M., Kimura, R., Sakuma, Y., and Saishoji, T. 2010. Sensitivity of Japanese *Fusarium graminearum* species complex isolates to metconazole. J. Pestic. Sci. 35:419-430.

Tateishi, T., Miyake, T., Mori, M., Sakuma, Y., and Saishoji, T. 2014. Effect of application timing of metconazole on Fusarium head blight development and mycotoxin contamination in wheat and barley. J. Pestic. Sci. 39(1):1-6.

Teich, A., and Nelson, K. 1984. Survey of Fusarium head blight and possible effects of cultural practices in wheat fields in Lambton County in 1983. Can. Plant Dis. Surv. 64:11-13.

Tekauz, A., Fernandez, M. R., Gilbert, J., Pearse, P. G., and Turkington, T. K. 2009. Differences in the *Fusarium* spp. affecting cereal crops in western Canada. Can. J. Plant Pathol. 31:128-129.

Tittlemier, S. A., Blagden, R., Chan, J., Roscoe, M., and Pleskach, K. 2020. A multi-year survey of mycotoxins and ergosterol in Canadian oats. Mycotoxin Res. 36(1):103-114.

Tittlemier, S. A., Arsiuta, J., Mohammad, U., Hainrich, C., Bowler, K., Croom, R., Olubodun, A., Blagden, R., Mckendry, T., Gräfenhan, T., and Pleskach K. 2020. Variable relationships between Fusarium damage and deoxynivalenol concentrations in wheat in western Canada in 2016, Can. J. Plant Pathol. 42(1):41-51.

Tittlemier, S. A., Gaba, D., and Chan, J. M. 2013. Monitoring of Fusarium trichothecenes in Canadian cereal grain shipments from 2010 to 2012. J. Agric. Food Chem. 61:7412– 8.

Valverde-Bogantes, E., Bianchini, A., Herr, J. R., Rose, D. J., Wegulo, S. N., and Hallen-Adams, H. E. 2019. Recent population changes of Fusarium head blight pathogens: drivers and implications, Can. J. Plant Pathol. 42(3): 315-329.

Van Der Fels-Klerx, H. J., Burgers, S. L. G. E., and Booij, C. J. H. 2010. Descriptive modelling to predict deoxynivalenol in winter wheat in the Netherlands. Food Addit Contam. 27:636-643.

Van Ginkel, M., Van Der Schaar, W., Zhuping, Y., and Rajaram, S. 1996. Inheritance of resistance to scab in two wheat cultivars from Brazil and China. Plant Dis. 80:863-7.

Vanderplank, J. E. 1984. Disease Resistance of Plants. Academic Press, New York, NY, USA.

Varga, E., Wiesenberger, G., Hametner, C., Ward, T. J., Dong, Y., Schöfbeck, D., McCormick, S., et al. 2015. New tricks of an old enemy: isolates of *Fusarium graminearum* produce a type A trichothecene mycotoxin. Environ. Microbiol. 17(8): 2588- 2600.

Vogelgsang, S., Beyer, M., Pasquali, M., Jenny, E., Musa, T., Bucheli, T. D., et al. 2019. An eight-year survey of wheat shows distinctive effects of cropping factors on different *Fusarium* species and associated mycotoxins. Eur. J. Agron. 105:62–77.

von der Ohe, C., Gauthier, V., Tamburic-Ilincic, L., Brule-Babel, A., Fernando, W. G. D., Clear, R., et al. 2010. A comparison of aggressiveness and deoxynivalenol production between Canadian *Fusarium graminearum* isolates with 3-acetyl and 15-acetyldeoxynivalenol chemotypes in field-grown spring wheat. Eur. J. Plant Pathol. 127(3):407–17.

Walker, S. L., Leath, S., Hagler Jr, W. M., and Murphy, J. P. 2001. Variation among isolates of *Fusarium graminearum* associated with Fusarium head blight in North Carolina. Plant Dis. 85:404-10.

Wanda, M. H., and Kenneth, A. V. 2013. Mycotoxins: Haschek and Rousseaux's Handbook of Toxicologic Pathology. Third edition. Elsevier. US.

Wang, C., Zhang, S., Hou, R., Zhao, Z., Zheng, Q., Xu, Q., Zheng, D., Wang, G., Liu, H., Gao, X., et al. 2011. Functional Analysis of the Kinome of the Wheat Scab Fungus *Fusarium graminearum*. PLoS Pathog 7(12):e1002460.

Wang, L., and Kutcher, H. R. 2018. Establishing LC-MS/MS based mycotoxin/deoxynivalenol (DON) platform to support FHB research and breeding programs. Soils and Crops Workshop 2018.

https://harvest.usask.ca/bitstream/handle/10388/8694/L.%20Wang%20and%20R.%20Kutcher,%202018.pdf?sequence=1

Wang, Y. Z., and Miller, J. D. 1988. Effect of *Fusarium graminearum* metabolites on wheat tissue in relation to Fusarium head blight resistance. J. Phytopathol. 122:118-125.

Ward, T. J., Bielawski, J. P., Kistler, H. C., Sullivan, E., and O'Donnell, K. 2002. Ancestral polymorphism and adaptive evolution in the trichothecene mycotoxin gene cluster of phytopathogenic Fusarium. Proceedings of the National Academy of Sciences USA 99:9278–9283.

Ward, T. J., Clear, R. M., Rooney, A. P., O'Donnell, K., Gaba, D., and Patrick, S. 2008. An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet. Biol. 45:473-484.

Wegulo, S. N., Baenziger, P. S., Hernandez, Nopsa. J., Bockus, W. W., and Hallen-Adams, H. 2015. Management of Fusarium head blight of wheat and barley. Crop Prot. 73:100–107.

Wegulo, S. N., Bockus, W. W., Hernandez, N. J., De Wolf, E. D., et al. 2011. Effects of integrating cultivar resistance and fungicide application on Fusarium head blight and deoxynivalenol in winter wheat. Plant Dis. 95:554-560.

Wenda-Piesik, A., Lemanczyk, G., Twaruzek, M., Blajet-Kosicka, A., Kazek, M., and Grajewski, J. 2017. Fusarium head blight incidence and detection of Fumarium toxins in wheat in relation to agronomic factors. Eur. J. Plant Pathol. 149:515–531.

Wilcoxon, R. D., Kommedall, T., Ozmon, E. A., and Windels, C. 1988. Occurrence of Fusarium species in scaby wheat from Minnesota and their pathogenicity to wheat. Phytopathology 78:586-589.

Willyerd, K. T., Li, C., Madden, L. V., Bradley, C. A., Bergstrom, G. C., and Sweets, L. E. 2012. Efficacy and stability of integrating fungicide and cultivar resistance to manage Fusarium head blight and deoxynivalenol in wheat. Plant Dis. 96(7):957-967.

Wong, L. S. L., Abramson, D., Tekauz, A., Leslie, D., and McKenzie, R. I. H. 1995. Pathogenicity and mycotoxin production of Fusarium species causing head blight in wheat cultivars varying in resistance. Can. J. Plant Sci. 75:261-267.

Xu, Y. G., and Chen, L. F. 1993. Wheat Scab: Theory and Practice on Control. Nanjing, China; Jiangsu Sci.-Tech. Publ. House.

Xue, A. G., Armstrong, K. C., Voldeng, H. D., and Babcock, C. 2004. Comparative aggressiveness of isolates of *Fusarium* spp. causing head blight on wheat in Canada. Can. J. Plant Pathol. 26:81-88.

Xue, A. G., Chen, Y., Seifert, K., Guo, W., Blackwell, B. A., et al. 2019. Prevalence of Fusarium species causing head blight of spring wheat, barley and oat in Ontario during 2001–2017. Can. J. Plant Pathol. 41(3):392-402.

Xue, A. G., Chen, Y., Seifert, G. W., Blackwell, B. A., Linda, J., Harris, L. J., and Overy, D. P. 2019. Prevalence of Fusarium species causing head blight of spring wheat, barley and oat in Ontario during 2001–2017. Can. J. Plant Sci. 41(3):392-402.

Yan, W., Li, H., Cai, S., Ma, H., Rebetzke, G., and Liu, C. 2011. Effects of plant height on type I and type II resistance to Fusarium head blight in wheat. Plant Pathol. 60:506-512.

Ye, Z., Brûlé-Babel, A. L., Graf, R. J., Mohr, R., and Beres, B. L. 2017. The role of genetics, growth habit, and cultural practices in the mitigation of Fusarium head blight. Can. J. Plant Sci. 97:316–328.

Yin, Y., Liu, X., Li, B., and Ma, Z. 2009. Characterization of sterol demethylation inhibitor-resistant isolates of *Fusarium asiaticum* and *F. graminearum* collected from wheat in China. Phytopathology 99:487-497.

Yli-Mattila, T., Parikka, P., Lahtinen, T., Ramo, S., Kokkonen, M., Rizzo, A., Jestoi, M., and Hietaniemi, V. 2009. Fusarium DNA levels in Finnish cereal grains. In Current Advances in Molecular Mycology; Gherbawy, Y., Mach, L., Rai, M., Eds.; Nova Science Publishers, Inc.: New York, NY, USA, pp.107–138.

Yli-Mattila, T., Paavanen-Huhtala, S., Jestoi, M., Parikka, P., Hietaniemi, V., Gagkaeva, T., Sarlin, T., Haikara, A., Laaksonen, S., and Rizzo, A. 2008. Real-time PCR detection and quantification of *Fusarium poae, F. graminearum, F. sporotrichioides* and *F. langsethiae* in cereal grains in Finland and Russia. Arch. Phytopathol. Pflanzenschutz. 41(4):1477-2906.

Yli-Mattila, T., Paavanen-Huhtala, S., Parikka, P., Hietaniemi, V., Jestoi, M., and Rizzo, A. 2004. Toxigenic fungi and mycotoxins in Finnish cereals. In An Overview on Toxigenic Fungi and Mycotoxins in Europe; Logrieco, A., Visconti, A., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, pp.83-100.

Yoshida, M., and Nakajima, T. 2010. Deoxynivalenol and nivalenol accumulation in wheat infected with *Fusarium graminearum* during grain development. Phytopathology 100:763-773.

Yoshida, M., Nakajima, T., Arai, M., Suzuki, F., and Tomimura, K. 2008. Effect of the timing of fungicide application on Fusarium head blight and mycotoxin accumulation in closed-flowering barley. Plant Dis. 92:1164-1170.

Yoshida, M., Nakajima, T., Tomimura, K., Suzuki, F., Arai, M., and Miyasaka, A. 2012. Effect of the timing of fungicide application on Fusarium head blight and mycotoxin accumulation in wheat. Plant Dis. 96:845-851.

Zecevic, V., Boskovic, J., Knezevic, D., and Micanovic, D. 2014. Effect of seeding rate on grain quality of winter wheat. Chilean J. Agric. Res. 74(1):23-28.

Zhang, H., van der Lee, T., Waalwijk, C., Chen, W., Xu, J., Xu, J., Zhang, Y., and Feng, J. 2012. Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. PLoS One 7(2):e31722.

Zhang, L., Luo, P., Ren, Z., and Zhang, H. 2011. Controlling fusarium head blight of wheat (*triticum aestivum* L.) with genetics. Adv. Biosci. Biotechnol. 2:263-270.

Zhang, Q., Axtman, J. E., Faris, J. D., Chao, S., Zhang, Z., Friesen, T. L., Zhong, S., Cai, X., Elias, E. M., and Xu, S. S. 2014. Identification and molecular mapping of quantitative trait loci for Fusarium head blight resistance in emmer and durum wheat using a single nucleotide polymorphism-based linkage map. Mol. Breed. 34:1677–1687.

APPENDICES

Appendix A. Table A.1. Comparison of cereal growth stage scales (BBCH vs Feekes vs Zadok) and description of the corresponding phenological development of cereals.

BBCH scale	Feekes scale	Zadok Scale	Description of growth stage
BBCH57	10.4	57	3/4 of inflorescence emerged
BBCH59	10.5	59	End of heading: inflorescence fully emerged
BBCH61	10.51	60	Beginning of flowering: first anthers visible
BBCH65	10.52	65	Anthesis half-way: 50% of anthers mature
BBCH69	10.53	69	End of flowering: all spikelets have completed flowering, but some dehydrated anthers may remain
BBCH71	10.54	71	Watery ripe: first grains have reached half their final size
BBCH73	11.1	73	Early milk

Appendix A. Table A.2. Summary of climatic conditions [temperature (°C) and monthly mean precipitation (mm)] for experimental sites at Saskatoon, Outlook and Melfort in 2016, 2017, and 2018.

Location/	Temperature (°C)						Precipitation (mm)						
year	Apr.	May	June	July	Aug.	Sep.	Apr.	May	June	July	Aug.	Sep.	Total
Saskatoon													
2016	5.5	15	18.5	19.3	16.9	11.8	3	43	47	77	70	25	265
2017	4.3	12.1	16.1	19.6	17.8	12.8	18.4	46.3	30.9	25.5	25.2	29.1	175.4
2018	-0.7	14.3	17.3	18.7	17.1	7.4	9.1	35	18.8	31.1	17.2	37.1	148.3
Outlook													
2016	6	13.5	17.5	18.6	16.9	12.1	9.5	55.7	45.8	194.6	69.9	24.4	399.9
2017	4.6	12.2	16.2	19.8	17.9	13.3	16.9	32.9	27.9	67.7	7	6.4	158.8
2018	0.9	14.9	17.4	18.5	17.5	8.2	5.9	24.9	12.9	35.2	12.6	25	116.5
Melfort													
2016	-	-	-	-	-	-	-	-	-	-	-	-	-
2017	2.9	10.8	15.2	18.6	17.8	12.5	23.6	46.4	44.1	33.3	23.1	13.2	183.7
2018	-3.4	13.9	16.7	17.5	15.8	6.9	5	38.5	46.6	69.5	43.2	42	244.8

Appendix A. Table A.3. *Fusarium graminearum* isolates along with the province of collection used to determine fungicide sensitivity (Chapter 4).

Province	Isolates
Alberta	NRRL-37401, NRRL-37408, NRRL-40909, NRRL-40954, NRRL-40965, NRRL-40966, NRRL-40967, NRRL-40969, NRRL-40984, NRRL-43011, NRRL-43013, NRRL-44618, NRRL-44627, NRRL-44633, NRRL-44636, NRRL-44709, NRRL-44762, NRRL-44778, NRRL-44785, NRRL-44835, NRRL-44877, NRRL-44878, NRRL-52429, NRRL-52441, NRRL-52512, NRRL-52600,
	DAOM-243247, DAOM-241166, DAOM-243206, DAOM-243207, DAOM-243209, DAOM-243210, DAOM-243244, DAOM-243245
Saskatchewan	101-e-1, 102-e-1, 103-a-1, 103-b-1, 104-c-1, 105-b-1, 106-a-1, 106-b-1, 108-b-1, 108-b-5, 109-a-1, 109-b-1, 110-a-5, 112-a-1, 112-b-1, 113-b-5, 114-e-4, 115-b-5, 115-e-4, 115-b-1, 116-a, 116-b, 116-a-1, 117-a, 118-a-3, 119-a-1, 120-a-1, 120-b-1, 126-a, 128-a-1, 131-c-1, 138-a-1, 139-a-1, 139-b-1, 140-b, 142-a-1, 146-a-1, 147-a-1, 154-a-1, 154-b-1, 155-a-1, 155-b-1, 156-a-5, 157-a-1, 157-b-1, 159-a-1, 159-b-1, 162-a-5, 163-a-5, 164-b-1, 171-b, 172-a-1, 172-b-1, 173-b-1, 173-b-5, 174-c-5, 174-b-1, 177-a-1, 177-b-1, 183-a-5, 207-c-5, 210-a-1, 214-a-5, 217-5-5, 217-b-5, 219-a-2, 220-a-5, 221-a-5, 224-a, 225-b, 227-a-1, 228-a, 228-b, 231-a-1, 231-b-1, 232-a-1, 233-c-5, 234-c-1, 234-d-1, 240-a-1, 240-b-1, 240-c-1, 241-b-1, 241-c, 242-a, 242-b-3, 243-a, 244-a, 244-b, 244-d, 245-b-2
	NRRL-37172, NRRL-37173, NRRL-37174, NRRL-37183, NRRL-37184, NRRL-37185, NRRL-37196, NRRL-37202, NRRL-37204, NRRL-37205, NRRL-37331, NRRL-37336, NRRL-37338, NRRL-37339, NRRL-37345, NRRL-37348, NRRL-37509, NRRL-39065, NRRL-39079, NRRL-39097, NRRL-39128, NRRL-39133, NRRL-39134, NRRL-39162, NRRL-39173, NRRL-39231, NRRL-44144, NRRL-44160, NRRL-44211, NRRL-44238, NRRL-44318, NRRL-52130, NRRL-52195,
	DAOM-233802, DAOM-233803, DAOM-233806, DAOM-233807, DAOM-233808, DAOM-233811, DAOM-241170, DAOM-243200, DAOM-243203, DAOM-243204, DAOM-243205, DAOM-250004, DAOM-250005, DAOM-250006, DAOM-250007, DAOM-250008,
Manitoba	HSW-15-2, HSW-15-5, HSW-15-6, HSW-15-7, HSW-15-8, HSW-15-9, HSW-15-10, HSW-15-15, HSW-15-17, HSW-15-24, HSW-15-33, HSW-15-35, HSW-15-36, HSW-15-37, HSW-15-38, HSW-15-41, HSW-15-45, HSW-15-

47, HSW-15-48, HSW-15-49, HSW-15-50, HSW-15-51, HSW-15-52, HSW-15-54, HSW-15-56, HSW-15-59, HSW-15-61, HSW-15-62, HSW-15-65, HSW-15-66, HSW-15-67, HSW-15-68, HSW-15-69, HSW-15-70, HSW-15-74, HSW-15-81, HSW-15-82, HSW-15-83, HSW-15-84, HSW-15-89, HSW-15-90, HSW-15-97, HSW-15-98, HSW-15-101, HSW-15-102, HSW-15-104, HSW-15-106, HSW-15-108, HSW-15-112,

NRRL-37425, NRRL-37450, NRRL-39407, NRRL-39414, NRRL-39441, NRRL-40201, NRRL-40205, NRRL-40208, NRRL-40210, NRRL-40310, NRRL-40350, NRRL-40375, NRRL-40406, NRRL-40408, NRRL-40450, NRRL-40461, NRRL-44385, NRRL-44414, NRRL-44485, NRRL-44513, NRRL-44515, NRRL-44558, NRRL-44591, NRRL-52005, NRRL-52008

DAOM-192130, DAOM-192131, DAOM-192132, DAOM-215947

DAOM - Canadian Collection of Fungal Cultures (DAOM), Ottawa, Ontario, Canada, NNRL - Northern Regional Research Laboratory

Appendix A. Table A.4. Wheat samples, year of collection, wheat class, % Fusarium damaged kernels (FDK), rural municipality (R.M.), wheat variety, seeding date, isolate identification and chemotype of *Fusarium graminearum* isolates**.**

Sample no.	Year	Wheat class	FDK (%)	R.M.	Variety	Seeding Date	Isolates	Chemotype
1	2014	CWAD	50	34	Kyle	09-Jun-14	101-b-1	3ADON
							101-e-1	3ADON
2	2014	CWRS	30	127	Laura	25-May-14	102-c-5	15ADON
							102-e-1	15ADON
3	2014	CWRS	18.4	129	Lillian	27-May-14	103-a-1	3ADON
							103-b-1	15ADON
4	2014	CWRS	30	159	Cadillac	31-May-14	104-b-3	3ADON
							104-c-1	3ADON
5	2014	CWRS	24.3	342	Mckenzie	28-May-14	105-a-1	15ADON
							105-b-1	3ADON
6	2014	CWAD	23.8	490		21-May-14	106-a-1	15ADON
							106-b-1	3ADON
7	2014	CWAD	8.3	319	AC Strongfield	10-May-14	107-a-3	3ADON
							107-f-3	3ADON
8	2014	CWAD	11.8	160	Brigade	-	108-b-1	15ADON
							108-b-5	3ADON
9	2014	CWAD	8	131	AC Strongfield	10-Jun-14	109-a-1	15ADON
							109-b-1	3ADON
10	2014	CWAD	7.1	160	Brigade	-	110-a-5	15ADON
							110-d-1	3ADON
11	2014	CPS	12.2	339	AC Andrew	03-Jun-14	111-a-3	15ADON
							111-d-4	15ADON
12	2014	CWAD	11	287	Brigade	24-May-14	112-a-1	15ADON
							112-b-1	15ADON
13	2014	CPS	18.2	370	AC Andrew	20-May-14	113-b-5	15ADON
							113-e-4	15ADON
14	2014	CWAD	7.1	191	AC Strongfield	03-Jun-14	114-e-3	3ADON
15	2014	CPS	6	369	AC Andrew	18-May-14	115-b-1	3ADON

							115-b-5	3ADON
							115-e-4	3ADON
16	2014	CWAD	9	131	Brigade	18-May-14	116-a	15ADON
							116-a-1	15ADON
							116-b	15ADON
17	2014	CWAD	18.2	287	Brigade	14-May-14	117-a	3ADON
18	2014	CPS	15.8	222	-	-	118-a-3	3ADON
							118-b-1	3ADON
							118-e-5	15ADON
19	2014	CWAD	13.6	160	Brigade	-	119-a-1	3ADON
							119-b-1	3ADON
20	2014	CPS	15.8	368	AC Andrew	20-May-14	120-a-1	3ADON
							120-b-1	3ADON
							120-d-5	15ADON
21	2014	CWAD	16.7	223	AC Transcend	-	121-d-5	3ADON
22	2014	CWAD	5.4	253	Brigade	23-May-14	122-b	15ADON
							122-c-1	3ADON
23	2014	CWRS	39.9	126	Lillian	24-May-14	123-c-6	15ADON
							123-d-3	3ADON
24	2014	CPS	15	318	-	-	124-a-2	15ADON
							124-b-5	15ADON
25	2014	CWAD	13.3	158	AC Transcend	17-May-14	125-c-2	3ADON
							125-c-5	15ADON
26	2014	CWRS	9	457	Prodigy	18-May-14	126-a	3ADON
							126-a-4	15ADON
27	2014	CPS	8.87	456	-	-	127-c-2	3ADON
							127-d	3ADON
28	2014	CWAD	11.6	157	Brigade	23-May-14	128-a-1	3ADON
29	2014	CWAD	10	126	Brigade	28-May-14	129-a-1	3ADON
30	2014	CPS	61.2	368	AC Andrew	17-May-14	130-a-5	15ADON
							130-b-7	15ADON

31	2014	CWRS	15.3	319	Mckenzie	14-May-14	131-a-2	15ADON
							131-c-1	3ADON
32	2014	CWAD	6.6	129	AC Strongfield	30-May-14	132-a-1	15ADON
							132-c-1	3ADON
33	2014	CWRS	15.1	126	Lillian	24-May-14	133-a-1	15ADON
							133-b-1	15ADON
34	2014	CWRS	7.2	91	-	-	134-2-a	15ADON
							134-b-2	15ADON
35	2014	CWAD	30.1	126	Brigade	28-May-14	135-c-5	3ADON
							135-d-3	15ADON
36	2014	CWRS	18	126	Lillian	24-May-14	136-d-2	15ADON
							136-e-3	3ADON
37	2014	CWAD	21.2	161	Brigade	07-Jun-14	137-a-2	3ADON
							137-f-4	3ADON
38	2014	CWRS	4.4	253	-	-	138-a-1	15ADON
							138-b-7	15ADON
39	2014	CPS	24.8	368	AC Andrew	17-May-14	139-a-1	3ADON
							139-b-1	3ADON
40	2014	CWAD	11	190	Verona	24-May-14	140-b	15ADON
41	2014	CWRS	25	186	Lillian	17-May-14	141-d-2	15ADON
							141-f-3	15ADON
42	2014	CWRS	12.8	157	Lillian	09-Jun-14	142-a-1	3ADON
							142-a-9	15ADON
43	2014	CWRS	13	126	-	-	143-a	3ADON
							143-b	15ADON
							143-d	15ADON
44	2014	CWAD	9.8	126	AC Strongfield	18-May-14	144-a-7	3ADON
							144-c-3	3ADON
45	2014	CWAD	11.2	126	Verona	21-May-14	145-b-2	3ADON
							145-e-3	3ADON
46	2014	CWAD	18.6	259	Verona	20-May-14	146-a-1	3ADON

							146-a-5	15ADON
47	2014	CWAD	83	35	Kyle	18-May-14	147-a-1	3ADON
							147-b-1	3ADON
48	2014	CWAD	37	137	Navigator	30-May-14	148-b-3	3ADON
							148-f-1	3ADON
49	2014	CWAD	7.2	160	AC Strongfield	10-Jun-14	149-a-9	3ADON
							149-d-2	3ADON
50	2014	CWAD	15	97			150-b-3	15ADON
51	2014	CWAD	6.8	45	AC Strongfield	05-Jun-14	151-c-2	15ADON
							151-d-4	3ADON
52	2014	CWRS	18	52	-	-	152-c-1	15ADON
							152-c-4	3ADON
53	2014	CWRS	11.5	126	-	-	153-b-1	15ADON
							153-d-2	3ADON
54	2014	CWRS	31	158	-	-	154-a-1	3ADON
							154-b-1	3ADON
55	2014	CWRS	64.9	252	-	-	155-a-1	3ADON
							155-b-1	15ADON
56	2014	CWRS	22	35	Prodigy	23-May-14	156-a-5	3ADON
							156-b-1	15ADON
57	2014	CWAD	10.5	128	Glen	15-May-14	157-a-1	3ADON
							157-b-1	3ADON
58	2014	CWAD	26.7	191	Brigade	-	158-b-7	3ADON
							158-d-4	3ADON
59	2014	CWRS	7.5	127	-	13-May-14	159-a-1	3ADON
							159-b-1	3ADON
60	2015	CPS	11.2	310	Sadash	24-May-15	160-c-5	15ADON
61	2015	CWAD	7	222	Brigade	18-May-15	161-d-2	3ADON
62	2015	CWRS	15	156	-	-	162-a-5	15ADON
							162-b-1	15ADON
							162-e-7	3ADON

63	2015	CWRS	10.8	458	Vesper	22-May-15	163-a-2	15ADON
							163-a-5	3ADON
64	2015	CWAD	6.8	36	Brigade	23-May-15	164-a-3	3ADON
							164-b-1	15ADON
65	2015	CWAD	6.9	157	Brigade	24-May-15	165-a-4	15ADON
							165-d-4	3ADON
66	2015	CWAD	17.8	158	Kyle	20-May-15	166-f-1	3ADON
							166-f-7	15ADON
67	2015	CWAD	21.4	129	Brigade	18-May-15	167-d-2	3ADON
							167-d-6	15ADON
68	2015	CWAD	13.7	159	Brigade	24-May-15	168-a-1	3ADON
							168-a-7	15ADON
69	2015	CWRS	10.5	371	-	-	169-a-6	3ADON
							169-b-1	3ADON
70	2015	CWAD	6.5	158	Verona	24-May-15	170-d-2	3ADON
							170-e-5	15ADON
71	2015	CWAD	4.8	158	-	-	171-a	3ADON
							171-b	3ADON
72	2015	CPS	17.6	125	-	-	172-a-1	15ADON
							172-b-1	15ADON
73	2015	CWAD	20.1	158	-	-	173-b-5	3ADON
74	2015	CWAD	10.3	160	AC Strongfield	10-Jun-15	174-b-1	3ADON
							174-c-5	15ADON
75	2015	CWAD	16.2	35	Brigade	27-May-15	175-a-5	15ADON
							175-d-2	15ADON
76	2015	CWAD	16.5	191	CDC Fortitude	15-May-15	176-a-1	3ADON
							176-b-1	3ADON
77	2015	CWAD	13.2	129	Brigade	-	177-a-1	3ADON
							177-b-1	3ADON
78	2015	CWAD	13.9	222	Brigade	24-May-15	178-c-2	3ADON
							178-d-6	15ADON

79	2015	CWAD	14.1	169	Brigade	10-May-15	179-a-6	3ADON
							179-d-2	3ADON
80	2015	CWRS	13.3	309	Laura	07-Jun-15	180-a-1	15ADON
							180-b-1	3ADON
81	2015	CWAD	11.1	130	Brigade	18-May-15	181-c-2	3ADON
							181-d-1	3ADON
82	2015	CWAD	10	287	CDC Verona	17-May-15	182-a	15ADON
							182-b-3	3ADON
83	2015	CPS	10.9	400	Sadash	16-May-15	183-a-1	3ADON
							183-a-5	3ADON
84	2015	CPS	14.8	339	-	-	184-c-5	15ADON
							184-e-3	3ADON
85	2015	CWAD	14.8	137	Verona	29-May-15	185-a-1	3ADON
							185-b-3	15ADON
86	2015	CWAD	9.3	137	Navigator	30-May-15	186-a-2	3ADON
87	2015	CWAD	7.3	45	AC Strongfield	05-Jun-15	187-c-4	3ADON
88	2016	CWAD	12.4	257	AC Strongfield	08-May-16	201-a-6	3ADON
							201-c-1	3ADON
89	2016	CWAD	10.1	2	AC Strongfield	23-May-16	202-a-4	15ADON
							202-c-4	3ADON
90	2016	CWAD	25.9	259	AC Strongfield	30-Apr-16	203-a-2	3ADON
							203-b-5	3ADON
							203-c-5	15ADON
91	2016	CWRS	10.7	344	-	-	204-a	3ADON
							204-b	15ADON
							204-c	15ADON
							204-d	3ADON
92	2016	CWAD	14.1	226	Verona	29-May-16	205-a-1	3ADON
							205-b-1	3ADON
							205-d-5	3ADON
93	2016	CWAD	14.4	228	Brigade	15-May-16	206-4-1	3ADON

							206-d-1	15ADON
							206-d-8	3ADON
94	2016	CWAD	15.9	288	Verona	01-May-16	207-c-5	3ADON
							207-d	3ADON
95	2016	CWRS	7.1	460	Utmost	21-May-16	208-a-9	3ADON
							208-b-4	15ADON
96	2016	CWAD	10.5	69	AC Strongfield	29-Apr-16	209-b-8	3ADON
							209-e-3	15ADON
97	2016	CWAD	7.7	371	Verona	15-May-16	210-a-1	3ADON
							210-b-1	3ADON
							210-e-5	3ADON
98	2016	CWAD	5.6	105	Brigade	05-May-16	211-a-3	3ADON
							211-a-6	15ADON
99	2016	CWAD	9.3	100	AC Strongfield	18-May-16	212-b-6	3ADON
							212-f-3	3ADON
100	2016	CWAD	7	168	AC Strongfield	16-May-16	213-a-1	3ADON
							213-c-1	15ADON
101	2016	CWAD	8.9	259	AC Strongfield	15-May-16	214-a-5	15ADON
							214-b-3	3ADON
102	2016	CWAD	12.3	287	Brigade	16-May-16	215-c-5	3ADON
							215-e-2	15ADON
103	2016	CWAD	6.4	287	Current	06-May-16	216-a-1	3ADON
							216-c-2	3ADON
104	2016	CWAD	49	287	Brigade	15-May-16	217-5-5	3ADON
							217-b-5	3ADON
105	2016	CWAD	5.6	169	Trancend	30-Apr-16	218-a	15ADON
							218-b-8	15ADON
106	2016	CWAD	16.8	257	Brigade	15-May-16	219-a-2	3ADON
							219-b-5	3ADON
107	2016	CWAD	15.1	257	CDC Fortitude	-	220-a-1	3ADON
							220-a-3	3ADON

							220-a-5	3ADON
108	2016	CWAD	11.5	288	Brigade	06-May-16	221-a-5	15ADON
							221-b-5	15ADON
109	2016	CWAD	11.6	257	Brigade	29-Apr-16	222-a-1	3ADON
							222-b-7	3ADON
110	2016	CWAD	11.2	342	-	12-May-16	223-a-4	3ADON
							223-a-7	15ADON
111	2016	CWAD	17.4	256	AC Strongfield	02-May-16	224-a	15ADON
							224-b-7	3ADON
112	2016	CWAD	24.1	253	AC Strongfield	19-May-16	225-a-2	3ADON
							225-b	3ADON
113	2016	CWAD	23.4	228	-	29-Apr-16	226-a-4	3ADON
							226-c-3	15ADON
114	2016	CWAD		162	Brigade	15-May-16	227-a-1	3ADON
115	2016	CWAD	7	259	-	-	228-a	3ADON
							228-b	3ADON
116	2016	CWRS	16.1	428	Utmost	09-May-16		
117	2016	CWAD	14.6	259	AC Strongfield	19-May-16	230-e-2	3ADON
							230-g-6	3ADON
118	2016	CWAD		162	Brigade	23-May-16	231-a-1	3ADON
							231-a-1	3ADON
							231-b-1	3ADON
119	2016	CWAD	16.4	228	Brigade	12-May-16	232-a-1	3ADON
							232-c-2	15ADON
120	2016	CWAD	8.3	286	Eurostar	29-Apr-16	233-b-3	3ADON
							233-c-5	3ADON
121	2016	CWAD	16.7	257	Brigade	N/R	234-c-1	3ADON
							234-d-1	3ADON
122	2016	CWAD	10.5	259	AC Strongfield	19-May-16	235-a-4	15ADON
							235-c-1	15ADON
123	2016	CWAD	5.3	259	AC Strongfield	04-May-16	236-a-1	3ADON

							236-b-1	15ADON
124	2016	CWAD	19.5	287	Brigade	02-May-16	237-a-2	3ADON
							237-d-4	3ADON
125	2016	CWAD	5.7	285	Verona	03-May-16	238-a-1	15ADON
							238-b-1	3ADON
126	2016	CWAD	9	254	Brigade	18-May-16	239-a-2	3ADON
							239-f-6	3ADON
127	2016	CWAD	7.4	257	Brigade	05-May-16	240-a-1	3ADON
							240-b-1	3ADON
							240-c-1	3ADON
128	2016	CWAD	5.6	257	Verona	05-May-16	241-b-1	3ADON
							241-c	3ADON
129	2016	CWAD	5.5	37	-	-	242-a	3ADON
							242-b-3	3ADON
130	2016	CWAD	12.6	257	AC Strongfield	15-May-16	243-a	15ADON
131	2016	CWAD	14.5	287	Marchwell	03-Jun-16	244-a	15ADON
							244-b	3ADON
							244-d	3ADON
132	2016	CWAD	24	228	AC Strongfield	18-May-16	245-b-2	15ADON

CWAD- Canada Western Amber Durum, CWRS- Canada Western Red Spring, CPS- Canada Prairie Spring.