Prevalence and Diversity of *Fusarium* Pathogens Causing *Fusarium* Head Blight on Oat in Manitoba

By

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ABSTRACT

In small grain cereals, *Fusarium* head blight (FHB), a serious plant disease caused by *Fusarium* spp., can cause significant yield losses and mycotoxin contamination. The most familiar *Fusarium* mycotoxins are trichothecenes of type-A (T-2, HT-2) and type-B (DON, NIV). Recent surveys conducted in western Canada have indicated that FHB was common in oat. So, this study focus is on the prevalence and diversity of *Fusarium* species found in commercial oat fields in Manitoba. A total of 168 oat samples were collected between 2016 and 2018. They were examined for the presence of *Fusarium graminearum, F. poae, F. avenaceum*, and *F. sporotrichioides* through morphological and molecular (conventional and real-time quantitative PCR) analyses. Our results showed that *F. poae* is the most common *Fusarium* species in oat, followed by *F. greaminearum* and *F. sporotrichiodies*. We used a phylogenetic approach to examine the relationship among *F. poae* strains from Manitoba using concatenate DNA sequences of translation elongation factor 1-α, TRI1 and TRI8. Furthermore, the level of mycotoxins in oat grain samples was analyzed using LC-MS/MS, and the correlation between *Fusarium* DNA and mycotoxin levels was investigated.

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 General Introduction

Oat (*Avena sativa*) is a cereal grain from the Poaceae grass family. The use of this grain as human food is increasing because of its unique nutritional value and health benefits for humans (Peterson, 1992). Over the last few decades, Canadian oat has turned from a domestic crop into a major export crop. Since then, Canadian farmers have regularly increased their share of the global oat market (www.poga.ca/marketaccess). Agriculture and Agri-Food Canada (AAFC) estimated that the 2019 oat production area in Canada was 3.6 million acres, representing an 18% increase over the 2018 cropping season (www.producer.com). Numerous reports demonstrated that the mycotoxin-producing fungal pathogens that cause *Fusarium* head blight could jeopardize oat products as healthy food (Clear et al. 1996, 2000; Tekauz et al. 2004, 2008; Gräfenhan et al. 2013; Banik et al. 2020). *Fusarium* spp. produce a wide spectrum of type A and B trichothecene mycotoxins, as well as various non-trichothecene mycotoxins, which affect grain quality and yield of numerous cereal crops, including oats (Groopman and Pestka, 2014; Binder 2007; Clear et al. 2000). The most frequent mycotoxins found in *Fusarium*-infected cereal grains are DON, its acetylated derivatives 3-ADON (3-acetyl deoxynivalenol), 15-ADON (15-acetyldeoxynivalenol), and NIV (nivalenol) (McCormick, 2003). These mycotoxins have long-term or short-term toxicity in animals, humans, and plants. Common symptoms associated with trichothecenes include dizziness, feed refusal, vomiting, vertigo, and bleeding. In North America, several *Fusarium* species such as *Fusarium graminearum*, *Fusarium poae*, *Fusarium sporotrichioides*, *Fusarium avenaceum,* and *Fusariumculmorum* are considered primary causative agents of FHB in all small grain cereals (Gilbert and Tekauz, 2000). On barley and wheat, *F. graminearum* has been the most common species

associated with FHB (Tekauz et al., 2000). FHB was not recognized as an oat disease or a public health hazard in Western Canada until 2002 (Tekauz et al. 2003a). However, Clear et al. (1996) had previously detected FHB symptoms and the occurrence of deoxynivalenol (DON) in oat grains from Manitoba. *F. poae* and *F. graminearum* were the primary *Fusarium* species isolated from affected oat kernels (Tekauz et al. 2004). Similarly, recent increases of FHB on oat in the prairie provinces have been documented, and the annual FHB disease survey reports confirmed the dominance of *F. poae* followed by *F. graminearum* and *F. sporotrichioides* in oat grains from Manitoba (Banik et al. 2016, 2017, 2018, 2019). In addition, the major *Fusarium* species in Saskatchewan and Ontario were determined to be *F. poae* and *F. graminearum* (Olson et al. 2015; Dyck et al. 2015; Xue et al. 2016, 2017, 2018).

Fusarium. poae is a weak pathogen on wheat compared to *F. graminearum*. Still, it is capable of producing mycotoxins, including trichothecenes of type A (T-2 and HT-2) and B (NIV), beauvericin (BEA), and enniatins (ENN) (Magliano et al. 2013). T-2/HT-2 and NIV are more toxic than DON (D'Mello et al. 1999; Nielsen et al. 2009). According to the European Food Safety Authority (EFSA, 2017), a tolerable daily intake (TDI) is about 0.02 μgkg^{-1} body weight for T-2/ HT-2, 1750 μgkg^{-1} body weight for DON and 1.2 μgkg^{-1}body weight for NIV. Compared to FHB on wheat and barley, the information on oat FHB and associated mycotoxins are very limited. It is crucial to have a detailed understanding of common *Fusarium* spp. and naturally occurring mycotoxins related with FHB on oat and other possible factors such as chemotype variability and genetic diversity of these *Fusarium* pathogens. This study aimed to (1) identify the dominant *Fusarium* species and mycotoxins related with FHB on oat in Manitoba; (2) investigate the correlation of mycotoxins with *Fusarium* species; (3) analyze the chemotypes of the collected *F. poae* and *F. graminearum* isolates; and (4) examine the genetic diversity of *F. poae* population in Manitoba.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Oat

2.1.1 Origin of Oat

Oat (*Avena sativa*) is a cereal crop of the Graminae family that originated from Western Asia and Eastern Europe (Hoffman et al., 1987). Oat has three ploidy levels, including diploid, tetraploid, and hexaploid. Most cultivated oats (*A. sativa*) are hexaploids (2n = 6x = 42), originating as an aggregation of three diploid genomes (AA, CC, DD) (Rines et al. 2006). Tournefort was the first person to describe oat and establish the genus Avena in 1700. Later, Linnaeus (Hoffman et al. 1987) described four oat species *Avena sterilis*, *A. fatua*, *A. sativa,* and *A. nuda*. In southern Europe, oats are grown as pasturage or forage crops. In North America, oats were brought from Europe and first settled in Florida, USA. In Canada, oats were first introduced in Quebec City, then Newfoundland in 1622 (Murphy et al. 1992). Oat is best adapted to cool regions for full seasonal growth and plump grain development. Oat cultivated in high temperatures often decreases productivity with low grain filling and poor quality due to the short growing season. Oat is often grown as a winter forage crop in mild temperate and subtropical regions like the southern USA, southern Europe, India, and east-central Australia (Rines et al. 2006).

2.1.2 Importance of Oat

Oats are mainly grown as forage, human food, and livestock feed. Oat grains consist of a hull (outer covering) and a groat (caryopsis). Oat groats are used in the milling industry to produce oat-based foodstuffs, such as oat flakes, oat flour, oat bran, oat cereals, and cereal bars. Oat hulls are primarily used for ruminant feeds. Some insoluble cellulosic fibres from hulls have also been incorporated into food products (Stevenson et al., 2011).

Historically, oats were considered an inconsequential coarse grain accounting for 5-7% of world coarse grain production. Most oat grains were processed for animal feeds (Welch et al., 2012). In the last decade, oat has become popular in many parts of the world. Oat production currently ranks sixth globally in terms of small grain cereal production, trailing corn, wheat, barley, sorghum, and millet.

The demand for oat-based food and pharmaceutical products has increased in the last decade due to their unique nutritional benefits and exceptional multifunctional characteristics. Oat provides a good source of dietary fibre, especially β-glucan, which moderates blood glucose and insulin peaks and lowers cholesterol levels (Brennan and Cleary, 2005). Oats are also a great source of protein and lipid. Because of their high water-holding capacity, oat flours are commonly used in baby food. (Welch, 1995). Oat and its by-products have become popular for diabetes and cardiovascular disorders treatment. Mainly, oat bran is high in vitamins B, protein, and fat. β-glucan has enormous functional properties, such as viscosity, reduction of plasma glucose and insulin responses, high transportation and excretion of bile acids for the intestinal tract and sinking of serum cholesterol levels. The combination of oat grains and oat bran may advance food products with nutrition and therapy against various illnesses (Butt et al., 2008).

2.1.3 Oat production and trade

Russia is the largest producer of oat, accounting for 20% of total global production. The total production of Russian oat was 4828 million tons from 2016 to 2019. They exported 140 million tons of oat to different countries, with 14.7% of economic growth in 2019. (https://www.indexmundi.com/agriculture). From 2004 to 2008, the United States and 27 EU member states and Australia contributed 77 % of the world's supply of oat grain, seed, and industrial-graded oat (Strychar et al. 2011).

World oat trade has changed significantly in the past 15 years, based on U.S. imports and Canadian and Scandinavian exports. Canada accounted for 64% of oat exports in 2005-2009, followed by Sweden and Finland (16%) and Australia (5%) (Strychar et al. 2011). Canada is a major commercial oat exporter, making up 15% of total global production and 60% of total global trades (Strychar et al., 2011). The average Canadian oat production was 3640 million tons from 2016 to 2019, and the annual average export was 1702 million tons (https://www.indexmundi.com/agriculture). The United States is the principal commercial importer of oats with two-thirds of all annual imports. Other minor oat importer countries are Japan and Mexico, less than 2% each year. However, Sweden and Finland are the main dominators of horse-quality markets in the United States.

2.1.4 Oat in Canada

Canada is among the world's leaders in the production and export of high-quality oats. Saskatchewan, Alberta, British Columbia, and Manitoba are the primary producer of oats. In 2019, Western Canada produced 3.7 MT of oats from a total Canadian production of 4 MT. Saskatchewan (2 MT) and Manitoba (0.7 MT) was the leading oat-producing province in Canada in 2019, followed by Alberta and British Columbia (0.8 MT) (Fig 2.1) (https://grainscanada.gc.ca). In 2019, 0.2 Mha of land w as used for Manitoba oat production to produce 0.7 MT of oat (https://grainscanada.gc.ca), which is approximately 18 to 19% of Canada's total oat production (Manitoba Agriculture).

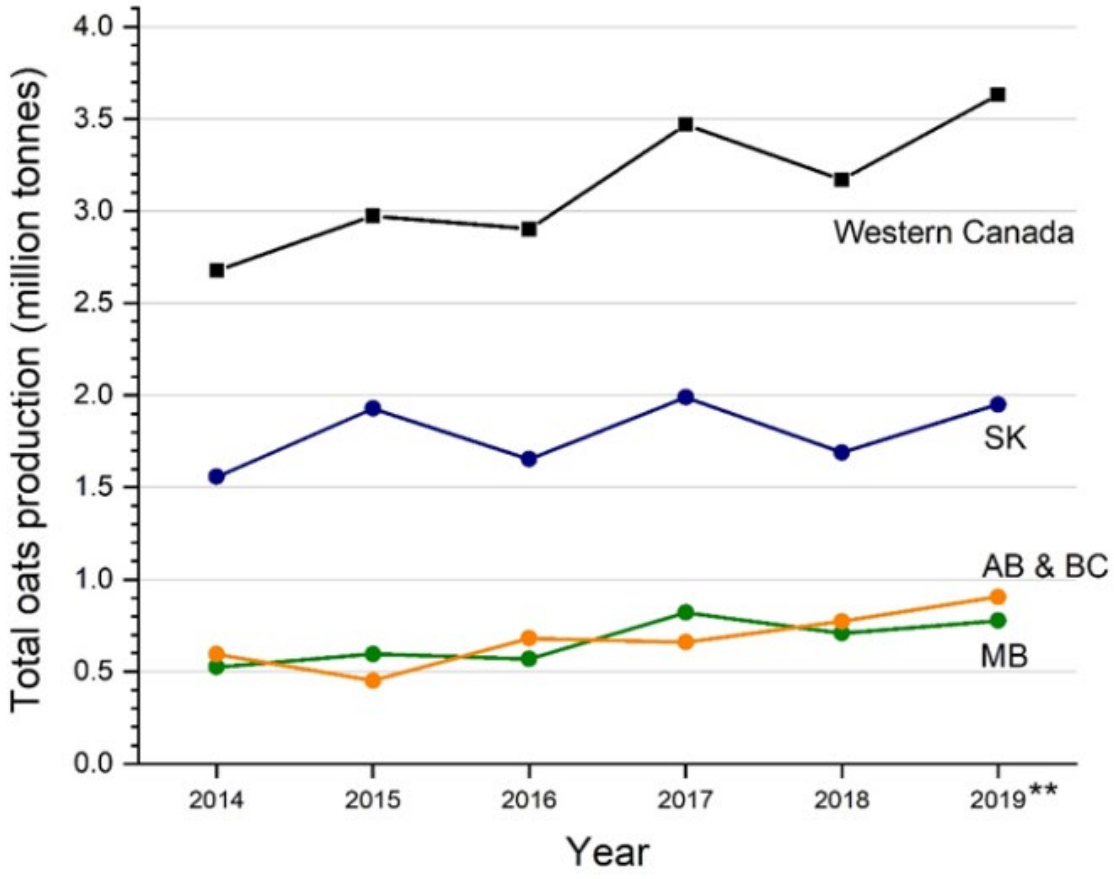

Fig 2. 1 Yearly comparison of total oat production in Canada

Canadian oats are mainly exported to the USA and Mexico. Ninety percentage of Canadian oats export go to the USA, followed by Mexico. Moreover, Canada has market access to China for pre-processed oats and oat seeds (for cultivation). In 2017, Canada exported CAD$4 million oat seeds and CAD$605,135 processed oats to China. Canadian oat export has increased from 1.4 to 2.4 MT annually, and most of this is exported to the United States (https://poga.ca/about-oats/market-access).

Canadian oats are used as milling oat, feed, forage, and hulless oat in the Canadian domestic market. The milling oats are used for oat flakes, oat flour, oat bran, oat cereals, and

cereal bars. From 2015-2019, 84% of Canadian milling oat was produced in Western Canada (Fig 2.2). An average of 6.5% oat was used as feed and forage for animals from 2015 to 2019 (https://grainscanada.gc.ca). The hulless oat has a niche market in the Canadian domestic market as well as the international market. This type of oat could be used for both seed and human consumption purposes.

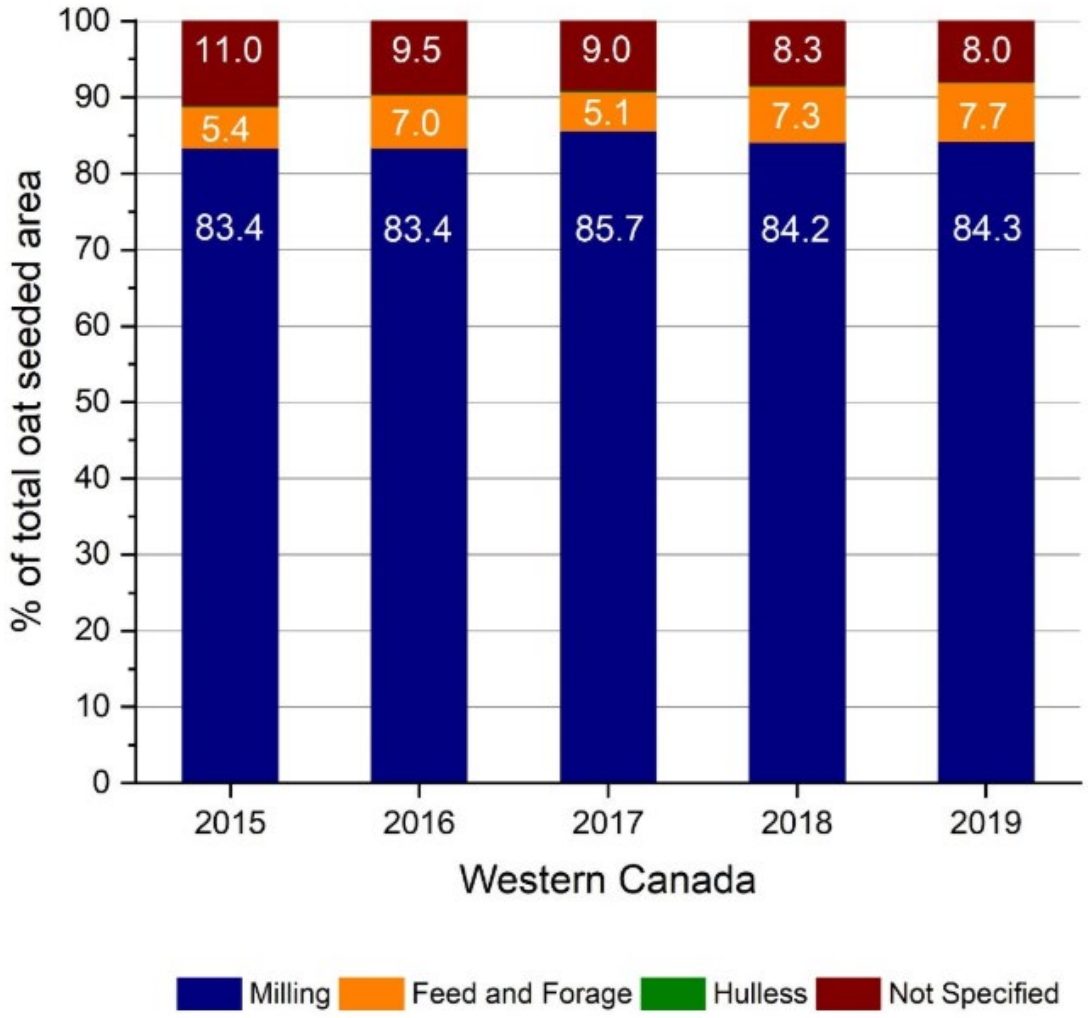

Fig 2. 2 Distribution of oat classes as percentage in western Canada from 2015 to 2019.

2.2 *Fusarium*

Fusarium is one of the most economically damaging genera of filamentous ascomycetes, including pathogens that cause head blight, seedling blights, wilts, cankers, and root rots in a wide range of commercially important crops (Ma et al., 2013, and Amarasinghe, 2011).

2.2.1 Origin and Classification

Fusarium is a genus of ascomycetous fungi belonging to the Nectriaceae family, Hypocreales order, Sordariomycetidae class, Ascomycota phylum, and Fungi kingdom (Guo, 2008). The genus *Fusarium* is split into 16 sections, four of which are important for *Fusarium* head blight disease, including Discolor, Gibbosum, Roseum, and Sporotrichiella (Liddell, 2003). *Fusarium graminearum*, *F. culmorum,* and *F. crookwellense* belong to section Discolor; *Fusarium equiseti*, *F. scirpi,* and *F. acuminatum* are contained in section Gibbosum; *Fusarium avenaceum* is from the section Roseum, and *F. sporotrichioides* , *F. tricinctum,* and *Fusarium poae*, are from the section Sporotrichiella (Guo, 2008).

2.2.2 Morphological characteristics of *Fusarium* species.

The formation of macroconidia, microconidia, and chlamydospores is an important morphological feature that distinguishes *Fusarium* spp. from other genera (Summerell et al., 2003). *Fusarium* spp. often differ in colony size, growth rate, pigmentation, the existence of macro and/or microconidia, and the size and number of chlamydospores (Windels, 1991).

Macroconidia are multi-septate, moon crest, or banana-shaped spores produced by *Fusarium* spp. within a sporodochium. *Fusarium* spp. produce macroconidia in a variety of shapes, ranging from straight to dorsiventrally curved. The shapes of macroconidia's end, apical, and basal cells are used to distinguish *Fusarium* species (Leslie and Summerell, 2006). The chlamydospores developed by *Fusarium* species are dense and loaded with spores

that transport the fungus overwinters in soil when a suitable host is not available (Nelson et al. 1994). Chlamydospores are typically formed in hyphae or conidiospores of older cultures. Usually, true chlamydospores have a warty appearance (verrucose) with a thick wall and light yellow-brown coloration. They can be found in a chain shape, either clumps or singly at the top of hyphae (Summerell et al., 2003). *Fusarium oxysporum*, *F. equiseti*, *and F. solani* produce chlamydospores for their survival (Nelson et al. 1994).

Fusarium graminearum (teleomorph: *Gibberella zeae*) produces vivid carmine-red mycelium and classic sickle-shaped macroconidia. Microconidia production is not reported in *F. graminearum* (Liddell, 2003). *Fusarium poae* belongs to section Sporotrichiella. On potato dextrose agar media, *F. poae* produces white to pink coloured, dense aerial mycelium with globose to piriform microconidia. The macroconidia are intermittent, spindle, and have a foot-shaped basal cell (Stenglein, 2009). The colony colour can range from white to yellow to deep carmine red depending on the culture age. The teleomorph of *F. poae* is unknown. *Fusarium culmorum*, *F. solani*, *F. equiseti*, *F longipes*, *F. avenaceumn* are also known as the producer of macroconidia. Some other *Fusarium* spp., such as *F. verticillioides*, *F. subglutinans* and *F. proliferatum,* are known to disperse through microconidia (Munkvold, 2003). These microconidia are present in conidiogenous cells that produce microconidia from the hyphae. There are two types of microconidia: monophialides with a single opening in the conidiogenous cell and polyphialides with two or more openings in the conidiogenous cell (Summerell et al., 2003).

2.2.3 Causal Agent of *Fusarium* Head Blight

Fusarium graminearum

FHB is a serious crop disease that affects small grains. It is caused by a *Fusarium* species complex, including *F. equiseti, F. poae, F. avenaceum, F. culmorum, F. graminearum* and *F. sporotrichioides* (Kosiak et al. 2003).

Fusarium graminearum is the most distinctive *Fusarium* species causing FHB in North America, Europe, and countries in other temperate climates (Desjardins, 2006). This pathogen can produce various B-trichothecene metabolites such as NIV, DON, 3-ADON, and 15-ADON (O'Donnell et al. 2004). DON is the most common mycotoxin found in *Fusarium*-infected cereals in North America. DON and NIV are the most frequent mycotoxins identified in cereal grains in Asia and Europe (Van der Lee et al. 2015).

FHB in North America was initially associated with *F. graminearum* isolates with 15-ADON chemotype. Since the early 2000s, a shifting of the *F. graminearum* population toward the 3-ADON chemotype has been reported (Guo et al., 2008). These 3-ADON chemotype isolates could pose a significant threat to the grain industries and farmers since they can supply more DON than 15-ADON chemotype isolates during the infection (Ward et al. 2005).

Fusarium poae

In different cereal surveys, *Fusarium poae* is the most frequently isolated FHB pathogen from several European and South American countries (Stenglein, 2009). In western Canada, *F. poae* has caused a level of infection that is very similar to *F. graminearum* in barley and oat in recent years. From 2011 to 2016, *F. poae* is the most frequent *Fusarium* species isolated in oat and barley in Canadian prairies and eastern Canada (www.phytopath.ca/publication/cpds/). Generally, *F. poae* is a weak pathogen compared to *F.*

graminearum and *F. culmorum*. It can produce a large number of trichothecenes type A and B mycotoxins, including BEA, Fus and Enn (Dinolfo et al. 2010). Although *F. poae* has no known sexual stage (Kerényi et al. 1997; Kerényi et al. 2004), it has *MAT1-1* and *MAT1-2* in its genome, known to be critical genetic fingerprints for the sexual cycle (Kerényi et al. 2004). The existence of repeat-induced point mutation (RIP), a defence mechanism against transposable elements (TEs), has recently been found in the *F. poae* genome (Vanheule et al., 2016).

Fusarium sporotrichioides

The morphology of *F. sporotrichioides* is similar to *F. poae,* but the overall profile of trichothecenes produced by *F. sporotrichioides* is very similar to that of *F. langsethiae* (Torp and Langseth, 1999; Torp and Nirenberg, 2004). Knutsen et al. (2004) described the main morphologically separating key characters among these three species. The lack of macroconidia, polyphialides and chlamydospores differentiates *F. poae* from *F. sporotrichioides*. The presence of monophialides and the occasional polyphialides/polyblastic conidiogenous, globose to napiform one-celled microconidia in *F. langsethiae* differentiates it from *F. sporotrichioides*.

Fusarium sporotrichioides is a potent producer of T-2/HT-2 mycotoxins. Between 1942 and 1947, *F. sporotrichioides* was identified as the most toxic *Fusarium* species in Russia, which contaminated overwintered grains with T-2/HT-2 toxin (Torp and Langseth, 1999). In the temperate regions of Northwest Europe, *F. sporotrichioides* is identified as the key contributor of T-2/HT-2 mycotoxins in oat (Thrane, 2001; Wilson et al., 2004).

Fusarium avenaceum

Fusarium avenaceum is a cosmopolitan plant pathogen that affects over 80 different plant genera (Leach and Hobbs, 2013). The anamorphic (*Gibberella avenacea*) state of *F.*

avenaceum is one of the most important species worldwide (Logrieco et al. 2002b). This species is widespread in Northern Europe and Canada (Parry et al. 1995; Yli-Mattila et al. 2004).

Fusarium avenaceum is usually a non-producer of trichothecenes (Langseth et al. 1999b, Edwards et al. 2001), and it can produce large amounts of toxic compounds including moniliformin (MON), BEA, ENN, and 2-Amino-14,16-dimethyloctadecan-3-ol (2-AOD-3-ol) (Uhlig et al. 2007). BEA and ENN are two well-known cyclic hexadepsipeptides that inhibit cholesterol acyltransferase. (Tomoda et al. 1992).

Fusarium avenaceum is morphologically and genetically diverse. *F. avenaceum* showed high genotypic diversity in European populations. For example, there were 67 haplotypes among 68 *F. avenaceum* isolates (recovered mainly from *Lupinus albus* L.) from the UK (Satyaprasad et al., 2000). *Fusarium avenaceum* is heterothallic, and each strain carries either the *MAT-1* or *MAT-2* idiomorph (Kerényi et al., 2004).

2.3 *Fusarium* Head Blight

Fusarium head blight (FHB) is a vital disease principally attacking wheat, barley, oat, and other grains worldwide. FHB poses a double threat to the grain industry since it decreases the grain yield and produces mycotoxins that are injurers to humans and animals. This disease has been recounted in all cereal-growing regions worldwide. Seventeen *Fusarium* species are associated with FHB (McMullen et al. 1997). In many countries, *F. graminearum* is regarded as the primary pathogen of FHB (McMullen et al., 1997; Gilbert and Tekauz, 2000). Other *Fusarium* species, including *F. culmorum*, *F. avenaceum*, *Microdochium nivale*, *F. moniliforme*, *F. oxysporum,* and *F. poae,* are also associated with FHB (Liddell, 2003).

Fusarium poae was the major *Fusarium* species isolated in England, Wales Poland (Logrieco et al. 2002). Several researchers have reported the predominance of *F. poae* in Manitoba, Canada (Gilbert and Tekauz, 2000).

2.3.2 Symptoms of *Fusarium* Head Blight

Fusarium head blight (FHB) symptoms are apparent before the grain matures and turns yellow in the field (Guo, 2008). At the base or apex of a panicle, a tan discoloration or water-soak appearance can be seen. Eventually, it spreads further from the point of invasion. The development of salmon pink to red-coloured mycelia can be seen at the base of the panicle and spreads across the entire head in moist condition. FHB can result in infected grains being bleached, shriveled, and chalky white, with black perithecia (Amarasinghe, 2011). *Fusarium*-damaged kernels (FDK) are also shrivelled, light, and dull greyish or pinkish (Bushnell et al., 2003).

2.3.3 *Fusarium* Head Blight Disease Cycle

Fusarium-infected crop residues on the soil surface serve as the most important sources of inoculum (Parry et al., 1995; Shaner, 2003; Bai and Shaner, 2004). Although macroconidia, chlamydospores, and *Fusarium* hyphal fragments can act as inoculum, airborne ascospores are the primary source of inoculum (Bai and Shaner, 2004; Fernando et al., 1997). In contrast, the secondary spread only has a minimal role in disease development. In most of the infested residues, the fungus produces asexual spores (macroconidia), dispersed to plants or plant debris by rain-splash or wind (Schmale et al., 2003). The amount of inoculum is fixed by the amount of *Fusarium*-infected crop residues on the soil surface

(Dill-Macky and Jones, 2000).

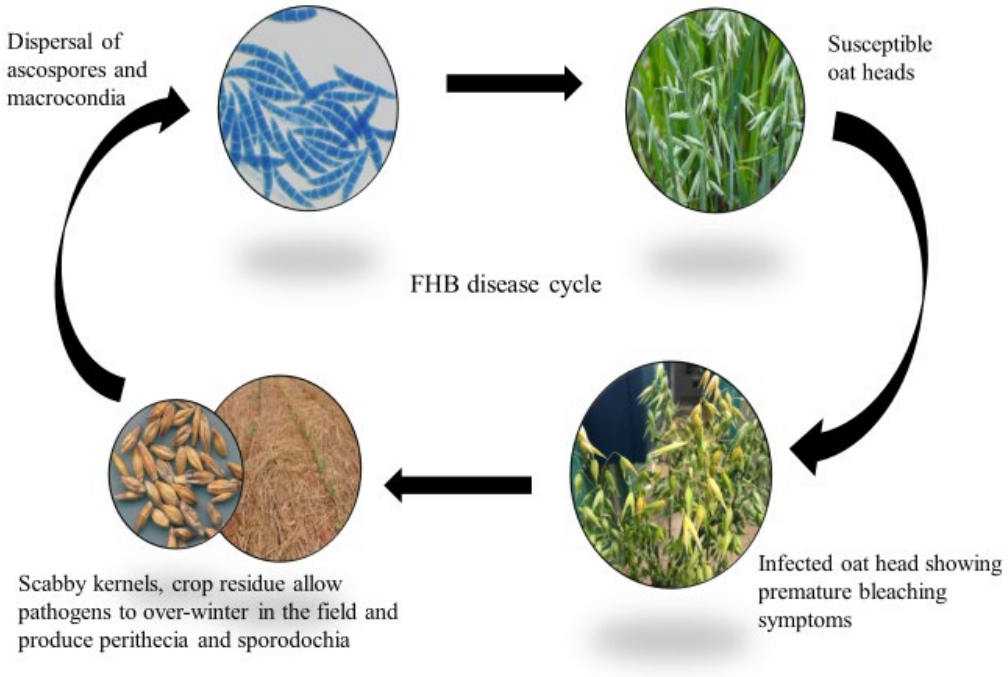

Fig 2. 3 The diagram depicts the *Fusarium* Head Blight disease cycle, starting with inoculum distribution and ending with oat harvest.

Fusarium spp. may live on both living and dead plant tissue and has a wide range of hosts (wheat, oat, barley, ray, maize). *Fusarium* infection is most sensitive during or immediately after the flowering stage of the host plant (Amarasinghe, 2011). The window for FHB infection in cereal is narrow, lasting only from anthesis to early grain development. Furthermore, when barley heads are exposed to *Fusarium* spores outside the leaf sheath, the barley plant becomes more vulnerable (McMullen et al., 2012). Visual signs first occur at the floret tip and then progress to the basal floret portions in oat (Tekle et al., 2012).

During infection, the thick-walled epidermal and hypodermal cells are the main penetration pathway for *Fusarium* pathogens that infect the external floret surface (Bushnell et al., 2003). Hyphal expansion on the outside surfaces allows fungal growth into stomachs

and invasion in glume or bloom of more sensitive sites. Once infected, florets do not produce grain or are not filled correctly (Bushnell et al., 2003).

For example, temperature and moisture have an impact on inoculum formation, dispersal, and the duration of the ideal infection environment. Warm and humid circumstances favor infection and disease development during flowering and the early phases of kernel development (Parry et al. 1995). FHB symptoms usually appear following a period of continuous moisture or rains and with a temperature between 10 °C to 25–30 °C during that anthesis (Chulze, 2013).

Wind or rain splashes disseminate Fusarium conidia. There is evidence of ascospores being discharged several hours or days following rainfall events, indicating that rainfall and high relative humidity influence ascospore dissemination (Fernando et al. 2000; Inch et al 2005). Gilbert and Tekauz (2000) reported that rainfall of >5mm and relative humidity of >80% inhibited ascospore release. During the flowering period of the host plant, a combination of 92-94 percent relative humidity and warm temperatures creates an optimum environment for FHB establishment and development (Sutton, 1982).

2.3.4 Infection Pathway of *Fusarium* species

The stomates are one of the infection pathways for FHB pathogens. Kang and Buchenauer (2000) demonstrated that *F. culmorum* hyphal penetrates through stomatal openings on the inner surface of wheat lemma. The thin-walled and photosynthetically active chlorenchyma provided an entry point for this fungus (Guo, 2008). Wheat pollen could also be colonized by *F. graminearum* and played a vital role in the infection of the florets. Guo (2008) reported that extruded anthers could be attacked and colonized by *F. graminearum* and made a path for the fungus into the florets. Moreover, a close relationship was found

between the infection and the presence of the extruded anthers. It was suggested that the secretions of the anther could stimulate the growth of *Fusarium* hyphae (Strange and Smith, 1971).

It has been demonstrated that *Fusarium* infections on oat occur on a single-spikelet basis. Infections rarely spread from spikelet to spikelet because of the long rachilla and rachis of the oat panicle (Tekle et al., 2012). The apical surfaces of oat florets have more lesions and mycelial colonies of *F. graminearum* (Tekle et al., 2012). The earliest visible symptoms appear at the floret tip and spread to the rest of the floret. The floret mouth is the primary channel for the fungus to enter the floret cavity, according to an FHB epidemic investigation in Norway (Tekle et al., 2012). For *Fusarium* pathogens, the cracks between the palea wing and the lemma wing near the floret mouth could represent an alternate entry point. Hyphae can colonize the caryopsis by growing from the apical sections of the lemma into the floret cavity and the exterior surfaces of the upper parts of the palea wings (Bjørnstad & Skinnes, 2008). During the early phases of *Fusarium* infection on oats, anthers appear to play a crucial role (Lewandowski et al. 2006). Because *Fusarium* spp. has a significant affinity for anthers, a high degree of anther ejection is significantly linked to reducing FHB on oat (Skinnes et al. 2010).

2.4 *Fusarium* Head Blight Control Strategies

2.4.1 Cultural control

Cultural control is a non-hazardous approach to managing FHB. Various cultural control strategies can be used, such as tillage and stubble management, crop rotation, weed control, planting dates, and sowing types with various heading dates. Tillage and stubble management have significant short-term as well as long-term effects on soil-borne fungi. *Fusarium* pathogens colonize in the upper 10 cm soil layer. The tillage depth could affect the

survival of *Fusarium* pathogens in soil (Steinkellner and Langer, 2004). Schmale et al. (2006) also showed that deep tillage reduces the abundance of *Fusarium* spp. at the soil surface.

Crop rotation is another method that can be used for the management of FHB. Non-hosts, such as canola, pulses, and forage legumes, minimize the likelihood of disease spread and increase, according to a three-year crop rotation research (Edwards, 2004). Rotation of wheat with non-host crops reduces the amount of infested wheat stubble (Guo, 2008). Compared to the corn-wheat rotation, Dill-Macky and Jones (2000) demonstrated a 25% reduction in DON contamination when wheat was planted after soybean.

Early or late planting is also an option to protect crops from *Fusarium* pathogens (McMullen, 2002). Another cultural practices that can be used to decrease FHB is weed control. Weed removal may make alternate FHB inoculum less available (Pirgozliev et al., 2003).

2.4.2 Biological control

Biological control is a way of controlling infections with microbial agents that is both environmentally benign and effective (Wegulo et al., 2015). By direct parasitism, competition for space and nutrition, antibiosis, inhibiting mycotoxins manufacturing, and activating host defence responses, these biological control agents (BCAs) can deteriorate or eradicate the pathogen (Agrios, 2005). In the biological management of FHB, spikelet infection, colonization, ascospore generation, and dissemination are possible locations of physical intrusion (Amarasinghe, 2011). In the infection trial of the head, the pathogen can be physiologically managed by blocking, halting, or delaying spore germination (Fernando et al., 2000). Various research teams have investigated using a wide range of microbes to prevent the spread of FHB (Table 2.1).

Table 2. 1 List of Biological Control Agents to control *Fusarium* Head Blight in small grain cereals

BCAs	**References**
Bacillus spp.	Luz et al. 2003; Fernando et al. 2002
Pseudomonas fluorescens	Khan and Doohan et al. 2009
Pseudomonas frederikbergensis	Khan and Doohan et al. 2010
Streptomyces spp.	Nourozian et al. 2006
Clonostachys rosea	Xue et al. 2009
Lysobacter enzymogenes	Jochum et al. 2006
Trichoderma harzianum	Inch et al. 2007; Matarese et al. 2012
Cryptococcus spp.	Khan et al. 2004; Schisler et al. 2011
Sclerotinia sclerotiorum	Fernando, 2003
Sporobolomyces roseus	Luz et al., 2003
Paenibacillus lentimorbus	Luz et al., 2003
Aureobasidium pullulans	Wachowska and Glowacka, 2014

2.4.3 Chemical control

Chemical control is one of the essential parts of an integrated FHB management approach. It is commonly used to manage both DON accumulation and disease severity. Several factors, such as the inoculum level, genetic resistance, climatic conditions, crop sensitivity, and yield potential, affect the effectiveness of fungicides in controlling FHB (Mesterházy, 2003b). The most widely used fungicides for the management of FHB are the demethylation inhibitor (DMI) class (McMullen et al., 2012). They include metconazole, propiconazole, prothioconazole, tebuconazole, and prothioconazole + tebuconazole. Tebuconazole, metconazole, and prothioconazole provide the most effective control of FHB in wheat (Paul et al. 2008; Pirgozliev et al. 2002). Meta-analyses of fungicide trials in the U.S. showed that prothioconazole + tebuconazole, metconazole, and prothioconazole were

superior to propiconazole and tebuconazole in suppressing FHB and DON, and all five fungicides resulted in significant yield and test weight increase and reduction in FHB and DON compared to the check (Paul et al. 2008, 2010). In addition, Tebuconazole had evidenced as the most effective fungicide against FHB in Europe (Mesterhazy et al., 2003). In Canada, several registered products, including Caramba ®, Folicur®, Proline®, Prosaro® are recommended for the management of FHB (Gilbert & Tekauz, 2011). Fungicides application through seed treatments could also be very effective against seedling blight to reduce fungal growth (Gilbert and Tekauz, 2000).

There are challenges associated with fungicide applications to suppress FHB and DON. One of the challenges is uneven maturity times (McMullen et al., 2012), which require multiple applications targeted at specific areas in a field. Extensive use of specific fungicides could also cause environmental concerns and induce pathogen resistance to that fungicide (Gilbert and Tekauz 2000). Moreover, without having accurate disease forecasting, it's challenging to reduce FHB severity and toxin accumulation through fungicide applications effectively.

2.4.4 Genetic control

Resistant cultivars are an essential part of FHB management, which is cost-effective and environmentally friendly. FHB resistance mechanisms are classified into five categories. Type I, tolerance to the initial infection; type II, resistance to pathogen spread within an infected spike; Type III, anti-kernel infection resistance; Type IV, disease tolerance, which means the tolerant oat cultivars may maintain yields even when the disease is present; type V, resistance to mycotoxin (Mesterhazy, 2003).

Type I resistance can be determined in the field after artificial inoculation. Type II resistance is often demonstrated under controlled environmental conditions through point

inoculation. The plants with this type of resistance have disease symptoms restricted to the inoculated florets (Gilbert and Tekauz, 2000). Studies have shown that cultivars with Type I and Type II resistance are more desirable as they are steadier and more resilient (Dweba et al., 2017).

Over the last two decades, much attention has been paid to quantitative trait loci (QTL) that confer FHB resistance. Over 100 QTL for FHB with variable levels of effectiveness have been identified. *Fhb1* is a major FHB QTL derived from the Chinese wheat cultivar 'Sumai 3', a widely used source of FHB resistance genes (Waldron et al. 1999). Subsequently, two novel FHB QTL, designated as *Fhb2* and *Fhb3*, were identified. *Fhb2* is located on chromosome 6B (Cuthbert et al. 2007). *Fhb3* was first discovered in *Leymus racemosus* and wheat-Leymus introgression lines. It is located on the short arm of chromosome 7Lr#1 (Qi et al. 2008). *Fhb5* (Qfhi.nau5A) is located on wheat chromosome 5A, controlling Type I resistance to FHB in wheat cultivar Wangshuibai (Xue et al. 2011). *Fhb6* was recently mapped on the sub-terminal region of the short arm of chromosome 1EIS#1S of a perennial grass *Elymus tsukushiensis* (Cainong et al. 2015).

In addition to QTL mapping, some alternative methods have been proposed to enhance host resistance to FHB. Haber et al. (2009) created a resistant wheat cultivar by manipulating critical resistance genes found in all wheat genotypes. A single seed of the doubled haploid Canadian wheat cultivar 'McKenzie' was used to inoculate multiple generations of selfed lines with Wheat streak mosaic virus (WSMV) (Amarasinghe 2011). These lines developed WSMV infection resistance, and some of them also showed improved FHB resistance, which was found to be persistent after three years of testing (Haber et al. 2009).

2.5 Types of *Fusarium* mycotoxins

Fusarium species produce extraordinary biologically diverse secondary metabolites known as mycotoxins (Desjardins, 2006). In 1903, *F. graminearum* and several other *Fusarium* species were associated with mycotoxins in animal farms (Chulze, 2013).

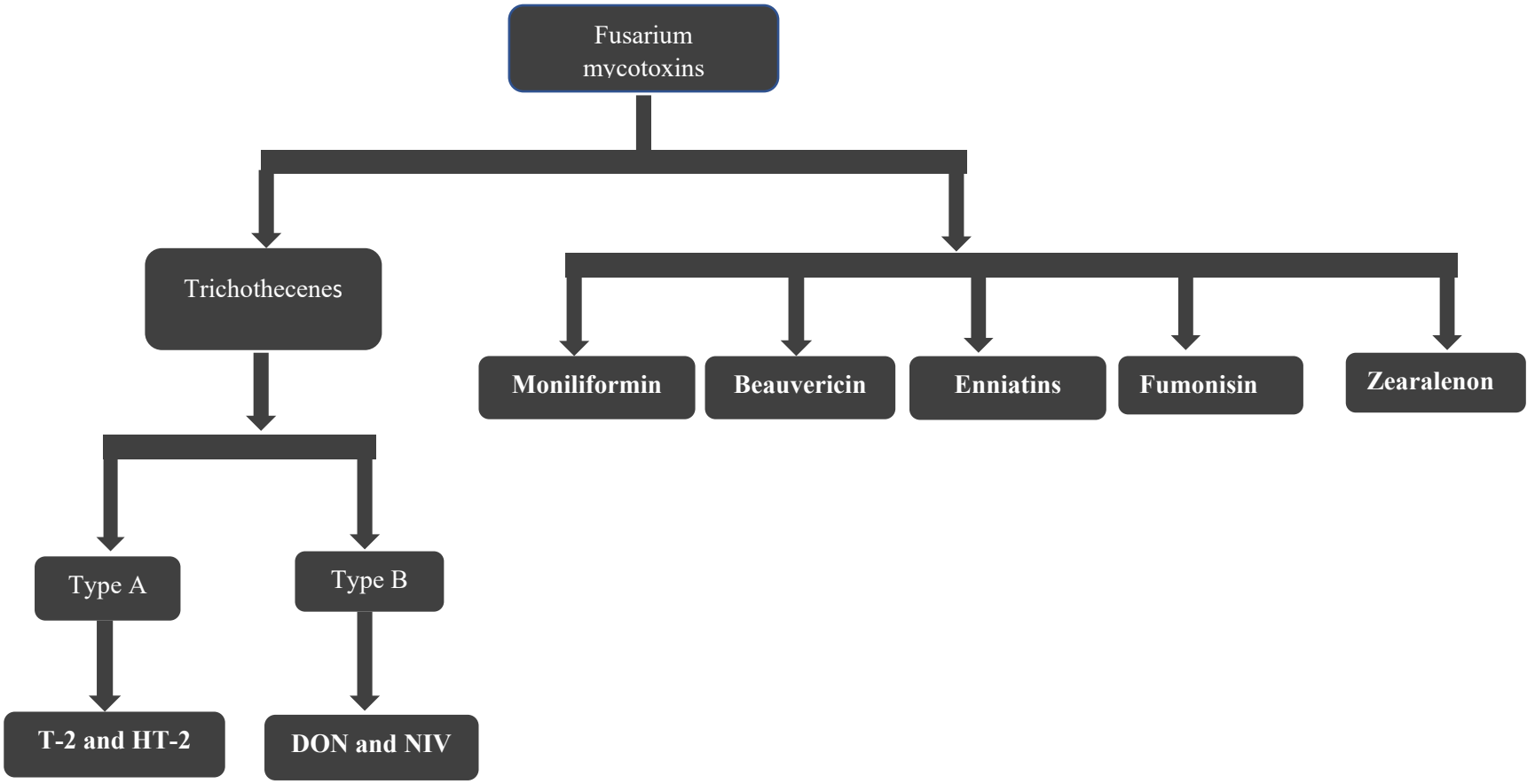

Fig 2.4 Mycotoxin produce by *Fusarium* spp

Bezuidenhout et al. 1988; Gelderblom et al. 1988; Pozzi et al. 2002; Hagler et al. 2001; Marin et al. 2013

2.5.1 Trichothecenes

Trichothecenes were first discovered as an antifungal metabolite in 1949 (Desjardins, 1993). Trichothecenes are a group of sesquiterpenoid metabolites produced by fungal pathogens from genera, including *Fusarium*, *Myrothecium*, *Phomopsis*, *Stachybotrys*, *Trichoderma*, and *Trichothecium* (McCormick et al. 2011). Trichothecenes are sesquiterpenoid alcohols that include the trichothecene tricyclic ring system, and they are biosynthesized through the farnesyl pyrophosphate pathway. The tricyclic ring construction contains a double bond at C-9, 10, and an epoxide group at C-13. The molecular weights of trichothecene typically range from 200-500 Da (Amarasinghe, 2011).

Trichothecenes prevent eukaryotic protein synthesis by inhibiting peptidyl transferase activity by acting selectively on ribosomal protein L3 within the 60S subunit (Rocha et al. 2005; Desjardins, 2006). *Fusarium* trichothecenes are classified into two types: type A and type B trichothecenes. Trichothecenes of types A and B differ by the absence (type A) or presence (type B) of a hydroxyl function at C-7. Type A trichothecenes (T-2 and HT-2) are also distinguished by the absence of a keto (carbonyl) function at carbon atom 8 (C-8). Type B trichothecenes (DON and NIV) are distinguished by the presence of a keto (carbonyl) function (Desjardins, 2009). Type-A trichothecenes are substantially more hazardous to humans and animals than type-B trichothecenes (Krska et al. 2001).

DON, NIV, and T-2/HT-2

The most common trichothecenes associated with *Fusarium* spp. are deoxynivalenol (DON), a type-B trichothecene. *Fusarium graminearum* and *F. culmorum* are potent producers of DON. Nivalenol (NIV) is another closely related type-B *Fusarium* trichothecenes (Desjardins, 2009; Nicholson et al., 2004). DON or its acetylated derivatives are less hazardous than NIV. As a result, NIV is the second most commonly occurring mycotoxin after DON in oat grain in Western Canada and Europe (Campbell et al., 2000;

Tittlemier et al. 2020; Schöneberg et al. 2018). Nivalenol can be produced by *F. graminearum, F. cerealis, F. poae* and *F. equiseti* (Marin et al., 2013). Several studies also documented the associations between *F. poae* and NIV content in cereal grains (Bosch et al., 1992; Christ et al., 2011; Vogelgsang et al., 2019; Tajrin, 2013).

Type-A trichothecenes commonly found in infected cereal grain are diacetoxyscirpenol (DAS), T-2, and HT-2 (Nicholson et al. 2004). T-2 and HT-2 are the main mycotoxins in oat, wheat, rye, and barley fields from Switzerland, Norway, and the UK (Schöneberg et al. 2018; Hofgaard et al. 2016; Hietaniemi et al. 2016; Bottalico et al. 2002; Morcia et al. 2016; Kosicki et al. 2020). However, T-2/HT-2 were not detected from samples collected from the Canadian Prairies collected between 2000 and 2002 (Clear et al. 2005). Contamination of T-2/HT-2 was detected (the maximum of 350ppb) in oat samples from southern Ontario (Tamburic-Ilincic, 2010). In most European countries, HT-2/T-2 and *F. langsethiae* always show a strong correlation in oat grains (Edwards et al. 2012; Tajrin, 2013; Schöneberg et al. 2018) though HT-2/T-2 showed a strong positive relation with *F. sporotrichioides* (Chapter 4).

For people and animals, these mycotoxins have various acute and chronic consequences (Zain, 2011). DNA, RNA synthesis, mitochondrial activity, cell division, and membrane effects are all inhibited by trichothecenes (Amarasinghe, 2011). Peptidyl transferase is a component of mammalian ribosomal subunit 60S, and these mycotoxins bind to it. They restrict membrane phospholipid metabolism and affect cell membranes (Omurtag, 2008; McCormick, 2003). Weight loss, reduced feed conversion, rejection, vomiting, bloody diarrhea, severe dermatitis, hemorrhage, lower egg production, abortions, and death are all signs of animal toxicity (Omurtag, 2008).

DON, also known as vomitoxin, disrupts cell function by binding to the ribosome, preventing protein synthesis. The main toxic effects of DON are immunosuppression or

immunostimulation. Low dietary DON consumption causes anorexia and stunting, whereas higher dietary DON consumption results in vomiting, immunotoxic effects, and changes in brain neurochemicals (Omurtag, 2008). The acute toxicity studies in animals showed that the DON's absorption, distribution, metabolism, and elimination differ from species to species. The maximum limits of *Fusarium*-produced mycotoxin such as deoxynivalenol (DON) in cereal grains range from 1.0 to 2.0 mg/kg (Health Canada, 2011; Tittlemier et al. 2013).

TRI **gene clusters**

The trichothecene biosynthesis is a complicated process involving 15 genes in the biosynthesis pathway (Desjardins, 2006). A cluster of genes complexes in the biosynthesis of trichothecene have been identified. One of the most studied secondary metabolite gene clusters in fungi is the trichothecene gene (*TRI*). The trichothecene biosynthetic gene cluster has been extensively studied in *F. graminearum* and *F. sporotrichioides*. These two species have a core cluster consisting of 12 genes responsible for synthesizing the trichothecene molecules (Proctor et al., 2018).

TRI5 was the first trichothecene biosynthetic gene discovered. This gene was first isolated as TOX5 from *F. sporotrichioides* (Hohn and Beremand, 1989). *TRI5* occurs as a single copy in the genome, and the interruption of *TRI5* will affect the biosynthesis of all trichothecene (Amarasinghe, 2011; McCormick, 2003; Desjardins, 2006). The cytochrome P450 monooxygenase genes *TRI4, TRI11*, and *TRI13*, the acyl transferase genes *TRI3* and *TRI7*, the esterase gene *TRI8*, the regulatory genes *TRI6* and *TRI10*, and the transporter gene *TRI12* are all members of the *TRI* cluster. *TRI9* and *TRI14* are also found in the core *TRI* cluster (Brown, 2002, 2003). Besides, *TRI1* is critical for the biosynthesis of the T-2 toxin. It was discovered after UV mutagenesis of *F. sporotrichioides* NRRL 3299, which resulted in mutants that could not generate T-2 toxin. *TRI1* mutant strain MB1716 accumulates 4,15-

diacetoxyscirpenol (4,15-DAS) and cannot add a hydroxyl group at C-8, demonstrating that *TRI1* is needed for hydroxylation at C-8 (Meek et al. 2003).

2.5.2 Zearalenone

Zearalenone (ZEA) is one of the most common mycotoxins produced by *Fusarium* spp. In America, Europe, and Asia (Omurtag, 2008). ZEAs are produced by many *Fusarium spp*., including *F. graminearum*, *F. culmorum*, *F. equiseti*, *F. pseudograminearum*, *F. semitectum* and *F. crookwellense*. *F. oxysporum F. poae* and *F. solani* do not produce zearalenones (Desjardins, 2006). High humidity and low temperatures conditions are favourable for ZEA production. ZEA is usually prevalent in infected corn, but it's also present in barley, oats, wheat, sorghum, millet, and rice to a lesser amount (Zinedine et al., 2007).

ZEAs are non-steroidal estrogens, and they have no active toxicity and no fatal mycotoxicosis associated with humans or animals. Nevertheless, ZEA, especially swine and sheep, can lead to infertilities, abortions or other breeding problems (Omurtag, 2008). According to the European Food Safety Authority (EFSA), the maximum consumption level of zearalenone in diets is 0.25 ppm body weight per day (European Food Safety Authority, 2014).

2.5.3 Fumonisin

Fumonisins are generated by several *Fusarium* species, including *Fusarium verticillioides* (formerly *Fusarium moniliforme=Gibberella fujikuroi*), *Fusarium proliferatum*, *Fusarium anthophilum*, *Fusarium nygamai*, and *Alternia alternata* f. sp. *Lycopersici*. Bezuidenhout et al. (1988) were the first to determine the chemical composition of fumonisins. Fumonisins structurally resemble sphingolipids and can alter sphingolipid biosynthesis. So far, twenty-eight fumonisins have been characterized, classified into four

groups (A, B, C, and P). FB1, FB2, and FB3 in group B are the major natural fumonisins contaminant found in cereals (Omurtag, 2008).

Fumonisins can induce leukoencephalamalacia, a fatal brain illness in horses, as well as porcine pulmonary edoema, a fatal condition in pigs. Furthermore, human esophageal cancer has been linked to FB1-contaminated grains, with many cases recorded in Africa, northern Italy, and Iran (Amarasinghe, 2011). The International Agency for Research on Cancer (IARC) evaluated fumonisin's cancer risk in humans and classified it as group 2B (probably carcinogenic). They poison animals, and at least one analog-like FB1 is carcinogenic in rodents (Omurtag, 2008). The European Food Safety Authority (EFSA) recommends a maximum of 2 ppm body weight per day for fumonisin consumption in diets.

2.5.4 Beauvericin

Beauvericin is a well-known mycotoxin produced by *Fusarium* spp such as *F. sporotrichioides*, *F. poae*, *F. langsethiae*, *F. avenaceum*, *F. subglutinans*, *F. proliferatum* (Marin et al. 2013). It has three D-hydroxyisovaleryl and three N-methylphenylalanyl residues in an alternating sequence. Beauvericin is an insecticide with antimicrobe and antiviral activities (Wang et al., 2012).

Beauvericin can trigger apoptosis and DNA fragmentation in human cell lines. In the case of the insulated guinea pig heart, beauvericin has adverse inotropical chronotropic effects (Logrieco et al., 2002). Moreover, beauvericin had significant antifungal activity against *Candida parapsilosis*, leading to high mortality rates in neonates (Zhang et al., 2007; Fukuda et al., 2004). Beauvericin is a mycotoxin, so its toxic effect on normal human cells and food safety has been investigated.

2.5.5 Enniatins

Enniatins (ENNs) are cyclohexadepsipeptides derived primarily from *Fusarium* species such as *F. avenaceum, F. tricinctum* (Marin et al., 2013). Twenty-nine enniatins, either as a single compound or as combinations of separate homologs, have been identified. However, only seven enniatins (A, A1, B, B1, B2, B3 and B4) have been found in cereal grains (Tittlemier et al. 2013). Enniatins are well-known for their ionophoric, phytotoxic, and anthelmintic properties, as well as their antibiotic activity and, more recently, their cytotoxic activity against cancer cell lines (Firakova et al. 2007). Furthermore, ENNs may have a genotoxic effect when several distinct ENNs are combinedly consumed (Krížová et al., 2021).

European Food Safety Agency (EFSA) calculated acute and chronic exposure of farm animals to ENNs (Krížová et al. 2021). In terms of chronic and acute exposure to ENNs, poultry had the most significant level (27.8 g/kg per day) and (113 g/kg per day). However, no concentration restrictions for ENNs have been defined (EFSA, 2014).

2.5.6 Moniliformin

Moniliformin (MON) is a common mycotoxin in cereals produced by *Fusarium avenaceum*, *F. proliferatum*, *F. subglutinans*, *F. tricinctum*, and *F. verticillioides* (Uhlig et al. 2004). MON is a tiny (98.0081 g/mol) acidic molecule with a strong polarity. It does not occur as an acid in nature due to the free acid's low pKa (0.5–1.7), but rather as water-soluble sodium or potassium salt (Herrera et al., 2017). Furthermore, very few investigations showed that MON is moderately heat stable during thermal food processing (Knutsen et al. 2018).

The toxicity of MON has been investigated both in vitro and in vivo. MON inhibits pyruvate dehydrogenase, transketolase, aldose reductase, glutathione peroxidase, and glutathione reductase in vitro. In ducklings and chickens, MON possesses acute toxicity

similar to T-2 toxin, with an LD_{50} ranging from 3.7 to 5.4 mg/kg body weight (Jestoi 2008). In farmed animals, MON causes weight loss, lower feed intake, higher mortality (chickens and turkeys), intestinal bleeding (rats), and pathological abnormalities in the heart and liver (Tuan et al., 2003).

2.6 Effects of *Fusarium* mycotoxins

2.6.1 Effects of trichothecenes in plants

Trichothecenes are phytotoxic, causing wilting, chlorosis, necrosis, and other symptoms in many plants (Cutler, 1988). DON's phytotoxic effects on plants include growth retardation, seedling inhibition, and reduction of green plant regeneration (Bruins et al. 1993). DON and 3-ADON are inhibitors of seed germination and the growth of root and leaf tissue. They are more phytotoxic than T-2 toxin, HT-2, and DAS and impede wheat coleoptile growth at low concentrations (Rocha, 2005). The principal phytotoxic effects of trichothecenes are inhibiting ribosomal protein synthesis, disruption of membrane integrity, and changes in electron and leaf chlorophyll concentration in plants (Amarasinghe, 2011).

2.6.2 Effects of mycotoxins on grain quality and processing

Milling oat is an important part of the overall oat-producing process. As a result, the presence of mycotoxins in milling oats should be taken seriously (Tekauz et al., 2004). Trichothecenes, such as nivalenol, HT-2, and T-2 toxins, are frequently discovered in food-grade oats (Langseth and Rundberget, 1999). *Fusarium*-associated mycotoxins were mainly present in the hull of contaminated oat grains. The investigation of naked and covered oats for bran and flakes production showed that the oat kernels of de-hulled oat had a lower level of mycotoxins (Adler et al., 2003). The heat treatment substantially reduces the level of DON in oat groats compared to the DON levels in raw oats (Tekauz et al., 2004).

Mycotoxins can also affect the malting quality of barley grain. Severely FHB-infected malts will lead to gushing during malting and brewing, affecting the finished beer product (Oliveira et al., 2012). The effect of pre-milling processes and the efficacy of mycotoxin removal are variable. Compared to unclean grains, the concentration of mycotoxins in cleaned wheat ranged from 7% to 63% for DON, 7% to almost 100% for NIV, and 7% to 40% for ZEA (Edwards et al. 2011; Lancova et al. 2008). Besides, the reduction of DON in debranded (removing the outer layer of wheat grains) wheat ranged from 15 to 78% (Cheli et al. 2010).

2.6.3 Trichothecenes as pathogenicity and virulence factors

Although the causes for the generation of mycotoxins by *Fusarium* infections are unknown, there is growing evidence that they may play a role in *Fusarium* pathogenesis. Pathogenicity is the capability of a pathogen to induce disease, and virulence is the quantity of disease caused by a pathogen (Desjardins, 2006). The severity of the disease triggered by a pathogenic strain on a susceptible host influences the disease's fate (Gilbert et al. 2001). Although DON is not required for pathogenicity, it may significantly impact the aggressiveness of *F. culmorum* (Manka et al., 1985; Proctor et al., 1995). Furthermore, a positive association was discovered between the virulence of *F. graminearum* and *F. culmorum* and DON in winter rye (Gang et al. 1998; Hestbjerg et al. 2002). According to Gilbert et al. (2002), aggressive *F. graminearum* strains can produce more mycotoxins than less aggressive strains. It has been hypothesized that the ability of *Fusarium* species to cause disease is related to trichothecene accumulation in the host during the infection process. The suppression, removal or degradation of mycotoxins in host plants could reduce the aggressiveness of *Fusarium* pathogen during the infection (Foroud and Eudes, 2009).

2.7 Chemotype diversity and geographical distribution patterns

2.7.1 *Fusarium graminearum*

DON is the most common trichothecene mycotoxin generated by *F. graminearum*. 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), and nivalenol (NIV) are all produced by *F. graminearum* (Gilbert et al. 2010). Ichinoe et al. (1983) initially recognized these chemotypes of *F. graminearum* as DON (which produced deoxynivalenol and 3-ADON) and NIV (which generated DON and 3-ADON) (nivalenol and 4-acetyl nivalenol). Following that, Miller et al. (1991) found two new *F. graminearum* chemotypes, 3-ADON and 15-ADON.

Fusarium graminearum DON and NIV chemotypes have been detected in numerous countries of Africa, Asia, Europe, and North America (Miller et al. 1991; Jennings et al. 2004; Mirocha et al. 1989). DON and 3-ADON were the most common *F. graminearum* chemotypes in Asia. In a global collection of 111 *F. graminearum* strains, 50% were 15-ADON chemotype, 35% were 3-ADON chemotype, and 15% were NIV chemotype. Significant differences in DON/NIV content were found between countries and between countries and their chemotype interactions. The 3-ADON isolates from Germany produced higher DON amounts in Germany than the isolates from Canada, China, Poland, and the UK (Amarasinghe et al., 2019).

In North America before to 1994, *F. graminearum* strains with the 15-ADON chemotype were the most common cause of FHB, but between 1998 and 2004, the prevalence of *F. graminearum* 3-ADON chemotype strain was documented (Gale et al. 2002; Ward et al. 2008). Surveillance studies revealed that in North America, the 3-ADON chemotype is replacing the 15-ADON chemotype (Gilbert et al., 2010). Ward et al. (2008) suggested that the recent shift in the population of *F. graminearum* was caused by transcontinental introgression. Goswami and Kistler (2004) suggested that the rapid invasion of *F.*

graminearum 3-ADON isolates in western Canada indicated certain selective advantages of *F. graminearum* isolates with 3-DON over 15-ADON chemotype, such as reproductive capacity, in vivo growth rate, and ability to use resources more efficiently (Ward et al. 2008).

2.7.2 *F. poae*

Fusarium poae is another important pathogen causing FHB on small grain cereals. *F. poae* was reported to produce beauvericin (BEA) in Norwegian and Finnish grain samples (Jestoi et al., 2004; Uhlig et al., 2006). In maize kernels contaminated with *F. poae*, the development of ENN A, B, and B1 and BEA has been documented (Chelkowski et al., 2007). Besides, *F. poae* DNA profiles and NIV content also showed a significant correlation in barley and wheat in Switzerland (Yli-Mattila et al. 2004b, 2008; Vogelgesang et al. 2008b). In another study, 14 *F. poae* isolates from Belgium and Italy produced NIV, DAS (diacetoxyscirpenol), NEO (neosolaniol), and FUS-X (fusarenon-X) (Vanheule et al., 2017). Thrane et al. (2004) reported 41 out of 49 isolates of *F. poae* from Europe produced NIV with DAS. Moreover, a single strain of *F. poae* can produce multiple mycotoxins (Richardson et al., 1989). For example, the *F. poae* strain BBA 64810 produces NIV, FX, 15-MAS (monoacetoxycirpenol), DAS, SCR (scirpentriol), BEA. *F. poae* isolate IBT 9924 produces NIV, FX, 15-MAS, DAS, SCR, T-2, HT-2 (Thrane et al. 2004).

2.8 Genetic diversity of *F. poae*

The identification and evolutionary history of *Fusarium* spp. are complex. *Fusarium* isolates within a particular species may vary considerably, morphologically (Leslie and Summerell, 2008) and genetically (Azor et al. 2009; Edel et al. 2001; Feng et al. 2010). Vanheule et al. (2016) and Fekete & Hornok (1997) found that *F. poae* has four core chromosomes and a set of supernumerary chromosomes, highly variable in size and composition. Recently, various molecular approaches based on the sequences of internal transcribed spacer (ITS), intergenic spacer region (IGS), β-tubulin (tub1), and translation

elongation factor 1ὰ (EF-1α) have been used to characterize genetic variations among *Fusarium* species (Knutsen et al. 2004; Torp and Nirenberg, 2004).

Kerenyi et al. (1997) found a highly complex composition of vegetative compatibility groups (VCGs) in 54 partially geographically diverse isolates of *F. poae*. *F. poae* exhibited a high level of intra-species variability using amplified fragment length polymorphism (AFLP) and multi-locus analysis (Kulik et al., 2011; Stenglein, 2010). Based on AFLP analysis, Vanheule et al. (2017) demonstrated that *F. poae* isolates from maize and barley fields of Belgium and Italy were divided into sub-clusters with no clear correspondence to either host or geographic origin. Similarly, Dinolfo et al. (2010) studied a total of 97 *F. poae* isolates from Argentina and England with inter simple sequence repeats (ISSR). High intraspecific variability was detected among these *F. poae* isolates. However, no clear relationship between the variability and the host or geographic origin was observed. Llorens et al. (2006) also showed that the clustering of *F. poae* based on the IGS region did not show any relationship with the host, geographic origin of the isolate, and mycotoxin-producing capacity.

CHAPTER 3

3.0 Occurrence of *Fusarium* Species Causing *Fusarium* Head Blight of Oats in Manitoba from 2016 to 2018

Abstract

Fusarium head blight (FHB), a devastating fungal disease caused by *Fusarium* species, can lead to significant yield losses and mycotoxin contamination in cereal production. The Fusarium species complex related with FHB on oat in Manitoba was investigated in this study. From 2016 to 2018, oat samples from 168 commercial fields in five crop districts in Manitoba were assessed for *Fusarium* infection. Microscopic analysis showed that oat seeds infected with *F. poae* (61%, 62% and 53%), followed by *F. graminearum* (54%, 29% and 40%) and 8%, 22% and 13% with *F. sporotrichioides* in 2016, 2017 and 2019 respectively. Conventional PCR showed that the oat fields were infested with *F. poae* (72%, 65% and 69% of fields), *F. graminearum* (28%, 25% and 48% of fields) and with *F. sporotrichioides* (9.3%, 6.7% and 15.4% of fields) in the respective years. The effect of geographical locations and crop rotation on the abundance of *Fusarium* DNA in oat grains was determined using quantitative PCR. One-way ANOVA revealed that the abundance of *F. poae* DNA in oat samples from five crop districts was similar. The concentrations of *F. greaminearum* and *F. sporotrichioides* DNA in oat samples from central and southwest crop districts differed from samples from northern Manitoba crop districts, except for *F. sporotrichioides* in 2018. Flax and cereal (wheat/barley) as the preceding crops significantly impacted the DNA abundance of all three *Fusarium* species in oat samples.

3.1 Introduction

Fusarium head blight (FHB) is an important disease of small grain cereal products in many parts of the world, threatening the cereal industry. The FHB etiology is complex. *Fusarium* spp. in one area is mainly distributed and dominated by climatic factors (for example, temperature, humidity, precipitation), agronomic practices (e.g. crop rotation, tillage and fertilizer), crop pathogens interacting as well as the competition among different *Fusarium* species (Pancaldi et al. 2010). Infections produced by *F. culmorum* and *F. avenaceum* are widespread in cooler geographic regions, such as northwestern Europe (Nielsen et al., 2014). In North America, the most common FHB causing *Fusarium* spp. are *F. graminearum*, *F. poae*, *F. sporotrichioides*, *F. avenaceum* and *F. culmorum* (Gilbert and Tekauz, 2000).

In oat, FHB symptoms start with water-soaked brownish patches at the base of the glumes and progress to blackening the glumen. Oat is supposed to be more resistant to FHB than wheat or barley because infected panicles have no visible symptoms. The long pedicles between spikes prevent the fungal mycelium from spreading throughout the grain (Bjørnstad et al., 2008; Tekauz et al., 2008). When *Fusarium* infections are present, the peduncle tissue turns dark or tan and is prematurely shrivelled (Parry et al., 1995). The pathogen invades the oat stem tissue, which causes the entire spike to blanch (Osborn and Stein, 2007). The *Fusarium*-damaged kernels (FDK) appear to have a shrunk colour with a decreased seed quality and weight (Osborne and Stein, 2007).

In Canada, oat is grown as both food and feed. Many milling oats are transported to the United States, where oats are used for breakfast cereals (Tekauz et al., 2004). Oats have many beneficial effects on human health, such as high β-glucan content, which reduces blood

glucose and insulin rises and decreases cholesterol levels. Furthermore, oats are rich in protein and have a good lipid profile. Oatmeal is frequently used in infant food due to its nutritious quality (Schöneberg et al., 2018).

FHB on oat was first identified in western Canada during the FHB epidemic in 1993 (Gilbert et al. 1994). The rare and localized occurrence of FHB on oat has since been documented in Québec, Prince Edward Island, New Brunswick, Nova Scotia, and Ontario (Tekauz et al., 2004; Tamburic-Ilincic 2008). *F. sporotrichioides*, *F. graminearum* and, *F. poae* were the most common *Fusarium* spp. found in oat kernels collected in Ontario (2005-2007) (Tamburic-Ilincic, 2010) and Manitoba (2002 to 2007) (Tekauz et al. 2008). *Fusarium poae* was also found on oat kernels from western Canada between 1995 and 1997 (Clear et al. 2000). There is a lack of information on FHB in oat compared to FHB in wheat and barley. A better understanding of the *Fusarium*-related complex associated with FHB on oat in Manitoba is required to assess FHB threats to commercial oats production and develop an appropriate management strategy for this disease.

This study aimed to determine the *Fusarium* spp. associated with oat FHB in Manitoba using morphological and conventional PCR analysis; to measure the abundance of *Fusarium* DNA in commercial oat grains and investigate the impact of regional climates and crop rotation on the abundance of *Fusarium* DNA in oat grains.

3.2 Materials and Methods

3.2.1 Collection of oat grain samples from 2016 to 2018

168 oat fields in Manitoba were assessed for FHB between July 18 and August 5 in 2016-2018 (43, 60, and 65 fields in 2016, 2017, and 2018, respectively) (Islam et al. 2021). The crop districts were divided into five regions, namely 1) central (CMB = 54 samples), 2) southwest (SWMB = 51 samples), 3) eastern (EMB = 13 samples), 4) northwest (NWMB = 22 samples), and 5) interlake (INMB = 27 samples) of Manitoba (Islam et al. 2021) (Fig. 3.1). Oat fields were surveyed throughout the growth stage between the late milk stage and full maturity (Islam et al. 2021). The fields were selected in regular intervals, depending on crop accessibility and availability, about 20 to 25 km along the survey route. Forty to sixty FHB infected oat panicles were collected randomly (blighted, shrivelled or bleached seeds) from each oat field and stored in paper envelopes. Data were collected on the preceding crops (immediate previous year) in those oat fields with their geographical locations.

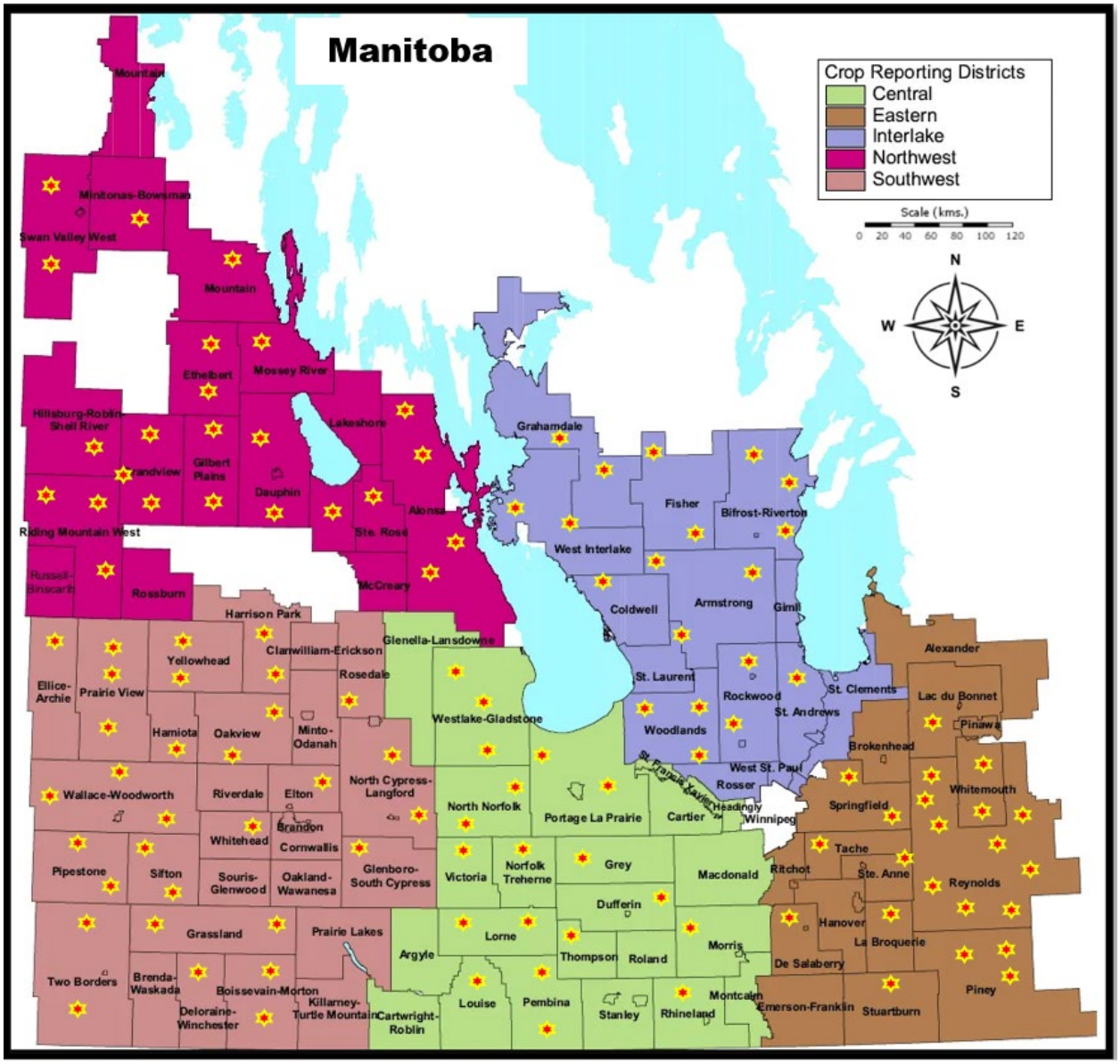

Fig 3. 1 Manitoba map shows the approximate location of oat fields where FHB infected oat samples were collected (2016 to 2018). *Ref.* www.gov.mb.ca/agriculture

3.2.2 Isolation and identification of *Fusarium* spp. (Microscopy)

Fusarium pathogens were isolated from fifty oat seeds per field using potato dextrose agar (PDA, Thermo Fisher) media. Before plating, grains were surface sterilized with 0.3% sodium hypochlorite (Islam et al. 2021). The PDA plates were kept at room temperature (20-

25 °C) for five days. The identification of *Fusarium* spp. was executed under the compound and

dissecting microscopy based on the morphology of conidia (Leslie and Summerell, 2008; Islam et al. 2021) (Fig. 3.2). Two *Fusarium* pathogens, *F. poae* and *F. graminearum* were transferred to another PDA plate for the pure culture and stored for further molecular analysis. The percentage of *Fusarium* infected seeds was estimated as described by Tekauz et al. **(**2004).

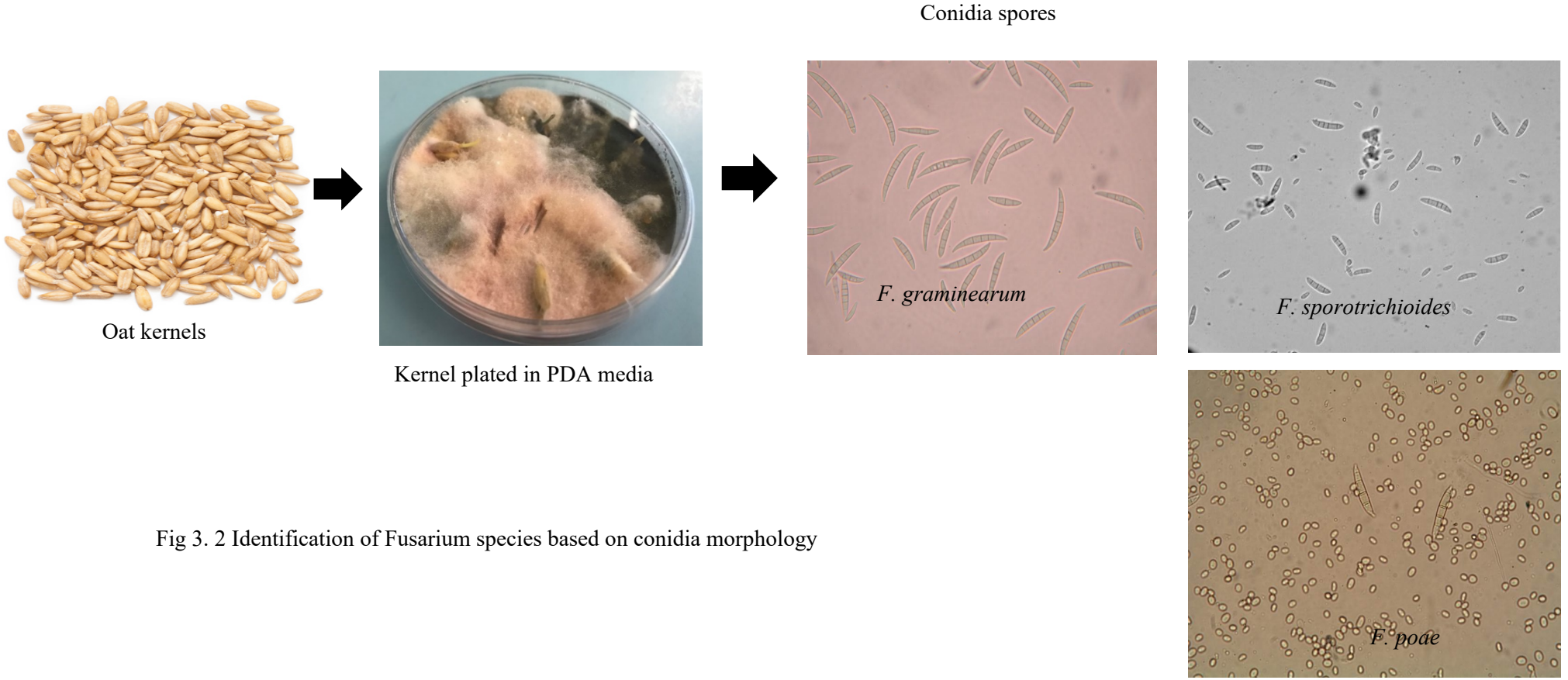

Fig 3. 2 Identification of Fusarium species based on conidia morphology

3.2.3 Conventional PCR

An oat grinder (Retsch ZM 200, Thermo Fisher Scientific.) was used to grind a sample of 20g oat grains per field. Following the manufacturers ' procedure, 1g grain flour was used to extract DNA using the QIAGEN DNeasy Mini Kit (QIAGEN Mississauga, ON, Canada) (Islam et al. 2021). The rest of the flour samples were kept at −20 °C for mycotoxin analyses. The concentration of DNA was determined using NanoDrop 2000 spectrophotometer (Thermo Fisher, Wilmington, DE, USA). The conventional polymerase chain reaction (PCR) was performed using a C1000 Touch™ Thermal Cycler (BioRad), and four *Fusarium* species-specific oligonucleotide primers were used to detect *Fusarium* spp. present in the oat flour (Table 3.1). The PCR conditions were as follows: initial denaturing step at 94°C for 15 min; 30 cycles at 95°C for 30 s; 50°C for 30 s; 72°C for 1 min 30 s and a final extension step at 72°C for 3 min (Islam et al. 2021). The amplification reactions were carried out in a volume of 25µl containing 10× PCR buffer (2.5µl), dNTPs(2µl), forward and reverse primer(1µl), Tag DNA polymerase (0.125µl), ddH2O (18.83µl) and template DNA (2.5µl). The PCR amplicons were divided electrophoretically on 2% agarose gels in the 1×TAE buffer, stained with GelRed (ThermoFisher) (Islam et al. 2021). The gel images were recorded using a Gel Doc™ EZ Imager (ThermoFisher, California, USA).

Target *Fusarium* spp.	Name of primer	Product size (bp)	Sequences (5'-3'), Demeke et al. 2005
F. poae	Fp82F	220	CAAGCAAACAGGCTCTTCACC
	Fp82R		TGTTCCACCTCAGTGACAGGTT
F. graminearum	Fg16F	450	CTCCGGATATGTTGCGTCAA

	Fg16R		GGTAGGTATCCGACATGGCAA
F. sporotrichioides	FspF	332	CGCACGTATAGATGGACAAG
	FspR		GTCAGAAGAGACGCATCCGCC
F. avenaceum	FaF	950	CAAGCATTGTCGCCACTCTC
	FaR		GTTTGGCTCTACCGGGACTG

Table 3. 1 Fusarium species-specific primers with amplicon size and sequences

3.2.4 The quantitative PCR analysis of the abundance of *Fusarium* DNA

Quantitative PCR (qPCR) was performed to determine the abundance of *Fusarium* DNA in oat grains. All reactions were analyzed in a CFX96™ real-time PCR detector system (BioRad, USA) (Islam et al. 2021). The triplicates of the standards and negative control (double distilled water) were used. PCR primers, based on the elongation factor 1α (EF1α) gene for three *Fusarium* species, *F. poae, F. graminearum* and, *F. sporotrichioides,* were used for the qPCR analysis (Table 3.2) (Islam et al. 2021). The qPCR was carried out in a total volume 20µl consisting of 10µl of SsoFast EvaGreenTM PCR Master Mix (BioRed), 1µl of each primer, 6µl ddH_2O, and 2µl of template DNA with a 37- cycle threshold (Ct) cut-off detection limit. The qPCR condition was as follows: initial preheating at 98ºC for 2 min followed by 40 cycles of 95°C for 15 s and 62°C for 1 min and dissociation curve analysis at 60°C to 95°C (Islam et al. 2021). Three technical replicates were performed for each sample.

Table 3. 2 *Fusarium* species-specific primers and sequences

Target *Fusarium* spp.	Name of primer	Sequences (5'-3'), Nicolaisen et al. 2009
F. poae	FpA51F	ACCGAATCTCAACTCCGCTTT
	FpA98R	GTCTGTCAAGCATGTTAGCACAAGT
F. graminearum	FgB397F	CCATTCCCTGGGCGCT
	FgB411R	CCTATTGACAGGTGGTTAGTGACTGG
F. sporotrichioides	FsA18F	GCAAGTCGACCACTGTGAGTACA
	FsA85R	CTGTCAAAGCATGTCAGTAAAAATGAT

3.2.5 Statistical analysis

The data homogeneity was verified prior to statistical analyses using the Kolmogorov-Smirnov test of normality, which measured the divergence of the field sample distribution. The skewness and kurtosis of each year's survey data were calculated and plotted to determine whether the distribution was normal (Islam et al. 2021). Outliers (extreme low and high values) were removed from the data set when necessary, and the data was transformed (log, square root, arcsine). The one-way analyses of variance (ANOVA) were performed using XLSTAT version 2019.4.2 (www.xlstat.com). The ANOVA determined the significance of the effect of survey years, field locations, and preceding crops for three dependent variables (the amount of *F. poae*, *F. graminearum,* and *F. sporotrichioides* DNA) (Table 3.6). Fisher's Least Significant Difference (LSD) test was used to compare the mean of different treatment groups (e.g., years, locations, and previous crops) for each variable within each survey year (Islam et al. 2021)

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3.3 Results

3.3.1 Spectrum of *Fusarium* species in oat grain samples

From 2016-2018, 8400 FHB oat seeds were grown on PDA plates and subjected to microscopic analysis. *F. poae* was the most common *Fusarium* species found in oat from Manitoba, and it was isolated from 61%, 62%, and 53% of oat grains collected in 2016, 2017, and 2018. Infection caused by *F. graminearum* was identified in 54% of oat grains in 2016, 29% of oat grains in 2017, and 40% of oat grains in 2018. Oat grains infected with *F. sporotrichiodes* were also detected at lower frequencies (8% in 2016, 22% in 2017, and 13% in 2018) (Table 3.3).

Conventional PCR was used to detect *Fusarium* infections in commercial oat fields (Table 3.4). *F. poae* was the most common *Fusarium* species detected between 2016 and 2018 was detected in 72%, 65%, and 69% of fields in 2016, 2017, and 2018, respectively. Infections caused by *F. graminearum* and *F. sporotrichiodes* were also detected. However, the percentage of fields infected with these two *Fusarium* species was lower than with *F. poae*. Moreover, oat fields infected with *F. aveneacum* were also detected (one field in 2016 and 3 fields in 2018) (Appendix Table 1a). The concentration of Fusarium DNA in oat samples was quantified using quantitative PCR (qPCR). The highest concentration of *F. poae* DNA was found in oat samples. *F. poae* DNA concentrations were twice as high as *F. graminearum* DNA concentrations in 2016 and 2017. This result agrees with our findings from microscopic and conventional PCR analyses. Although the average DNA concentration of *F. graminearum* was lower than that of *F. poae*, it was significantly higher than that of *F. sporotrichiodes*. The one-way ANOVA analysis was used to study the effect of survey years

on the mean concentration of *F. poae*, *F. graminearum*, and *F. sporotrichioides* in oat samples (Table 3.6). The mean concentration of *F. graminearum* and *F. sporotrichioides* on oat grains were similar among the three survey years. However, the mean concentrations of *F. poae* DNA in oat samples in 2016 and 2017 were higher than those collected from 2018. In all three years, the concentrations of *F. poae* DNA in oat samples were highest, followed by *F. graminearum* DNA and *F. sporotrichioides* DNA.

Table 3. 3 *Fusarium* species complex found in oat kernels from commercial fields in Manitoba during 2016-2018.

Crop districts	**Year**	***Fusarium* spp.**					
		Fusarium poae		***Fusarium graminearum***		**Fusarium sporotrichioides**	
		X/n	% of infected seeds	X/n	% of infected seeds	X/n	% of infected seeds
Average Manitoba (combined five districts)	2016	1312/2150	61	1174/2150	54	180/2150	8
	2017	1875/3000	62	872/3000	29	682/3000	22
	2018	1750/3250	53	1350/3250	40	670/3250	20
CMB	2016	720/900	80	684/900	76	180/900	20
	2017	864/1200	72	506/1150	44	144/1200	12
	2018	390/650	60	500/650	75	52/650	8
EMB	2016	160/400	40	48/400	12	0/400	0
	2017	0	0	0	0	0	0
	2018	0	0	0	0	0	0
INMB	2016	0	0	0	0	0	0
	2017	171/500	34	54/450	12	18/500	7

	2018	800/1000	80	100/1000	10	266/700	38
NWMB	2016	16/200	8	0/200	0	0/200	0
	2017	0	0	0	0	0	0
	2018	48/300	6	600/800	75	256/800	32
SWMB	2016	416/650	65	442/650	68	0/650	0
	2017	840/1300	64	312/1300	24	520/1300	40
	2018	512/800	64	100/800	12	96/800	12

Notes: X=number of seeds infested with respective *Fusarium* spp. n= total number of FHB infected seeds used in PDA plates. 50 FHB infected seeds were used for each sample or field. Total number of seed samples (n=168): central/CMB=18 (2016), 24 (2017), 13(2018); eastern/EMB=8(2016), interlake/INMB=10(2017), 20(2018); north-west/NWMB=4(2016), 16(2018), south-west/SWMB=13(2016), 26(2017), 16(2018). No data available for EMB in 2017 and 2018; INMB in 2016 and NWMB in 2017.

Table 3. 4 Conventional PCR analysis showing the percentages of oat fields infested with *Fusarium* spp. in three survey years (2016-2018) across Manitoba.

		***Fusarium* spp.**							
Manitoba crop districts (CDs)	**Year**	***F. poae***			***F. graminearum***		***F. sporotrichioides***		***F. aveneacum***
		X/n	**% field**	**X/n**	**% field**	**X/n**	**%field**	**X/n**	**% field**
CMB	2016	15/18	83	8/18	44	1/18	5.5	0	0
	2017	13/24	54	6/24	25	3/24	12	0	0
	2018	5/13	38	10/13	76	4/13	30	0	0
EMB	2016	4/8	50	3/8	37	2/8	25	0	0
	2017	0	0	0	0	0	0	0	0
	2018	0	0	0	0	0	0	0	0
INMB	2016	0	0	0	0	0	0	0	0
	2017	6/10	60	2/10	20	0/10	0	0	0
	2018	18/20	90	6/20	30	3/20	15	2/20	10
NWMB	2016	4/4	100	0/4	0	0/4	0	0	0
	2017	0	0	0	0	0	0	0	0
	2018	12/16	75	8/16	50	1/16	0	0	0

SWMB	2016	8/13	61	1/13	7.6	1/13	15	1/13	7.6
	2017	20/26	76	7/26	26	1/26	3.8	0	0
	2018	10/16	62	7/16	43	2/16	12	1/16	6.2
Average Manitoba (combined five CDs)	2016	31/43	72	12/43	28	4/43	9.3	1/13	7.6
	2017	39/60	65	15/60	25	4/60	6.7	0	0
	2018	45/65	69	31/65	48	10/65	15.4	3/36	8.3

Notes: X=number of fields infested with respective *Fusarium* spp. n= total number of FHB infested fields tested with *Fusarium* spp. specific primers/conventional PCR. 1g powder of FHB infected seeds per oat field was used to extract DNA for species detection. Total number of seed samples/fields: CMB=18 (2016), 24 (2017), 13(2018); EMB=8(2016), INMB=10(2017), 20(2018); NWMB=4(2016), 16(2018), SWMB=13(2016), 26(2017), 16(2018). No data is available for EMB in 2017 and 2018, INMB in 2016 and NWMB in 2017.

Table 3. 5 RT-qPCR analysis summarizing the range and mean concentrations of the DNAs for *F. poae, F. graminearum*, and *F. sporotrichioides* in three-year (2016-2018) across the five crop districts of Manitoba.

	The concentration of *Fusarium* spp. (ng of fungal DNA/ng of total gDNA[a])					
Survey years	2016(n=43)		2017(n=60)		2018(n=65)	
	range	Mean[b]	range	Mean	range	Mean
F.poae	0.01-1.58	0.13±0.05 **a**	.005-1.93	0.12±0.008 **a**	0.005 - 0.38	0.08±0.002 **a**
F.graminearum	0.02-1.35	0.05±0.07 **b**	0.007-0.80	0.07±0.007 **b**	0.007 - 0.05	0.07±0.003 **b**
F.sporotrichioides	0.002-1.37	0.02±0.03 **c**	0.006-0.31	0.01±0.004 **c**	0.001 – 0.15	0.01±0.003 **c**

[a]gDNA refers to genomic DNA (total of fungal/Fusarium and oat-seed DNA).

[b]Mean±SE refers to Manitoba mean (combining average concentrations of five crop districts/fields). The average concentration of each crop district is presented in Figures 3.5 and 3.6. Means denoted by a different letter indicate significant differences between years (P<0.05). n=Total number of fields.

Table 3. 6 P-values for the effect of survey years, regional field locations, and preceding crops on the concentrations of three *Fusarium* spp. DNA in oat grain samples in Manitoba.

Data set/survey years	Source of variation	*Fusarium* spp. DNA (ng of fungal DNA/µl of total gDNA)		
		P-values[a]		
		F. poae	*F. graminearum*	*F. sporotrichioides*

Combined years (2016, 2017, and 2018)	Years	0.39	**0.048**** [b]	0.080
2016	field locations	0.899	**0.011****	**0.037****
2017	field locations	0.826	**0.020****	**0.042****
2018	field locations	0.745	**<0.001****	0.077
2016	previous crops	0.347	0.807	0.412
2017	previous crops	**0.036****	**0.001****	**0.002****
2018	previous crops	0.588	0.411	**0.013****

[a] P-values are determined using one-way ANOVA
[b] the letters in bold represented a statistical difference ($P \leq 0.05$).

3.3.2 Variations in the abundance of *Fusarium* spp. DNA in oat samples from different crop districts of Manitoba.

Fusarium DNA abundance in oat grains from five Manitoba crop districts (CDs), including central (CMB), southwest (SWMB), eastern (EMB), northwest (NWMB), and interlake (INMB), was analyzed using One-way ANOVA for the regional effect on the concentration of *Fusarium* DNA found in oat samples. The result showed that the Manitoba crop district did not have a significant ($P \leq 0.05$) impact on the concentrations of *F. poae* DNA in oat samples (Table 3.6). However, the concentrations of *F. graminearum* (2016, 2017, and 2018) and *F. sporotrichioides* (2016 and 2017) were significantly ($P \leq 0.05$) impacted by the sample locations. The concentrations of *F. graminearum* DNA were higher in CMB and EMB regions samples than other Manitoba CDs. Similarly, the concentrations of *F. sporotrichioides* appeared higher in CMB and SWMB, almost two times higher on average than samples from other CDs in Manitoba (Fig. 3.3).

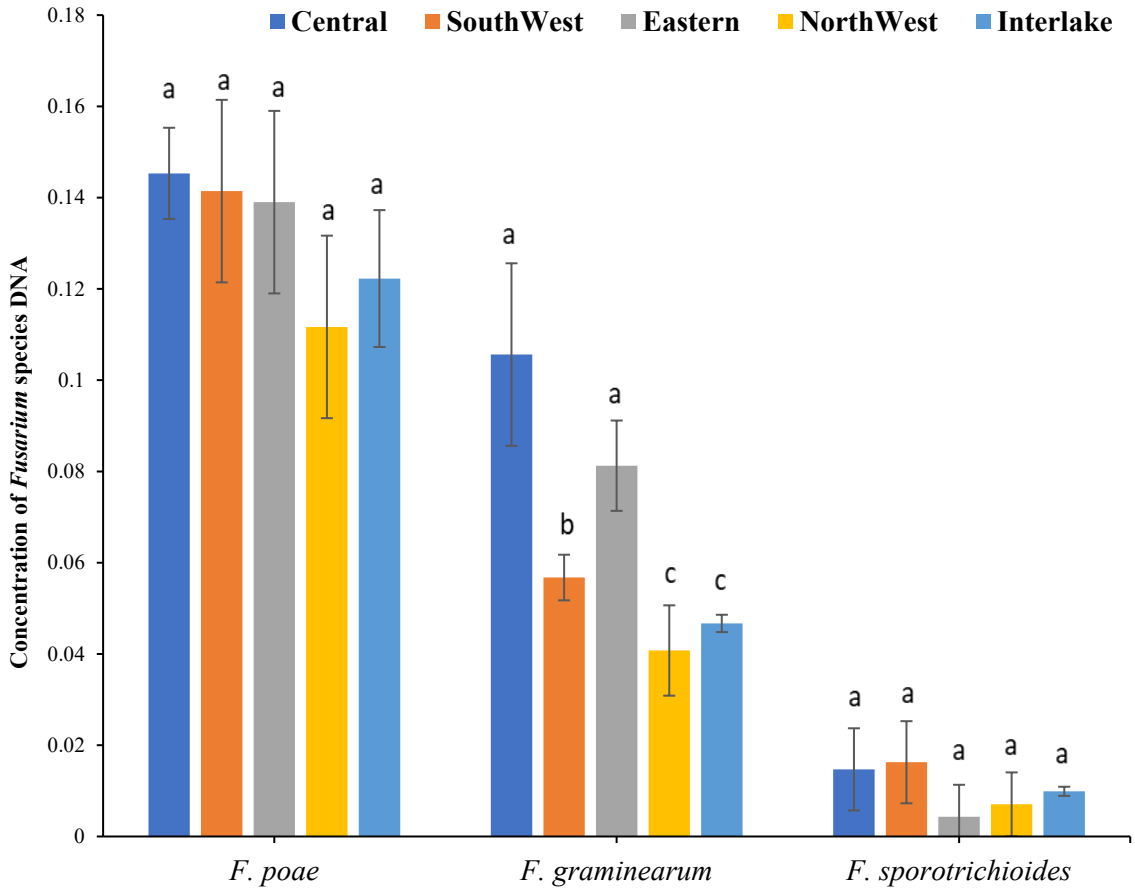

Fig 3. 3 Effect of regional field locations of Manitoba on the abundance of three Fusarium spp. DNA in oat grain samples. FHB infected grains were collected from four locations (no samples from INMB) in 2016, three locations in 2017 (no fields in EMB and NWMB), and four locations (no samples from EMB) in 2018.

3.3.3 Effect of crop rotation on the abundance of *Fusarium* spp. in oat grain samples

The impacts of preceding crops on the abundance of *Fusarium* DNA in oat samples were evaluated using ANOVA analysis. Crop rotation had a significant effect on *Fusarium* spp. DNA concentrations in oat samples (Table 3.6).

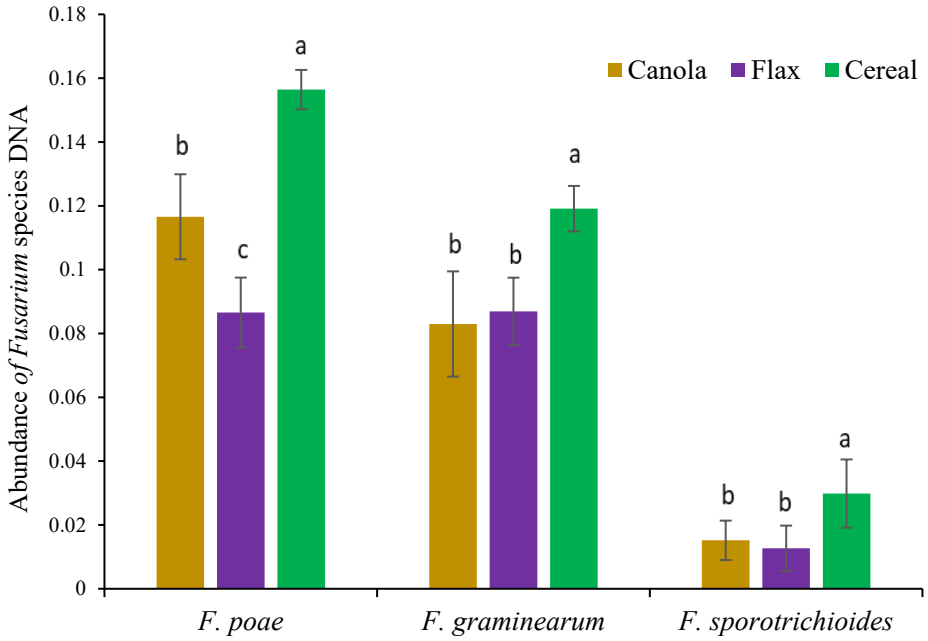

Fig 3. 3 The quantity of *Fusarium* spp. DNA in oat grain samples across Manitoba was affected by crop rotation.

The abundance of *F. poae*, *F. graminearum* and *F. sporotrichioides* DNA was significantly higher ($P \leq 0.05$) in oat grains from the fields where the preceding crop was cereals (Fig. 3.4). The concentration of *Fusarium* spp DNA in oat grain was nearly half when the preceding crops were flax or canola, compared to cereals previously.

3.4 Discussion

In the current study, *Fusarium* infection was common in oat grain collected in Manitoba. *Fusarium poae* was the pre-dominant *Fusarium* species causing FHB on oat. This outcome agrees with recent reports published by the Canadian Grain Commission, which showed that FHB incidence had substantially increased in oat-growing regions of Manitoba in 2016 compared to the previous years (CGC, 2017). Several previous studies have shown that FHB on oat in Canadian prairie provinces and eastern Canada is caused by *F. poae*, *F. graminearum*, *F. sporotrichioides* and *F. avenaceum* (Tekauz et al. 2000). *Fusarium poae* was the predominate *Fusarium* sp. in oat by Xue et al. (2019) in oat samples from Ontario from 2001 to 2017. Besides, in 2013 and 2014, *F. poae* was identified as the most common seed-borne *Fusarium* spp. affecting oat production in Canada (Guo et al. 2018). *F. poae* has been recognized as the primary contributor to FHB in Argentina (González et al. 2008) and several European countries, including France, Hungary, Ireland, Poland, Slovakia, Switzerland, and the United Kingdom (Xue et al. 2019).

It is well documented that *F. graminearum* is the pre-dominant *Fusarium* species on wheat in North America (Tekauz et al., 2000; Vogelgsang et al., 2019). It was also the predominant *Fusarium* species in commercial barley fields in western Canada from 2016 to 2018 (Banik et al., 2017; Ziesman et al., 2018 & 2019). Furthermore, *F. graminearum* is the dominant *Fusarium* spp. barley and in wheat in Europe (Schöneberg et al. 2016; Vogelgsang et al. 2009).

The abundance of *F. poae* in oat samples from various regions of Manitoba shows no significant variation. However, the concentration of *F. graminearum* and *F. sporotrichioides* DNA was significantly higher in the CMB, EMB, and SWMB than NWMB and INMB cropping districts (Fig. 3.3). These results partially agree with Grafenhan et al.

(2013), which showed that *F. graminearum* and *F. poae* were most frequently isolated in oat samples from the CMB and SWMB crop. The mean concentrations of fungal DNA from these two species in grain samples were significantly lower in NWMB and INMB regions than in other Manitoba regions (Fig. 3.3). Regional weather conditions during anthesis are the most critical factors in *Fusarium* infection. The weather data indicated that oat fields located in Manitoba's northwest and interlake regions received relatively higher temperatures and lower precipitation during flowering/anthesis in July (Table 3.7), which could reduce the growth and proliferation of *F. graminearum* and *F. sporotrichioides*. This result is coincided with the remark by Clear et al. (2000) that *F. graminearum* replaced *F. avenaceum*, the primary pathogen from wheat-FHB. Between 1993 and 1997, cooler weather conditions favoured the growth of *F. avenaceum* over *F. graminearum* in eastern Saskatchewan (Nelson et al., I981).

Crop rotation appears to have a big impact on the amount of Fusarium DNA in oat grains. In this study, higher concentrations of *Fusarium* DNA were found in oat samples from fields with cereal planted as the previous crop. On the other hand, canola and flax as preceding crops resulted in a significantly lower amount of *Fusarium* DNA in oat grains. These results agree with the study done by Golkari et al. (2008), where a reduced amount of *Fusarium* inoculum was isolated from pea and canola stubble. A study was conducted from commercial wheat fields across southwestern Ontario to determine the relative importance of previous and adjacent crop, tillage, field size, and sampling direction on the abundance of *F. graminearum* (Schaafsma et al. 2005). The results showed that the planting of wheat after corn or wheat with minimum or no-tillage practices increased the severity of FHB across southwestern Ontario. Similarly, Dill-Macky et al. (2000) also showed that corn-wheat rotation increases the incidence and severity of FHB, whereas soybean as crop residue

decreases the severity of FHB. Likewise, wheat and barley residues produced more ascospores of *F. graminearum* than corn or other residues (Pereyra & Dill-Macky, 2008).

Currently, the information on the effect of crop residues on the abundance of *F. poae* is minimal. It has been shown that cereal-cereal rotation does not affect the abundance of *F. poae,* but it increases the abundance of *F. langsethiae* in grains (Schöneberg et al., 2018). With the increased importance of *F. poae* in the pathogen complex causing FHB on oat, more detailed studies are warranted to better understand this pathogen.

3.5 Conclusion

This study confirmed that FHB is common on oat in Manitoba. *Fusarium poae, F. graminearum* and *F. sporotrichioides* are the three most common *Fusarium* pathogens in commercial oat fields. These *Fusarium* pathogens are affected by several regionally linked parameters like environments (i.e., temperature and precipitation) and crop rotation. Further long-term epidemiological surveys are critical to understanding better *Fusarium* species complex associated with FHB on oat.

CHAPTER 4

4.0 Occurrence of *Fusarium*-Produced Mycotoxins in Oats across Manitoba Crop Districts from 2016 to 2018

Abstract

This study investigated naturally occurring mycotoxins in oat grain from commercial fields in Manitoba between 2016 and 2018. The analysis of *Fusarium*-associated mycotoxin showed that DON was the most abundant mycotoxin in oat from Manitoba, followed by NIV and HT-2/T-2. The principal component and regression analysis of *Fusarium* DNA and the level of mycotoxins indicates that *F. graminearum* is the principal contributor of DON in oat. In contrast, BEA and NIV are associated with *F. poae*. T-2 and HT-2 are likely due to the infection caused by *F. sporotrichioides*. Analysis of variance (ANOVA) revealed a significant variation of mycotoxin levels among Manitoba crop districts. Furthermore, crop rotation also has a major impact on the level of mycotoxins in oat. Oat grains from the field with cereal as the previous crop contain significantly higher DON, BEA, T-2, and HT-2.

4.1 Introduction

Mycotoxins are secondary metabolites produced by *Fusarium*, *Aspergillus*, and *Penicillium* fungi (Sherif et al., 2009). Aflatoxin, fumonisin, trichothecenes, zearalenone (ZEA), and ochratoxins are the most frequent mycotoxins (Binder et al. 2007; Filtenborg et al. 2000). Trichothecene, including T-2, HT-2, deoxynivalenol (DON), 3-ADON, 15-ADON, and nivalenol (NIV), are frequent mycotoxins generated by *Fusarium* infections. It is well documented that *F. graminearum* and *F. culmorum* are ZEA and type B trichothecenes producers, including DON and NIV (Bottalico and Perrone, 2002). Besides, *F. langsethiae* and *F. sporotrichioides* are the producers of type A trichothecenes, HT-2, and T-2 (Thrane et al. 2004). *F. poae* produces NIV and other non-trichothecene mycotoxins, such as beauvericin (BEA) and diacetoxyscirpenol (DAS). Furthermore, *F. avenaceum* and *F. tricinctum* are often associated with the contamination of moniliformin (MON), enniatins (ENN), and BEA in small grains (Thrane et al. 2004).

Many of these mycotoxins have oestrogenic, teratogenic, mutagenic, and carcinogenic effects on humans and animals (Binder et al., 2007; D'Mello et al., 1999). Trichothecenes impair DNA, RNA, mitochondrial activity, cell division and have membrane effects, among other things. T-2/HT-2 and NIV have higher toxicity than DON (D'Mello et al. 1999; Nielsen et al. 2009).

In 2003, the Food and Agriculture Organization (FAO) set legislative limits for main mycotoxins produced by *Fusarium* species in cereals and cereal foodstuffs projected for human consumption (FAO Food and Nutrition Paper 81, 2003). The legislation includes DON with a limit of 2000 ppb for human consumption and 5000 ppb for cattle and poultry consumption. The indicative limits for HT-2 and T-2 were 100ppb for cattle and poultry and 25 ppb for dairy animals. According to the Canadian Food Inspection Agency (CFIA), the

maximum tolerated levels of DON, NIV, HT-2, and T-2 are 2000 ppb, 50 ppb, 100 ppb, <1000 ppb, respectively (Charmley and Trenholm 2017) (see Appendix table 2a).

Oat contains β-glucan, known to moderate blood glucose and insulin and lower cholesterol levels. It is a high-value cereal (Brennan and Cleary, 2005). Oat is also a good protein and lipid source (Welch, 1995). Oatmeal is well-known for its nutritional profile and other beneficial properties such as stability due to its high-water retention (Welch, 1995). However, for safe consumption, especially as baby food, oats must be free of mycotoxins (Lombaert et al., 2003). Therefore, the knowledge of *Fusarium*-associated mycotoxins in commercial oat grains is critical for lowering the risk of contaminated oat grains entering the supply chain.

FHB on oat was first detected in western Canada during the FHB outbreak in the eastern prairie in 1993, where *F. graminearum*, *F. avenaceum*, *F. poae* and *F. sporotrichioides* were commonly detected in oat grains (Gilbert et al. 1994; Tekauz et al. 2004). Since then, the sporadic and localized presence of FHB in oat has been reported in Quebec, Prince Edward Island, New Brunswick, Nova Scotia, and Ontario (Tekauz et al. 2004; Tamburic-Ilincic 2008). *Fusarium sporotrichioides*, *F. graminearum* and, *F. poae* were the most common *Fusarium spp*. in oat kernels from Ontario (Tamburic-Ilincic, 2010) and Manitoba (Tekauz et al. 2008). Clear et al. (2000) showed the presence of *F. poae* on oat kernels in western Canada from 1995 to 1997.

In Norway, *F. poae*, *F. graminearum*, *F. sporotrichioides* and *F. avenaceum* were frequently isolated from commercial oat grains (Kosiak et al., 2003). *F. poae* was the predominant *Fusarium* spp. in oat fields in Switzerland (Schöneberg et al. 2018). This pathogen has also been recognized as an important contributor to FHB in Argentina (González et al. 2008) and several European countries, including France, Hungary, Ireland,

Poland, Slovakia, Switzerland, and the United Kingdom (Xue et al. 2019). On the other hand, *F. graminearum* has emerged as a significant threat to oat production in Brazil (Martinelli et al., 2014).

The main objectives of this study were i) to quantify the naturally occurring mycotoxins in oat samples collected from commercial fields in Manitoba; ii) to identify the main contributor of mycotoxins in oat grains; iii) to demonstrate the consequence of geographical location and crop rotation on the mycotoxin level in oat.

4.2 Materials and Methods

4.2.1 Sample collection and oat grinding

A total of 168 oat fields in Manitoba were surveyed for FHB from 2016 to 2018. All data were also collected on the previous crops in those fields with their geographical location. At each survey site, forty to sixty FHB-infected panicles were collected and preserved in paper envelopes. A subsample of 20g de-hulled oat grains was ground with an oat grinder (Retsch ZM 200, Scientific Inc.). One-gram grain flour was used for mycotoxin analysis. The rest of the flour samples were kept at −20 °C until further analysis.

4.2.2 Sample preparation for LC-MS/MS

Ground oat samples were investigated for 11 mycotoxins, including DON, BEA, NIV, HT-2, T-2, MON, ENN A, ENN A1, ENN B, ENN B1, and DAS (Islam et al. 2021). One-gram powder of oat grains was used to extract mycotoxins using a combination of acetonitrile, methanol, and water in a ratio of 75:10:15 (Islam et al. 2021). All samples were sonicated for 30 minutes and shaken for 90 minutes in a rotary shaker at 40 rpm. After centrifugation at 4000×g for 30 min, 10 ml of the extract was filtered with a 0.2 µm nylon syringe filter (ThermoFisher) (Islam et al. 2021). In a nitrogen evaporator, the cleaned

extracts were dried under a warm stream of nitrogen gas (RapidVap Labconco, USA) for 90 min at 70°C. Each sample was then re-suspended in 1 mL of 50:50 Mobile Phase A (water + 0.1% formic acid + 5mM ammonium format): B (methanol + 0.1% formic acid + 5mM ammonium format) and transferred to a corresponding 1.5 ml LC vial. Liquid Chromatography: Mass Spectrometry (LC-MS/MS) (ORBITRAP ID-X, ThermoFisher) was used to quantify different mycotoxins at the Mycotoxin Lab, Morden Research and Development Center, AAFC, Morden, MB (Islam et al. 2021). Recovery experiments of spiked samples validated the method. The recovery range for the measured trichothecenes, Nivalenol (NIV), Deoxynivalenol (DON), diacetoxyscirpenol (DAS), T-2, and HT-2 was between 50% and 100%. The limits of quantification (LOQ) and detection (LOD) were set at 50 and 10 parts per billion (ppb), respectively.

4.2.3 Quantification of *Fusarium* biomass using qPCR

The materials and methods for quantitative PCR (qPCR) were described in Chapter 3 (section 3.2.4). Briefly, all reactions were analyzed in a CFX96™ Real-Time PCR Detector System (BioRed, USA). All standards and the negative control (double distilled water/ddH_2O) were run as triplicates. The qPCR was carried out in a volume of 20µl consisting of 10µl SsoFast EvaGreen® PCR Master Mix (BioRed), 1µl of each primer, 6µl ddH2O, and 2µl template DNA with a 37-cycle threshold (Ct) cut-off detection limit (Islam et al. 2021). The qPCR reactions were performed in duplicate on all samples.

4.2.4 Statistical analysis

The data homogeneity was verified prior to statistical analyses using the Kolmogorov-Smirnov test of normality, which measured the divergence of the field sample distribution. The skewness and kurtosis of each year's survey data were calculated and plotted to

determine whether the data distribution was normal. Outliers (extreme low and high values) were removed from the data set when necessary, and the data was transformed (log, square root, arcsine) (Islam et al. 2021). The one-way analyses of variance (ANOVA) were performed using XLSTAT. The ANOVA determined the significance of the effect of sampling (survey) years, regional climates (field locations) and preceding crops (previous crops) of oat fields for dependent variables (mycotoxin). Fisher's Least Significant Difference (LSD) test was used to compare the mean of different treatment groups (e.g., years, locations, and previous crops) for each variable within each survey year (Islam et al. 2021). In addition, the principal component (PCA) analysis was performed to analyze the relationship between the amount of fungal DNA and the amount of mycotoxin using SAS.

4.3 Results

4.3.1 *Fusarium*-produced mycotoxins in Manitoba oat grains

DON and NIV were the two most abundant mycotoxins found in oat grains collected between 2016 to 2018 from Manitoba. The concentrations of DON in these samples ranged from 0 to 4143 ppb, 0 to 1881 ppb, and 0 to 632 ppb in 2016, 2017 2018, respectively (Table 4.1). The maximum mean DON concentration was in 2016 (604 ppb) and the lowest was in 2018 (253 ppb). The contamination of oat samples with NIV was also common. From 2016 to 2018, the percentage of oat grains contaminated with NIV (above LOD) ranged between 78-100%, higher than DON. Nevertheless, means of NIV in oat samples were lower than DON. T-2, HT-2, BEA, and MON contamination was frequently detected in oat but only at much lower concentrations, compared to DON and NIV. The sporadic contamination of oat grains with DAS and ENNs (A, A1, B, and B1) was also detected (Table 4.1). Overall, the naturally occurring mycotoxins in oat samples from 2016 and 2017 were slightly higher than in 2018. However, it is statistically insignificant based on the one-way ANOVA analysis

except for BEA and MON. One-way ANOVA revealed that the mean concentrations of two mycotoxins, namely BEA and MON, were significantly different among three survey years ($P \leq 0.05$) (Table 4.2). BEA had the highest mean concentration in 2017. In contrast, the concentration of MON in oat samples was lower in 2018, compared to 2016 and 2017 ($P \leq 0.05$) (Table 4.2).

Table 4. 1. Naturally occurring mycotoxins in oats, grains collected from 2016-2018 in Manitoba

Years	2016				2017				2018			
N-samples	43				60				65			
	% of the fields above LOQ	% of the fields above LOD	maximum (ppb)	mean (ppb)	of fields above LOQ	of fields above LOD	Maximum (ppb)	mean (ppb)	% of the fields above LOQ	% of the fields above LOD	maximum (ppb)	mean (ppb)
DON	83	85	4143	604±87	41	41	1881	569±104	38	40	632	253±20
NIV	78	100	865	252±27	69	78	795	234±16	78	98	581	145±12
T-2	40	77	1155	43±18	81	81	973	53±16	25	25	794	22±12
HT-2	18	35	1100	60±29	13	13	419	26±10	10	10	654	23±11
BEA	90	90	119	25±4	70	80	159	41±4	71	71	169	24±5
MON	25	32	349	31±11	20	20	533	30±11	11	11	209	12±5
DAS	0	15	25	8±1.1	7	7	25	7±0.6	5	5	25	6±0.5
ENN-A	0	0	0	0±0.3	0	0	0	0±0.1	0	0	0	0±0.1
ENN-A1	0	3	25	0.6±0.6	4	4	25	2±0.6	2	1	25	1±0.4
ENN-B	8	8	1605	51±37	3	24	93	9±2	8	8	85	4±1
ENN-B1	5	32	166	14±4	11	11	25	5±0.9	8	8	128	5±2

Note: % of field determined by mycotoxin infected fields/total number of fields. Mycotoxins presented here are at concentrations above the limit of quantitation (LOQ) and above the Limits of Detection (LODs) are presented here, LOQ set at 50 µg/kg (ppb) and set at ten µg/kg (ppb). Mycotoxin concentrations below the respective LOQ or LOD were calculated as LOQ/2 or LOD/2, respectively. ± standard error.

Table 4. 2 Summary statistics (one-way ANOVA) for the effect of survey years, geographical locations, and preceding crops on major mycotoxin concentrations in oat grains across Manitoba

		Fusarium producing major mycotoxins					
		P-value					
Data set/Survey years	Source of variation	DON	NIV	BEA	T-2	HT-2	MON
2016, 2017 and 2018	Years	0.457	0.174	**0.018***	0.460	0.718	0.054
2016	Field location	0.249	0.264	**0.006***	0.141	**0.010***	**0.027***
2017	Field location	**0.025***	**0.004***	0.058	**0.0332***	0.120	0.383
2018	Field location	0.176	0.389	**0.000***	**0.015***	**0.012***	0.057
2016	Previous crop	**0.026***	0.766	**0.040***	0.050	**0.001***	0.697
2017	Previous crop	0.052	0.078	**0.011***	0.252	.157	0.060
2018	Previous crop	0.342	**0.037***	**0.010***	**0.010***	**0.033***	**0.009***

*significant (P≤0.05)

4.3.2 Impact of Manitoba crop districts on the mean concentration of mycotoxins

The effect of geographical locations on the concentrations of DON, NIV, BEA, T-2, HT-2, and MON in oat samples was analyzed using one-way ANOVA (Fig. 4.1). On average, the concentrations of DON were significantly (P≤0.05) higher in samples from the central and eastern regions of Manitoba (Fig. 4.1). The concentrations of NIV were slightly higher in samples from central and southwest Manitoba. Similarly, the T-2, HT-2, MON, and BEA concentrations were the highest in the sample from central Manitoba (Fig. 4.1).

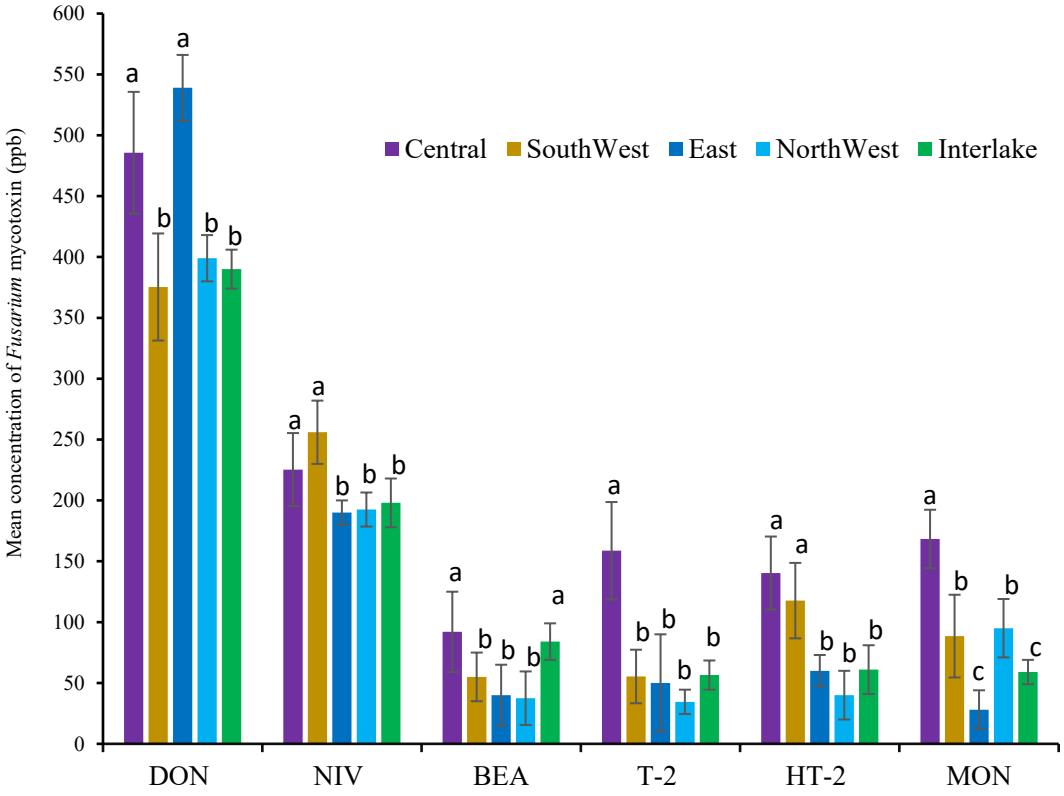

Fig 4. 1 Effect of Manitoba crop districts (locations) on the mean concentration of mycotoxins in oat grains surveyed from 2016 to 2018.

4.3.3 Impact of preceding crops of oat on the mean concentration of mycotoxins

The impact of crop rotation on the concentration of mycotoxins was analyzed using one-way ANOVA. On average, the concentrations of DON, BEA, and T-2 were significantly ($P \leq 0.05$) higher in oat samples from fields previously planted with cereals, compared to fields with canola and flax as previous crops (Fig. 4.2). We didn't observe a similar effect for NIV and MON levels. They were statistically identical in samples from fields with wheat or canola as previous crops. However, samples from fields with flax as the previous crop had a lower concentration of NIV and MON. Overall, samples from fields with flax/canola-cereal rotation appeared to have lower means of mycotoxins in oat grains than those fields with cereal-cereal rotation.

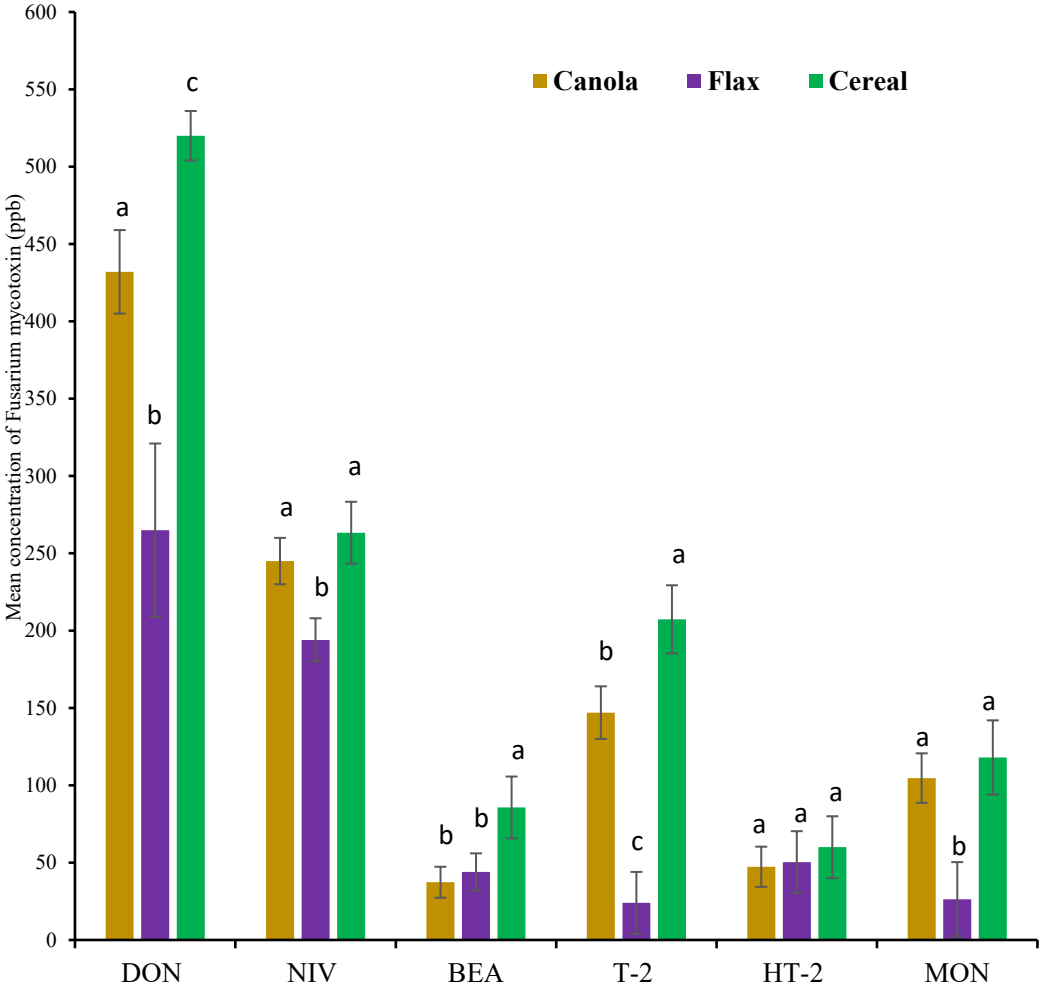

Fig 4. 2 Effect of preceding crops of oat on the mean concentration of mycotoxins in oat grains surveyed from 2016 to 2018.

4.3.4 Relationship between the abundance of *Fusarium* spp. and mycotoxins

Principal component analysis (PCA) was used to detect the primary producers associated with various mycotoxins found in oat grains. The result is shown in Figure 4.3. The first two dimensions described for 53.13% of variabilities using three *Fusarium* spp. found in grain samples (n=168). The x-axis described 30.84% of the variability, and the y-axis represented an additional 22.39% of the original variability. A high correlation was found between the level of *F. graminearum* (FG) DNA and DON as they clustered in the same quadrant. Similarly, the F. poae (FP) DNA, NIV, and BEA levels showed a close association. Furthermore, *F. sporotrichioides* (FS) DNA and T-2/HT-2 showed the maximum correlation within oat samples collected from 2016 to 2018.

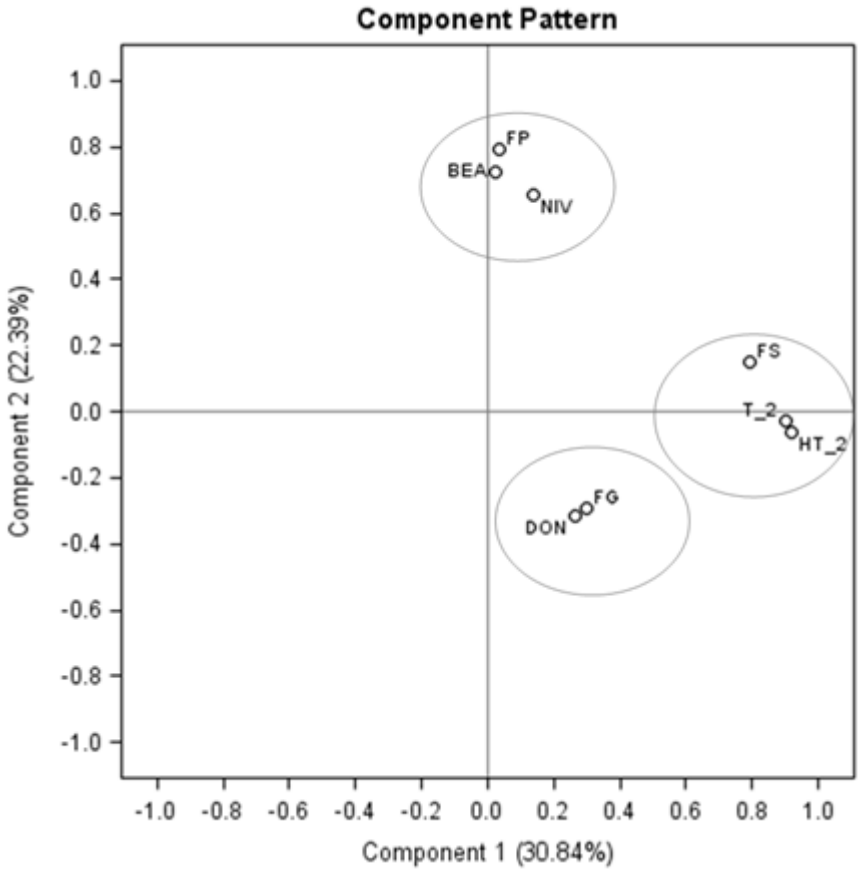

Fig 4. 3 Principal component analysis of *Fusarium* species DNA and mycotoxin concentration in infected oat samples. FG, FP, and FS denote *F. graminearum, F. poae*, and *F. sporotrichioides*, respectively; mycotoxin DON, deoxynivalenol; mycotoxin NIV, nivalenol; mycotoxin BEA, beauvericin; mycotoxin MON, moniliformin; mycotoxin T-2 and mycotoxin HT-2.

4.4 Discussion

In this study, DON was the most predominant mycotoxins found in oat grains in Manitoba. Clear et al. (2000) previously measured DON in oat samples from Manitoba in 1995 and 1996. A maximum of 340 ppb of DON was found in those samples. Gräfenhan et al. (2013) demonstrated that DON was the most frequently detected mycotoxins in oat samples from western Canada. Similarly, a recent survey conducted in the eastern prairies and the province of Québec also showed the predominance of DON in oat grains collected from 2014 to 2017 (Tittlemier et al. 2020).

NIV was another major mycotoxin found in oat samples from Manitoba. This result agrees with the results of Campbell et al. (2000) and Tittlemier et al. (2020), in which NIV was found to be the second most important mycotoxins after DON in oat sample from western Canada. In a Swedish oat survey, NIV was the second most occurring mycotoxin after DON in oat grain (Schöneberg et al., 2018). The contamination of cereal grains with NIV has also been reported in several other EU countries (Edwards, 2009; Alkadri et al., 2014). Furthermore, a low concentration of BEA was detected in over 95% of oat samples on oat samples in Manitoba. Also, Tittlemier et al. (2020) documented the abundance of BEA in the harvested oat samples during a similar period.

Several studies have shown that T-2 and HT-2 are the main mycotoxins in oat samples from Switzerland, Norway, and the UK (Schöneberg et al., 2018; Hofgaard et al., 2016; Hietaniemi et al., 2016). In addition, T-2/HT-2 mycotoxins were also commonly detected in grain samples from wheat, rye, and barley fields in Europe (Bottalico et al., 2002; Morcia et al., 2016; Kosicki et al. 2020). In our study, T-2/HT-2 mycotoxins were found in oat grains from different regions of Manitoba but only at very low concentrations. Similarly, Tamburic-Ilincic (2010) reported the contamination of T-2/HT-2 (the maximum of 350ppb) in oat samples from southern Ontario. Interestingly, T-2/HT-2 were not detected in the composite grains samples from Canadian Prairies collected between 2000 and 2002 (Clear et al. 2005).

This study found positive relationships between *F. graminearum* - DON, *F. poae* - NIV, and *F. sporotrichioides* - T-2/HT-2 (Fig. 4.3). Several studies have investigated the relationship between *Fusarium* spp. and mycotoxin production. The correlations between *F. graminearum* and DON content, *F. poae,* and NIV content in cereal grains infected by *Fusarium* pathogens are well documented (Bosch et al. 1992; Christ et al. 2011; Vogelgsang

et al. 2019; Tajrin, 2013). The correlations between *F. poae* and BEA, *F. poae* and NIV, have been found in Swedish wheat and oat fields (Lindblad et al. 2013; Fredlund et al. 2013). The strong correlations between *F. graminearum* and DON, *F. avenaceum* and ENN/MON, and *F. poae* and NIV have been detected from southern Belgium (Hellin et al. 2016; Jestoi et al. 2004; Kiecana et al. 2002). NIV and DON also show strong correlations with *F. poae* and *F. graminearum,* respectively*,* in malting barley from Argentina (Nogueira et al., 2018).

Several studies have indicated that *F. graminearum* could be a potent producer of NIV (Boutigny et al., 2014; Pasquali et al., 2016). In our study, *F. graminearum* isolates with NIV chemotypes were not detected in samples used in this study. To the author's best knowledge, the presence of *F. graminearum* isolates with NIV chemotypes has not been detected in cereal grains in Western Canada. On the other hand, *F. poae* isolates with NIV chemotype were commonly detected in oat from Manitoba in this study. The abundance of *F. poae* DNA showed a close relationship with NIV concentration in oat samples. As a result, we conclude that *F. poae* was the primary source of NIV in Manitoba oat. In most European countries, HT-2/T-2 and *F. langsethiae* always show a strong correlation in oat grains (Edwards et al. 2012; Tajrin, 2013; Schöneberg et al. 2018). In the current study, no isolates of *F. langsethiae* were found in oat samples from Manitoba. To date, no reports of *F. langsethiae* have been made in Canada.

Significant differences were found between the crop district and mycotoxin levels in oat. Previous studies have shown that DON concentration in prairie oats differed significantly over years and regions (Clear et al. 1996, 2000; Campbell et al. 2000; Martos et al. 2010). The regional difference in mycotoxin levels in oat samples is likely due to the local weather conditions. For example, the cooler and more humid conditions are more suited for the growth of *F. graminearum* (Nelson et al. 1981), where hot and dry weather conditions will

promote the growth of *F. poae* (Xu et al. 2014). Additionally, the variations in mycotoxin levels could also be due to agronomic factors such as delaying grain harvesting and host genetic resistance (Edwards et al. 2018).

One-way ANOVA revealed crop rotation has a significant impact on the level of mycotoxins in oat grains. It is known that crop rotation has a significant impact on the level of DON in wheat and cereal over cereal rotation stimulates both the rate and level of DON contamination (Schaafsma et al. 2001). A 4-year study conducted by Pageau et al. (2008) showed that the highest DON contents in barley were obtained when cereal were seeded in crop rotation other than dry pea crop, suggesting that preceding crops (e.g., cereals) can impact *Fusarium* species spectrum and the level of mycotoxins in cereal grains.

4.5 Conclusion

DON was the most common *Fusarium* mycotoxin followed by NIV and T-2/HT-2 in oat samples from 2016 to 2018 in Manitoba. Strong correlations between *F. graminearum* and DON, *F. poae* and NIV and BAE, and *F. sporotrichioides* of T-2/HT-2 were detected in this study. Geography and crop rotation also had a significant impact on mycotoxin levels in oat, as the levels of DON, NIV, and HT-2 were significantly higher in the central region of Manitoba**.** Mycotoxin levels were higher in oat samples from fields previously planted with cereal compared to canola or flax. The mycotoxin profiles in oat obtained in this study would contribute to future research on the management of FHB on oat.

CHAPTER 5

5.0 Chemotype Characterization of *Fusarium* Species and Genetic Diversity of *Fusarium poae* on Oat in Manitoba.

Abstract

Fusarium head blight (FHB) is a common cereal disease that causes yield loss and mycotoxin contamination caused by a group of *Fusarium* species. This study investigated chemotypes of *Fusarium* pathogens found in oat samples from Manitoba during 2016 and 2018. Among *F. graminearum* strain, 3-ADON and 15-ADON chemotypes were frequently identified. In comparison, nearly 100% of *F. poae* strain were of NIV chemotype. We also performed the phylogenic analysis of the *F. poae* strain using partial DNA sequences derived from the translation elongation factor-1 alpha (*EF-1α*), *TRI1,* and *TRI8* genes. Our result showed a high degree of intra-species variations among *F. poae* isolates from Manitoba. Four groups of *F. poae* isolates were identified in the analysis, and the grouping of *F. poae* isolates showed no clear correlation with sampling locations and crop rotation.

5.1 Introduction

The main trichothecene mycotoxin generated by *F. graminearum* is deoxynivalenol (DON). *Fusarium graminearum* can also produce 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), and nivalenol (NIV) (Gilbert et al. 2010). Production of these trichothecenes is an important trait of *F. graminearum*. *F. graminearum* isolates with 3-ADON chemotype produce both DON and 3-ADON, and strain of 15-ADON chemotype can produce both DON and 15-ADON (Goswami and Kistler, 2004; Ward et al. 2008).

Historically, *F. graminearum* 15-ADON chemotype was the predominant chemotype in North America (Miller et al. 1991). In western Canada, a shift from 15-ADON to 3-ADON chemotype isolates of *F. graminearum* in wheat fields has been reported since 2000 (Ward et al., 2008).

Fusarium poae is another major pathogen in the FHB complex. The main trichothecenes produced by *F. poae* are NIV, fusarenon-X (FX), monoacetoxyscirpenol (MAS), diacetoxyscirpenol (DAS), and scirpentriol (SCR) (Stenglein, 2009). *F. poae* can also generate mycotoxins other than trichothecenes, such as beauvericin (BEA) (Jestoi et al. 2004; Uhlig et al. 2006), and enniatin A, B, and B1 (Chelkowski et al. 2007).

Fusarium poae is one of the primary pathogens associated with oat FHB in Manitoba (Clear et al. 2000 a, b; Tekauz et al. 2004; Grafenhan et al. 2013). According to Leslie and Summerell (2008), isolates within *F. poae* may vary morphologically and genetically. *Fusarium poae* produce both sexual and asexual reproduction. It possesses transposable elements (TEs), a significant risk for sustainable disease management (Vanheule et al., 2017). Various multi-loci barcoding techniques have been used to detect intra-species variability within different *Fusarium* species, including phylogenetic analyses with the ribosomal internal transcribed spacer (ITS) regions and intergenic spacer (IGS) region, genes encoding β-tubulin (Yli-Mattila et al. 2004), and *EF-1α* (Knutsen et al. 2004), as well as AFLP fingerprints (Schmidt et al. 2004). Moreover, genes from the *Fusarium TRI* gene cluster are also used to study the genetic variability within *Fusarium* species (Vanheule et al., 2017). For example, in *F. graminearum*, the variations in the *TRI1* gene led to the characterization of a new group of isolates producing NX-2, a new Type A trichothecene (Ramdass et al. 2019). Several other genes in the *TRI* cluster (*TRI1, TRI4, TRI5, TRI8, TRI11*) have also been used to study *Fusarium* spp. complex found in cereals from Europe, Turkey, and North America (Villafana et al. 2020; Manganiello et al. 2019; Villani et al. 2016).

. The main objectives of this study were to investigate i) the main *Fusarium* chemotypes in oat samples collected from commercial fields in Manitoba and ii) the genetic variability of the *F. poae* population in Manitoba.

5.2 Materials and Methods

5.2.1 Collection and Processing of Survey samples

A total of 168 oat fields in Manitoba were surveyed for FHB from 2016 to 2018. Oat fields were surveyed during the growth stage between the late milk stage and any point up to full maturity. Depending on crop availability and accessibility, fields were chosen at regular 20–25 km intervals along the survey routes. At each survey location, forty to sixty affected spikes were collected and stored in paper wrappers. An oat grinder was used to grind a subsample of 20g oat grains (Retsch ZM 200, Scientific Inc.). To extract DNA, 1g of grain flour was utilized. The remaining flour samples were held at 20 °C until they were tested again.

5.2.2 Isolation of *Fusarium* species

Fifty grains per field sample was used to isolate *Fusarium* spp. using potato dextrose agar (PDA) media (Islam et al. 2021). The grains were surface sterilized with 0.3 % sodium hypochlorite before plating. The PDA plates were incubated at room temperature (20-25°C) for five days. The identification of *Fusarium* spp. was done under the compound and dissecting microscopes based on the morphology of conidia (Leslie and Summerell, 2008). The conidia of *F. poae* and *F. graminearum* were transferred into another PDA media for the pure culture of these isolates (Islam et al. 2021).

5.2.3 Chemotypes of *F. graminearum* and *F. poae* found in oat samples

Genomic DNA was extracted from the oat flour using QIAGEN DNeasy Mini Kit (QIAGEN Mississauga, ON, Canada) following the manufacturer's procedure (Islam et al. 2021). The concentrations of DNA were determined using NanoDrop 2000 spectrophotometer (Thermo Fisher, Wilmington, DE, USA). Multiplex PCR (C1000 Touch™ Thermal Cycler, USA) was performed to determine *F. graminearum* 3-ADON, *F. graminearum* 15-ADON, *F. graminearum* NIV, and *F. poae* NIV chemotypes using primer sets described in Table 5.1 (Islam et al. 2021). PCR assay was accomplished by using the following cycle parameters: initial denaturing step at 94°C for 15 min; 30 cycles at 95°C for 30 s; 50°C for 30 s; 72° for 1 min 30 s and a final extension step at 72°C for 3 min (Islam et al. 2021). PCR amplicons were detached on 2% agarose gels in 1×TAE buffer and stained with GelRed (Islam et al. 2021). Gel images were scanned using a Gel Doc™ EZ Imager.

Table 5. 1 PCR primers used to detect different chemotypes of *Fusarium* pathogens.

Primer name	**Amplicon size**	**Primer sequence (5'-3') Ward et al. 2008 and Dinolfo et al. 2012**
15ADON (*F.graminearum*)	610	TCATCAATCAATCTTTTTCACTTT
		AATCTAACAAACTCATCTAAATAC
3ADON (*F.graminearum*)	243	CTTTAATCCTTTATCACTTTATCA
		TACACTTTCTTTCTTTCTTTCTTT
NIV (*F.graminearum*)	840	CTTTTCATCTTTTCATCTTTCAAT
		CTTTATCAATACATACTACAATCA
NIV (*F.poae*)	296	TATCCTTGCATGGCAATGCC
		AAATGGCGATACGAGTATTGA

5.2.4 Monosporic *F. poae* isolation and DNA extraction

The streak method was used to generate the monosporic culture of *F. poae*. A10 mL spore suspension was prepared and serially diluted to a very low spore concentration (Islam et al. 2021). Then, a loop was used to create a streak around the PDA media. The PDA plates were kept at room temperature (20-25 °C) for 48hr. A single spore was selected under the dissecting microscope and transferred onto another PDA plate for further growth (Islam et al. 2021). Genomic DNA was extracted from *F. poae* mycelium using the QIAGEN DNeasy® Mini Kit following the manufacturer's protocol. The genomic DNA concentration was measured with a NanoDrop 2000 spectrophotometer (Thermo Fisher, Wilmington, DE, USA). The quality of DNA was checked using gel electrophoresis (2% agarose gel and gel-red).

5.2.5 PCR protocol

Three genes (*TEF-1α, TRI1,* and *TRI8*) were amplified directly from *F. poae* genomic DNA (Islam et al. 2021). (Table 5.2). The PCR reactions were performed with a C1000 Touch™ Thermal Cycler system in a volume of 25µl containing 2.5µl of 10× PCR buffer, 15.88µl of water, 2µl of dNTP mix (2.5 mM), 1µl of each primer (10 pmol), 0.125µl of Taq polymerase (5 U/µL), and 2.5µl of template DNA (100 ng/µL) (Islam et al. 2021). Amplification was carried out using annealing temperature 56ºC for 30 sec, and PCR assay was completed by using the following cycle parameters: initial denaturing step at 94°C for 15 min; 30 cycles at 95°C for 30 s; 50°C for 30 s; 72° for 1 min 30 s and a final extension step at 72°C for 3 min (Islam et al. 2021). The PCR products were separated electrophoretically on 1.5% agarose gels in 1×TAE buffer and stained with GelRed. The amplified bands were cut under UV light (UVITEC) (Islam et al. 2021). The purification of amplicons was performed using the Qiagen gel extraction kit (Toronto, Canada) following the manufacturer's protocols (Islam et al. 2021). The intensity of the products was checked using a NanoDrop 2000

spectrophotometer (ThermoFisher, Wilmington, USA). The cleaned PCR amplicons were sequenced at National Research Council (Saskatoon, Canada) using Sanger sequencing protocols (ABI 3730xl DNA sequencers).

Table 5. 2 Sequences and names of primers

Primer name	Sequence (5'-3') Dr. Linda Harris (unpublished)*
TEF1αF	ATGGGTAAGGAGGAGAAGACT
TEF1αR	GGAAGTACCAGTGATCATGTT
Tri1F	CGGGCCTGTGGACATCT
Tri1R	GGGTTTCTTGAGCCAATGGAAT
Tri8F	ACAACATCCTCTATCGCACAAC
Tri8R	GTGATATCCCATGATGCCTTCC

* Ottawa Research and Development Centre Agriculture and Agri-Food Canada.

5.2.6 Phylogenetic analysis of *F. poae* strains based on sequences of *TRI1*, *TRI8,* and *TEF-1α*

The raw Sanger sequences were processed using MEGAX (Kumar et al. 2018) to clean ambiguous nucleotides (average quality >50). The dataset was cleaned of extremely long and short homopolymers, as well as low-quality and chimeric sequences (Islam et al. 2021). The alignments of *TEF-1α, TRI1*, and *TRI8* were created using MUSCLE3.8.31 (Islam et al. 2021; Edgar, 2004) with default settings. We used TrimAl 1.4 (Capella-Gutiérrez et al. 2009) to trim the alignments and remove positions with gaps in 10% or more of the sequences (Islam et al. 2021). The concatenated sequences of *TEF-1α*, *TRI1*, and *TRI8* for each *F. poae* strain were created using MEGAX10.2.6. The concatenated sequence of *TEF-*

1α, *TRI1,* and *TRI8* from *F. graminearum* PH-1 (European Nucleotide Archive, accession number PRJEB5475) was also created and included as an outgroup in the analysis (Islam et al. 2021). The locations of distinct genes in the concatenated alignment were documented in a partition file. The optimal exchange model for each partition (-m MFP) was found using IQTree (version 1.6.12) and a consensus tree was inferred using maximum likelihood and rapid bootstrapping (n = 1000).

5.3 Results

5.3.1 Chemotype determination of *Fusarium* species

Among three *F. graminearum* chemotypes (3-ADON, 15-ADON, and NIV) previously reported in Canada, both 3-ADON and 15-ADON were detected in Manitoba oat fields. However, *F. graminearum* isolates with NIV chemotype were not found in any of the oat samples from Manitoba. Overall, *F. graminearum* 3-ADON isolates are more common than 15-ADON isolates in Manitoba. The provincial percentages of oat fields infected with *F. graminearum* isolates with 3-ADON and 15-ADON chemotypes were 30% and 23%, respectively. The percentage of oat fields infected with *F.s graminearum* 3-ADON/15-ADON chemotypes varied among different crop districts. In CMB, the percentage of fields infected with *F. graminearum* 3-ADON isolates was higher than 15-ADON isolates in 2017 and 2018 but not in 2016. In SWMB, *F. graminearum* isolates with 3-ADON chemotype were more common than 15-ADON isolates in all three years.

In comparison, the provincial average of oat fields infected with *F. poae* isolates with NIV chemotype was 86% (Table 5.3). These *F. poae* chemotype isolates were detected in 100% of fields from CMB between 2016 to 2018, INMB in 2016, and SWMB in 2016 (Table 5.3).

Table 5. 3 Percentage of the acetyl ester derivative of DON at 15- and 3-position oxygen (15ADON and 3ADON, respectively) and NIV chemotypes of *Fusarium* species for different locations in Manitoba from 2016 to 2018.

Crop districts	Years	3-ADON(Fg)	15-ADON(Fg)	Ratio	NIV(Fg)	NIV(Fp)
		%IF	%IF	3ADON:15ADON	% IF	% IF
All crop districts*	Average three-years	30	23	30:23	0	86
CMB	2016	6	39	5:7	0	100
	2017	33	21	8:5	0	100
	2018	31	38	6:5	0	100
EMB	2016	25	25	1:1	0	75
	2017	0	0	0	0	0
	2018	0	0	0	0	0
INMB	2016	0	0	0	0	
	2017	40	20	2:1	0	100
	2018	11	24	1:1	0	90
NWMB	2016	0	0	0	0	0
	2017	0	0	0	0	0
	2018	13	44	5:7	0	75
SWMB	2016	62	0	8:0	0	100

2017	26	27	7:7	0	76
2018	13	6	2:1	0	62

Notes: *% IF: Infected field. Total number of oat fields (n=168): CMB=18 (2016), 24 (2017), 13 (2018); EMB=8 (2016), INMB=10 (2017), 20 (2018); NWMB=4 (2016), 16 (2018), SWMB=13 (2016), 26 (2017), 16 (2018), no data available for EMB in 2017 and 2018; INMB in 2016 and NWMB in 2017. % IF denotes percent of infected oat fields.

5.3.2 Phylogenetic analysis of *F. poae* isolates collected in Manitoba

The maximum likelihood technique was used to infer evolutionary relationships among 95 *F. poae* strains using the concatenated nucleotide sequences of *TEF-1*, *TRI1*, and *TRI8* genes (ML). The results of phylogenetic analysis revealed that all the *F. poae* strains studied belonged to a single monophyletic lineage (Figure 5.1). The consensus tree from ML analysis suggested four groups of *F. poae* strains, with a good bootstrap value support (>75 percent). There was no evident link between the clustering of these strains and sampling location/crop rotation, according to our findings.

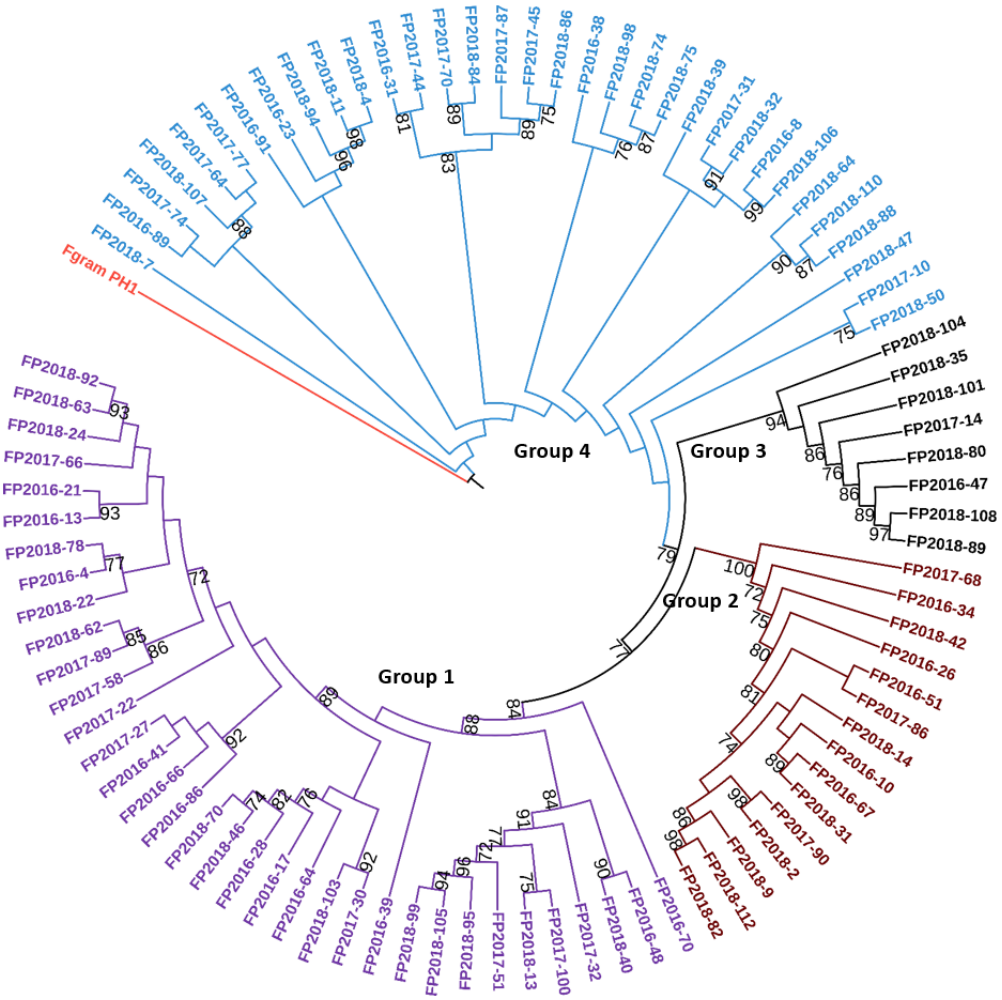

Fig 5. 1 A maximum likely hood phylogenetic tree of 95 *F. poae* strains collected in Manitoba. The phylogenetic tree was inferred from the concatenated sequences of *TEF-1α, TRI1* and *TRI8* genes. Relevant bootstrap values (expressed as a percentage of 1000 replicates) are shown at branch points.

The concatenated sequence of *F. graminearum* strain PH1 obtained from GenBank was treated as the outgroup.

5.4 Discussion

A very useful method for assessing the toxigenic potential of fungal isolates is the chemotypic characterization of *Fusarium* isolates. Our study presented that the infection caused by the *F. graminearum* 3-ADON strain is more common than the 15-ADON strain in oat fields of Manitoba. Similarly, Amarasinghe et al. (2015) documented the predominance of *F. graminearum* 3-ADON over 15-ADON chemotype in wheat fields of Manitoba. Before 1994, *F. graminearum* isolates with 15-ADON chemotype were the principal pathogens causing FHB on wheat in North America. However, shifting to *F. graminearum* isolates with 3-ADON chemotype were reported between 1998 and 2004 (Gale et al., 2002; Ward et al., 2008). Several other studies also showed higher levels of 3-ADON than 15-ADON in wheat from western Canada (Goswami and Kistler, 2004; Gilbert et al., 2010). It is believed that eastern Canada was the original point of entry for *F. graminearum* 3-ADON populations in North America which subsequently spread west across Canada and into the Upper Midwest of the United States (Amarasinghe, 2011). The shifting of *F. graminearum* 15-ADON to 3-ADON could be due to environmental conditions and various agricultural practices. According to Guo et al. (2008), seed shipment and long-distance transportation of spore was the reason behind chemotype shifting of *F. graminearum* 3-ADON and 15-ADON in Manitoba.

In the PCR analysis, we didn't detect any *F. graminearum* NIV chemotype isolates. This result is not surprising. NIV-producing *F. graminearum* isolates have not yet been reported in Canada (Amarasinghe et al., 2015). *F. graminearum* isolates with NIV chemotype have been found in several countries of Africa, Asia, and Europe (Miller et al., 1991; Jennings

et al., 2004; Mirocha et al., 1989). *F. graminearum* NIV chemotype isolates are preferentially found in wheat, maize, oat, and barley crop (Pasquali et al., 2016).

Fusarium poae isolates with NIV chemotype are prevalent in oat fields of Manitoba. This result is in line with the results presented in Chapter 4 (Section 4.3.4), suggesting that F. poae is a significant contributor to NIV in oat samples from Manitoba. The presence of *F. poae* isolates with NIV chemotype has been reported in various cereal crops from Europe and Southern America. For instance, Pettersson et al. (1995) mentioned *F. poae* as NIV producing pathogens in Swedish cereal grains (oat, barley, and wheat) between 1987 and 1990. In another study, *F. poae* isolates with NIV chemotype were found in wheat, barley, and maize samples collected from Belgium and Italy (Vanheule et al., 2017).

The phylogenetic analysis using the concatenated sequences of *TEF-1α/ TRI1* and *TRI8* showed high intra-species variability within *F. poae* isolates from Manitoba. Four groups of *F. poae* isolates were identified. There was no evident link between the clustering of these *F. poae* strains and sample locations/previous hosts when they were grouped. In a prior study published by Vanheule (et al. 2017), the genetic diversity of a set of *F. poae* isolates from Europe was analyzed using AFLP. The clustering of these *F. poae* isolates showed no clear correspondence to host and geographic origins. On the other hand, Vogelgsang et al. (2011) used microsatellite markers to characterize *F. poae* isolates from Switzerland. Two groups of *F. poae* isolates from distinct geographic areas within Switzerland were identified. Moreover, Dinolfo et al. (2014) also demonstrated that the intraspecific variability of *F. poae* isolates from Argentina, Belgium, Canada, England, Finland, France, Germany, Hungary, Italy, Luxembourg, Poland, Switzerland, and Uruguay was substantial, with a partial grouping of *F. poae* isolates connected to their geographic origins. Kerenyi et al. (1997) discovered a highly complicated pattern of vegetative compatibility groups (VCGs) in 54 partially geographically diverse isolates of *F. poae*. The disparities in these results may be partly explained by the different techniques used

and the specific genome composition of *F. poae*. *F. poae* isolates feature four core chromosomes (the "core genome") plus a set of supernumerary chromosomes that differ significantly in size, content, and number. Their presence has been found to cause severe chromosomal rearrangements inside the core chromosomes (Vanheule et al., 2016).

The way fungal pathogens live could impact their epidemiology and their ability to adapt and evolve. Because *F. poae* is thought to mix sexual and asexual reproduction and contains an active set of TEs on its chromosomes, it has been documented that *F. poae* could have a lot of genetic variation and pose a significant threat to humans (Vanheule et al., 2017). Therefore, future studies involving genome typing by sequencing and RNA-seq analysis will better understand this pathogen.

5.5 Conclusion

The chemotype analysis of *F. graminearum* isolates in commercial oat fields in Manitoba showed that *F. graminearum* strains with 3-ADON were more common than 15-ADON chemotype. The increasing level of more aggressive *F. graminearum* 3-ADON isolates will continue to pose risks to oat production in Manitoba, especially in epidemic years. Although we didn't detect any *F. graminearum* isolates with NIV chemotype in Manitoba oat fields, there is a widespread presence of *F. poae* isolates with NIV chemotype in oat. Since *F. poae* is a potent producer of NIV, a mycotoxin that is four times more toxic than DON, a more detailed study will be required to understand its role in the *Fusarium* complex on small grain cereals. The phylogenetic analysis of *F. poae* isolates revealed a high intra-species variability. More research is required to understand better the pathogenic properties and adaptations of *F. poae* on oat, a pathogen with increasing importance to oat industries in western Canada.

CHAPTER 6

6.0 GENERAL DISCUSSION AND CONCLUSIONS

6.1 General discussion and conclusions

In the current study, we investigated *Fusarium* species and naturally occurring mycotoxins in oat grains from Manitoba. *F. poae* is by far the major *Fusarium* species in oats, followed by *F. graminearum* and *F. sporotrichioides*. The *Fusarium* pathogen complex affecting oat is somewhat different from those found in wheat and barley, in which *F. graminearum* is considered the most dominant species (Osborne et al., 2007; Banik et al., 2017; Ziesman et al. 2018 & 2019).

Fusarium graminearum isolates with the 3-ADON chemotype are more frequently detected than those with the 15-ADON chemotype in commercial oat fields. It has been well documented that there are higher levels of 3-ADON than 15-ADON in wheat from western Canada (Goswami and Kistler, 2004; Gilbert et al., 2010). *Fusarium graminearum* isolates with NIV chemotype have been found in many countries of Africa, Asia, and Europe (Amarasinghe, 2011; Pasquali et al., 2016) in wheat, maize, oat, and barley. The presence of *F. graminearum* strain with NIV chemotype has not been stated in Manitoba to date. Our studies show that *F. poae* strain with NIV is common in oat from Manitoba. The principal component analysis of *Fusarium* biomass and mycotoxin content in oat grains indicated that *F. poae* primarily produces NIV and BEA in oat grains. As a result, we infer that *F. poae* is the primary source of NIV found in Manitoba oat samples.

DON and NIV are the two most common mycotoxins found in oat grains from Manitoba. Although *F. poae* is the most abundant *Fusarium* species found in oat, the concentration of DON found in oat grains is often higher than NIV. *Fusarium graminearum* is likely a more potent producer of mycotoxins than *F. poae* during infections. Nevertheless,

previous studies have shown that NIV is far more toxic than DON to humans and animals. Currently, the information on the role of *F. poae* in the *Fusarium* complex affecting small grain cereal is limited. Therefore, a better understanding of *F. poae* and the potential impact of NIV in oat or other cereal grains on end-users will be very important to evaluate the risk of this pathogen to the grain industries.

Crop rotation has a significant impact on the abundance and mycotoxin levels in oat grains. Our result suggests that the cereal-cereal rotation often results in a higher abundance of *Fusarium* biomass and mycotoxin levels in oat grains than cereal-oilseed or cereal-pulse rotations. Higher levels of contamination with DON from fields with cereals as the previous crop was also observed by Schaafsma et al. (2001) and Pageau et al. (2008) in barley, corn, and wheat. The preceding crops can affect FHB epidemics by acting as suitable host plants for *Fusarium* pathogens. They can increase the number of inoculum and produce large amounts of crop debris suitable for the saprophytic survival of these pathogens (Beyer et al., 2006).

The preliminary phylogenetic analysis of *F. poae* isolates using concatenated sequences of *EF-1α/ TRI1* and *TRI8* revealed high intra-species variability among *F. poae* isolates from Manitoba *F. poae* is producing sexual and asexual reproduction, and it also has an active arsenal of TEs, which are the principal challenges for disease management and among critical traits of a successful pathogen. A better understating of genetic diversity and genomics of this pathogen will be significant in maintaining the effectiveness of current FHB management strategies.

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Appendix

Appendix 1a. Morphological, conventional PCR and qPCR analysis to identify *Fusarium* head blight (FHB) pathogens from oat survey samples from 2016 to 2018.

Survey 2016

	Morphological analysis(infected seed %)	Conventional PCR analysis	qPCR analysis (ng/µl)

Regional Location	Field ID	Fp	Fg	Fs	Fp	Fg	Fs	Fp	Fg	Fs
CMB	4	2	0	0	†	–	–	0.06913	0.01607	0.00804
CMB	8	2	0	2	†	–	–	0.28925	0.16039	0.08577
CMB	9	4	0	0	–	†	–	0.04636	0.02634	0.00243
CMB	10	2	2	4	†	–	–	0.26472	0.27753	0.05831
CMB	12	8	4	0	†	†	–	0.03680	0.06623	0.00469
CMB	13	0	2	0	†	–	–	0.12647	0.12725	0.00000
CMB	15	4	2	0	–	–	–	0.07780	0.37220	0.00605
CMB	17	2	4	0	†	–	–	0.04232	0.21511	0.00157
CMB	18	2	2	0	†	–	–	0.07507	0.00594	0.00164
CMB	21	4	2	0	†	†	–	0.01036	0.03921	0.01710
CMB	23	0	2	0	†	†	†	0.13360	0.05963	0.00637
CMB	25	2	2	0	†	–	–	0.05291	0.08406	0.03210
CMB	26	0	2	0	†	–	–	0.33748	0.03803	0.00307
CMB	28	2	0	4	†	†	–	0.47835	0.13501	0.04323
CMB	31	4	0	0	†	†	–	0.17004	0.01853	0.00233
CMB	34	0	6	0	†	†	–	0.08079	0.17008	0.00942
CMB	36	2	4	0	–	†	–	0.00809	0.01955	0.00000
CMB	38	0	4	0	†	–	–	0.05139	0.25657	0.05045
SWMB	39	4	6	0	†	–	–	0.29291	0.02965	0.01394

SWMB	41	4	0	0	†	†	–	0.01340	0.01764	0.00810
SWMB	47	8	2	0	†	–	–	0.02105	0.00710	0.00211
SWMB	48	2	2	0	–	–	–	0.25354	0.31775	0.00482
SWMB	51	0	4	0	†	–	–	0.05753	0.51170	0.00682
SWMB	53	4	0	0	†	–	–	0.07641	0.04592	0.01519
SWMB	56	2	4	0	–	–	–	0.00786	0.00939	0.00000
SWMB	58	0	2	0	–	–	–	0.01171	0.00981	0.00000
SWMB	60	2	4	0	–	–	–	0.00807	0.00784	0.00000
SWMB	64	0	4	0	–	–	–	0.01510	0.03151	0.00135

SWMB	66	4	2	0	†	–	†	0.03602	0.02196	0.00408
SWMB	67	2	2	0	†	–	–	0.10645	0.01287	0.00000
SWMB	70	0	2	0	†	–	–	0.06397	0.01461	0.00300
EMB	72	2	0	0	†	†	†	0.07436	0.01011	0.00000
EMB	73	4	0	0	†	†	–	0.06710	0.00839	0.00377
EMB	74	2	4	0	†	–	–	0.02145	0.00083	0.00000
EMB	75	4	0	0	–	†	–	0.06547	0.01836	0.00232
EMB	76	0	0	0	–	–	–	0.01663	0.00651	0.00175
EMB	78	2	0	0	†	–	–	0.09652	0.00551	0.00000
EMB	81	6	0	0	–	–	–	0.00712	0.05513	0.00000
EMB	83	0	2	0	–	–	†	0.00785	0.02877	0.00000
NWMB	86	0	0	0	†	–	–	0.12595	0.06448	0.01652
NWMB	87	0	0	0	†	–	–	0.23206	0.28810	0.00311
NWMB	89	2	0	0	†	–	–	0.06637	0.02257	0.00000
NWMB	91	2	0	0	†	–	–	0.05354	0.05317	0.00000

Appendix 1b.

Survey 2017

		Morphological analysis (Infected seed %)			Conventional PCR analysis			qPCR analysis (ng/µl)		
Regional Location	Field ID	Fp	Fg	Fs	Fp	Fg	Fs	Fp	Fg	Fs
CMB	02	2	0	0	†	–	–	0.03160	0.05404	0.00000
CMB	05	1	0	0	–	–	–	0.07902	0.11711	0.00316
CMB	07	2	0	2	–	–	†	0.27040	0.00816	0.00084
CMB	09	2	4	0	†	†	–	0.01261	0.26916	0.00000
CMB	10	1	0	0	†	–	–	0.06323	0.03254	0.01234
CMB	13	1	0	0	–	–	–	0.02630	0.03605	0.00203
CMB	14	2	0	0	†	–	–	0.01414	0.05457	0.00123
CMB	16	0	0	2	–	–	†	0.03910	0.06929	0.00000
CMB	19	2	0	0	†	–	–	0.05697	0.36956	0.00342
CMB	21	1	0	0	–	–	–	0.10042	0.02883	0.00915
CMB	22	2	2	0	–	†	–	0.11154	0.04779	0.00616

CMB	24	2	0	0	†	–	–	0.20758	0.10063	0.00000
CMB	27	1	0	0	–	–	–	0.04859	0.03685	0.00000
CMB	28	2	4	0	†	†	–	0.01465	0.25441	0.00982
CMB	30	2	0	0	†	–	–	0.02415	0.32392	0.00000
CMB	31	1	0	0	–	–	–	0.20950	0.21415	0.00000
CMB	32	2	4	0	–	†	–	0.01679	0.32284	0.00284
CMB	33	1	0	0	†	–	–	0.01367	0.08488	0.01537
CMB	34	1	0	0	†	–	–	0.21342	0.19048	0.00000
CMB	35	2	4	0	†	†	–	0.31465	0.19932	0.05180
CMB	37	2	0	0	–	–	–	0.03258	0.03482	0.00000
CMB	40	1	0	0	†	–	–	0.01953	0.32577	0.00000
CMB	44	2	0	2	†	–	†	0.80080	0.07005	0.00481
CMB	45	1	4	0	–	†	–	0.15206	0.18146	0.00000
SWMB	47	1	0	0	†	–	–	0.23815	0.11709	0.00000
SWMB	49	2	0	0	†	–	–	0.21828	0.54818	0.00786
SWMB	51	2	0	2	–	–	–	0.14796	0.05115	0.00079
SWMB	52	1	0	0	†	–	–	0.13085	0.08999	0.00591
SWMB	53	2	0	0	†	†	–	0.02540	0.22466	0.00167
SWMB	55	1	0	0	†	–	–	0.01839	0.05042	0.00000
SWMB	57	2	2	2	†	†	–	0.17276	0.29064	0.00407

SWMB	58	1	0	0	†	–	–	0.02163	0.32851	0.00510
SWMB	59	2	2	2	†	†	–	0.22992	0.31963	0.00102
SWMB	60	1	0	0	–	–	–	0.01527	0.02965	0.02871
SWMB	61	1	2	2	–	†	–	0.52673	0.81038	0.05424
SWMB	62	1	0	0	†	–	–	0.01621	0.40402	0.01618
SWMB	64	2	0	2	†	–	–	0.02730	0.62162	0.00000
SWMB	66	0	0	0	†	–	–	0.01889	0.25249	0.00443
SWMB	67	1	0	0	–	–	–	0.04710	0.41610	0.00992
SWMB	68	2	2	0	†	†	–	0.02763	0.18848	0.01011
SWMB	70	1	0	0	†	–	–	0.01454	0.18815	0.01175
SWMB	74	2	0	0	†	–	–	0.02599	0.27556	0.01049
SWMB	76	2	2	2	†	†	–	0.06700	0.01527	0.00557
SWMB	77	1	0	0	†	–	–	0.01309	0.02867	0.00241
SWMB	79	2	2	2	†	†	†	0.08017	0.01006	0.00000
SWMB	84	0	0	2	–	–	–	1.37131	0.03590	0.00000
SWMB	86	0	0	0	†	–	–	0.75224	0.02104	0.00000
SWMB	87	2	0	2	†	–	–	0.01159	0.06826	0.07984
SWMB	89	2	0	2	–	–	–	0.20088	0.04456	0.00332
SWMB	90	1	0	0	†	–	–	0.55991	0.00986	0.00080
INMB	91	2	0	0	–	–	–	0.08288	0.19508	0.00571

INMB	92	2	0	0	†	–	–	0.02530	0.03005	0.06187
INMB	94	1	2	0	†	†	–	0.08288	0.02305	0.01264
INMB	95	3	0	0	†	–	–	1.43407	0.04993	0.00222
INMB	98	2	0	2	†	–	–	0.50531	0.03470	0.00000
INMB	99	3	0	0	–	–	–	1.04284	0.01176	0.00306
INMB	100	1	0	0	–	–	–	0.03157	0.03034	0.05764
INMB	102	3	4	0	†	†	–	0.16088	0.04597	0.00764
INMB	104	2	0	0	†	–	–	0.33960	0.03814	0.01545

Appendix 1c.

Survey 2018

		Morphological analysis (Infected seed %)			Conventional PCR analysis				qPCR analysis (ng/µl)		
Regional Location	Field ID	Fp	Fg	Fs	Fp	Fg	Fs	Fa	Fp	Fg	Fs
CMB	02	8	0	0	–	†	–	–	0.62533	0.12359	0.01151
CMB	04	20	2	0	†	†	–	–	0.10433	0.12694	0.00000
CMB	07	18	2	0	–	†	†	–	0.05053	0.31607	0.15960

CMB	09	4	0	0	†	†	–	–	1.29985	0.24012	0.00703
CMB	11	18	0	0	†	†	–	–	0.43163	0.02095	0.00237
CMB	12	30	0	0	†	†	–	–	0.08250	0.19140	0.01627
CMB	13	0	2	0	–	†	†	–	0.35102	0.28495	0.00798
CMB	14	2	2	4	–	–	†	–	0.00466	0.07714	0.02390
CMB	18	16	2	0	†	†	–	–	0.35800	0.10060	0.00341
CMB	19	14	0	0	–	†	†	–	1.01931	0.03375	0.00437
CMB	20	14	0	0	–	†	–	–	0.10164	0.01547	0.00211
CMB	21	10	0	0	–	–	–	–	0.41105	0.01473	0.00000
CMB	22	0	0	0	–	–	–	–	0.19124	0.08460	0.00000
SWMB	24	4	0	0	–	–	–	–	0.70693	0.00686	0.00000
SWMB	26	10	0	0	–	–	–	–	0.02183	0.01435	0.01289
SWMB	30	4	2	0	†	†	–	–	0.38370	0.05555	0.00000
SWMB	31	10	0	0	†	–	–	–	0.01576	0.02062	0.01358
SWMB	32	18	0	0	†	–	–	–	0.57273	0.00897	0.00000
SWMB	35	0	0	0	†	†	–	–	0.01309	0.24786	0.00159
SWMB	36	6	0	2	–	†	–	†	0.02109	0.07541	0.00506
SWMB	37	4	0	2	–	†	–	–	0.00647	0.03591	0.00877
SWMB	39	4	0	0	–	†	–	–	0.11877	0.02183	0.00514
SWMB	40	12	0	2	†	–	–	–	0.02109	0.01558	0.00000

SWMB	41	2	0	0	†	†	–	–	0.08089	0.17365	0.00464
SWMB	42	4	0	0	–	†	–	–	0.27017	0.02477	0.00000
SWMB	43	0	0	0	†	–	–	–	0.40845	0.11250	0.00000
SWMB	46	10	0	0	†	–	–	–	0.49461	0.02549	0.00000
SWMB	47	6	0	0	†	–	†	–	0.42825	0.02190	0.00000
SWMB	48	20	0	0	†	–	†	–	0.18559	0.01014	0.00951
NWMB	50	18	0	0	†	†	–	–	0.31983	0.13214	0.00298
NWMB	52	24	0	2	†	–	†	–	0.18358	0.05028	0.03358
NWMB	53	18	0	0	†	†	–	–	0.04627	0.05416	0.00000
NWMB	56	14	0	6	†	–	–	–	1.56846	0.01341	0.00000
NWMB	57	32	0	4	–	†	–	–	0.63507	0.01619	0.00167
NWMB	60	24	0	0	†	†	–	–	0.71294	0.11146	0.00056
NWMB	62	10	0	0	†	†	–	–	0.83950	0.18702	0.00066
NWMB	63	4	2	2	–	†	–	–	0.03864	0.16350	0.00096
NWMB	64	2	4	0	–	†	–	–	0.93960	0.01647	0.00271
NWMB	70	8	0	0	†	–	–	–	0.10060	0.04344	0.00265
NWMB	72	4	4	2	†	–	–	–	0.61390	0.02316	0.00000
NWMB	74	10	0	0	†	†	–	–	0.40350	0.08947	0.00000
NWMB	75	18	0	0	†	–	–	†	0.06700	0.01527	0.00557
NWMB	78	22	0	0	†	–	–	–	0.01309	0.02867	0.00241

NWMB	80	26	2	0	–	–	–	–	0.08017	0.01006	0.00000
NWMB	82	12	0	0	†	–	–	–	1.37131	0.03590	0.00000
INMB	84	2	0	0	†	–	–	–	0.75224	0.02104	0.00000
INMB	86	6	0	0	†	†	–	–	0.01159	0.06826	0.07984
INMB	88	8	0	0	†	–	–	–	0.20088	0.04456	0.00332
INMB	89	4	0	0	†	–	–	†	0.55991	0.00986	0.00080
INMB	92	8	2	0	–	†	–	–	0.08288	0.19508	0.00571
INMB	94	2	0	0	†	–	†	–	0.02530	0.03005	0.06187
INMB	95	2	0	0	†	–	–	–	0.08288	0.02305	0.01264
INMB	96	20	0	0	†	–	–	–	1.43407	0.04993	0.00222
INMB	97	20	0	0	†	†	–	–	0.50531	0.03470	0.00000
INMB	98	22	0	0	†	–	–	–	1.04284	0.01176	0.00306
INMB	99	6	0	0	†	–	†	–	0.03157	0.03034	0.05764
INMB	101	14	0	8	†	–	–	–	0.16088	0.04597	0.00764
INMB	103	0	0	0	†	–	–	†	0.33960	0.03814	0.01545
INMB	104	22	0	0	†	†	–	–	0.24786	0.07155	0.03694
INMB	105	18	0	0	–	–	–	–	0.15673	0.02197	0.00000
INMB	106	28	0	0	†	†	–	–	0.83508	0.03542	0.00000
INMB	107	22	0	0	†	–	–	–	0.37437	0.03430	0.01337
INMB	108	4	0	4	†	–	†	–	0.17430	0.04050	0.12581

INMB	110	6	0	0	†	†	–	–	0.37437	0.03430	0.00562
INMB	112	18	0	2	†	–	–	–	0.06426	0.04050	0.02427

Appendix 1d.

Climatic properties (temperature and precipitation) across Manitoba crop districts in July during the FHB survey years (2016-2018).

Manitoba regional locations	July average temperature(°C)				July average precipitation			
	2016	2017	2018	Combined 3-years	2016	2017	2018	Combined 3-years
Central (CMB)	19.2	18.4	18.7	18.7	33.8	20	16.7	23
Southwest (SWMB)	18.1	17.7	17.6	17.8	34.8	15.8	14.1	21.3
Eastern (EMB)	19.5	18.4	18.7	18.8	25.2	15.3	15.9	18.8
Northwest (NWMB)	17.7	17.4	17.7	17.6	20	26.5	22.7	23
Interlake (INMB)	18.4	17.5	18.7	18.2	14.1	18.7	21.4	18

Note: Manitoba local climate data retrieved from www.gov.mb.ca/agriculture/weather/

Appendix 2a. Naturally occurring mycotoxins in oats, grains collected from 2016-2018 in Manitoba.

Survey 2016

Mycotoxin analysis(ppb)

Field ID	Regional Location	Previous Crop	DON	NIV	T2	HT2	DAS	ENN-A	ENN-A1	ENN-B	ENN-B1	Beauvericin	Moniliformin
4	CMB	Canola	59.6	88.1	5.3	72.4	< LOD	< LOD	< LOD	< LOD	< LOQ	44.2	0
8	CMB	Canola	836.4	410.3	248.8	595.9	< LOD	< LOD	< LOD	121.2	< LOQ	14.7	316.7
9	CMB	Flax	1167	253.4	<100	0	< LOD	0.0	0.0	< LOD	< LOD	5.2	0
10	CMB	Canola	1114.3	204.2	23.4	< LOQ	< LOD	< LOD	< LOD	< LOQ	< LOQ	29.7	< LOQ
17	CMB	Canola	<LOD	< LOQ	0	0	< LOD	0.0	< LOD	< LOD	< LOD	15.3	0
18	CMB	Canola	142.6	< LOQ	<100	0	< LOD	< LOD	< LOQ	108.0	166.3	49	0
23	CMB	Canola	300.7	81.2	5.8	0	< LOD	< LOD	< LOD	< LOQ	103.3	29.8	49.3
26	CMB	Canola	<LOD	170.1	< LOQ	< LOQ	< LOD	0.0	0.0	< LOD	< LOQ	27.9	< LOQ
28	CMB	Flax	269.7	< LOQ	< LOQ	< LOQ	< LOD	< LOD	< LOD	< LOD	< LOQ	15.4	< LOQ
31	CMB	Flax	166.5	< LOQ	<100	0	< LOD	0.0	0.0	< LOD	< LOQ	0	0
66	CMB	Cereal	0	157.1	11.6	0	< LOD	0.0	< LOD	< LOQ	< LOQ	16.2	0
67	CMB	Cereal	0	83.1	< LOQ	< LOQ	< LOD	0.0	0.0	< LOD	< LOQ	58.6	348.5
81	CMB	Cereal	730.1	118.3	0	0	0.0	0.0	0.0	0.0	0.0	7.8	0
83	CMB	Cereal	544.3	50.0	0	0	0.0	0.0	0.0	0.0	0.0	15.2	0
86	CMB	Cereal	638.0	327.2	274.6	301.7	0.0	0.0	0.0	0.0	0.0	77.6	152

87	CMB	Cereal	136.5	592.1	0	<LOD	0.0	0.0	0.0	0.0	0.0	119.2	<LOD
89	CMB	Cereal	214.6	186.0	81	166.6	0.0	0.0	0.0	0.0	0.0	11.2	0
91	CMB	Cereal	247.0	153.1	0	0	0.0	0.0	0.0	0.0	0.0	18.7	0
12	SWMB	Flax	448.3	365.3	5.3		< LOQ	0.0	0.0	< LOD	< LOD	78.8	69.1
13	SWMB	Flax	3143	257.6	0	0	< LOD	0.0	0.0	1604.5	< LOD	17.6	0
21	SWMB	Flax	94.3	< LOQ	21	0	< LOD	0.0	< LOD	< LOQ	< LOD	0	12.9
25	SWMB	Cereal	<LOD	336.5	91.9	168.4	< LOD	0.0	< LOD	< LOD	< LOD	11.3	24.4
34	SWMB	Canola	578.5	180.7	0	0	< LOD	0.0	0.0	< LOD	< LOD	5.7	20.9
36	SWMB	Canola	105.2	< LOQ	0	0	< LOD	0.0	< LOD	< LOD	< LOD	9.7	0
38	SWMB	Canola	<LOD	103.2	1154.6	1100.9	< LOD	0.0	0.0	0.0	0.0	15	0
39	SWMB	Cereal	4143.2	864.6	< LOQ	< LOQ	< LOD	0.0	0.0	0.0	0.0	0.0	0
47	SWMB	Corn	197.8	168.9	4	0	< LOD	0.0	0.0	< LOD	0.0	13.1	0
48	SWMB	Canola	60.3	< LOQ	< LOQ	< LOQ	< LOD	0.0	0.0	< LOD	< LOD	0.0	0
51	SWMB	Canola	<LOD	133.2	13.1	53	< LOD	0.0	0.0	0.0	< LOD	6.8	11.5
53	SWMB	Canola	176.4	144.9	4.1	0	0.0	0.0	< LOD	0.0	< LOD	12.7	0
56	SWMB	Cereal	96.3	110.6	0	5	< LOD	0.0	< LOD	< LOD	< LOD	6.9	0
15	EMB	Flax	194.1	< LOQ	0	0	< LOD	< LOD	< LOD	< LOQ	< LOQ	42.1	0
70	EMB	Cereal	0	167.5	<100	0	< LOD	0.0	0.0	0.0	0.0	22.1	0
72	EMB	Canola	730.1	118.3	<101	1	< LOD	0.0	0.0	< LOD	< LOD	22.4	0

73	EMB	Cereal	544.3	< LOQ	0	0	< LOD	< LOD	< LOD	< LOQ	< LOQ	28.2	0
74	EMB	Cereal	638	327.2	<100	0	< LOQ	0.0	< LOD	< LOD	< LOD	15.4	113.6
75	EMB	Canola	136.5	592.1	<100	0	< LOQ	0.0	< LOD	< LOD	< LOD	12.2	0
76	EMB	Cereal	214.6	186	7.7	0	< LOQ	0.0	0.0	0.0	0.0	17.9	0
78	EMB	Cereal	247	153.1	<100	0	< LOD	0.0	0.0	< LOD	< LOD	85.4	0
41	NWMB	Canola	1549	164.3	3.1	0	< LOD	0.0	< LOD	< LOD	< LOD	5.6	0
58	NWMB	Cereal	< LOQ	234.6	<100	0	< LOQ	< LOD	< LOD	< LOQ	< LOD	0	0
60	NWMB	Cereal	<LOD	156.5	<101	1	< LOD	< LOD	< LOD	< LOQ	< LOQ	0	0
64	NWMB	Cereal	91.8	375.7	0	0	< LOQ	0.0	0.0	0.0	0.0	0	0

Appendix 2b.

Survey 2017

Mycotoxin analysis(ppb)

Field ID	Regional Location	Previous Crop	DON	NIV	T2	HT2	DAS	ENN-A	ENN-A1	ENN-B	ENN-B1	Beauvericin	Moniliforminn
02	CMB	Canola	440.3	<LOD	0	5	<LOD	0.0	0.0	0.0	<LOD	24.5	533.3
05	CMB	Canola	412.8	93.2	4.6	<LOD	<LOD	0.0	0.0	5.0	<LOD	17.5	0
07	CMB	Canola	862.4	123.8	0	0	<LOD	0.0	0.0	0.0	0.0	0	0
09	CMB	Canola	<LOD	102.9	<100	0	<LOD	5.0	<LOD	5.0	<LOD	24.3	0
10	CMB	Canola	<LOD	160.9	91.3	83.5	<LOD	0.0	0.0	5.0	0.0	48.8	0
21	CMB	Canola	316.8	119.5	0	0	<LOD	0.0	0.0	5.0	<LOD	0	242.1
22	CMB	Canola	<LOD	50	14.3	0	<LOD	0.0	0.0	5.0	<LOD	16	0
31	CMB	Canola	<LOD	109.6	<100	0	<LOD	0.0	0.0	5.0	<LOD	61.5	0
32	CMB	Canola	<LOD	67	<100	0	<LOD	0.0	0.0	0.0	0.0	35.1	0
33	CMB	Canola	<LOD	164.7	<100	0	<LOD	0.0	0.0	5.0	0.0	15.5	0
34	CMB	Canola	509	77	0	0	<LOD	0.0	0.0	0.0	0.0	20	0

35	CMB	Canola	929	140.5	973.2	418.7	<LOD	0.0	0.0	5.0	0.0	20.6	0
37	CMB	Canola	0	303.9	<100	0	<LOD	0.0	0.0	0.0	0.0	8.2	0
40	CMB	Canola	<LOD	130.7	<100	0	<LOD	0.0	0.0	0.0	0.0	0	0
47	CMB	Canola	531.8	277.2	0	0	<LOD	5.0	<LOQ	25.0	<LOD	13.1	0
49	CMB	Cereal	858.7	381.8	0	0	<LOD	0.0	0.0	0.0	0.0	99.8	0
74	CMB	Canola	<LOD	<LOQ	<100	0	<LOD	0.0	0.0	5.0	0.0	28.2	0
84	CMB	Cereal	<LOD	427.4	108	92.5	<LOQ	0.0	0.0	5.0	<LOD	52.4	32.2
86	CMB	Canola	<LOD	180.8	12.6	0	<LOD	0.0	0.0	5.0	0.0	23.6	0
87	CMB	Flax	<LOD	194.1	<100	0	<LOD	0.0	0.0	0.0	0.0	37.8	0
89	CMB	Canola	<LOD	220.7	9.4	0	<LOD	0.0	0.0	5.0	0.0	86.6	0
90	CMB	Cereal	305.1	132.8	4.8	0	<LOD	0.0	0.0	5.0	<LOD	18.1	0
91	CMB	Canola	705.9	488.5	5.1	0	<LOD	0.0	0.0	0.0	0.0	26.6	0

92	CMB	Canola	<LOD	241.9	<100	0	<LOD	0.0	<LOD	5.0	<LOD	69.3	0
13	SWMB	Canola	<LOD	157.5	<100	0	<LOD	0.0	0.0	0.0	0.0	9.5	0
14	SWMB	Canola	<LOD	121.8	<100	0	<LOD	0.0	0.0	5.0	<LOD	5.4	0
16	SWMB	Canola	<LOD	264.7	0	0	<LOD	0.0	0.0	0.0	0.0	17.1	0
19	SWMB	Canola	176.4	406.5	4.4	0	<LOD	0.0	<LOD	85.3	<LOQ	85.6	28
24	SWMB	Canola	591.9	94.8	47.9	291.7	<LOD	5.0	<LOD	25.0	<LOQ	8.5	10.8
28	SWMB	Canola	<LOD	57	<100	0	<LOD	0.0	<LOD	5.0	5.0	31.2	0
44	SWMB	Canola	4470.7	149.6	0	0	<LOD	0.0	0.0	0.0	0.0	8.1	0
45	SWMB	Canola	537.7	93.4	0	0	<LOD	5.0	<LOD	25.0	<LOQ	49.5	119
51	SWMB	Wheat	239.4	178.1	0	0	<LOD	0.0	0.0	0.0	0.0	17.7	0
52	SWMB	Canola	223.3	60.4	0	0	<LOD	0.0	0.0	0.0	0.0	4.4	0
53	SWMB	Canola	<LOD	406.7	<100	0	<LOQ	0.0	0.0	25.0	<LOD	29.3	0

55	SWMB	Canola	<LOD	212.6	<100	0	<LOD	0.0	0.0	5.0	<LOD	9.9	0
57	SWMB	Flax	242.6	110.3	0	0	<LOD	0.0	<LOD	93.3	<LOQ	22.8	0
58	SWMB	Canola	<LOD	136.9	<100	0	<LOD	0.0	0.0	25.0	<LOD	0	0
59	SWMB	Flax	111.1	221.2	<100	0	<LOD	0.0	<LOD	0.0	<LOD	26.5	66.2
60	SWMB	Cereal	<LOD	120.6	6.2	0	<LOD	0.0	0.0	0.0	0.0	0	0
61	SWMB	Cereal	927.8	266.8	63.6	299.5	<LOD	0.0	<LOD	25.0	<LOD	159.2	0
62	SWMB	Flax	0	174.1	63.7	67.3	<LOD	0.0	0.0	5.0	<LOD	64.1	262.6
64	SWMB	Canola	<LOD	77.3	<100	0	<LOD	0.0	0.0	0.0	0.0	10.8	0
66	SWMB	Canola	<LOD	176.6	<100	0	<LOD	0.0	0.0	0.0	<LOD	14.2	0
67	SWMB	Canola	<LOD	126.9	<100	0	<LOD	0.0	<LOD	5.0	<LOD	62.3	0
68	SWMB	Canola	<LOD	169	12.6	0	<LOD	0.0	0.0	0.0	0.0	8.5	0
70	SWMB	Flax	0	61.7	<100	0	<LOD	0.0	0.0	25.0	0.0	9.7	0

76	SWMB	Cereal	250	206.9	275.8	194	<LOD	0.0	0.0	5.0	<LOD	81.3	0
77	SWMB	Cereal	<LOD	25	9.2	0	<LOD	0.0	0.0	0.0	0.0	16.3	0
79	SWMB	Canola	499.4	206.6	5	0	<LOD	0.0	0.0	70.9	<LOD	152.9	135.9
27	INMB	Cereal	<LOD	116.1	<100	0	<LOD	0.0	0.0	5.0	<LOD	0	0
30	INMB	Canola	438.7	66.9	<100	0	<LOD	0.0	0.0	0.0	0.0	0	0
94	INMB	Cereal	3835.9	238.5	4.3	0	<LOD	5.0	<LOQ	25.0	<LOQ	12.7	34.1
95	INMB	Canola	<LOD	169.5	<100	0	<LOQ	5.0	0.0	5.0	<LOD	35.8	211.7
98	INMB	Flax	<LOD	202.2	<100	0	<LOQ	0.0	0.0	5.0	<LOD	49.8	0
99	INMB	Canola	<LOD	174.9	<100	0	<LOD	0.0	<LOD	25.0	<LOD	62.3	0
100	INMB	Canola	<LOD	190.1	<101	1	<LOD	0.0	0.0	0.0	0.0	27.5	0
102	INMB	Canola	1374.6	127.6	3.9	0	<LOD	0.0	<LOD	25.0	<LOQ	52.3	0
104	INMB	Canola	1881.5	795	0	0	<LOD	0.0	0.0	5.0	0.0	41	0

105	INMB	Corn	<LOD	341.9	<100	0	<LOD	0.0	0.0	0.0	0.0	23.9	0

Appendix 2c.

Survey 2018

Mycotoxin analysis(ppb)

Field ID	Regional Location	Previous Crop	DON	NIV	T2	HT2	DAS	ENN-A	ENN-A1	ENN-B	ENN-B1	Beauvericin	Moniliformin
02	CMB	Flax	0	89.5	0	<LOD	<LOD	0.0	0.0	<LOD	<LOD	0	0
04	CMB	Canola	296.7	66.3	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	14.1	0

07	CMB	Cereal	516	133.4	794.3	654.4	<LOD	0.0	0.0	<LOD	0.0	14.8	0
09	CMB	Flax	526.8	66.5	<LOD	<LOD	<LOD	0.0	<LOD	<LOQ	<LOD	0	162.3
11	CMB	Corn	<LOD	50	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	0	0
12	CMB	Canola	632.2	319.7	<LOD	0	<LOD	0.0	0.0	0.0	0.0	9.5	0
13	CMB	Canola	323.5	99.7	<LOD	<LOQ	<LOD	0.0	0.0	0.0	0.0	7.1	0
18	CMB	Canola	247.1	159.7	<LOD	0	<LOD	0.0	0.0	0.0	0.0	4.9	0
19	CMB	Flax	<LOD	75.9	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	11.8	0
20	CMB	Flax	<LOD	127.3	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	0	0
21	CMB	Canola	<LOD	99.3	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	0	0
89	CMB	Canola	80.1	221.9	96.3	282.3	<LOD	0.0	0.0	0.0	0.0	0	0
92	CMB	Canola	5	98.4	6.3	0	<LOD	0.0	0.0	0.0	0.0	4.9	0
14	SWMB	Canola	117.7	221.1	0	0	<LOD	0.0	0.0	0.0	0.0	41.4	0
30	SWMB	Canola	<LOD	117.8	<LOD	<LOD	<LOQ	0.0	0.0	0.0	0.0	34.7	0
31	SWMB	Corn	<LOD	126.2	<LOD	<LOD	<LOD	0.0	<LOD	<LOD	<LOQ	0	0
32	SWMB	Canola	<LOD	208.7	<LOD	<LOD	<LOQ	0.0	0.0	<LOD	<LOD	15.7	0
50	SWMB	Canola	<LOD	99.1	0	<LOD	<LOD	0.0	0.0	0.0	0.0	52.4	0
52	SWMB	Canola	<LOD	<LOQ	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	10.6	0
53	SWMB	Flax	<LOD	63.1	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	5.5	0
56	SWMB	Flax	<LOD	25	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	6.6	0
57	SWMB	Canola	<LOD	142.6	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	23.6	0

63	SWMB	Canola	<LOQ	<LOQ	57.2	106.2	<LOD	0.0	0.0	0.0	0.0	7.2	0
64	SWMB	Canola	541.1	61.1	0	<LOD	<LOD	0.0	0.0	0.0	0.0	0	0
70	SWMB	Canola	217.7	<LOD	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	4.7	0
72	SWMB	Cereal	0	<LOQ	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	0	0
74	SWMB	Canola	<LOD	<LOQ	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	0	0
75	SWMB	Canola	<LOD	50	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	20.1	0
78	SWMB	Canola	123	51.2	<LOD	0	<LOD	0.0	0.0	0.0	0.0	0	0
22	NWMB	Flax	239.8	63	0	<LOD	<LOD	0.0	0.0	<LOD	<LOD	14.3	0
24	NWMB	Canola	<LOD	92.7	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	0	0
26	NWMB	Canola	<LOD	91.4	32.6	0	<LOD	5.0	<LOD	<LOQ	<LOQ	0	208.5
35	NWMB	Cereal	374.3	188.7	<LOD	<LOD	<LOD	0.0	0.0	<LOD	<LOD	0	0
36	NWMB	Corn	68.8	287.8	<LOD	<LOD	<LOD	0.0	0.0	<LOD	<LOD	14.8	88
37	NWMB	Canola	<LOD	188.1	3.1	0	<LOD	0.0	0.0	0.0	0.0	6.1	0
39	NWMB	Canola	<LOD	172	0	<LOD	<LOD	0.0	0.0	<LOD	<LOD	26.8	0
40	NWMB	Canola	<LOD	159.4	<LOD	<LOD	<LOD	5.0	<LOQ	84.5	128.0	38.4	109.9
41	NWMB	Canola	178	<LOQ	66.2	0	<LOD	0.0	0.0	<LOD	<LOD	0	0
43	NWMB	Canola	358.7	86.4	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	13.4	0
48	NWMB	Canola	<LOD	<LOQ	<LOD	<LOD	<LOD	0.0	<LOD	<LOQ	<LOQ	13.6	78.4
80	NWMB	Canola	<LOD	137.9	<LOD	<LOD	<LOD	0.0	0.0	<LOD	0.0	29.8	41.3
82	NWMB	Canola	54.7	130.2	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	9.9	0

84	NWMB	Canola	<LOD	107.3	<LOD	<LOD	<LOD	0.0	0.0	0.0	<LOD	4.5	0
86	NWMB	Canola	<LOD	<LOQ	<LOD	<LOD	<LOD	0.0	<LOD	<LOD	0.0	17.7	0
88	NWMB	Canola	<LOD	<LOQ	0	<LOD	<LOD	0.0	0.0	0.0	0.0	0	0
42	INMB	Canola	72.3	137.5	0	<LOD	<LOD	0.0	0.0	0.0	0.0	4.6	0
46	INMB	Flax	245.8	87.6	<LOD	<LOD	<LOD	0.0	<LOD	<LOQ	<LOQ	5.8	11.1
47	INMB	Corn	<LOD	146.1	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	0	0
95	INMB	Corn	389.8	<LOQ	0	<LOD	<LOD	0.0	0.0	0.0	0.0	24	0
96	INMB	Corn	<LOD	215.9	118.3	200.3	<LOD	0.0	0.0	0.0	0.0	169.4	0
97	INMB	Canola	<LOD	134.2	5.1	0	<LOD	0.0	0.0	<LOD	0.0	100.1	0
98	INMB	Canola	74.7	106.2	<LOD	0	<LOD	0.0	<LOD	<LOD	<LOD	132.7	0
99	INMB	Canola	<LOD	<LOQ	0	<LOD	<LOD	0.0	0.0	0.0	0.0	11.8	0
101	INMB	Cereal	<LOD	223.9	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	94.4	0
103	INMB	Cereal	<LOD	<LOQ	9.5	0	<LOD	0.0	0.0	<LOD	<LOD	0	0
104	INMB	Canola	<LOD	204.4	27.8	0	<LOD	0.0	<LOD	0.0	0.0	32.8	0
105	INMB	Canola	<LOD	180.1	30.9	74.3	<LOD	0.0	0.0	<LOD	0.0	118.3	0
106	INMB	Canola	<LOD	581.3	8.4	0	<LOQ	0.0	0.0	<LOD	<LOD	116.5	0
107	INMB	Canola	<LOD	<LOD	<LOD	<LOD	<LOD	0.0	0.0	0.0	0.0	0	0
108	INMB	Cereal	78.7	50.2	<LOD	0	<LOD	0.0	0.0	<LOD	<LOD	45	0
110	INMB	Canola	70.2	73.2	3.9	0	<LOD	0.0	0.0	0.0	0.0	46.4	0
112	INMB	Canola	<LOD	260	77	91.5	<LOD	0.0	<LOD	<LOD	<LOD	73.7	0

Appendix 3. Climatic properties (temperature and precipitation) across Manitoba crop districts in July during the FHB survey years (2016-2018).

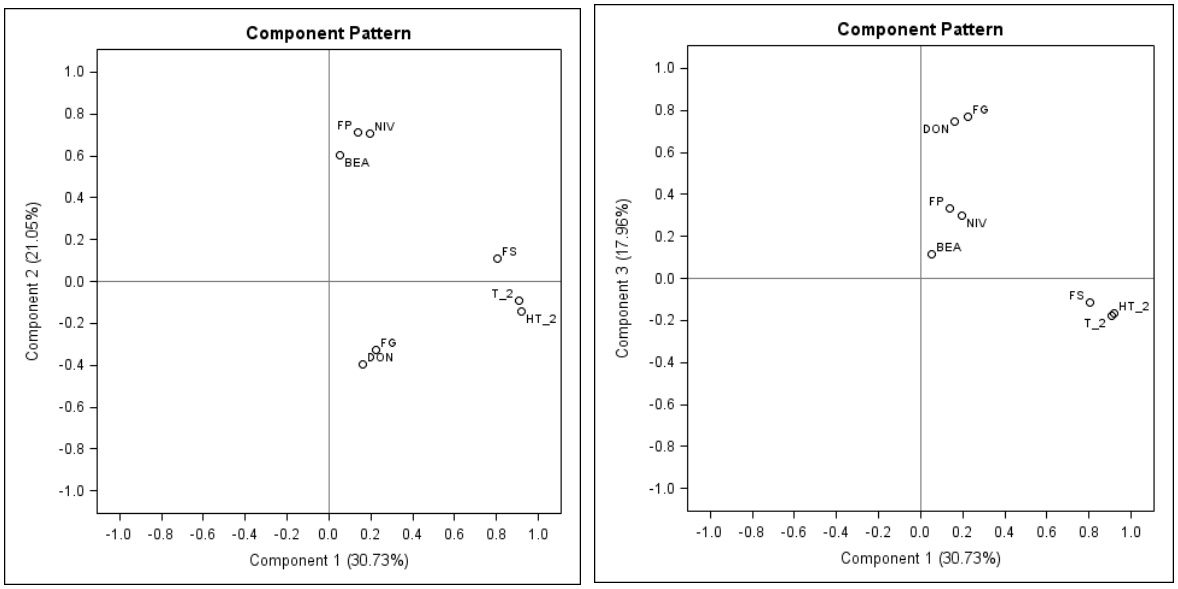
Manitoba regional locations	July average temperature(°C)				July average precipitation			
	2016	2017	2018	Combined 3-years	2016	2017	2018	Combined 3-years
Central (CMB)	19.2	18.4	18.7	18.7	33.8	20	16.7	23
Southwest (SWMB)	18.1	17.7	17.6	17.8	34.8	15.8	14.1	21.3
Eastern (EMB)	19.5	18.4	18.7	18.8	25.2	15.3	15.9	18.8
Northwest (NWMB)	17.7	17.4	17.7	17.6	20	26.5	22.7	23
Interlake (INMB)	18.4	17.5	18.7	18.2	14.1	18.7	21.4	18

Note: Manitoba local climate data retrieved from www.gov.mb.ca/agriculture/weather/

Appendix 4a. PCA and correlation coefficient

In the principal component (PC) analysis of the relationships between the measured parameters and eigenvalue of eight factors, the first three PC accounted for 69.7% of the total variance. The first PC (30.7% of the total variance) was associated with Fs (r=0.806, p=<0.0001), T_2 (r=0.906, p=<0.0001) and Ht_2 (r=0.922, p=<0.0001), the second PC (21.1% of the total variance) was associated with Fp (r=0.714, p= <0.0001), BEA (r=0.603, p= <0.0001) and NIV (r=0.709, p=<0.0001), and the third PC (17.9% of the total variance) was associated with Fg (r=0.772, p=<0.0001) and DON (r=0.744, p=<0.0001). Based on the PC and variable correlation pattern Fs, T-2 and Ht_2 is in one group; Fp, BEA and NIV in another group while Fg and DON are in the other group (Fig. 1).

Appendix Fig. 1 Principal component analysis based on the amount of DNA of three *Fusarium* species and five toxins production in oat grains.



Appendix 4b. Descriptive statistics of Principle component analysis.

The SAS System

The CORR Procedure

3 With Variables: Prin1 Prin2 Prin3

8 Variables: FP FG FS BEA NIV DON T_2 HT_2

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum	Label
Prin1	141	0	1.56791	0	-0.88662	10.44817	
Prin2	141	0	1.29782	0	-3.38346	4.84190	
Prin3	141	0	1.19853	0	-1.87211	4.46474	
FP	142	0.22388	0.22888	31.79094	0.00466	1.04284	Fp
FG	142	0.08625	0.10680	12.24734	0.0008332	0.52673	Fg
FS	142	0.00844	0.01922	1.19832	0	0.15960	Fs
BEA	142	38.89366	32.70651	5523	4.40000	185.00000	BEA
NIV	142	245.01408	181.66380	34792	50.00000	864.60000	NIV
DON	142	237.05211	410.88440	33661	0	3143	DON
T_2	141	44.95674	144.25023	6339	0	1155	T_2
HT_2	142	35.85211	131.38072	5091	0	1101	HT_2

Pearson Correlation Coefficients

Prob > |r| under H0: Rho=0

Number of Observations

	FP	FG	FS	BEA	NIV	DON	T_2	HT_2
Prin1	0.13546	0.22471	0.80618	0.05251	0.19636	0.16354	0.90619	0.92213
	0.1092	0.0074	<.0001	0.5363	0.0196	0.0527	<.0001	<.0001
	141	141	141	141	141	141	141	141
Prin2	0.71485	-0.32581	0.10818	0.60325	0.70934	-0.39898	-0.09294	-0.14350
	<.0001	<.0001	0.2017	<.0001	<.0001	<.0001	0.2730	0.0896
	141	141	141	141	141	141	141	141

Prin3	0.33264	0.77246	-0.11289	0.11543	0.30035	0.74402	-0.18003	-0.16397
	<.0001	<.0001	0.1826	0.1729	0.0003	<.0001	0.0327	0.0520
	141	141	141	141	141	141	141	141

Appendix 5a. Percentage of the acetyl ester derivative of DON at 15- and 3-position oxygen (15ADON and 3ADON, respectively) and NIV chemotypes of Fusarium species for different locations in Manitoba from 2016 to 2018.

	Survey 2016			
Regional Location	3-ADON	15-ADON	Fg Nivalenol	Fp-Nivalenol
CMB	-	-	-	+
CMB	-	+	-	+
CMB	-	-	-	+
CMB	+	-	-	+
CMB	-	-	-	+
CMB	-	-	-	+
CMB	-	+	-	+
CMB	-	-	-	+
CMB	+	+	-	+
CMB	-	-	-	+
CMB	-	-	-	+
CMB	-	+	-	+
CMB	-	-	-	+
CMB	-	-	-	+
CMB	-	+	-	+
CMB	-	-	-	+
CMB	-	+	-	+

CMB	+	+	-	+
SWMB	+	+	-	+
SWMB	+	-	-	+
SWMB	+	-	-	+
SWMB	-	-	-	+
SWMB	+	+	-	+
SWMB	-	+	-	+
SWMB	+	+	-	+
SWMB	-	-	-	+
SWMB	-	-	-	-
SWMB	+	+	-	+
SWMB	+	+	-	+
SWMB	+	-	-	+
SWMB	-	-	-	-
EMB	+	+	-	+
EMB	-	-	-	+
EMB	-	-	-	+
EMB	-	-	-	+
EMB	-	+	-	+
EMB	-	-	-	+
EMB	-	-	-	+
EMB	-	-	-	+
NWMB	+	-	-	+
NWMB	-	-	-	-
NWMB	-	-	-	+

NWMB	-	-	-	+

Appendix 5b.

	Survey 2017			
Regional Location	3-ADON	15-ADON	Fg Nivalenol	Fp-Nivalenol
CMB	-	-	-	+
CMB	+	-	-	+
CMB	+	-	-	-
CMB	+	-	-	+
CMB	-	-	-	+
CMB	-	-	-	+
CMB	+	+	-	+
CMB	-	-	-	+
CMB	-	+	-	+
CMB	-	-	-	+
CMB	+	+	-	+
CMB	+	+	-	+
CMB	-	-	-	+
CMB	-	-	-	+
CMB	-	+	-	+
CMB	-	+	-	-
CMB	-	+	-	+
CMB	-	-	-	+
CMB	-	-	-	+
CMB	-	-	-	+
CMB	-	-	-	+
CMB	-	+	-	+
CMB	-	-	-	+
CMB	-	-	-	+
SWMB	-	-	-	+

SWMB	-	-	-	-
SWMB	-	-	-	+
SWMB	-	-	-	+
SWMB	+	-	-	+
SWMB	-	-	-	+
SWMB	+	+	-	+
SWMB	-	-	-	+
SWMB	-	-	-	+
SWMB	-	+	-	+
SWMB	-	+	-	+
SWMB	-	-	-	+
SWMB	+	+	-	+
SWMB		-	-	+
SWMB	+	+	-	+
SWMB	-	-	-	+
SWMB	+	-	-	+
SWMB	-	-	-	+
SWMB	-	+	-	+
SWMB	-	+	-	+
SWMB	-	-	-	+
SWMB	-	-	-	+
SWMB	-	+	-	+
SWMB	+	-	-	+
SWMB	-	-	-	+
SWMB	+	+	-	+
INMB	-	-	-	+
INMB	+	-	-	+
INMB	+	+	-	-
INMB	-	-	-	+
INMB	-	-	-	+
INMB	-	-	-	+
INMB	-	-	-	-

INMB	+	+	-	+
INMB	-	-	-	+
INMB	+	-	-	+

Appendix 5c.

	Survey 2018			
Regional Location	3-ADON	15-ADON	Fg Nivalenol	Fp-Nivalenol
CMB	-	-	-	-
CMB	-	+	-	+
CMB	-	-	-	-
CMB	+	+	-	+
CMB	+	+	-	+
CMB	+	+	-	+
CMB	+	-	-	+
CMB	-	-	-	-
CMB	-	-	-	+
CMB	-	-	-	+
CMB	-	+	-	+
CMB	-	-	-	+
CMB	-	-	-	-
SWMB	-	-	-	-
SWMB	-	-	-	+
SWMB	-	-	-	+
SWMB	-	-	-	+
SWMB	+	-	-	+
SWMB	-	+	-	-
SWMB	-	+	-	-
SWMB		-	-	+
SWMB	+	-	-	+
SWMB	-	-	-	no DNA
SWMB	-	+	-	-
SWMB	-	-	-	no DNA

SWMB	-	-	-	+
SWMB	-	-	-	+
SWMB	-	-	-	-
SWMB	-		-	+
NWMB	-		-	
NWMB	-	+	-	No DNA
NWMB	-	-	-	+
NWMB	-	+	-	+
NWMB	-	-	-	-
NWMB	-	-	-	-
NWMB	-	-	-	+
NWMB	-	-	-	+
NWMB	+	+	-	+
NWMB	-	+	-	+
NWMB		+	-	no DNA
NWMB	-	+	-	+
NWMB	-	-	-	+
NWMB	-	-	-	+
NWMB	-	+	-	+
NWMB	-	-	-	-
INMB	+	+	-	+
INMB	-	+	-	-
INMB	+	-	-	+
INMB	-	-	-	-
INMB	-	-	-	+
INMB	-	-	-	+
INMB	-	-	-	+
INMB	-	-	-	+
INMB	-	-	-	+
INMB	-	-	-	no DNA
INMB	-	-	-	+
INMB	-	-	-	+

INMB	-	-	-	+
INMB	-	-	-	+
INMB	-	-	-	+
INMB	-	-	-	+
INMB	-	-	-	+

Appendix 6. Phylogenetic tree analysis

Isolate name	Cluster	Crop district	Previous crop
FP2018-92	Group 1	CMB	Canola
FP2018-63		SWMB	Canola
FP2018-24		NWMB	Canola
FP2017-66		SWMB	Canola
FP2016-21		SWMB	Flax
FP2016-13		SWMB	Flax
FP2018-78		SWMB	Canola
FP2016-4		CMB	Canola
FP2018-22		NWMB	Flax
FP2018-62		SWMB	Canola
FP2017-89		CMB	Canola
FP2017-58		SWMB	Canola
FP2017-22		CMB	Canola
FP2017-27		INMB	Cereal
FP2016-41		NWMB	Canola
FP2016-66		CMB	Cereal
FP2016-86		CMB	Cereal
FP2018-70		SWMB	Canola
FP2018-46		INMB	Flax
FP2016-28		CMB	Flax
FP2016-17		CMB	Canola
FP2016-64		NWMB	Cereal
FP2018-103		INMB	Cereal

FP2017-30		INMB	Canola
FP2016-39		SWMB	Sunflower
FP2018-99		INMB	Canola
FP2018-105		INMB	Canola
FP2018-95		INMB	Corn
FP2017-51		SWMB	Wheat
FP2018-13		CMB	Canola
FP2017-100		INMB	Canola
FP2017-32		CMB	Canola
FP2018-40		NWMB	Canola
FP2016-48		SWMB	Canola
FP2016-70		EMB	Cereal
FP2017-68	Group 2	SWMB	Canola
FP2016-34		SWMB	Canola
FP2018-42		INMB	Canola
FP2016-26		CMB	Canola
FP2016-51		SWMB	Canola
FP2017-86		CMB	Canola
FP2018-14		SWMB	Canola
FP2016-10		CMB	Canola
FP2016-67		CMB	Cereal
FP2018-31		SWMB	Corn
FP2017-90		CMB	Cereal
FP2018-2		CMB	Flax
FP2018-9		CMB	Flax
FP2018-112		INMB	Canola
FP2018-82		NWMB	Canola
FP2018-104	Group 3	INMB	Canola
FP2018-35		NWMB	Cereal
FP2018-101		INMB	Cereal
FP2017-14		SWMB	Canola
FP2018-80		NWMB	Canola

FP2016-47		SWMB	Corn
FP2018-108		INMB	Cereal
FP2018-89		CMB	Canola
FP2018-7	Group 4	CMB	Cereal
FP2016-89		CMB	Cereal
FP2017-74		CMB	Canola
FP2018-107		INMB	Canola
FP2017-64		SWMB	Canola
FP2017-77		SWMB	Cereal
FP2016-91		CMB	Cereal
FP2016-23		CMB	Canola
FP2018-94		CMB	Canola
FP2018-11		CMB	Corn
FP2018-4		CMB	Canola
FP2016-31		CMB	Flax
FP2017-44		SWMB	Canola
FP2017-70		SWMB	Flax
FP2018-84		NWMB	Canola
FP2017-87		CMB	Flax
FP2017-45		SWMB	Canola
FP2018-86		NWMB	Canola
FP2016-38		SWMB	Canola
FP2018-98		INMB	Canola
FP2018-74		SWMB	Canola
FP2018-75		SWMB	Canola
FP2018-39		NWMB	Canola
FP2017-31		CMB	Canola
FP2018-32		SWMB	Canola
FP2016-8		CMB	Canola
FP2018-106		INMB	Canola
FP2018-64		SWMB	Canola
FP2018-110		INMB	Canola

FP2018-88	NWMB	Canola
FP2018-47	INMB	Corn
FP2017-10	CMB	Canola
FP2018-50	SWMB	Canola