Evaluation of fusarium head blight and rust reaction in rye and fungicide efficacy in fusarium head blight mitigation

by

Duoduo Wang

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Department of Plant Science

University of Manitoba

Winnipeg

Abstract

In Canada, very little is known about fusarium head blight (FHB) and leaf rust on rye. The overall goal of the thesis is to provide critical and practical information on rye reaction to FHB, leaf rust, and ergot, and the effect of fungicide application on controlling FHB. Fall rye spikes were collected from naturally infected fields in Manitoba. Five *Fusarium* species (*Fusarium graminearum*, *F. avenaceum*, *F. poae*, *F. sporotrichioides*, and *F. equiseti*) were isolated and identified from these samples. *Fusarium graminearum* was the principal *Fusarium* species recovered from affected kernels. It was the most pathogenic species among the four *Fusarium* species (*F. graminearum*, *F. avenaceum*. *F. sporotrichioides*, and *F. poae*) evaluated on six fall rye genotypes and two wheat checks in the greenhouse. Two sets of fall rye genotypes were assessed through artificial inoculation in multi-environmental trials. Rye was less affected by FHB and more resistant to the disease than winter wheat. Disease levels and deoxynivalenol (DON) varied among fall rye genotypes tested for their reactions to *F. graminearum*. Fourteen open-pollinated genotypes, nine hybrids, and eight testcross hybrids showed high FHB resistance. Few genotypes had low ergot severity under natural infection. The fungicide Prosaro™ (prothioconazole and tebuconazole) application timing for managing FHB on rye was investigated through six treatments, including application at heading, 10% anthesis, 80% anthesis, six days after 50% anthesis, and inoculated-untreated and uninoculated-untreated controls. The highest percent control of FHB incidence, severity, and index occurred when fungicide was applied at 10% anthesis, while fungicide applied at 80% anthesis resulted in the highest efficacy for reducing *Fusarium*-damage kernels and DON. Fungicide treatments significantly increased thousand kernel weight, test weight, and yield under a high mean FHB index. Two sets of fall rye genotypes to leaf rust was evaluated in rust nurseries through artificial inoculation. Most of the open-pollinated genotypes were moderately susceptible or susceptible to leaf rust. Several hybrids showed moderate resistance or resistance to leaf rust with low disease severity. The integrated information from this study will assist rye growers manage FHB, leaf rust, and ergot, and provides vital information for resistance breeding.

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Chapter 1

General introduction

Rye (*Secale cereale* L.) is a relatively major cereal crop grown in northern and eastern Europe, but is a minor cereal in Canada (Small, 1999; Dreyer, 2018). On a global scale, rye acres have substantially decreased since the 1970s, and rye production is less than five percent of wheat or rice production (Bushuk, 2001; Dreyer, 2018). However, rye has better tolerance to drought, salt, or aluminum stress compared to other small grain cereals (Geiger and Miedaner, 2009; Dreyer, 2018). Rye is the most productive cereal crop on infertile, sandy, or acid soil landscapes (Bushuk, 2001; Geiger and Miedaner, 2009). In addition, rye has an excellent ability to compete with weeds and therefore has a low requirement for herbicides, which is environment friendly and reduces weed control costs (Dreyer, 2018). As a multiple-use crop, rye can be utilized as a cereal grain, forage, or pasture (Bushuk, 2001). In Canada, rye is used primarily for animal feed with a small amount used for alcohol distilling and food (Rye sector, MB, 2011). There are two types of varieties, conventional open-pollinated varieties and hybrids, commonly grown today (Meija and Krams, 2019). Because of its high yield, uniformity, and lodging tolerance, hybrid rye is increasingly popular in northern Europe and has been introduced into western Canada since 2014 (King, 2017; Meija and Krams, 2019). Availability of low-input, high-yield, hybrid rye dramatically increased rye production on the prairies (King, 2017; Barber, 2019). In 2021, seeded rye acres in Canada were 40% higher than the previous five-year average (Statistics Canada).

Fusarium head blight (FHB) is a devastating worldwide disease in cereal crops, which can cause grain yield losses and quality reduction (Sutton, 1982; Bai and Shaner, 1994; Parry et al., 1995; McMullen et al., 1997; Osborne and Stein, 2007). Some *Fusarium* species produce mycotoxins, such as deoxynivalenol (DON) in infected grain, which are harmful to human and animal health when consumed in food or feed products (Parry et al., 1995; D'Mello and Macdonald, 1997; D'Mello et al., 1999; Placinta et al., 1999; Gilbert and Tekauz, 2000). Mycotoxin contamination could lead to downgrading, or even rejection, of grain at marketing (Sutton, 1982; Snijders, 1990; Parry et al., 1995; McMullen et al., 2012). The economic losses caused by FHB in wheat were estimated to be over $1.5 billion in Canada since the 1990s (Innovation express, 2014). In Manitoba, the outbreak of FHB in cereal crops could be traced to 1984 (Tekauz et al., 2000).

Although the disease was most common in Manitoba, it was later found in Saskatchewan and Alberta (Fernandez et al., 1999; Clear and Patrick, 2000). The incidence of FHB, as measured by the frequency of *Fusarium*-damaged kernels (FDK) in grain samples, has been appreciably increasing in Saskatchewan and Alberta in the last decade (Canadian Grain Commission, 2019). Much research has been carried out in western Canada on FHB in wheat and barley (Gilbert and Haber, 2013; Arseniuk et al., 1999). However, very little is known about the FHB reaction in fall rye.

Fusarium graminearum is the predominant species causing FHB in North American and many other parts of the world, especially on wheat (Gale et al., 2003; Shaner, 2003; Goswami and Kistler, 2004; Osborne and Stein, 2007; Gilbert and Tekauz, 2011). In Manitoba, more than 90% of the isolations from infected kernels of spring wheat are *F. graminearum*, and it produces mainly DON (Gilbert et al., 2008; Nielsen et al., 2011; Shah et al., 2018). Other species such as *F. poae*, *F. sporotrichioides*, and *F. avenaceum* account for a more significant proportion of the species causing FHB on oat and barley across the prairies (Gilbert and Tekauz, 2011; Banik et al., 2020a, 2020b, 2021). *Fusarium graminearum* and *F. culmorum* produce the mycotoxin, DON, but *F. avenaceum* produces primarily moniliformin (Liddell, 2003; Yli-Mattila et al., 2006; Grafenhan et al., 2013). *Fusarium sporotrichioides* and *F. Poae* produce primarily T-2 toxin and nivalenol, respectively (Liddell, 2003; Thrane et al., 2004). While DON is less acutely toxic than other trichothecenes such as nivalenol and T-2 toxin, it is the most common mycotoxin contaminating cereal grains used for food and feed (Pettersson and Hedman, 1997; Placinta et al., 1999; Binder, 2007; Sobrova et al., 2010).

Several strategies are used to manage FHB and DON contamination in cereal crops, including cultural practices, fungicide application, and host resistance (Parry et al., 1995; Osborne and Stein, 2007; Gilbert and Tekauz, 2011; Gilbert and Haber, 2013; Dweba et al., 2017). Cultural strategies for FHB management mainly reduce sources of primary inoculum available for dispersal to cereal spikes (Bai and Shaner, 1994; Parry et al., 1995; McMullen et al., 2012). Several fungicide products, including Caramba™, Folicur™, Proline™, and Prosaro™, have been registered and are currently available for FHB suppression (Gilbert and Tekauz, 2011). However, fungicide efficiency for reduction of FHB and DON content in wheat is variable due to the complexity of the causal pathogen, problems associated with application timing, and fungicide

resistance in pathogen populations (Parry et al., 1995; Lehoczki-Krsjak et al., 2010; Mesterhazy et al., 2011). Host resistance has the potential to provide economical and sustainable strategies for controlling FHB (Gilbert and Fernando, 2004). Compared to wheat and barley, only a limited number of studies have been conducted on breeding FHB resistance into other small grain cereal crops, such as rye (Parry et al., 1995). Overall, a single management strategy is unlikely to protect crops from FHB completely, and thus an integrated approach is required to maintain yield potential, reduce the risk of mycotoxin contamination, and increase producer returns (Krupinsky et al., 2002; Osborne and Stein, 2007; Gilbert and Tekauz, 2011; Gilbert and Haber, 2013; Dweba et al., 2017).

Leaf diseases of rye generally involve leaf and stem rust (Schlegel, 2013b). Leaf rust is one of the most common diseases on rye in Germany (Miedaner and Sperling, 1995; Miedaner et al., 2002; Roux et al., 2004; Wilde et al., 2006; Miedaner et al., 2012). In continental climates, leaf rust epidemics cause up to 40% yield losses on rye (Kobylanski and Solodukhina, 1983 cited by Miedaner and Sperling, 1995). In Canada, leaf rust is also common in cereal crops and is species-specific (Fetch et al., 2011). As with FHB, little information is available about the reaction of the rye cultivars to prevalent races of leaf rust in western Canada. Although rye is usually considered less susceptible to several pathogens than wheat and barley, ergot is a common disease on rye because of its cross-pollinated nature (Helm and Schneiter, 1991; Miedaner et al., 2012; Schlegel, 2013b). Ergot disease causes yield reduction and produces ergosterol, which is toxic to humans and livestock (Helm and Schneiter, 1991).

With the introduction of new hybrid rye cultivars and the potential expansion of rye production in western Canada, it is essential to evaluate the FHB reaction of rye and consider disease control on rye (Lyseng, 2017). The study was designed to provide critical and practical information about FHB on rye and the reaction of rye to rust and ergot in western Canada. The data will assist producers in selecting fall rye cultivars to grow in regions prone to FHB or rust epidemics. Furthermore, the resistant germplasms will help breeders develop resistant cultivars. The objectives for the research were to: 1) determine the primary causal agent of FHB on rye in Manitoba, 2) compare the pathogenicity of four *Fusarium* species causing FHB on six rye and two winter wheat genotypes under controlled conditions, 3) assess FHB reaction in commercial and breeding populations of rye, including hybrid and open-pollinated genotypes, through multi-environments in southern Manitoba, 4) evaluate the efficacy of fungicide with different application

timings to mitigate FHB and DON contamination on rye, and 5) assess rye reaction to leaf rust in field inoculated nurseries.

Chapter 2

Literature review

2.1 Rye

Rye (*Secale cereale* L.) is an annual, cross-pollinated cereal with self-incompatibility (Lundqvist, 1956). Like many traditional small grain crops, it was believed to originate in the Fertile Crescent of the Near East (Hillman, 1978; Geiger and Miedaner, 2009). In its main growth areas, such as Turkey, Lebanon, Syria, Iran, Iraq, and Afghanistan, rye was never cultivated as a crop but grew as a weed with wheat and barley (Hillman, 1978; Geiger and Miedaner, 2009). Compared to wheat crop, the growth of weed rye was less impeded by the wetter conditions and more acidic soils when it spread northward or into higher altitudes (Hillman, 1978). Rye was domesticated in the region around the Caspian Sea at about 3000-4000 BCE and then brought to eastern Europe and later to western Europe (Geiger and Miedaner, 2009). The English and Dutch introduced cultivated rye to the western hemisphere during the sixteenth and seventeenth centuries (Oelke et al., 1990; Bushuk, 2001).

2.1.1 Rye production in the world

Compared to wheat and barley, the production areas of rye are limited (Miller magazine: World rye and oat market). World rye acreages declined due to the extensive breeding progress in wheat and barley (Geiger and Miedaner, 2009; FAO, 2018). From 1986 to 2016, the harvested area of rye decreased from 15.4 million hectares to 4.4 million hectares worldwide and production declined 57%, from 30 to 13 million tonnes (FAO, 2018). Current rye production is 12 to 14 million tons per year (Miller magazine: World rye and oat market; USDA FAS). According to the Food and Agriculture Organization of the United Nations (FAO), the majority of rye is produced in Russia, Germany, Poland, Belarus, and Ukraine.

2.1.2 Rye production in Canada

Rye acreage and production in Canada has fluctuated over the last ten years (USDA FAS). Western Canada is the main region of rye production in Canada (Rye sector, MB, 2011).

Saskatchewan usually has the highest rye acreage among the three prairie provinces, followed by Manitoba and Alberta (Rye sector, MB, 2011). In 2019, Manitoba was the leading province for rye production with 132,500 tonnes, compared to 102,800 tonnes in Saskatchewan, 43,100 tonnes in Ontario, 26,800 tonnes in Alberta, and 21,000 tonnes in Quebec (Statistics Canada). Along with the increase in rye consumption and the introduction of high-yield hybrid rye, rye production in Ontario and Quebec has increased from 2014 to 2018 (Pearce, 2015; Hallick, 2018). Although rye is a relatively minor cereal in Canada, Canada was the highest exporter of rye in the world in 2016/17 (Miller magazine: World rye and oat market). Higher sales to the United States enhanced Canadian rye exports over the five-year average (Small, 1999; Raine, 2018).

2.1.3 Rye consumption

In Europe, rye is mainly used for bread making, which takes up about 50-75% of the harvest (Small, 1999; Geiger and Miedaner, 2009). Rye flour can be used alone to make traditional dark bread consumed extensively in North Germany, Finland, the Baltics, Poland, Belarus, and the Russian Federation (Bushuk, 2001; Geiger and Miedaner, 2009; Rye sector, MB, 2011). It can also be mixed with wheat flour to make "light-rye" bread (Bushuk, 2001; Rye sector, MB, 2011). In Canada, livestock feed is the main area for the domestic consumption of rye (Rye sector, MB, 2011). Although rye has a relatively low feed value compared to other feed grains, the price of rye is attractive (Bushuk, 2001; FAO, 2018). Only a small amount of rye is used for bread making in Canada (Allen, 1922). Additionally, rye grain can be used for distilling (Allen, 1922; Small, 1999). In the United States, less than 50% of rye area is grown for grain, with the remainder used as a forage in the form of pasture and hay, or as a cover or green manure crop (Oelke et al., 1990; Small, 1999). Rye has some advantages to be used as forage because of its stress tolerance, such as winter hardiness, and rapid growth in the spring (GRDC Grownotes, Cereal rye; Oelke et al., 1990; FAO, 2018). Fall rye is usually harvested seven to ten days earlier than winter wheat or winter triticale, and is readily grazed when other crops are not available in spring and fall (Oelke et al., 1990; Bushuk, 2001; FAO, 2018). As a cover crop, rye can compete effectively with weeds and reduce soil erosion (GRDC Grownotes, Cereal rye; Bushuk, 2001; FAO, 2018). The remainder of rye harvest is used to produce paper or bioenergy, such as bioethanol (Geiger and Miedaner, 2009; FAO, 2018).

2.1.4 Hybrid rye

Hybrid rye was developed in Germany in 1986 (Barber, 2019). More than half of hybrid rye in Germany is used for animal feed due to its extremely high yields and good energy content, which results in a low cost of production for feed producers (Barber, 2019). Hybrid rye has a long growing season and good nutrient and water use efficiency, making it fit for Canadian growers (Barber, 2019). Therefore, the rye breeding company KWS LOCHOW GMBH, Bergen, Germany partnered with FP Genetics in Regina, Saskatoon, Canada, and SeedNet in southern Alberta, and introduced hybrid rye into Canada (Barber, 2019). FP Genetics has introduced five different KWS hybrid rye cultivars into Canada since 2015 (Barber, 2019). Brasetto was the first hybrid cereal crop that came to western Canada, which was registered in Canada in 2015 (Heppner, 2014; King, 2017; Wilde et al., 2017; Barber, 2019). It had a 20 to 25% higher yield than the check variety Hazlet (Heppner, 2014). KWS Bono had a higher yield than Brasetto and was registered in Canada in 2016 (Variety registration office; Barker, 2015; King, 2017; Barber, 2019). KWS Gatano had a 50% improved ergot resistance over previously registered hybrids and was registered in Canada in 2018 (Variety registration office; Barber, 2019). KWS Trebiano had a 40 to 50% ergot resistance over KWS Gatano, and Progas was the first new forage hybrid (Barber, 2019). There were two other hybrids, Guttino and KWS Daniello, registered in Canada by SeedNet in 2015 and 2017, respectively (Variety registration office; King, 2017). In addition to high yields, hybrid rye has more uniform maturity and better quality than open-pollinated rye (Barker, 2015). A unique pollen technology was used to develop hybrid rye and narrowed down the pollination window with increased pollen production, limiting the chance for ergot spores to attach to the flowers (Barber, 2019; Schlobohm, 2019). Hybrid rye is expected to be a competitive alternative crop for the feed industry in Canada, which has been demonstrated in Europe (King, 2017; Barber, 2019). Unlike conventional open-pollinated rye, hybrid rye is not grown in infertile lands, but is grown and managed as other productive crops (Barber, 2019). In comparison with open-pollinated rye, a higher falling number makes hybrid rye be better for milling (Barker, 2015; King, 2017; Wilde et al., 2017).

2.2 Fusarium head blight

Fusarium head blight (FHB) is a fungal disease in cereals, such as wheat, barley, oats, rye, corn, canary seed, and forage grasses (Canadian Grain Commission, 2019). It is caused by several *Fusarium* species such as *Fusarium graminearum, F. culmorum, F. poae, F. avenaceum, F. sporotrichioides, F. verticillioides,* and *F. equiseti* (Nicholson et al., 2003; Osborne and Stein, 2007). It was first reported on wheat, barley, and rye grass in England in 1884 (Smith, 1884). Most of the early FHB records came from the USA in the 1890s (Parry et al., 1995). From 1927 to 1980, moderately widespread FHB was reported in wheat about once every nine years in southwestern Ontario, Canada (Sutton 1982). Fusarium head blight appeared to be found anywhere in the cereal growing areas in eastern Canada (Canadian Grain Commission, 2019). Since the early 1990s, FHB became one of the most significant diseases limiting wheat production in Canada and the United States (Haile et al., 2019). In 1993, there was a severe FHB epidemic in the spring wheat in Manitoba, Canada, and the northern United States, which led to research of FHB management, especially genetic resistance in wheat (Bai et al., 1994; Gilbert and Haber, 2013). In western Canada, the black soil zone with its accompanying plentiful rainfall is the most likely region for FHB epidemics (Canadian Grain Commission, 2019). In addition to North America, the occurrence of FHB and significant losses caused by FHB in wheat have been reported in many areas, such as Europe, China, and South America (Bai et al., 1994; Parry et al., 1995; Torres et al., 2019). Accordingly, the International Maize and Wheat Improvement Center (CIMMYT) treated FHB as a vital disease that damages wheat production in numerous parts of the world (Dubin et al., 1997).

2.3 *Fusarium* species

Several *Fusarium* species can result in FHB and be cultured from naturally infected wheat, barley, or rye spikes (Parry et al., 1995; Grafenhan et al., 2013; Foroud et al., 2014). Most records of *Fusarium* species associated with FHB include *F. graminearum, F. culmorum, F. poae, F. avenaceum, F. sporotrichioides,* and *Microdochium nivale* (Fr.) Samuels & I.C. Hallett (Osborne and Stein, 2007).

Previously, two populations of *F. graminearum* were recognized as Group 1 and Group 2 according to the difference in morphology and culture (Francis and Burgess, 1977). Based on the later phylogenetic analyses, Group 1 strains were identified as *F. pseudograminearum* (Aoki and O'Donnell, 1999; Scott and Chakraborty, 2006). A multilocus phylogenetic analysis indicated that Group 2 was a monophyletic species complex consisting of at least nine geographically structured lineages (O'Donnell et al., 2000, 2004). Along with the analysis of additional isolates, five more species were added, and there are 14 lineages in the *F. graminearum* complex currently (Starkey et al., 2007; O'Donnell et al., 2008; Yli-Mattila et al., 2009; Sarver et al., 2011). The prevalent species causing FHB in North America was lineage 7 designated as *F. graminearum sensu stricto*, which was broadly distributed globally, especially in temperate wheat growing regions (Sutton, 1982; Glenn, 2007; Osborne and Stein, 2007; Gilbert and Tekauz, 2011). In Manitoba, *F. graminearum* accounted for more than 90% of isolates cultured from *Fusarium* infected kernels (Gilbert et al., 2008). *Fusarium asiaticum*, known as *F. graminearum* lineage 6, was the most important phylogenetic species in China and other Asia countries (O'Donnel et al., 2000; Gale et al., 2002; O'Donnel et al., 2004).

The distribution of *Fusarium* species in FHB is mainly related to climatic conditions, especially temperature (Parry et al., 1995; Saremi et al., 1999; Doohan et al., 2003). Climatic conditions affect the incidence of *Fusarium* species probably through the mode of pathogen reproduction or through soil and vegetation type (Saremi et al., 1999; Doohan et al., 2003). *Fusarium culmorum* was found frequently in cooler regions of northwest Europe (Miller, 1995; Parry et al., 1995; Desjardin, 2006). It was a dominant *Fusarium* species in wheat, rye, and triticale, which occupied 30% of total *Fusarium* isolates cultured from infected kernels in Poland (Chelkowski and Manka, 1983; Snijders, 1990; Miedaner et al., 1993, 2001). Based on phylogenetic analysis of the β-tubulin sequence, *F. culmorum* is closely related to *F. graminearu*m (Glenn, 2007). *Fusarium graminearum* and *F. culmorum* are the most aggressive *Fusarium* species associated with FHB in the world (Stack and McMullen, 1985; Wong et al., 1995; Brenan et al., 2003). Other *Fusarium* species, including *F. poae*, *F. avenaceum*, and *F. sporotrichioides*, were detected on cereals grown west of Manitoba and associated with FHB on wheat and barley in Europe (Tekauz et al., 2000, 2004; Osborne and Stein, 2007; Tekauz et al., 2009). *Fusarium avenaceum* is prevalent in cool, temperate regions, such as the western part of the Canadian prairies

and some European countries (Gilbert and Tekauz, 2000; Liddell, 2003). Although some *Fusarium* species are less aggressive than *F. graminearum*, they are also important since they may result in the accumulation of mycotoxins in the absence of apparent symptoms (Salas et al., 1999). *Fusarium poae* produces many mycotoxins, including trichothecenes, beauvericin, and enniatins, which affect food and feed safety (Stenglein, 2009). In addition, *F. poae* appears to promote FHB development through early colonization of hosts before *F. graminearum* or *F. culmorum* (Sturz and Johnston, 1983; Bai et al., 2003; Gale, 2003; Mesterhazy, 2003).

2.4 *Fusarium* mycotoxins

Fusarium species can produce several important mycotoxins involving trichothecene, zearalenone, fumonisins, moniliformin, and butenolide in host tissue (Miller, 1995; Mirocha et al., 2003; Desjardins and Proctor, 2007; Harris et al., 2007). Based on the structural components, trichothecenes associated with FHB are classified as type A and type B (Krska et al., 2001; Foroud and Eudes, 2009). The major type A trichothecenes include T-2 toxin and HT-2 toxin, which are highly toxic (Ueno, 1983; Krska et al., 2001; Mirocha et al., 2003). Deoxynivalenol (DON), the acetylated forms 3-acetyldeoxynivalenol (3-ADON) and 15-acetyldeoxynivalenol (15-ADON), nivalenol (NIV), and fusarenon-X belong to type B trichothecenes (Pirgozliev et al., 2003; van der Lee et al., 2015). Although DON is less toxic than other trichothecenes, it is the most prevalent mycotoxin produced by *Fusarium* species and consequently contributes a significant economic impact on grain production (Placinta et al., 1999; Desjardin, 2006). *Fusarium graminearum* and *F. culmorum* produce mainly DON, NIV, and their derivatives, while also producing zearalenone (Perkowski et al., 1988; Faifer et al., 1990; Muller and Schwadorf, 1993; Hussein and Brasel, 2001; Miedaner et al., 2001; Liddell, 2003). *Fusarium sporotrichioides, F. poae,* and *F. equiseti* are type A trichothecenes producers (Krska et al., 2001; Liddell, 2003). Moniliformin is a mycotoxin produced by *F. avenaceum*, the predominant species causing cereal FHB in Poland (Chelkowski et al., 1987; Arseniuk et al., 1999). The number of *Fusarium* species, or strains of the same species, affect mycotoxin production (Xu et al., 2007). The competition between *Fusarium* species may result in the synergistic effect on mycotoxin production (Xu et al., 2007).

2.4.1 Geographic distribution of *Fusarium graminearum* chemotype

The distribution of *F. graminearum* chemotypes varied between different world regions (Torres et al., 2019). In China, Korea, Australia, New Zealand, and Northern Europe, most isolates of *F. graminearum* produced DON and 3-ADON (Miller et al., 1991; Yli-Mattila et al., 2009; Wegulo, 2012; van der Lee et al., 2015). In North America, Southern America, central Europe, and southern Russia, the 15-ADON chemotypes were dominant in *F. graminearum* populations (Miller et al., 1991; Yli-Mattila et al., 2009; Wegulo, 2012; van der Lee et al., 2015). The NIV chemotype was found in China, Japan, and other Asian countries, but was not common in Europe, South Africa, and the United States (Miller, 1995; Suga et al., 2008; Gale et al., 2011; Zhang et al., 2012; van der Lee et al., 2015). Based on isolates collected between 1984 and 2004, the highest frequency of 3-ADON chemotypes was observed in the maritime province Prince Edward Island, close to 100%, and the frequency of 3-ADON chemotypes lower in other provinces from East to West across Canada (Ward et al., 2008). Since the early 1980s, the 15-ADON chemotype has constantly been predominant in Ontario (Kelly et al., 2015; Tamburic-Ilincic et al., 2015; Crippin et al., 2019; Oghenekaro et al., 2019). In western Canada, a significantly increased percentage of 3-ADON chemotypes was observed in the *F. graminearum* isolates collected between 1998 and 2004 (Ward et al., 2008). In 2018 and 2019, the 3-ADON chemotype replaced the 15-ADON chemotype in Manitoba and Saskatchewan (Oghenekaro et al., 2019). The increase of 3-ADON chemotypes was also observed in the eastern United States (Puri and Zhong, 2010; Gale et al., 2011; Schmale et al., 2011). In addition, a novel trichothecene toxin, NX-2, produced by the strain of *F. graminearum*, was detected in rice cultures in the United States (Varga et al., 2015). The new chemotype was not found outside North America (Liang et al., 2014; Kelly et al., 2016). Trichothecene mycotoxins influence the aggressiveness, or virulence, of *F. graminearum* on wheat (Wegulo et al., 2015). The 3-ADON chemotype was reported to produce more mycotoxins and grow faster in culture than the 15-ADON chemotype (Ward et al., 2008; Gilbert et al., 2010; Puri and Zhong, 2010). Greater disease severity caused by the 3-ADON chemotype was observed on one susceptible and one moderately resistant wheat cultivar compared to the 15-ADON chemotypes (Puri and Zhong, 2010).

2.4.2 Food and feed safety impacted by *Fusarium*

Mycotoxins can contaminate human food and animal feed, resulting in feed refusal and vomiting in humans and animals (Chelkowski et al., 1987; Perkowski et al., 1988; Faifer et al., 1990). A critical mechanism for trichothecenes is that they can inhibit protein synthesis in eukaryotic cells (Krska et al., 2001; Desjardins and Proctor, 2007). Zearalenone has a similar structure to estrogen and accordingly causes similar mammalian responses (Hidy et al., 1977). Due to the toxic effects of mycotoxins, several countries established advisory limits for the maximum levels of DON in finished products of wheat intended for human consumption and animal feeds (van Egmond, 1989; Torres et al., 2019). For example, the maximum level of DON suggested by the Food and Drug Administration (FDA) is one part per million (ppm) in finished wheat products for human consumption in the United States (Pirgozliev et al., 2003; Wegulo, 2012). Grain used for swine and cattle feed in the United States should not have DON over 5 and 10 ppm, respectively (Pirgozliev et al., 2003; Wegulo, 2012). Health Canada recommends that DON levels in uncleaned soft wheat for use in non-staple foods and baby foods should not exceed 2 ppm and 1 ppm, respectively (Health Canada, 2020). The feeding guidelines established by Agriculture and Agri-Food Canada are 1 ppm for swine, dairy cattle, and horses and 5 ppm for poultry and growing beef cattle and sheep in the finished feed (Wegulo, 2012). In the European Union, the recommended advisory limits of DON are 0.5 ppm for retail products, including bread, pastries, biscuits, cereal snacks, and breakfast cereals and 0.75 ppm for flour and grain (Pricket et al., 2000; Torres et al., 2019).

2.5 Disease cycle of fusarium head blight

Fusarium species survive and overwinter as saprophytic mycelia on infected crop debris (Goswami and Kistler, 2004). In the following spring, overwintered crop residues, such as maize stem nodes and grains, provide the principal inoculum source for FHB (Atanasoff, 1920; Sutton, 1982; Khonga and Sutton, 1988; Parry et al., 1994; Fernando et al., 1997). Infested seed is probably an additional source of inoculum (Inch and Gilbert, 2003b). Although planting an infected wheat seed can only cause seedling blight, but not FHB on the spike, *F. graminearum* can survive on seed for two to three years even buried in the soil (Khonga and Sutton, 1988; Parry et al., 1995; Gilbert et al., 2003; Inch and Gilbert, 2003b; Gilbert and Fernando, 2004). Depending on the

Fusarium species, the form of inoculum can be macroconidia, chlamydospores, ascospores, or hypha (Parry et al., 1995; Osborne and Stein, 2007). Ascospores are an important form of inoculum for *Gibberella zeae* caused by *F. graminearum* (Parry et al., 1995).

2.5.1 Spore production and dispersal

Specific temperature ranges and wetness are required for FHB to develop (Parry et al., 1995). Perithecia do not adequately develop at temperatures that are lower than 15 °C or higher than 30 ºC (Tschanz et al., 1976; Dufault et al., 2002a, 2002b). The optimum temperature ranges for ascospore discharge and macroconidia production are from 25 to 28 ºC and 28 to 32 ºC, respectively (Tschanz et al., 1976; Sutton, 1982). Accordingly, warm and wet conditions favor the formation of inoculum on the host and residues in fields (Sutton, 1982). Both wind and rain play an essential role in dispersing the *Fusarium* inoculum (Parry et al., 1995; Gilbert and Fernando, 2004). Rain appears to be more important for the dispersal of macroconidia than wind, since macroconidia are only trapped during rain events (Parry et al., 1995; Fernando et al., 1997; Gilbert and Tekauz, 2000). Although ascospores are dispersed by wind, rain or heavy dews are necessary for the initial discharge of ascospores from the ostiole of the perithecia (Parry et al., 1995). In addition, insects and birds can transfer spores or hypha in cereal fields (Sutton, 1982; Parry et al., 1995). Inoculum dispersal was thought to occur over a limited distance, such as a few meters from an inoculum focus (Fernando et al., 1997). However, Maldonado-Ramirez et al. (2005) showed that ascospores could spread over many kilometers with the wind.

2.5.2 Infection

Fusarium head blight infection begins with the germination of macroconidia or ascospores deposited on, or inside, the spike (Parry et al., 1995; Bushnell et al., 2003). *Fusarium graminearum* cannot penetrate the glume, lemma, or palea directly due to thick-walled epidermal cells (Kang and Buchenauer, 2000; Bushnell et al., 2003). Mycelium development on exterior surfaces of florets, or glumes, is a significant first step for invasion (Lewandowski and Bushnell, 2001; Bushnell et al., 2003). Mycelia grow over the glume's abaxial surface to its adaxial surface to reach stomata or other entry sites within a spike (Lewandowski and Bushnell, 2001; Bushnell et al., 2003). Subcuticular hyphal growth on the glume, lemma, or palea provides adequate nutrients

for the fungus and facilitates hyphal development on epidermal surfaces (Bushnell et al., 2003). Spikes are most susceptible to infection at the flowering stage due to anther extrusion (Sutton, 1982; Bushnell et al., 2003). Some compounds from anthers are thought to stimulate the infection of FHB (Parry et al., 1995; Osborne and Stein, 2007). Direct penetration can also happen through wounds, gaps between the palea and lemma within the floret or spikelet, and the thin-walled epidermis at the base of the glumes (Lewandowski and Bushnell, 2001; Bushnell et al., 2003; Goswami and Kistler, 2004).

Fusarium graminearum usually spreads between florets within a spikelet and between spikelets through vascular bundles in rachis tissues of wheat (Pugh et al., 1933; Ribichich et al., 2000; Brown et al., 2010). Fungus colonization in vascular bundles adversely affects the function of xylem and phloem tissues, which results in the premature death of spikelets (Goswami and Kistler, 2004). Moreover, the mycotoxin DON excreted by *F. graminearum*, which suppresses a jasmonate-related defense reaction, can also induce plant cell death (Pusztahelyi et al., 2015; Bonnighausen et al., 2019). Although DON is not necessary for initial infection by *F. graminearum*, it is a virulence factor that stimulates the rapid spread of the fungus into the rachis from florets in wheat (Bai et al., 2001; Jansen et al., 2005; Boenisch and Schafer, 2011). However, DON does not appear to support the pathogen to break through the rachis node and rachilla in barley (Jansen et al., 2005). Consequently, the spread of *F. graminearum* hyphae between florets is limited through the phloem in barley (Jansen et al., 2005). Overall, a brief biotrophic stage appears to be present in the interaction with the host, and a necrotrophic phase is the primary stage for this fungus to enlarge colonization (Bushnell et al., 2003; Goswami and Kistler, 2004; Brown et al., 2010). Therefore, *F. graminearum* is considered as a hemibiotroph (Trail, 2009; Torres et al., 2019).

2.6 Symptoms of fusarium head blight

Generally, symptoms of FHB are similar in all small grain cereal crops (Parry et al., 1995). Initial visible symptoms in wheat typically appear on florets, located in the middle of a spike (Atanasoff, 1920; Pugh, 1933; Andersen, 1948). The first symptoms develop at the point of infection as small water-soaked spots at the base, or middle, of the glume, or on the rachis (Atanasoff, 1920; Pugh, 1933; Parry et al., 1995; Bushnell et al., 2003; Guenther and Trail, 2005;

Trail, 2009). More water-soaked lesions appear in veins rather than the area between them. Lesions then spread to the whole spikelet and then spikelets above and below the primary infection site under warm and wet conditions (Atanasoff, 1920; Pugh, 1933). Later, lesions change color to slightly brown, purplish-brown, or bleached (Atanasoff, 1920; Bennett, 1931; Pugh, 1933; Trail, 2009). In some cases, the first symptom may have a slightly pinkish tint produced by the fungus on exposed anthers or the floret surface, especially under favorable conditions (Atanasoff, 1920; Pugh et al., 1933). The fungus spreads over infected areas and develops a layer of macroconidia on the glume or floret surface (Atanasoff, 1920; Pugh et al., 1933). Eventually, a mass of spores change color from slightly pinkish to dark salmon-orange, and pink sporodochia, or purple black perithecia, may be visible on the glume or at the base of spikelet (Atanasoff, 1920; Pugh et al., 1933; Parry et al., 1995; Goswami and Kistler, 2004;). The entire spikelet turns to a bleached color, and finally, the whole spike may be prematurely bleached (Pugh, 1933; Bushnell et al., 2003; Goswami and Kistler, 2004; Osborne and Stein, 2007). Awns usually become deformed and curved downward (Goswami and Kistler, 2004). Infected grains are usually shrunken or shriveled with chalky pinkish discoloration (Andersen, 1984; Parry et al., 1995; McMullen et al., 1997; Goswami and Kistler, 2004). Except for apparent water-soaked lesions, FHB symptoms in rye are similar to those in wheat (Atanasoff, 1920). Infected spikes of wheat have the same shape and size as healthy spikes. In contrast, rye spikes damaged by FHB are usually narrower than healthy spikes, and spikelets in infected parts are smaller with pink to orange pustules (Chelkowski, 1989).

2.7 Fusarium head blight management

The prevalence of FHB is generally influenced by several factors, including crop resistance, previously grown crop, frequency of host crop, tillage practice, and environment (Guo et al., 2010). Individual strategies cannot effectively protect crops from FHB (Gilbert and Tekauz, 2011; Gilbert and Haber, 2013). A combination of several management strategies involving cultural practices, fungicide application, and resistant cultivars effectively reduces the risk of yield losses and mycotoxin contamination caused by FHB (Gilbert and Tekauz, 2011; Gilbert and Haber, 2013). A durum wheat study in North Dakota showed the highest yields, lowest DON accumulation, and lowest disease severity following use of moderately resistant cultivar, rotation with canola, and fungicide application at anthesis (McMullen et al., 2008). The combination of tillage, resistant wheat cultivar use, and fungicide application significantly decreased DON contamination

compared to a susceptible wheat variety without tillage and fungicide application (Blandino et al., 2012). No information is available for rye about integrated management strategies to reduce FHB.

2.7.1 Cultural practices

Some cultural practices used to control FHB reduce inoculum-borne residues, which provide a substrate for perithecia to overwinter in the field (Gilbert and Haber, 2013). Tillage operations that bury crop residues below the soil surface speed the decomposition and reduce the number of perithecia, and consequently decrease the source of inoculum for the following crop (Khonga and Sutton, 1988; Miller et al., 1998; Pereyra et al., 2004; Gilbert and Tekauz, 2011). Different tillage strategies show significantly different efficacy for FHB control (Dill-Macky and Jones, 2000; Krebs et al., 2000). Moldboard plowing reduced FHB disease levels more effectively than chisel plowing (Dill-Macky and Jones, 2000). However, conventional tillage is not commonly used on the prairies in Canada (Gilbert and Tekauz, 2011; McMullen et al., 2012). In addition, tillage cannot bury all residues, and seeding operations can bring buried residues to the soil surface again (Gilbert and Tekauz, 2011). Chopping residues with a straw chopper may be another option to accelerate decomposition and decrease the source of inoculum (Gilbert and Tekauz, 2011; Gilbert and Haber, 2013). Burning of crop residues can also reduce the level of inoculum, but is not environmentally acceptable (Salas and Dill-Macky, 2005; McMullen et al., 2012).

Crop rotations play an important role in reducing the risk of severe FHB (McMullen et al., 1997, 2012). Management of infected residue through crop rotation reduces FHB and DON by up to 30% (McMullen et al., 2012). Avoiding planting wheat following wheat or maize, which are hosts of *F. graminearum*, reduces the chance of exposure to inoculum produced on residues in the field (Teich and Nelson, 1984; Parry et al., 1995; Krebs et al., 2000; Pereyra and Dill-Macky, 2008). However, *F. graminearum* is not host-specific and can infect many species (Inch and Gilbert, 2003a; Pereyra and Dill-Macky, 2008; Shah et al., 2018). Some *Fusarium* species can also grow on roots of other non-cereal crops, including pulses and oilseeds (Gilbert et al., 2003). Further, wind-borne ascospores, which are the primary inoculum, may be transported for many kilometers (Maldonado-Ramirez et al., 2005). In cereal growing regions where corn predominates, spores that cause FHB on wheat spikes come primarily from regional atmospheric sources rather than within-field debris (Bergstrom et al., 2010, 2011). Accordingly, crop rotation alone is insufficient to

control FHB, especially in environments where atmospheric spore loads are high (Gilbert and Tekauz, 2011; McMullen et al., 2012).

Altering sowing dates may be associated with FHB control because the flowering stage plays a crucial role in FHB infection (McMullen et al., 2012; Shah et al., 2018). Choo et al. (2014) showed that early planting resulted in less DON contamination and higher yield than late planting in barley. Early sowing may bring the flowering stage ahead of the favorable period for spore development and make the crop escape the attack of FHB (Shah et al., 2018). In addition, staggering planting dates or using cultivars differing in maturity reduces the risk that the flowering stage of the entire crop will occur under favorable conditions for FHB infection (McMullen et al., 2012). Use of cultural practices to reduce risk of FHB are crucial in regions where high-yielding cultivars do not have moderate resistance to FHB (McMullen et al., 2012).

2.7.2 Chemical control

Fungicide application is a vital strategy to suppress FHB, especially in FHB prevalent regions and areas lacking resistant cultivars (Parry et al., 1995; McMullen et al., 1997; Jones, 2000). Fungicide treatment mainly has two purposes for FHB control: one is that it reduces disease levels; another is that it controls toxin contamination of grains (Mesterhazy, 2003).

2.7.2.1 Efficacy of various types of fungicides

Several fungicides involving triazoles, carbendazim, hexaconazole, mancozeb, benomyl, prochloraz, and triadimenol, were used for FHB management (Yuan and Zhou, 2005; Spolti et al., 2014; Dweba et al., 2017). Among these fungicides, triazole fungicides, such as tebuconazole, metconazole, prothioconazole, and propiconazole, and benzimidazole fungicides, such as carbendazim, contributed to effective control of FHB in wheat (Pirgozliev et al., 2002; Paul et al., 2008; Machado et al., 2017). The triazoles are in the demethylation inhibitor (DMI) class and have been widely used for FHB control (McMullen et al., 2012; Wegulo et al., 2015). A multivariate meta-analysis was used to combine results from over 100 uniform fungicide trials that were conducted over 11 years from 14 different locations in the United States (Paul et al., 2008). It indicated that the most effective fungicide for FHB suppression on wheat was the combination of prothioconazole and tebuconazole (52% reduction in disease index relative to the untreated check),

followed by metconazole (50%), prothioconazole (48%), tebuconazole (40%), and propiconazole (32%) (Paul et al., 2008). Paul et al. (2008) also reported that metconazole (45% reduction in DON accumulation compared to untreated check) was the most effective fungicide for suppressing DON accumulation on wheat, followed by prothioconazole (43%), the combination of prothioconazole and tebuconazole (42%), tebuconazole (23%), and propiconazole (12%). Machado et al. (2017) reported that tebuconazole and carbendazim performed better than propiconazole for controlling FHB on wheat in Brazil. Benzimidazole fungicide, such as carbendazim and benomyl, have a longer history of us for FHB control on wheat than triazoles (Machado et al., 2017). After more than three decades, this type of fungicide was still utilized against FHB, especially in China, because of its relatively low cost (Machado et al., 2017). Prochloraz, an imidazole derivate, was widely used in several European countries (Ramirez et al., 2004; Mateo et al., 2011; Kotowicz et al., 2014). At low to medium infective pressure, prochloraz provided effective control of FHB in durum wheat (Menniti et al., 2003). However, fungicides in a quinone outside inhibitor (QoI) class, such as the strobilurin fungicide azoxystrobin, were generally ineffective in suppressing FHB and even might increase DON contamination in grains compared to untreated wheat (Simpson et al., 2001; Pirgozliev et al., 2002; Mullenborn et al., 2008; Gilbert and Haber, 2013). Currently, several fungicide products have been registered for managing FHB in Canada, including Caramba™ (metconazole), Folicur™ (tebuconazole), Proline™ (prothioconazole), and Prosaro™ (prothioconazole and tebuconazole) (Gilbert and Tekauz, 2011).

2.7.2.2 Factors affected fungicide efficacy

Although fungicides, such as tebuconazole, metconazole, and prothioconazole, were reported to be effective against FHB in wheat, complete control of FHB was not achieved by any type of fungicide (Pirgozliev et al., 2002; Paul et al., 2008; Dweba et al., 2017). The effectiveness of fungicides is affected by several factors, such as the level of cultivar resistance, climate, fungicide type, fungicide coverage, the rate of application, and the timing of application (Parry et al., 1995; Mesterhazy et al., 2003; Edwards, 2004; Henriksen and Elen, 2005; Mesterhazy et al., 2011; Dweba et al., 2017; Acs et al., 2018; Mesterhazy et al., 2018a, 2018b). Fungicide application timing plays a critical role in FHB management (Mesterhazy, 2003; Shah et al.,2018). Since anthers were found to have a role in facilitating infection, spraying fungicide on anthers was essential to obtain good control of FHB (Mesterhazy, 2003). Mauler-Machnik and Suty (1997)

reported that tebuconazole fungicide sprayed within four days before or after inoculation at Zadoks growth stage GS 55 had 40 to 95% efficacy. However, fungicide applied five to ten days before or after the inoculation decreased the efficacy to 30 to 50% (Mauler-Machnik and Suty, 1997). Therefore, the optimal timing of fungicide application for FHB control in wheat is during anthesis, while fungicide treatment up to six days after anthesis also can reduce disease levels and DON accumulation (D'Angelo et al., 2014; Paul et al., 2018). In general, fungicide has significantly greater efficacy for FHB and DON suppression in spring wheat than in winter wheat (Hershman and Milus, 2003; Paul et al., 2008; McMullen et al., 2012; Paul et al., 2018). In comparison with spring wheat, winter wheat has a more prolonged flowering stage, which appears to reduce fungicide efficacy (Hershman and Milus, 2003; Paul et al., 2008). However, uneven flowering times in fields pose a challenge in fungicide application (Wegulo et al., 2015). The short application window makes it difficult for growers to apply fungicides if the anthesis period coincides with the rain (Mesterhazy, 2003; Wegulo et al., 2015). In the field, FHB infection usually occurs after rainfall, and fungicide application may be delayed for several days until the soil dries, which misses the optimum application time and reduces fungicide efficacy (Mesterhazy, 2003). If the optimum spraying time is missed and FHB disease occurs, later fungicide treatment will not prevent DON accumulation (Yuen and Schoneweis, 2007). Fungicide efficacy was better on resistant cultivars than susceptible ones in some studies in barley and wheat (Mesterhazy et al., 2003; Horsley et al., 2006; Mesterhazy et al., 2011). Mesterhazy et al. (2003) showed that FHB and DON accumulation were better controlled in a moderately wheat resistant cultivar than in a susceptible cultivar. In contrast, Koch et al. (2006) reported that fungicide treatment reduced DON concentration slightly in a moderately resistant wheat cultivar, but reduced the DON concentration significantly in a susceptible cultivar. When tebuconazole fungicide was applied to 13 barley genotypes with different resistance levels, FHB severity and DON accumulation varied (Horsley et al., 2006). Accordingly, fungicide efficiency is generally variable and often insufficient for FHB control under field conditions (Lehoczki-Krsjak et al., 2010; Mesterhazy et al., 2011).

2.7.2.3 Fungicide resistance phenomenon

Overuse of the same fungicide can result in fungicide resistance in the pathogen population (Dweba et al., 2017; Torres et al., 2019). The first reported carbendazim resistant isolate of *F. graminearum* was detected in China in 1992 (Zhou et al., 1994). The frequency of resistant isolates

in the pathogen population of some regions in China gradually increased since 1988, and as a result, the efficacy of carbendazim fungicide in FHB control decreased (Wang et al., 2002). In the United States, resistance to a DMI fungicide, tebuconazole, was found in *F. graminearum* isolates (Spolti et al., 2014). The development of fungicide resistance in the pathogen population is a challenge for FHB management using fungicide (Spolti et al., 2014). Mixtures of different triazole fungicides or triazole with strobilurins were recommended to obtain the sustainable control of FHB (Ramirez et al., 2004; McMullen et al., 2008; Gilbert and Haber, 2013).

2.7.3 Host resistance

Genetic resistance is the most cost-effective management strategy for FHB (Ruckenbauer et al., 2001). Resistance to FHB of wheat is quantitative and conferred by many minor genes and a few major ones (Bai and Shaner, 2004; Osborne and Stein, 2007; Brar et al., 2019). The quantitative characteristic of FHB resistance results in wide variation in phenotypic reaction and environmental response (Osborne and Stein, 2007). The stability of phenotypic reaction over environments depends on the level of resistance to FHB (Miedaner, 1997). Accordingly, it is necessary to assess genotypes properly for FHB resistance across several environments (Miedaner, 1997). Five types of resistance to FHB were described: I) resistance to initial infection; II) resistance to disease spread within infected spikes; III) resistance to mycotoxin accumulation; IV) resistance to kernel infection; V) tolerance (Schroeder and Christensen, 1963; Miller et al., 1985; Mesterhazy, 1995; Mesterhazy et al., 1999). Type I resistance was described based on disease incidence through spray inoculation at anthesis (Mesterhazy, 1995). Several morphological traits provided potential resources to improve type I resistance to FHB (Haile et al., 2019). The morphological characteristics related to initial infection involve plant height, spike density, anther extrusion or retention, and absence or presence of awns (Mesterhazy, 1995; Buerstmayr et al, 2019). The relationship between plant height and FHB resistance was observed in many wheat populations and further supported by reports of co-localization of quantitative trait loci (QTLs) for FHB resistance with QTLs for plant height (Mesterhazy, 1995; Gervais et al., 2003; Somers et al., 2003; Haberle et al., 2009). The researchers mainly explored type II resistance based on the percentage of symptom spread through point inoculation within a spike under controlled conditions (Mesterhazy, 1995; Gilbert and Tekauz, 2000). Type III resistance represented the level of DON (Shah et al., 2018). Type IV resistance could be measured as the percentage of damaged

kernels defined as *Fusarium*-damage kernel (FDK) (Cowger et al., 2009). Although most studies associated resistance to FHB focused on reducing FHB development in crops, resistance to FDK and DON accumulations was increasingly important, along with economic penalties and food safety concerns. (McMullen et al., 2012). Tolerance to FHB could be measured by comparing yield between infected and uninfected plants (Fakhfakh et al., 2011).

In general, the development of resistant cultivars was achieved through two pathways toward resistance: utilization of FHB resistance from adapted germplasm or incorporation of exotic resistance genes through crosses (Stack and Frohberg, 2000; Anderson et al., 2001; Bai and Shaner, 2004; Mergoum et al., 2007; Mckendry, 2008; Yu et al., 2008a; Kollers et al., 2013). Since 1993, the existing cultivars of hard red spring wheat, durum wheat, and barley were screened based on disease levels in the field, FDK, DON, yield, and test weight (McMullen and Stack,1994; Busch, 1995; Wiersma et al., 1996). In bread wheat, the Chinese cultivar Sumai 3 was a significant source of resistance and has been used extensively in breeding programs worldwide (Niwa et al., 2014). Chinese landrace Wangshuibai was another line with a high level of FHB resistance (Lin et al., 2004; Jia et al., 2005; Yu et al., 2008b). In addition to Chinese resistant germplasm, some spring wheat cultivars with different levels of resistance to FHB were also detected in germplasm from Japan, Brazil, and the United States (Mesterhazy, 1997; Bai and Shaner, 2004; Browne, 2007). Although the primary emphasis was originally on spring wheat, in the last decade, more winter wheat populations were screened for FHB resistance (Abate et al., 2008; Wilde et al., 2008; Haberle et al., 2009; Zhang et al., 2012). Several winter wheat cultivars with FHB resistance were identified in Europe and the United States (Abate et al., 2008; Haberle et al., 2009; Zhang et al., 2012). Rye was the most resistant to FHB in several studies of wheat, triticale, and rye (Miller et al., 1985; Gaikpa et al., 2019). However, there are no evaluations on FHB resistance in rye germplasm.

In addition, exotic sources of resistance were screened and incorporated into wheat or barley cultivars with desired yield and quality characteristics (McMullen et al., 1997). The extensive screening of exotic sources of resistance did not achieve a higher level of resistance than the screening of native sources of resistance (McMullen et al., 2012). Less than 1% of interspecific crosses from other grass species increased levels of resistance to FHB (McMullen et al., 1997). Rare recombination events were selected to reduce linkage drag when resistance genes were

incorporated from exotic sources into adapted cultivars (Prat et al., 2014; Brar et al., 2019). Consequently, the development of resistant cultivars to FHB was a relatively slow process (Wegulo et al., 2015). Over time, incorporating FHB resistance into adapted cultivars of bread wheat and durum wheat became a broadly effective strategy (McMullen et al., 2012). Intensive research of QTL mapping resulted in the identification of over 100 QTLs related to different levels of FHB resistance in wheat (Waldron et al., 1999; Cuthbert et al., 2007; Buerstmayr et al., 2009; Xue et al., 2010; Cainong et al., 2015; Bai et al., 2018). One of the major QTL *Fhb1*, derived from Sumai 3, has been widely used as a source of type II resistance in commercial lines (Waldron et al., 1999; Buerstmayr et al., 2003; Liu and Anderson, 2003; Legge et al., 2004; Anderson, 2007). In addition, spring wheat cultivars, such as the Brazilian cultivar Frontana, have been used as a source of FHB resistance in winter wheat (Pandeya et al., 1996).

The situation in durum wheat is entirely different from that of bread wheat (McMullen et al., 2012). It is challenging to develop FHB resistance for durum wheat due to rare sources of resistance (Legge et al., 2004; McMullen et al., 2012; Prat et al., 2017). Only a limited number of QTLs with minor effects for FHB resistance have been identified in durum (Buerstmayr et al., 2014; Prat et al., 2014). Most durum wheat populations mapped were based on exotic sources of resistance (Prat et al., 2014, 2017). The durum cultivar Divide was released in 2006 and showed partial resistance and occupied an average of 27% of North Dakota hectares in 2011 (McMullen et al., 2012). Since 2014, several new studies detected FHB resistance QTLs derived from durum wheat (Haile et al., 2019). However, QTLs identified in durum wheat provided a lower level of resistance compared to the effect of *Fhb1* in bread wheat (Prat et al., 2017). In addition to FHB resistance derived from durum wheat, FHB resistance from bread wheat was successfully transferred into durum lines, including some major QTLs, such as *Fhb1*, Qfhb.ndwp-5A, and Qfhb.ndwp-7A (Giancaspro et al., 2016; Prat et al., 2017; Zhao et al., 2018). Barley had a similar situation with durum wheat in FHB resistance studies (McMullen et al., 2012). The newly released barley cultivar, Quest, obtained the resistance from exotic barley cultivars that traced back to China and Switzerland (McMullen et al., 2012).

2.7.4 Biological control

Biological control agents (BCAs) are suggested to be used as part of integrated management and may help to reduce the amount of chemical fungicide application (Xue et al., 2009; Gilbert and Tekauz, 2011; Gilbert and Haber, 2013). A wide array of organisms, including fungi and bacteria, can be used as BCAs for FHB (Palazzini et al., 2007; Xue et al., 2009). The most commonly investigated bacterial strains, including *Bacillus* spp., *Streptomyces* spp., and *Pseudomonas* spp., successfully resulted in the reduction of FHB disease levels in fields (Khan et al., 2001; Schisler et al., 2002, 2006; Wang et al., 2015; Palazzini et al., 2017). The fungal BCAs, including *Cryptococcus* spp. and *Clonostachys* spp., also contributed to the decrease of FHB incidence and severity in field trials (Khan et al., 2001; Schisler et al., 2002; Luongo et al., 2005; Xue et al., 2009; Palazzini et al., 2013; Xue et al., 2014). Fewer yeast strains were identified as effective BCAs against FHB compared to bacteria and fungi (Gilbert and Haber, 2013).

Mechanisms of the interaction between BCAs and the pathogen include the direct interaction, such as parasitism or antibiosis, and the indirect interaction, such as competition, disease resistance induction, or plant growth promotion (Legrand et al., 2017). Antibiosis appears to be the most common mechanism that bacterial BCAs utilize to suppress the growth of *F. graminearum* (Legrand et al., 2017). Fengycin and iturin are major inhibitory compounds mainly produced by *Bacillus* spp. against *F. graminearum* (Wang et al., 2007; Dunlap et al., 2013; Zhao et al., 2014). Interestingly, the induction of a resistance mechanism via a heat-stable elicitor was observed in *Lysobacter enzymogenes* strain C3, a potential BCA for FHB (Jochum et al., 2006). Biological control agents can be used as a spikelet treatment at anthesis to decrease FHB disease levels and reduce mycotoxin contamination; they can also be used as a residue treatment to reduce pathogen survival on residue and limit primary inoculum of *Fusarium* in fields (Palazzini et al., 2013; Wegulo et al., 2015; Legrand et al., 2017). Application of BCAs during flowering may reduce or delay germination of *F. graminearum* spores on spikes and result in FHB control (Gilbert and Fernando, 2004). *Streptomyces* sp. 87B was found to be a potential BCA to control FHB at different times in the *F. graminearum* life cycle, contributing to *F. graminearum* suppression at anthesis in spikes and in wheat stubble (Palazzini et al., 2017). In addition, *Clonostachys* spp. was notably used on wheat straw to reduce the production of *F. graminearum* inoculum (Luongo et al., 2005; Palazzini et al., 2013).

Biological control is more environmentally friendly than fungicide application, but it cannot wholly replace chemical control in FHB management (Gilbert and Haber, 2013; Shah et al., 2018). Field application of BCAs was less effective than greenhouse application (Palazzini et al., 2013; Torres et al., 2019). Luongo et al. (2005) obtained saprophytic fungus isolates from different sources of necrotic plant tissues and selected some of them that could inhibit colonization and sporulation of *F. graminearum* on wheat straw. Eleven of 135 isolates screened in laboratory conditions consistently reduced sporulation of *F. graminearum* on wheat straw (Luongo et al., 2005). However, the results of *F. graminearum* suppression were inconsistent when these isolates were tested in field conditions (Luongo et al., 2005). The fluctuating environmental conditions, such as desiccation, osmotic stress, UV radiation, and extreme temperature, limited the effectiveness and survival of BCAs in fields (Dweba et al., 2017). Some biotic factors, such as host resistance, could also affect the effectiveness of BCAs (Gilbert and Fernando, 2004; Gilbert and Haber, 2013; Xue et al., 2014). Accordingly, the identified BCAs were applied in a limited number of field trials, but did not show complete FHB control (Dweba et al., 2017).

2.8 Cereal rusts

Rust diseases are caused by pathogenic biotrophic fungi (Fetch et al., 2011). They are highly specialized on the hosts they infect, known as *formae speciales* or f. sp. (Fetch et al., 2011). For example, *Puccinia graminis* f. sp. *secalis* causes stem rust of rye while *P. graminis* f. sp. *tritici* causes stem rust of wheat (Steffenson et al., 1983; Fetch et al., 2011). There are divergent opinions for the scientific classification of leaf rust pathogens (Anikster et al., 1997). One group classified the broad group of leaf rust pathogens as a single species, *P. recondita*, and it was subdivided into *formae speciales* based on the host (Anikster et al., 1997). Another group classified these fungi into several species (Savile, 1984; Anikster et al., 1997). Accordingly, leaf rust of rye is caused by *P. recondita* f. sp. *secalis*, and leaf rust of wheat is caused by *P. recondita* f. sp. *tritici* based on the broad species definition, but another group considered leaf rust pathogens of rye and wheat as two distinct species, *P. recondita* in rye and *P. triticina* in wheat (Savile, 1984; Swertz, 1994). Within forms, rust diseases are also specialized for the host genotypes they attack, known as physiological races (Zillinsky, 1983; Fetch et al., 2011). The occurrence of new physiological races in fungi creates a threat to host resistance maintenance (Zillinsky, 1983).

2.8.1 Symptoms of leaf and stem rust

Leaf rust generally infects leaves, but may also be found on glumes and awns (Dyck and Kerber, 1985; Peksa and Bankina, 2019). Small circular to oval yellow spots appear first and then develop into orange-red to dark red pustules scattered on the upper surfaces of the leaf (Zillinsky, 1983). The pustules erupt through the epidermis, without causing loose epidermal tissue at the margins, and produce many spores (Zillinsky, 1983).

The symptoms of stem rust usually appear on stems and leaf sheaths, but occasionally they may be found on leaf blades and glumes (Zillinsky, 1983; Leonardo and Szabo, 2005; Miedaner et al., 2016). A tiny chlorotic fleck appears a few days after infection (Leonardo and Szabo, 2005). Later, a mass of reddish-brown spores produces pustules that rupture the host epidermis (Zillinsky, 1983; Leonardo and Szabo, 2005). The uredinial pustules are generally diamond-shaped and larger than leaf rust pustules (Zillinsky, 1983; Leonardo and Szabo, 2005). They can be observed on both the upper and lower surfaces of the leaf (Zillinsky, 1983; Schumann and Leonard, 2000).

2.8.2 Disease cycle of leaf and stem rust

The life cycle of cereal rusts is macrocyclic and includes five distinct stages of urediniospores, teliospores, basidiospores, pycniospores, and aeciospores (Leonard and Szabo, 2005; Kolmer, 2013). The first three spore forms develop on cereal hosts, while the last two are produced on alternate hosts (Kolmer, 2013). Among these spores, urediniospores enable continuous cycles of infection on cereal hosts, as the asexual cycle (Roelfs, 1985; Schumann and Leonard, 2000; Kolmer, 2013). In mild climates, the asexually reproducing urediniospores survive winter on cereal plants (Roelfs, 1985). In cold areas where no host tissues are available during winter, inoculum for rust infection comes from urediniospores that are transported long distances by the wind from milder climates to the cropping region (Hirst and Hurst, 1967; Roelfs, 1985). Teliospores developed from telia as the first stage in the sexual cycle (Zillinsky, 1983). After overwintering, the teliospores germinate and produce wind-borne basidiospores to infect the alternate host, generally on the upper leaf surface (Zillinsky, 1983; Roelfs, 1985; Schumann and Leonard, 2000; Leonard and Szabo, 2005). The alternate host for *P. triticina* is the *Thalictrum speciosissiumum* species in the Ranunculaceae family, while the alternate host for *P. recondita*

includes *Lycopsis arvensis* species in the Boraginaceae family (Anikster et al.,1997; Kolmer, 2013). The pycnial structures develop on the infected alternate host and produce pycniospores by movement in water by insects or rain splashing (Leonard and Szabo, 2005; Kolmer, 2013). The hyphae fertilized by pycniospores grow down through the leaf where they eventually produce aecia on the underside of the leaf surface (Roelfs, 1985; Schumann and Leonard, 2000; Leonard and Szabo, 2005; Kolmer, 2013). The aeciospores are released from the matured aecium, and wind disperses them to their cereal host, causing infection (Kolmer, 2013). The infection results in the development of uredinium to produce urediniospores or teliospores, thus completing the life cycle (Schumann and Leonard, 2000; Kolmer, 2013).

2.8.3 Disease management of leaf and stem rust

The use of resistant cultivars and eradication of the alternate host were the most effective strategies to control rust disease for many years (Zillinsky, 1983). Host resistance to rust includes both race-specific and non-race-specific types (Roelfs, 1985). Genetic resources with different stem rust resistance levels were reported for rye cultivars from several countries (Solodukhina and Kobylyanskij, 2003; Miedaner et al., 2016). Leaf rust resistance genes were found on all rye chromosomes except for chromosome 5R (Singh and McIntosh, 1990; McIntosh et al., 1995; Wehling et al., 2003; Roux et al., 2004; Wilde et al., 2006). Resistance genes from rye constituted a valuable genetic resource for leaf and stem rust resistance in wheat and triticale (Roelfs et al., 1992; Roux et al., 2004; Crespo-Herrera et al., 2017).

The eradication of the alternate host of wheat and rye stem rust happened as early as 1660 and started in Rouen, France (Roelfs, 1985). It reduced the initial inoculum and eliminated the sexual cycles, decreasing the number of pathogen races and stabilizing the pathogen phenotypes (Roelfs, 1985). In Canada, the role of barberry in the life cycle of wheat and rye stem rust has been eliminated due to the eradication programs that started around 1916 (Fetch et al., 2011). For wheat leaf rust in North America, the alternative host does not play a significant role in the origin of new races because of the rare natural infection of native *Thalictrum* species (Samborski, 1985).

Currently, cultural methods for cereal rust control largely depend on the use of early maturing cultivars and early seeding (Roelfs, 1985; McCallum et al., 2007). In western Canada,

early planting of spring wheat may avoid severe rust infection since the epidemics typically occur in late July and early August (McCallum et al., 2007). However, too early planting of winter cereal may increase the chance of rust infection in fall (Roelfs, 1985). Some fungicides used for fusarium head blight management, such as Tilt™ and Folicur™, effectively control rust diseases (McCallum et al., 2007). Fungicide application gives an added input cost and are less economical than using resistance cultivars (Dickson, 1959; Roelfs, 1983; Zillinsky, 1983). However, they are valuable for epidemic situations when new races develop while new resistant cultivars are unavailable (Zillinsky, 1983; Samborski, 1985).

2.9 Ergot

Ergot is a fungal disease of the inflorescence caused by *Claviceps* species (Schumann and Uppala, 2000; Pazoutova, 2002; Wegulo and Carlson, 2011; Miedaner and Geiger, 2015). There are about 40 species of *Claviceps* in the world (Pazoutova, 2002; Miedaner and Geiger, 2015). The most important one is *Claviceps purpurea* Tulasne, which is distributed across most temperature regions of the world (Pazoutova, 2002; Miedaner and Geiger, 2015). *Claviceps purpurea* can infect many host plants, including about 400 grass species (Miedaner and Geiger, 2015). Among these hosts, rye is the most economically significant host (Miedaner and Geiger, 2015). Yield reduction is one of most significant effects caused by ergot, while the presence of toxic alkaloids present in the sclerotia is the main concern for food and feed safety (Schumann and Uppala, 2000). The toxic alkaloids contribute to severe health problems for humans and animals (Schumann and Uppala, 2000).

2.9.1 Symptoms of ergot

The first sign of ergot infection in the plant is the appearance of honeydew, a yellow-sugary slime, on the spike during, or after, flowering (Schumann and Uppala, 2000; Wegulo and Carlson, 2011; Friskop et al., 2018). Later, a purplish-black ergot (sclerotium) replaces the kernel and protrudes from the glumes on the spike (Schumann and Uppala, 2000; Wegulo and Carlson, 2011). The shape of the ergot resembles a horn, and the size is generally several times larger than the regular seed (Wegulo and Carlson, 2011).

2.9.2 Disease cycle of ergot

Claviceps purpurea overwinters in the soil, or on the soil's surface, as sclerotia that developed on previous cereal crops or grasses (Tenberge, 1999; Wegulo and Carlson, 2011). Four to eight weeks of 0-10 ºC temperatures are required for the sclerotia to be vernalized before germination (Schumann and Uppala, 2000; Menzies and Turkington, 2015). In spring, sclerotia germinate and form stroma with fruiting structures known as perithecia (Schumann and Uppala, 2000; Wegulo and Carlson, 2011). Ascospores are released from the perithecia and dispersed by the wind and rain splash (Bove, 1970; Colotelo and cook, 1977; Alderman, 2003; Wegulo and Carlson, 2011). The fungus cannot penetrate through closed glumes and only infects the ovary of host plants rather than other parts of the plant during flowering (Schumann and Uppala, 2000). Accordingly, cool, wet weather that prolongs the flowering period increases the chance for ascospores to land on the stigma (Wegulo and Carlson, 2011). Ascospores are the primary inoculum and germinate and infect the ovary within 24 h of contact with the flower (Bove, 1970; Wegulo and Carlson, 2011; Miedaner and Geiger, 2015). A grayish-white mycelial mass (stroma) grows in the ovaries following the germination of ascospores (Bove, 1970; Luttrell, 1980; Wegulo and Carlson, 2011). The pathogen produces the sticky fluid honeydew which is then excreted from the ovary (Tenberge, 1999; Schumann and Uppala, 2000; Wegulo and Carlson, 2011; Menzies and Turkington, 2015). The honeydew contains the conidia developed and released from the stroma (Luttrell, 1980; Wegulo and Carlson, 2011). Conidia as the secondary inoculum are spread to other florets through honeydew by direct contact, rain splash, farming equipment, and especially insect activity (Tenberge, 1999; Wegulo and Carlson, 2011; Menzies and Turkington, 2015; Miedaner and Geiger, 2015). Dew or rain are required to dilute the honeydew leading to the germination of conidia (Schwartingl and Hiners, 1945). The stroma grows in the ovaries and mature into sclerotia within four to five weeks, which completes the disease cycle (Luttrell, 1980; Wegulo and Carlson, 2011; Miedaner and Geiger, 2015). A hard purple-black surface layer of sclerotium protects the inner fungal mycelial tissue and storage cells in the center of the sclerotia (Bove, 1970; Tenberge, 1999; Schumann and Uppala, 2000).

2.9.3 Disease management of ergot

A single strategy cannot entirely control ergot, although there are several different strategies for ergot management (Menzies and Turkington, 2015). Cultural practices play a significantly important role in ergot management in cereal crops due to the lack of economical chemical control and genetic resistance (Schumann and Uppala, 2000). Some fungicides were assessed on ergot control in the UK and the United States (Menzies and Turkington, 2015). However, most of the fungicides used in the trials were not registered for use in Canada (Menzies and Turkington, 2015). Accordingly, no fungicide is officially recommended for ergot management in cereals (Miedaner and Geiger, 2015). Genetic resistance of ergot in cereals has not been found (Miedaner and Geiger, 2015).

Cultural practices focus on managing the ergot spore load in the field (Menzies and Turkington, 2015). Tillage is an agronomic practice that buries sclerotia in the soil, and as a result, ascospores are not formed and cannot be dispersed on host plants (Miedaner and Geiger, 2015). However, along with the expansion of no-till soil management, crop rotation is another agronomic practice to limit spore load (Miedaner and Geiger, 2015). Since sclerotia usually only survive for one year in the soil, rotation with nonhost crops reduces the infection pressure (Mitchell and Cooke, 1968; Miedaner and Geiger, 2015). Use of sclerotia-free seed or certified seed that contains ergot at a low level can also reduce ergot severity in the field (Bailey et al., 2003; Wegulo and Carlson, 201; Miedaner and Geiger, 2015). Control of weedy grasses is important for ergot management in crop cereals due to the initial source of inoculum from grasses (Mantle et al., 1977). Grasses also serve as a source of honeydew if they are infected before crop flowering (Mantle et al., 1977). Uniform stands of crops limit the number of late tillers and, as a result, reduce the flowering period, which decreases the risk of infection (Miedaner and Geiger, 2015). Based on scouting for ergot in the field, heavily infected parts should be harvested separately from others (Bailey et al., 2003; Wegulo and Carslon, 2011; Miedaner and Geiger, 2015).

2.9.4 Food and feed safety of ergot infected grain

Ergot infection of cereals threatens food and feed safety (Miedaner and Geiger, 2015). Three major groups of ergot alkaloids, Clavine alkaloids, D-lysergic acid and its derivatives, and

ergopeptines, can cause severe health problems in humans and animals (Miedaner and Geiger, 2015). Ergot thresholds are generally determined as a percentage of sclerotia weight per grain weight (Miedaner and Geiger, 2015; Friskop et al., 2018). All unprocessed cereals should not have ergot over 0.05% for human consumption and 0.1% for animal feed in Europe (Miedaner and Geiger, 2015; Miedaner et al., 2021). Ergot threshold is 0.3% for rye in the United States (Wegulo and Carlson, 2011; Friskop et al., 2018). Limits of ergot contamination in cereal grains vary with the grade, from 0.05 to 0.33% for rye in Canada (Canadian Grain Commission, 2004). Canada is one of only two countries with regulations for ergot alkaloids in feed, and there are no guidelines for food (Scott, 2019). In Canada, guideline limits of ergot alkaloids in feed are 6 ppm for swine, 3 ppm for cattle, sheep, and horses, and 9 ppm for chicks (FAO, 2004).

Chapter 3

Fusarium species associated with fusarium head blight of fall rye (*Secale cereale* L.) in Manitoba

3.1 Abstract

Fusarium head blight (FHB) is a common spike disease in small grain cereals caused by several *Fusarium* species. *Fusarium graminearum* is generally predominant in Manitoba, especially on wheat. In this study, rye spike samples were collected from naturally infected fall rye fields or research plots from 2016 to 2018 in Manitoba. *Fusarium* species were recovered from 23.57% of the kernel samples that were plated. Five *Fusarium* species were identified, including *F. graminearum*, *F. avenaceum*, *F. poae*, *F. sporotrichioides*, and *F. equiseti*, using species-specific PCR. The results showed that *F. graminearum* was the most frequently isolated *Fusarium* species from fall rye samples collected in Manitoba, which was in 66.67% of *Fusarium* isolates. Other *Fusarium* species frequently detected on rye included *F. avenaceum* (19.91%) and *F. poae* (7.79%). *Fusarium sporotrichioides* (3.46%) and *F. equiseti* (2.16%) were detected in low frequencies. Among the *F. graminearum* isolates, more 15-ADON chemotype isolates were isolated from fall rye spike samples than 3-ADON chemotype isolates. No NIV chemotype isolates were detected among fall rye spike samples tested.

3.2 Introduction

Several *Fusarium* species cause fusarium head blight (FHB) in small grain cereals, including wheat, barley, oat, and rye (Tekauz et al., 2000, 2004; Gilbert and Tekauz, 2011; Grafenhan et al., 2013; Xue et al., 2019). *Fusarium graminearum*, *F. culmorum*, and *F. avenaceum* were the three predominant species reported internationally (Parry et al., 1995). *Fusarium graminearum* predominates in North America, especially on wheat, but is not always the prime cause of FHB in every region of western Canada (Gilbert and Tekauz, 2011). In Manitoba, *F. graminearum* was the main cause of FHB in spring and winter wheat, accounting for more than 90% of isolations collected from 2003 to 2007 (Gilbert et al., 2008; Tekauz et al., 2009). In Saskatchewan and Alberta, *F. poae* and *F. avenaceum* play a significant role as an agent of FHB in spring wheat (Tekauz et al., 2009; Gilbert and Tekauz, 2011).

Environmental conditions and crop types could contribute to the variable distribution of *Fusarium* species causing FHB (van Eeuwijk et al., 1995; Xu et al., 2008). Geographical distribution of *Fusarium* species is also related to temperature requirements (Parry et al., 1995). In warmer regions of the world, including some areas of the United States, Canada, Australia, and central Europe, *F. graminearum* is generally the most important cause agent of FHB (Parry et al., 1995). The optimal growth temperature for *F. graminearum* is 25 ℃ (Popovski and Celar, 2013). *Fusarium culmorum*, *F. poae*, and *F. avenaceum* tend to predominate in cooler regions (Parry et al., 1995). In wheat, *F. graminearum* was the most prevalent *Fusarium* species causing FHB in Manitoba from 2016 to 2018 (Henriquez et al., 2017, 2018, 2019). However, based on FHB survey in Manitoba, *F. poae* predominated in infected barley and oat fields (Banik et al., 2018a, 2018b, 2019a, 2019b). However, no study has evaluated the occurrence and prevalence of *Fusarium* species associated with FHB on rye from 2016 to 2018 in Canada.

Deoxynivalenol (DON), commonly known as vomitoxin, is one of the most important trichothecene mycotoxins produced by *F. graminearum* (Desjardins, 2006). The mycotoxin inhibits protein biosynthesis in eukaryotes and causes health problems in animals, such as feed refusal, diarrhea, emesis, alimentary hemorrhaging, and contact dermatitis (Desjardins, 2006). Strains of *F. graminearum* usually exhibit one of three primary trichothecene profiles: (1) DON and 15-acetyldeoxynivalenol (15-ADON) (15-ADON chemotype); (2) DON and 3-acetyldeoxynivalenol (3-ADON) (3-ADON chemotype); (3) nivalenol (NIV) and its acetylated derivatives (NIV chemotype) (Ward et al., 2002; Desjardins, 2006; Torres et al., 2019). The distribution of different chemotypes across wheat growing regions is related to the geographical origins of *F. graminearum* (Mirocha et al., 1989). The 15-ADON chemotype was dominant in North America, central Europe, southern Russia, and South America, while a greater percentage of the 3-ADON chemotype was present in northern Europe, China, Korea, Australia, and New Zealand (Mirocha et al., 1989; Yli-Mattila et al., 2009; van der Lee et al., 2015). The NIV chemotype is usually isolated in Asian countries and less frequently in Europe, South Africa, and the United States (Suga et al., 2008; Gale et al., 2011; Zhang et al., 2012; van der Lee et al., 2015). In 2015, a fourth chemotype was detected in the United States which produces NX-2, a trichothecene with a chemical structure similar to 3-ADON (Varga et al., 2015).

In Canada, an increased frequency of the 3-ADON chemotype isolates was observed between 1998 and 2004 in western Canada (Ward et al., 2008). Between 2005 and 2007, the frequency of the 3-ADON chemotype continued to increase significantly in the prairies, and in 2007, the 3-ADON chemotype predominated in Manitoba (Kelly et al., 2015). In 2018 and 2019, the 3-ADON chemotype accounted for about 75% of isolates collected from Manitoba and Saskatchewan (Oghenekaro et al., 2021). However, the 15-ADON chemotype remained predominant in Ontario (Tamburic-llincic et al., 2006; Amarasinghe et al., 2009; Kelly et al., 2015; Tamburic-llincic et al., 2015; Crippin et al., 2019; Oghenekaro et al., 2021). The stability of the 15-ADON genetic background in Ontario seems to be related to corn production as regions with greater proportions of the 15-ADON chemotype tend to grow more corn (Tamburic-llincic et al., 2015; Crippin et al., 2019).

The objectives of this study were to investigate the occurrence and prevalence of *Fusarium* species causing FHB on rye and to detect the chemotypes of *F. graminearum* isolated from fall rye spike samples collected in Manitoba between 2016 and 2018. The single spore isolates obtained from the study provided inoculum for additional research on rye.

3.3 Materials and methods

3.3.1 Sample collection

Fall rye spikes were collected from 16 naturally infected fall rye fields in 2016 and five in 2017 in Manitoba from June to July by Dr. Maria Antonia Henriquez at the Morden Research and Development Centre, Agriculture and Agri-Food Canada (AAFC) (**Figure 3.1**). Among these 21 fall rye fields, two fields had the same GPS locations, but the samples were collected from two different varieties at different growth stages. Additionally, in 2016, fall rye spikes with FHB symptoms were collected from 32 fall rye registration trial plots at the University of Manitoba Ian N Morrison Research Farm in Carman, MB and 16 fall rye registration trial plots in the Point field research station in Winnipeg, MB. In 2018, fall rye spikes with FHB symptoms were collected from 15 naturally infected fall rye registration trial plots in Brandon, MB (GPS 50.0233846, -99.8791567) and 13 fall rye registration trial plots in the Point field research station in Winnipeg, MB. Approximately ten spikes were collected based on visible FHB symptoms at each sampling site.

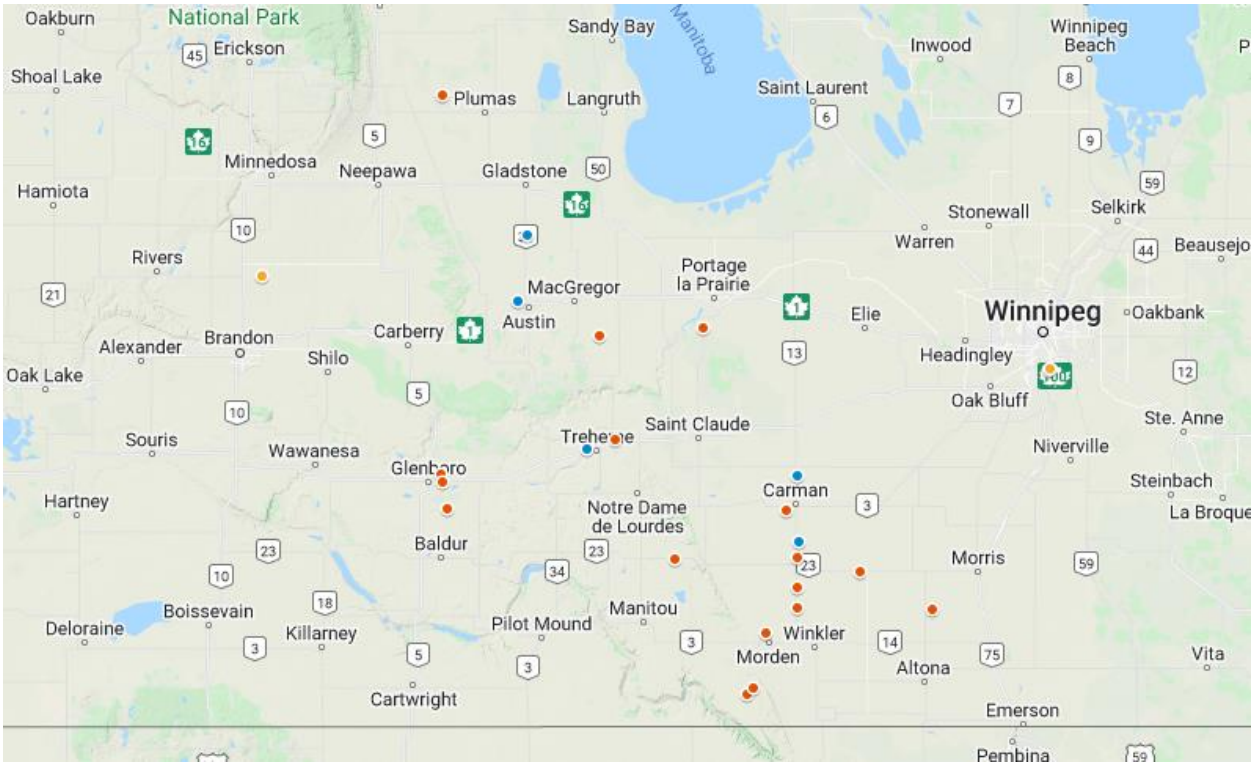

Figure 3.1 A map showing 20 fall rye fields in Manitoba, Canada, from which spikes infected with *Fusarium* were collected in 2016 (red dots), 2017 (blue dots), and 2018 (orange dots).

3.3.2 Sample isolation

Five kernels with FHB symptoms and five without FHB symptoms were randomly taken from spikes collected from each field or experimental plot. According to the protocol provided by Dr. Maria Antonia Henriquez (personal communication), kernels were surface disinfected in a 6% bleach solution of 6% sodium hypochlorite for 1 min and dried on sterile filter paper. All five kernels were placed on a 150mm-diameter petri dishes containing spezieller nährstoffarmer agar (SNA) (**Appendix 1**). The plates were put in the dark in an incubator (VWR International LLC., USA) set at 21℃ for seven days. For samples with *Fusarium* species growth, glass slides were made of mycelia taken from developed sporodochia on SNA and dyed with acid fuchsin. Their morphological features were observed under 40× magnification using a Fisherbrand™ Stereomaster™ microscope (Fisher Scientific International Inc., USA). The developed sporodochia in a SNA medium were washed using autoclaved distilled water and spread over a water agar (WA) medium (**Appendix 1**). After 18 to 20 hours of incubation at 21 ℃ in dark, a single germinating macroconidium was isolated and transferred onto a potato dextrose agar (PDA)

medium (**Appendix 1**). All plates were stored at 4 ℃ after culturing at room temperature (21 to 24 ℃) for seven days under fluorescent light. These single spore isolates were also preserved on sterile filter paper and stored at -20 ℃.

3.3.3 DNA extraction

DNA extraction was carried out according to the protocol described by Henriquez et al. (2020). Mycelia were harvested from a fresh culture on PDA and macerated with 1000 µL TES extraction buffer (4 M NaCl, 1 M Tris pH 7.5, and 0.5 M EDTA pH 8.0). The mycelia were broken into small pieces in a 1.5 mL microcentrifuge tube using a sterile pellet pestle. Then 100 µL 1% SDS and 2 µL proteinase K (10 mg/mL stock concentration) were added to the tube. The mixture was vortexed and incubated in an Isotemp™ digital dry bath (Fisher Scientific International Inc., USA) at 65 ℃ for 60 min. After incubation, 350 µL of 7.5 M ammonium acetate were added. The mixture was vortexed, incubated at room temperature for 7 min, and centrifuged for 10 min at 12000 rpm. The supernatant (800 µL) was transferred into a new tube. The supernatant was added with 300 µL of Chloroform: Isoamyl alcohol (24:1) and then vortexed and centrifuged for 10 min at 12000 rpm. The top layer was transferred into a new tube, and the previous step was repeated. DNA was precipitated by adding isopropanol up to the 1.5 mL mark, followed by vortex and incubation at -20 ℃ for at least 2 hours. The DNA pellet was obtained by centrifuging for 10 min at 12000 rpm after incubation and washed using 800 µL of cold 70% ethanol, followed by vortex and centrifuging at 12000 rpm for 5 min. After air-drying for a minimum of 15 min, the pellet was suspended in 100 µLTE buffer (10 mM Tris-HCL pH 7.5 and 1 mM EDTA) and treated with 5 µL of RNase A (1 mg/mL initial concentration). The DNA was incubated at 37 ℃ for a minimum of 45 min and stored at -20 ℃.

3.3.4 Identification of *Fusarium* species using PCR

To identify the *Fusarium* species, species-specific PCR primers described by Demeke et al. (2005) were used (**Table 3.1**). The PCR was performed in a 25 µL volume containing 10× buffer (1.5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl pH8.3), 0.2 mM dNTPs, 0.4 µM of each primer (**Table 3.1**), 0.75 units of Taq DNA polymerase (Applied Biosystems, USA), and 50 ng template DNA. The DNA amplification was conducted using an initial 3 min denaturation at

95°C, followed by 38 cycles of 30 s at 95°C, 20 s at 62°C, and 45 s at 72°C, and a final extension of 72°C for 5 min. Products of PCR were separated on 2% agarose gels and visualized using the Gel Doc XR+ System (Bio-Rad Laboratories Ltd., USA) (Demeke et al., 2005).

Table 3.1 List of forward (F) and reverse (R) primer sequences used to determine *Fusarium* species of samples collected in Manitoba, Canada from 2016 to 2018 and the expected DNA fragment length (Demeke et al., 2005)

Primer name	Species	Sequence (5'-3')	Size (bp)
FaF	*F. avenaceum*	CAAGCATTGTCGCCACTCTC	920
FaR		GTTTGGCTCTACCGGGACTG	
FC01F	*F. culmorum*	ATGGTGAACTCGTCGTGGC	570
FC01R		CCCTTCTTACGCCAATCTCG	
FEF1	*F. equiseti*	CATACCTATACGTTGCCTCG	400
FER1		TTACCAGTAACGAGGTGTATG	
Fg16F	*F. graminearum*	CTCCGGATATGTTGCGTCAA	450
Fg16R		GGTAGGTATCCGACATGGCAA	
FP82F	*F. poae*	CAAGCAAACAGGCTCTTCACC	220
FP82R		TGTTCCACCTCAGTGACAGGTT	
AF330109CF	*F. sporotrichioides*	AAAAGCCCAAATTGCTGATG	332
AF330109CR		TGGCATGTTCATTGTCACCT	

3.3.5 Chemotype analysis

Chemotypes of *F. graminearum* were identified using chemotype-specific PCR described by Ward et al. (2002, 2008). A four-primer mixture, 3CON (5'-TGGCAAAGACTGGTTCAC-3'), 3D15A (5'-CGCATTGGCTAACACATG-3'), 3D3A (5'-CGCATTGGCTAACACATG-3') and 3NA (5'-GTGCACAGAATATACGAAGC-3') was applied in the PCR. The 3-ADON chemotype was identified through primers 3D3A/3CON producing a 243 bp fragment; the 15-ADON chemotype was confirmed through primers 3D15A/3CON producing a 610 bp fragment; the NIV chemotype was identified by using primers 3NA/3CON producing an 840 bp fragment (Ward et al., 2008).

3.4 Results

3.4.1 *Fusarium* isolate identification

Two hundred and thirty-one single spore isolates were isolated from a total of 980 plated rye kernel samples and identified as *F. graminearum*, *F. poae*, *F. avenaceum*, *F. equiseti*, or *F. sporotrichioides*, based on morphological features described in the Fusarium Laboratory Manual (Leslie and Summerel, 2006) and species-specific PCR assays (**Table 3.2**). There were 36.94% of plated kernel samples with no pathogen growth, and 39.49% of plated kernels produced growth from non-*Fusarium* pathogens. *Fusarium graminearum* was the most frequently isolated *Fusarium* species from rye samples collected in 2016 and 2018. *Fusarium avenaceum* was the only species isolated from 2017 rye samples. Overall, 154 of 231 single spore isolates were identified as *F. graminearum*, which accounted for 66.67% of *Fusarium* isolates. During 2016 to 2018, 46 isolates were identified as *F. avenaceum*, with a frequency of 19.91% in *Fusarium* isolates. *Fusarium poae* was one additional species frequently detected on rye. Eighteen kernel samples were infected with *F. poae*, and its frequency in *Fusarium* isolates was 7.79%. Other species recovered at a low frequency, including *F. sporotrichioides* (3.46%) and *F. equiseti* (2.16%). Only eight isolates were identified as *F. sporotrichioides*, and five were *F. equiseti*. There were situations where more than one species was found in a kernel sample.

Table 3.2 *Fusarium* species collected from fall rye fields in Manitoba, Canada from 2016 to 2018

Year	Field code	Number of Samples	F. g.	F. s.	F. p.	F. e.	F. a.
2016	FR-16-01	10	0	0	0	0	0
	FR-16-02	10	0	0	0	0	1
	FR-16-03	10	4	0	0	0	0
	FR-16-04	10	4	0	0	0	0
	FR-16-05	10	1	0	0	1	0
	FR-16-06	10	0	0	0	0	0
	FR-16-07	10	0	0	0	0	1
	FR-16-08	10	0	0	0	0	0
	FR-16-09	10	3	0	0	0	0
	FR-16-10	10	4	0	0	0	0
	FR-16-11	10	0	0	0	0	0
	FR-16-12	10	0	0	0	0	0
	FR-16-13	10	2	0	1	0	0
	FR-16-14	10	0	0	0	0	0
	FR-16-15	10	4	0	0	0	0
	FR-16-16	10	5	0	0	0	0
	FR-16-17	10	0	0	0	0	0
	Carman	320	69	5	10	3	24
	Winnipeg	160	52	1	4	1	13
2017	FR-17-01	10	0	0	0	0	0
	FR-17-02	10	0	0	0	0	0
	FR-17-03	10	0	0	0	0	0
	FR-17-04	10	0	0	0	0	0
	FR-17-05	10	0	0	0	0	3
2018	Brandon	150	2	2	3	0	4
	Winnipeg	130	4	0	0	0	0
	Total	980	154	8	18	5	46

F. g.=*F. graminearum*, F. s.=*F. sporotrichioides*, F. p.=*F. poae*, F. e.= *F. equiseti*, F. a.=*F. avenaceum*.

3.4.2 Chemotype of *Fusarium graminearum*

Only two chemotypes, 3-ADON and 15-ADON, were detected among *F. graminearum* isolates. Of the isolates collected from naturally infected fall rye in Winnipeg from 2016 to 2018, 53.57% of *F. graminearum* isolates were the 3-ADON chemotype and 46.43% of the isolates were

the 15-ADON chemotype (**Table 3.3**). However, the proportion of *F. graminearum* isolates with the 15-ADON chemotype was higher than the 3-ADON chemotype (62.32% vs. 37.68%) in Carman in 2016. Among the eight fall rye fields with *F. graminearum* detected in 2016, five of them had more 15-ADON chemotypes isolates than 3-ADON. Only two *F. graminearum* isolates were collected from Brandon in 2018 and both were the 15-ADON chemotype. In total, the frequency of 3-ADON chemotypes in southern Manitoba was 43.51% from 2016 to 2018.

Table 3.3 Number of *Fusarium graminearum* samples collected from fall rye fields in Manitoba, Canada from 2016 to 2018 that were either 15-ADON or 3-ADON chemotype

Year	Field code	Number of Samples	F. g.	15-ADON	3-ADON
2016	FR-16-03	10	4	4	0
	FR-16-04	10	4	0	4
	FR-16-05	10	1	1	0
	FR-16-09	10	3	0	3
	FR-16-10	10	4	4	0
	FR-16-13	10	2	0	2
	FR-16-15	10	4	3	1
	FR-16-16	10	5	4	1
	Carman	320	69	43	26
	Winnipeg	160	52	25	27
2018	Brandon	150	2	2	0
	Winnipeg	130	4	1	3
	Total	850	154	87	67

F. g.=*F. graminearum.*

3.5 Discussion

In general, it was not easy to collect fall rye spikes with FHB symptoms in naturally infected fields, as disease levels of FHB on fall rye in Manitoba were low from 2016 to 2018. In 2016, the mean disease incidence of FHB on rye was 6.70% (range 0-19.51%) in Manitoba, according to the survey provided by Dr. Maria Antonia Henrique (**Appendix 3**). Infection and development of FHB depend on the weather conditions that coincide with the flowering period (Sutton, 1982; McMullen et al., 1997). In southern Manitoba, fall rye usually started to flower in the second week of June when the conditions were less conducive to FHB infections, based on

annual FHB reports from Manitoba Agriculture. Therefore, fall rye has a high potential to escape FHB infections. In 2017 and 2018, the disease levels of FHB on wheat, barley, and oat in Manitoba were much lower compared to the previous ten years (Banik et al., 2018a, 2018b; Henriquez et al., 2018; Banik et al., 2019a, 2019b; Henriquez et al., 2019). The growing conditions throughout Manitoba were drier than usual, especially in 2017, which were not favorable for FHB development in fields (Banik et al., 2018a, 2018b). Consequently, only a few isolates were cultured from fall rye spike samples collected in 2017 and 2018.

In the current study, 23.57% of plated kernel samples collected in Manitoba during 2016 to 2018 were infected with at least one of five *Fusarium* species. A study of rye samples collected in 2010 showed that 14.75% of plated rye kernel samples from Manitoba were contaminated with *Fusarium* (Grafenhan et al., 2013). Different frequency of *Fusarium* species isolated from plated kernels in the two studies may be due to variation in epidemics of FHB in different years and regions. The higher frequency of *Fusarium* infected kernels in the current study may be due to the existence of FHB nurseries around experimental plot where some rye spike samples were collected. During 2016 to 2018, 66.67% of isolated *Fusarium* species from rye kernels were *F. graminearum*, and 19.91% were *F. avenaceum*. Grafenhan et al. (2013) reported that 33 of 59 seeds (55.93%) were contaminated with *F. graminearum*, and 22 of 59 seeds (37.29%) were contaminated with *F. avenaceum* in rye samples collected in 2010 from Manitoba. Other species, including *F. poae*, *F. sporotrichioides*, and *F. acuminatum*, were identified at low frequencies (Grafenhan et al., 2013). *Fusarium poae* and *F. sporotrichioides* were also found at low frequencies in rye samples collected from 2016 to 2018 (**Table 3.2**). It indicated that *F. graminearum* was the predominant species causing FHB on rye in Manitoba. However, only *F. avenaceum* was identified in rye samples collected from Alberta, and it also was the most frequently isolated species in Saskatchewan (Grafenhan et al., 2013). Therefore, *F. graminearum* and *F. avenaceum* appear to be the most prevalent species causing FHB in rye in Manitoba and other parts of the prairies.

In Manitoba, the FHB index for winter wheat was 2.7% in 2016, which was much higher than 2017 (0.06%) and 2018 (0.1%) (Henriquez et al., 2017, 2018, 2019). *Fusarium graminearum* was the most prevalent *Fusarium* species causing FHB not only on fall rye, but also on barley and oat in 2016 (Banik et al., 2017a, 2017b). However, in 2017, *F. avenaceum* was the only *Fusarium* species isolated from fall rye samples collected in five rye fields. In 2018, *F. graminearum* was

not the most frequently isolated species from rye samples collected in Brandon, where there were no artificially inoculated FHB nurseries nearby. Disease surveillance data from 2017 to 2018 in Manitoba indicated *F. poae*, not *F. graminearum*, accounted for a greater proportion of the species on barley and oat, followed by *F. graminearum*, *F. sporotrichioides*, *F. avenaceum*, and *F. equiseti* (Banik et al., 2018a, 2018b, 2019a, 2019b). Epidemics of FHB in wheat seems to influence the predominant species on other crops due to the increased levels of *F. graminearum* inoculum on wheat (Tekauz et al., 2000). Xue et al. (2019) showed that the isolation frequency of *F. graminearum*, but not other resident *Fusarium* species from wheat and barley, increased significantly during the FHB epidemic years.

Additionally, environmental conditions could affect the predominant causal agents of FHB in fields (Xu et al., 2008). *Fusarium graminearum* initially did not cause high levels of FHB in barley (Tekauz et al., 2000). However, excessive moisture during the growing season could favor *F. graminearum* over *F. poae* (Tekauz et al., 2000). Drier weather during the growing season in 2017 could have favored *F. poae* over other species causing FHB on barley and oat (Xu et al., 2008; Banik et al., 2017a, 2017b). In this study, only a small number of isolates could be cultured from fall rye kernel samples in 2017 and 2018 due to low disease levels. As a result, strong conclusions on the effect of weather on *Fusarium* species causing FHB in fall rye cannot be made.

In fall rye fields, the frequency of the 15-ADON chemotype was higher than the 3-ADON chemotype (56.49% vs. 43.51%) in Manitoba from 2016 to 2018. The predominant chemotype was not consistent across different sampling sites. In contrast, *F. graminearum* 3-ADON chemotypes were more common than 15-ADON chemotypes in Manitoba oat fields according to samples collected from 2016 to 2018 (Tabassum, 2021). However, in 2016, the 15-ADON chemotype was predominant on oat in central Manitoba (Tabassum, 2021). Similarly, the 15-ADON chemotype was also dominant on rye in 2016. No *F. graminearum* isolates were isolated from rye spike samples collected from 2017. Of the small number of rye samples with *F. graminearum* collected in 2018, equal numbers of the 15-ADON and 3-ADON chemotypes were observed. The 3-ADON chemotype frequency of the isolates collected from spring wheat in Manitoba was 72.6% and 75.5% in 2018 and 2019, respectively (Oghenekaro et al., 2021). The 3-ADON chemotype was predominant in spring wheat fields in southern Manitoba, central Manitoba, and the Interlake region of Manitoba (Oghenekaro et al., 2021). The difference of locations where

rye spike samples were collected could result in different results of chemotype distribution in rye and wheat. Moreover, only a few isolates were available for some fall rye sampling sites due to low levels of FHB infection. Therefore, the number of isolates collected from fall rye fields was too small to confirm the distribution of *F. graminearum* chemotypes in Manitoba. In the current study, no *F. graminearum* isolates with NIV chemotype were detected in rye samples. In Canada, NIV-producing *F. graminearum* isolates have not yet been reported (Gilbert and Haber, 2013; Amarasinghe et al., 2015).

Different *Fusarium* species could produce different mycotoxins, including the most important ones: trichothecenes, zearalenone, moniliformin, and enniatin (Yli-Mattila et al., 2010). *Fusarium graminearum* typically produces type B trichothecenes, while *F. poae* and *F. sporotrichioides* are known to accumulate type A trichothecenes (Geraldo et al., 2006; Krska et al., 2001; Liddell, 2003). However, *F. avenaceum* has been reported to produce moniliformin, enniatin analogs, and beauvericin (Beccari et al., 2018). The species composition of FHB pathogens plays an important role in the mycotoxin contamination of crops (Valverde-Bogantes et al., 2020). Deoxynivalenol could be the main mycotoxin contaminant in rye, as *F. graminearum* is the principal pathogen of the crop in Manitoba. Also, the chemotype shift in the *F. graminearum* population influences the mycotoxin contamination of crops and aggressiveness of isolates (Ward et al., 2008). It is important to continue monitoring species associated with FHB to understand current mycotoxin contamination potential within the rye grain supply chain.

Chapter 4

Comparative pathogenicity of four *Fusarium* species causing fusarium head blight on rye

4.1 Abstract

Fusarium head blight (FHB) is a significant disease of small grain cereals in Canada. There is little information about how fall rye reacts to different *Fusarium* species. Six fall rye genotypes and two winter wheat checks were inoculated with distilled water and four *Fusarium* species, *Fusarium graminearum, F. avenaceum, F. sporotrichioides*, and *F. poae*, collected from naturally infected fall rye fields. They were spray inoculated at 30%, 50%, and 70% anthesis with a mixture of two single spore isolates for each species in a greenhouse. Symptoms of FHB were rated as the proportion of infected spikelets within a spike 14, 18, 23, and 27 days after the first inoculation. Disease incidence was calculated based on the number of infected spikes among inoculated spikes per plant, and disease severity was assessed as mean proportion of infected spikelets among inoculated spikes per plant 18 days after the first inoculation. The experiment was repeated four times. Two plants for each treatment from the first two runs were randomly selected for fungal biomass analysis. Fungal biomass was quantified in two of four spikes collected 18 days after the first inoculation using real-time quantitative PCR. *Fusarium graminearum* caused significantly higher disease incidence, disease severity, and fungal biomass than the other three *Fusarium* species. Therefore, *F. graminearum* was the most pathogenic species causing FHB on rye, followed by *F. avenaceum*. *Fusarium sporotrichioides* and *F. poae* were considered weakly pathogenic. Moreover, *F. graminearum* infected and spread quickly in the spikes of the most susceptible genotypes. Almost no disease spread in the spikes of all genotypes inoculated with *F. poae*. For the resistant genotypes, *F. graminearum* caused low disease severity and fungal biomass, and these genotypes showed very low levels of FHB disease from the other *Fusarium* species. Fungal biomass was significantly correlated with mean proportion of infected spikelets inoculated with *F. graminearum* and *F. avenaceum*, but not with the other two species.

4.2 Introduction

Fusarium head blight (FHB) is a destructive disease affecting the spikes of small grain cereals, such as wheat, barley, oat, and rye (Parry et al., 1995; McMullen et al., 1997; Gilbert and

Tekauz, 2000). The disease reduces grain yield and quality by damaging kernel development (McMullen et al., 1997, 2012). Under epidemic conditions, the disease may cause yield losses of up to 70% in some fields (Parry et al., 1995). Fusarium head blight led to a cumulative economic loss of two billion USD loss between 1993 and 2001 in the United States and 520 million CAD in Canada in the 1990s (McMullen et al., 2012; Xia et al., 2020). Additionally, many *Fusarium* species have the potential to produce mycotoxins such as deoxynivalenol and zearalenone in grain (Parry et al., 1995). Mycotoxin contaminated grains pose a health concern if they are used for animal feed or human food (Snijders, 1990; Parry et al., 1995; Abramson, 1998; Placinta et al., 1999).

Several *Fusarium* species, such as *Fusarium graminearum*, *F. culmorum*, *F. sporotrichioides*, *F. avenaceum*, *F. poae*, and *F. equiseti*, have been commonly isolated from FHB infected wheat kernels (Duthie et al., 1986, Wong et al., 1992; Gilbert et al., 2001; Xue et al., 2002). Geography and climate influence *Fusarium* species frequencies (van Eeuwijk et al., 1995). *Fusarium graminearum* is the most prevalent *Fusarium* species causing FHB on wheat in Canada (Sutton, 1982; Wong et al., 1992; Clear and Patrick, 2000; Gilbert and Tekauz, 2000; Fernandez et al., 2001). In western Canada, *F. avenaceum* and *F. graminearum* were the most frequently isolated species from rye samples collected in 2010 (Grafenhan et al., 2013). Fusarium head blight of rye is mainly caused by *F. graminearum* and *F. culmorum* in Europe (Miedaner et al., 1993). *Fusarium graminearum* and *F. culmorum* were more pathogenic and aggressive than other *Fusarium* species on wheat (Stack and McMullen, 1985; Wilcoxson et al., 1988; Wong et al., 1992, 1995; Stack et al., 1997).

Various *Fusarium* species produce mycotoxins in grain (Snijders, 1990). The most significant classes of *Fusarium* mycotoxins that adversely effect human and animal health include trichothecenes, fumonisins, moniliformin (MON), and zearalenone (ZEA) (D'Mello et al., 1999). *Fusarium graminearum* predominately produces type B trichothecenes, including deoxynivalenol (DON), 3-acetyledeoxynivalenol (3-ADON), 15-acetyledeoxynivalenol (15-ADON), and nivalenol (NIV) (Pirgozliev et al., 2003; Popovski and Celar, 2013). *Fusarium sporotrichioides* is a producers of type A trichothecenes, including T-2 toxin, HT-2 toxin, neosolaniol, and diacetoxyscirpenol, while *F. poae* produces primarily NIV (Thrane et al., 2004; Popovski and Celar, 2013). *Fusarium avenaceum* produces the less well-known MON (Liddell, 2003; Yli-

Mattila et al., 2006; Grafenhan et al., 2013). Deoxynivalenol plays an important role in the aggressiveness of *F. graminearum* and *F. culmorum* isolates (Gang et al., 1998; Mesterhazy, 2002). However, *F. graminearum* isolates that were unable to produce DON and 3-ADON in vitro still had the ability to cause disease on wheat, rye, and triticale seedlings (Manka et al., 1985). Accordingly, DON is not necessary for pathogenicity of *F. graminearum* (Manka et la., 1985; Alexander et al., 1997).

Resistance to FHB has been organized into five distinct categories: resistance to the initial infection (type I), resistance to disease spread within a spike (type II), resistance to mycotoxin accumulation in grain (type III), resistance to kernel damage (type IV), and tolerance (type V) (Schroeder and Christensen, 1963; Miller et al., 1985; Mesterhazy, 1995; Mesterhazy et al., 1999). Type I resistance can be measured as disease incidence through spray inoculation, while type II resistance can be assessed as disease severity through point inoculation within a spike under controlled environments (Mesterhazy, 1995; Gilbert and Tekauz, 2000). Resistance to FHB caused by *F. graminearum* and *F. culmorum* is highly correlated on both wheat and rye (Miedaner et al., 1993; Mesterhazy et al., 1999). In winter wheat, resistance to FHB caused by *F. graminearum*, *F. culmorum*, and *F. nivale* is species nonspecific (van Eeuwijk et al., 1995). Nevertheless, some host preferences were observed among *Fusarium* species (Arseniuk et al., 1993).

Disease levels can be quantified through several parameters in studies of host resistance and fungal pathogenicity (Nicholson et al., 2003). Some studies used ergosterol content to determine fungal colonization of *F. graminearum* and *F. culmorum* in fall rye (Miedaner and Perkowski, 1996; Gang et al., 1998; Miedaner et al., 2000). However, the ergosterol assay estimates the total amount of all fungi present, but not the amount of a specific species (Nicholson et al., 2003). Real-time quantitative PCR (qPCR) enables sensitive and reliable detection and quantification of individual *Fusarium* species in infected grain or plant tissues (Nicholson et al., 2003). Most qPCR assays are designed for one, or a few, *Fusarium* species targeting regions with limited sequence variability (Niessen, 2008). The specificity of qPCR assays is improved by targeting unique genes such as toxin genes, but toxin genes are only presented in some *Fusarium* species (Nicolaisen, 2009). The translation elongation factor 1α (EF1α) is well characterized for many *Fusarium* species and presents sufficient sequence variability to distinguish between species (Kristensen et al., 2005).

Some studies evaluated the aggressiveness of some isolates of *F. graminearum* or *F. culmorum* in fall rye (Gang et al., 1998; Miedaner et al., 2000). However, little is known about how fall rye reacts to different *Fusarium* species and the effect of fall rye genotype on the pathogenicity of the pathogens. The objectives of this study were to:

1) compare the pathogenicity of the four *Fusarium* species, *F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, and *F. poae*, frequently isolated on fall rye in Manitoba
2) compare the disease severity of six fall rye genotypes and two winter wheat genotypes inoculated with four *Fusarium* species
3) determine the fungal biomass produced by the four *Fusarium* species in infected rye spikes using qPCR assay based on EF1α
4) determine correlations between disease severity and fungal biomass in infected spikes.

4.3 Materials and methods

4.3.1 Rye genotypes

Six fall rye genotypes, including KWS-H10110 (Resistant (R)), KWS-H161 (R), Amilo (Intermediate (I)), Pearl (I), Vitallo (I), and Dakota (Moderate susceptible (MS)), were used. They represented different levels of FHB resistance in rye based on field evaluations in Chapter 5. Winter wheat genotypes 32C*17 (R) and Caledonia (Susceptible (S)) were used as the resistant and susceptible checks.

4.3.2 Fungal inoculum

Eight isolates identified from work reported in Chapter 3 were selected to be used in this study, based on the rate of mycelia growth on a potato dextrose agar (PDA) medium (**Appendix 1**) and identification through sequencing, done by Debbie Miranda from Dr. Maria Antonia Henriquez's group at the Morden Research and Development Centre, Agriculture and Agri-Food Canada (AAFC) (**Appendix 2**). Sequences were read through the software DNASTAR (DNASTAR Inc., USA) and analyzed using the Basic Local Alignment Search Tool (BLAST). Each of the four *Fusarium* species included two single spore isolates: *F. graminearum* (FR-16-03-

2-A and 114-4-A), *F. avenaceum* (FR-16-13-7 and 215-5-A), *F. sporotrichioides* (112-4-A and 302-5-C), and *F. poae* (FR-16-11-2-A and 102-4-A).

To prepare the inoculum, a single spore culture on a PDA medium was transferred onto a spezieller nährstoffarmer agar (SNA) medium (**Appendix 1**). Plates were placed under fluorescent light at room temperature (21 to 24℃) for one week. Then about ten plates of SNA media were divided into sections and added into the flask with aerated liquid carboxy methyl cellulose (CMC) media (**Appendix 1**). Seven days after incubation at room temperature under fluorescent light, the conidiospore suspension was collected from the CMC medium by filtering through two layers of sterile cheesecloth. The number of conidiospores per milliliter was determined using a hemocytometer (Hausser Scientific, USA). Equal concentrations of two isolates for the same *Fusarium* species were combined and adjusted to 5×10^5 spores/mL with distilled water for inoculation. The surfactant Tween™ 20 (VWR International LLC., USA) was added at a volume of 2 mL per liter of inoculum.

4.3.3 Greenhouse experiment

The experiment was repeated four times over two years in 2018 and 2019 in consideration of labour capability. Each time was termed as a run. There were five plants per genotype for each treatment. Plants were grown in root trainers (one seed per one 14 × 3.5 × 2.5 cm cell) with Sunshine™ soil mix #4 (Sungro™ Horticulture Ltd., USA) and placed in a growth room at 23 ℃ for 16 h with light and 18 ℃ for 8 h in dark. Two weeks after seeding when plants were at the three leaf stage, they were transferred into a vernalization room at 4℃ for 16 h with light and 8 h in dark. After nine weeks vernalization, plants were transferred into one-gallon pots (one plant per pot) and maintained in the greenhouse of the Crop Technology Centre, University of Manitoba, Winnipeg. Supplemental light was set for 16 h daylight, and the temperature ranged from 18 to 25 ℃. Controlled-release fertilizer (13-12-12) (Master Plant-Prod Inc., Canada) was applied once following transplanting at a rate of 20 g per pot.

The first four spikes from each plant were inoculated three times at 30%, 50%, and 70% anthesis by using a spray bottle. The inoculum was sprayed on the spike until it ran down the awns and florets. Plants were transferred into a humidity chamber for 12 h after each inoculation and

returned to the greenhouse bench. The humidity chamber was maintained at or near 100% relative humidity by the continuous operation of two ultrasonic humidifiers (Sunbeam, USA). Symptoms of FHB were rated as the percentage of infected spikelets per spike. Infected spikelets of one spike per plant was assessed 14, 18, 23, and 27 days after the first inoculation to determine the optimum timing of disease evaluation. As disease progression was much faster and reached a maximum much earlier, infected spikelets of the spike by *F. graminearum* were only assessed 14 and 18 days after the first inoculation (**Figure 4.7**). The other three spikes were assessed and collected 18 days after the first inoculation, and stored at -20 ℃. Disease incidence was calculated as the percentage of infected spikes among inoculated spikes per plant, and disease severity of a plant was recorded as mean proportion of infected spikelets among inoculated spikes (Stack and McMullen, 1998). Plants sprayed with distilled water served as checks to detect any drift of inoculum in the greenhouse.

4.3.4 DNA extraction

Two spikes from each of two plants per genotype for each treatment in the first two repeated trials were used for DNA extraction. According to the method described by Henriquez et al. (2020), the spike was freeze-dried and ground in liquid nitrogen with a mortar and pestle. One hundred milligrams of ground spike samples were mixed with 1000 μL TES extraction buffer (4 M NaCl, 1 M Tris pH 7.5, and 0.5 M EDTA pH 8.0) in a 1.5 mL microcentrifuge tube. Then 100 μL 1% SDS and 2 μL proteinase K (10 mg/mL stock concentration) were added to the tube. The mixture was vortexed and incubated in an Isotemp™ digital dry bath (Fisher Scientific, USA) at 65°C for 60 min. After incubation, 350 μL of 7.5 M ammonium acetate was added. The mixture was vortexed, incubated at room temperature for 7 min, and centrifuged for 10 min at 12000 rpm. The supernatant (800 μL) was transferred into a new tube. The supernatant was added with 300 μL of Chloroform: Isoamyl alcohol (24:1) and then vortexed and centrifuged for 10 min at 12000 rpm. The top layer was transferred into a new tube, and the previous step was repeated. DNA was precipitated by adding isopropanol up to the 1.5 mL mark, followed by vortex and incubation at -20°C for 2 hours at least. The DNA pellet was obtained by centrifuging for 10 min at 12000 rpm after incubation and washed using 800 μL of cold 70% ethanol, followed by vortex and centrifuging at 12000 rpm for 5 min. After air-drying for a minimum of 15 min, the pellet was

suspended in 100 µL TE buffer (10 mM Tris-HCL pH 7.5 and 1 mM EDTA) and treated with 5 µL of RNase A (1 mg/mL initial concentration). The DNA was incubated at 37°C for a minimum of 45 min and stored at -20°C. The DNA concentration was measured using a NanoDrop™ 2000 spectrophotometers (Thermo Fisher Scientific Inc., USA).

Fungal DNA from isolates FR-16-03-2-A, 114-4-A, FR-16-13-7, 215-5-A, 112-4-A, 302-5-C, FR-16-11-2-A, and 102-4-A were utilized to generate a standard curve to quantify the fungal DNA in a spike. Mycelia of each isolate were harvested from a fresh culture on PDA and macerated with 1000 µL TES extraction buffer (4 M NaCl, 1 M Tris pH 7.5, and 0.5 M EDTA pH 8.0). They were broken into small pieces in a 1.5 mL microcentrifuge tube using a sterile pellet pestle, and followed the DNA extraction protocol described above.

4.3.5 Real-time quantitative PCR

Fusarium species-specific primers were used to quantity fungal DNA in infected spike samples based on the EF1α gene (Nicolaisen et al., 2009) (**Table 4.1**). The qPCR mixture consisted of 5 µL Applied Biosystems™ PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific Inc., USA), 0.5 µL of forward primer (10 µM), 0.5 µL of reverse primer (10 µM), and 4 µL template DNA (25 ng/µL). The qPCR was performed in a CFX96™ Real-Time System PCR machine (Bio-Rad Laboratories Ltd., USA) using the following thermal cycling protocol: 2 min at 50 ℃; 95 ℃ 2 min; 40 cycles of 95 ℃ for 15 s, and 60 ℃ for 1 min followed by dissociation curve analysis at 60 to 95 ℃ (Nicolaisen et al., 2009).

Table 4.1 Sequences of *Fusarium* species-specific primers used to determine fungal biomass in two spikes from each of two plants per genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Pearl, Vitallo, 32C*17, and Caledonia) for each treatment (*F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and distilled water) collected 18 days after the first inoculation in the first two repeated trials

Fusarium species	Primer	Sequence (5'-3')
F. graminearum	FgramB379 fwd	CCATTCCCTGGGCGCT
	FgramB411 rev	CCTATTGACAGGTGGTTAGTGACTGG
F. avenaceum	Fave574 fwd	TATGTTGTCACTGTCTCACACCACC
	Fave627 rev	AGAGGGATGTTAGCATGATGAAG
F. sporotrichioides	FspoA18 fwd	GCAAGTCGACCACTGTGAGTACA
	FspoA85 rev	CTGTCAAAGCATGTCAGTAAAAATGAT
F. poae	FpoaeA51 fwd	ACCGAATCTCAACTCCGCTTT
	FpoaeA98 rev	GTCTGTCAAGCATGTTAGCACAAGT

4.3.6 Quantification of *Fusarium* DNA in rye spikes

A standard curve for each *Fusarium* species was prepared using a ten-fold dilution series from 100 ng/µL to 1 pg/µL of the mixture of DNA from two single spore isolates per *Fusarium* species. The amount of fungal DNA in rye spike samples was calculated from cycle threshold (Ct) using the standard curve run in parallel reactions in three replicates. The *Fusarium* biomass was evaluated in terms of picograms of fungal DNA per nanograms of rye-*Fusarium* DNA. Additionally, DNA of spike samples sprayed with distilled water was randomly divided into four groups to serve as checks for each *Fusarium* species assay.

4.3.7 Data analysis

Statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., USA). Plant means were calculated from individual spike scores and used in all subsequent analyses. Disease severity data were transformed by using square root transformation. The data of each run and combined runs were evaluated using PROC UNIVARIATE. The shapiro-wilk values were over 0.9, and, therefore, they were close to a normal distribution. The distribution of the residuals was symmetrical, which suggested that data of four runs could be pooled. The analysis

of variance (ANOVA) was conducted using the PROC MIXED procedure. The effects of treatment, genotype, and their interaction served as fixed effects. The effects of run and plant were assumed to be random variables. The least significant difference (LSD, p=0.05) was used for comparisons of means for main effects and their interaction for disease incidence, disease severity, and fungal biomass at a 5% probability. Correlation coefficients between infected spikelets and fungal biomass were accessed using the PROC CORR procedure.

4.4 Results

4.4.1 Pathogenicity of *Fusarium* species

The effects of treatment, genotype, and their interaction were significant for disease incidence and severity, assessed 18 days after the first inoculation (**Table 4.2**). There was also a significant run × genotype interaction for disease incidence.

Significant differences were observed in disease incidence and severity among the four *Fusarium* species and the distilled water treatment (**Figure 4.1** and **Figure 4.2**). *Fusarium graminearum* produced significantly higher mean disease incidence (93.75%) and severity (74.61%) averaged across all genotypes than the other *Fusarium* species and thus, was considered the most highly pathogenic among the four *Fusarium* species. *Fusarium avenaceum* resulted in 83.28% disease incidence and 27.50% disease severity and was moderately pathogenic. *Fusarium sporotrichioides* had significantly higher disease incidence (52.19%) than *F. poae* (21.25%). The difference of disease severity between *F. sporotrichioides* (9.69%) and *F. poae* (4.91%) was also significant. They were considered to be weakly pathogenic.

Table 4.2 Combined analyses of variance for disease incidence and disease severity of four runs of greenhouse experiments conducted on six fall rye genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Pearl, and Vitallo) and two wheat genotypes (32C*17 and Caledonia) assessed 18 days after the first inoculation with *F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and distilled water

Source	DF	Disease incidence (%)		Disease severity (%)	
		Mean Square	Pr > F	Mean Square	Pr > F
Trt	4	240669.00	<.0001	1743.94	<.0001
Geno	7	8292.75	0.0005	60.96	<.0001
Trt*Geno	28	1782.98	<.0001	9.65	<.0001
Run	3	1150.78	0.4495	8.18	0.1552
Run*Trt	12	544.66	0.3117	2.90	0.1628
Run*Geno	21	1159.71	0.0168	3.09	0.2367
Run*Trt*Geno	84	462.07	0.4792	2.01	0.0927
Plant(Geno)	32	354.69	0.8332	1.92	0.7251
Run*Plant(Geno)	96	550.52	0.1246	2.06	0.0614
Trt*Plant(Geno)	128	401.07	0.8228	1.90	0.1283

Trt=treatment; Geno=genotype; DF=degree of freedom.
Note: the experiment was repeated four times, and each time was termed as one run.

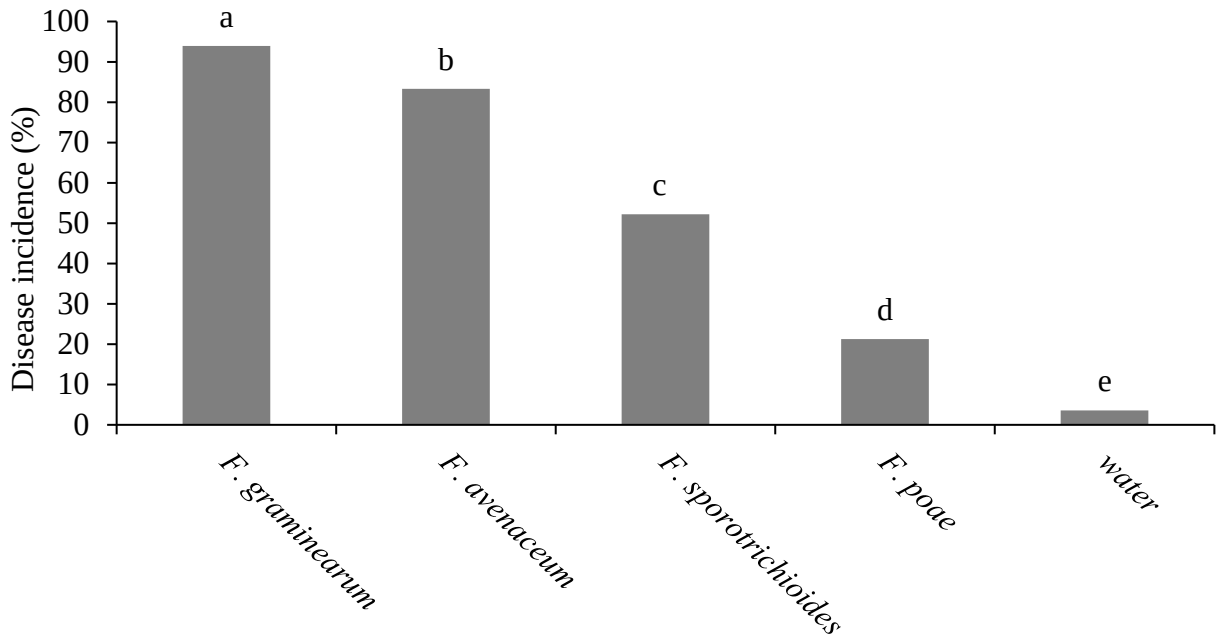

Figure 4.1 Mean disease incidence (%) of five treatments (*F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and distilled water) across six fall rye genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Pearl, and Vitallo) and two wheat genotypes (32C*17 and Caledonia) assessed 18 days after the first inoculation. Treatments with the same letter above columns are not significantly different at p=0.05 (LSD).

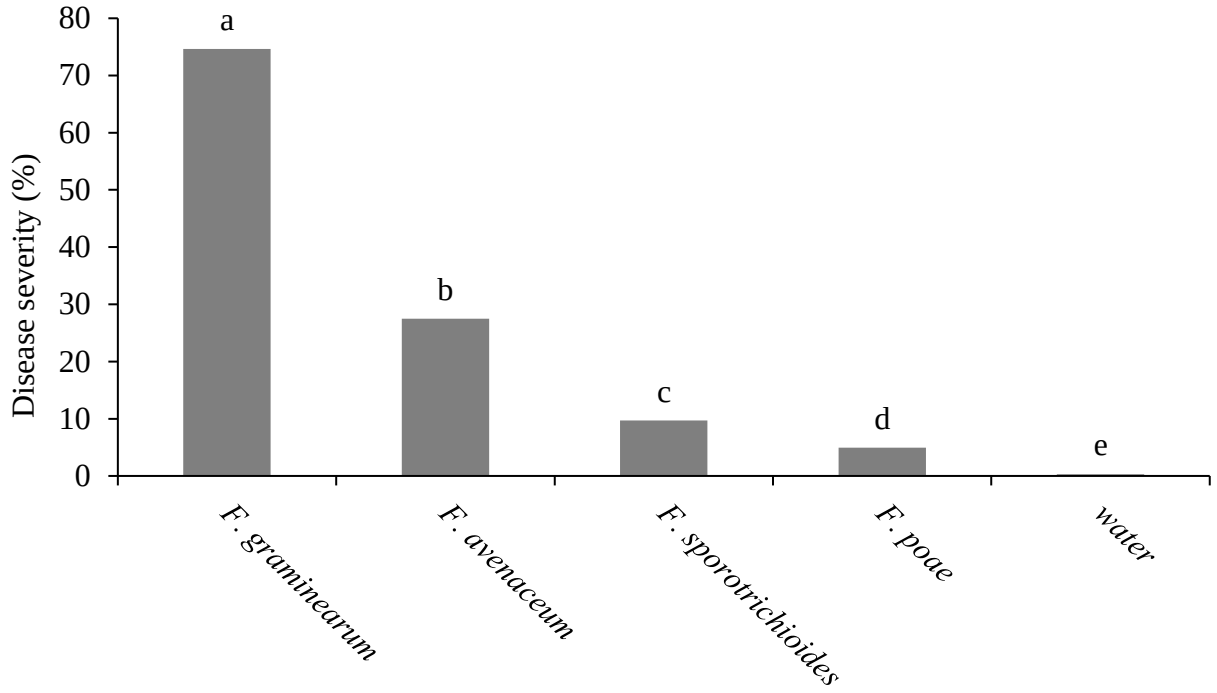

Figure 4.2 Mean disease severity (%) of five treatments (*F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and distilled water) across six fall rye genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Pearl, and Vitallo) and two wheat genotypes (32C*17 and Caledonia) assessed 18 days after the first inoculation. Treatments with the same letter above columns are not significantly different at p=0.05 (LSD).

The susceptible wheat check, Caledonia, had significantly higher mean disease incidence (69%) and severity (34.83%) than other genotypes tested (**Figure 4.3** and **Figure 4.4**). Two hybrids, KWS-H161 (41.25%) and KWS-H10110 (41.25%) had significantly lower disease incidence than Dakota (52.25%), Pearl (56.25%), and Vitallo (52.5%), were not significantly different from Amilo (45%) and 32C*17 (49%) (**Figure 4.3**). KWS-H161 (13.42%) and KWS-H10110 (15.73%), had significantly lower disease severity than the four open-pollinated rye genotypes, Amilo (23.31%), Dakota (26.26%), Pearl (28.93%), and Vitallo (25.16%), and the two wheat checks, 32C*17 (19.57%) and Caledonia (34.83%) (**Figure 4.4**).

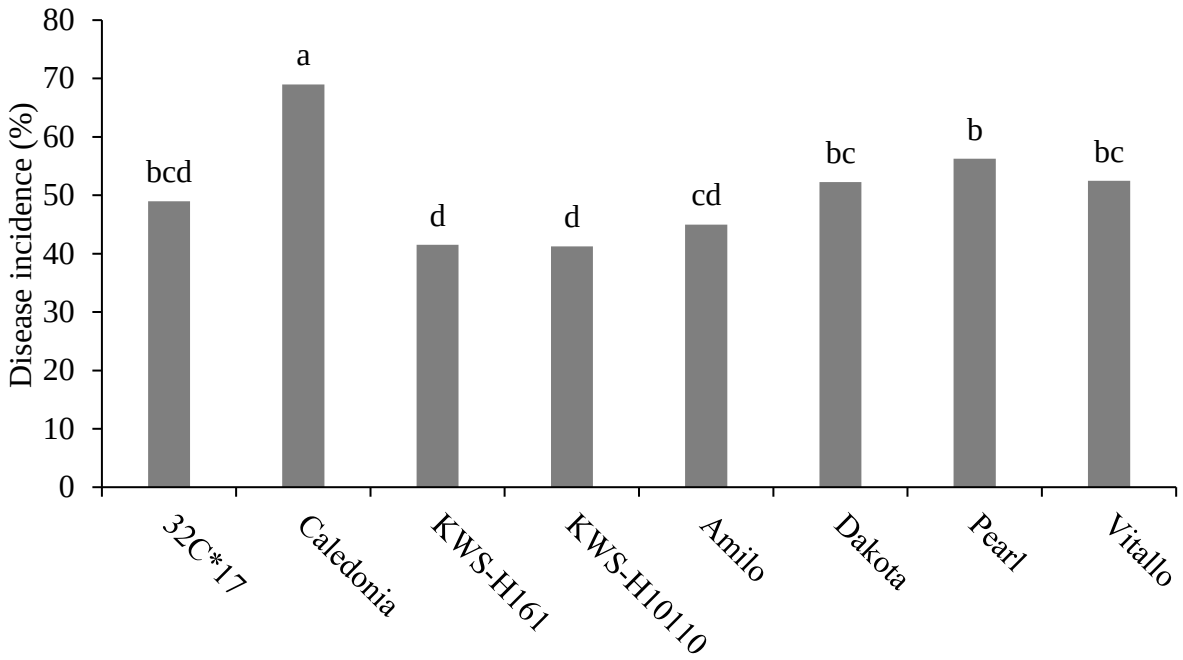

Figure 4.3 Mean disease incidence (%) of six fall rye genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Pearl, and Vitallo) and two wheat genotypes (32C*17 and Caledonia) across five treatments (*F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and distilled water) assessed 18 days after the first inoculation. Genotypes with the same letter above columns are not significantly different at p=0.05 (LSD).

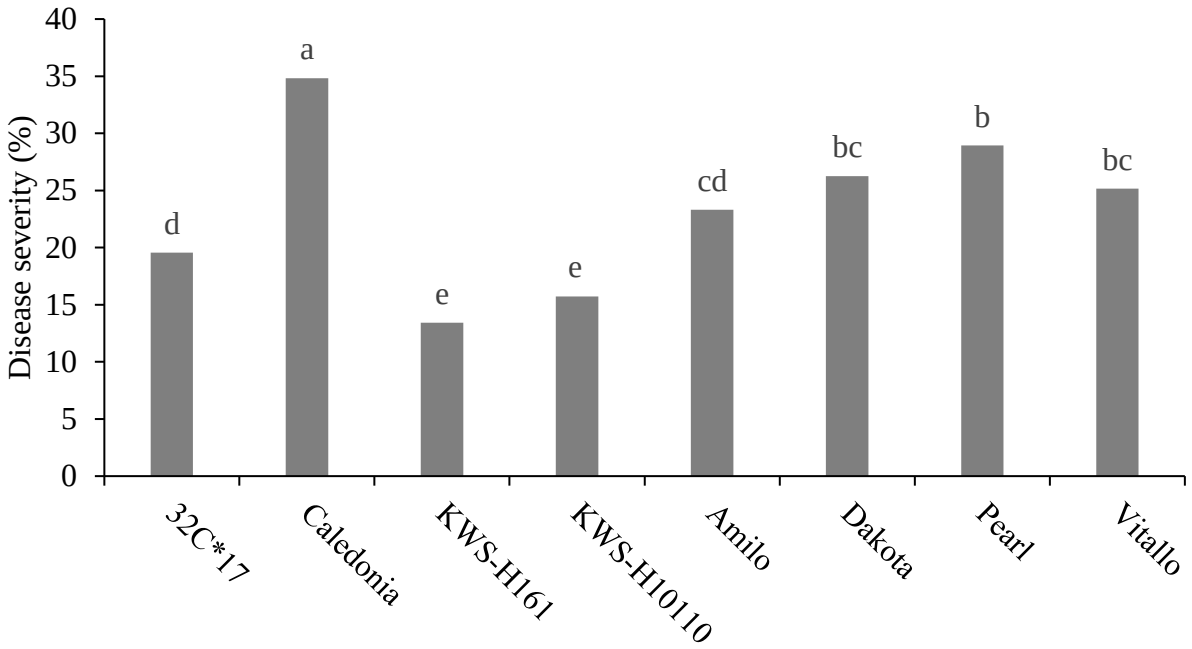

Figure 4.4 Mean disease severity (%) of six fall rye genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Pearl, and Vitallo) and two wheat genotypes (32C*17 and Caledonia) across five treatments (*F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and distilled water) assessed 18 days after the first inoculation. Genotypes with the same letter above columns are not significantly different at p=0.05 (LSD).

Fall rye and winter wheat genotypes differed in disease incidence for *F. avenaceum*, *F. sporotrichioides*, *F. poae*, but not *F. graminearum* (**Figure 4.5**). All genotypes inoculated with *F. graminearum* had disease incidence over 85%. However, they differed in disease severity for *F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, and *F. poae* (**Figure 4.6**). The ranking of disease severity of the six fall rye and two wheat genotypes in reaction to *F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, and *F. poae* were similar. The resistant wheat genotype 32C*17 had disease severity of 5.74% (*F. poae*) to 56.11% (*F. graminearum*), while the susceptible wheat check Caledonia had disease severity ranging from 11.31% (*F. poae*) to 84.87% (*F. graminearum*). Fusarium head blight spread quickly on spikes of the susceptible and moderately susceptible genotypes when inoculated with *F. graminearum*. The mean disease severity of the susceptible wheat check, Caledonia, and the intermediate to moderately susceptible fall rye genotypes Amilo,

Dakota, Pearl, and Vitallo were over 80% when inoculated with *F. graminearum*. The resistant wheat check, 32C*17, and the resistant fall rye genotypes KWS-H161 and KWS-H10110 also had moderately high mean disease severity of over 50% when inoculated with *F. graminearum*. When inoculated with *F. avenaceum*, KWS-H161 and KWS-H10110 had significantly lower disease incidence and severity than the four open-pollinated genotypes and the susceptible wheat check (**Figure 4.5** and **Figure 4.6**).

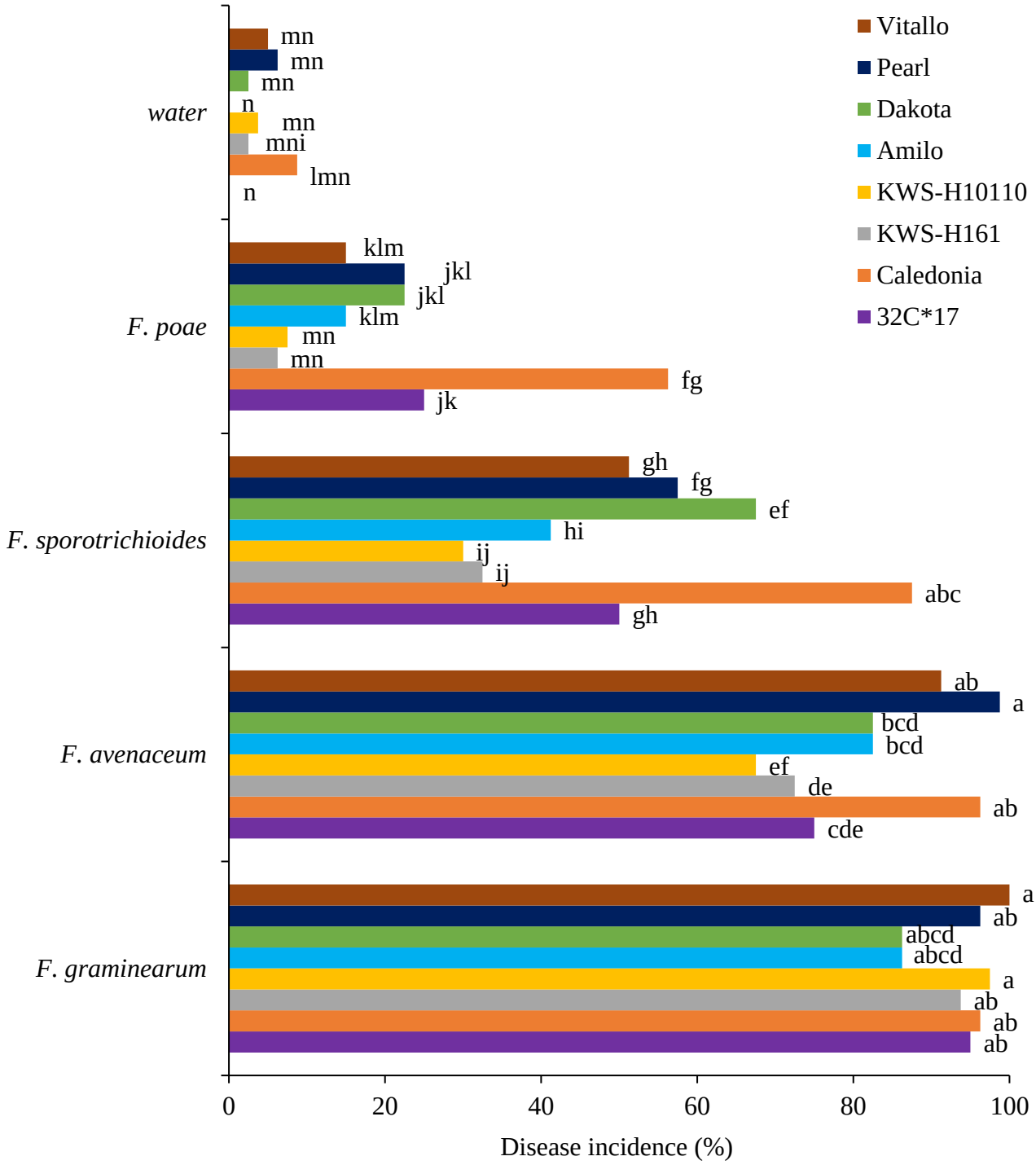

Figure 4.5 Mean disease incidence (%) of six fall rye genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Vitallo, and Pearl) and two wheat genotypes (32C*17 and Caledonia) assessed 18 days after the first inoculation with *F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and distilled water. Genotype × treatment means with the same letter next to the columns are not significantly different at p=0.05 (LSD).

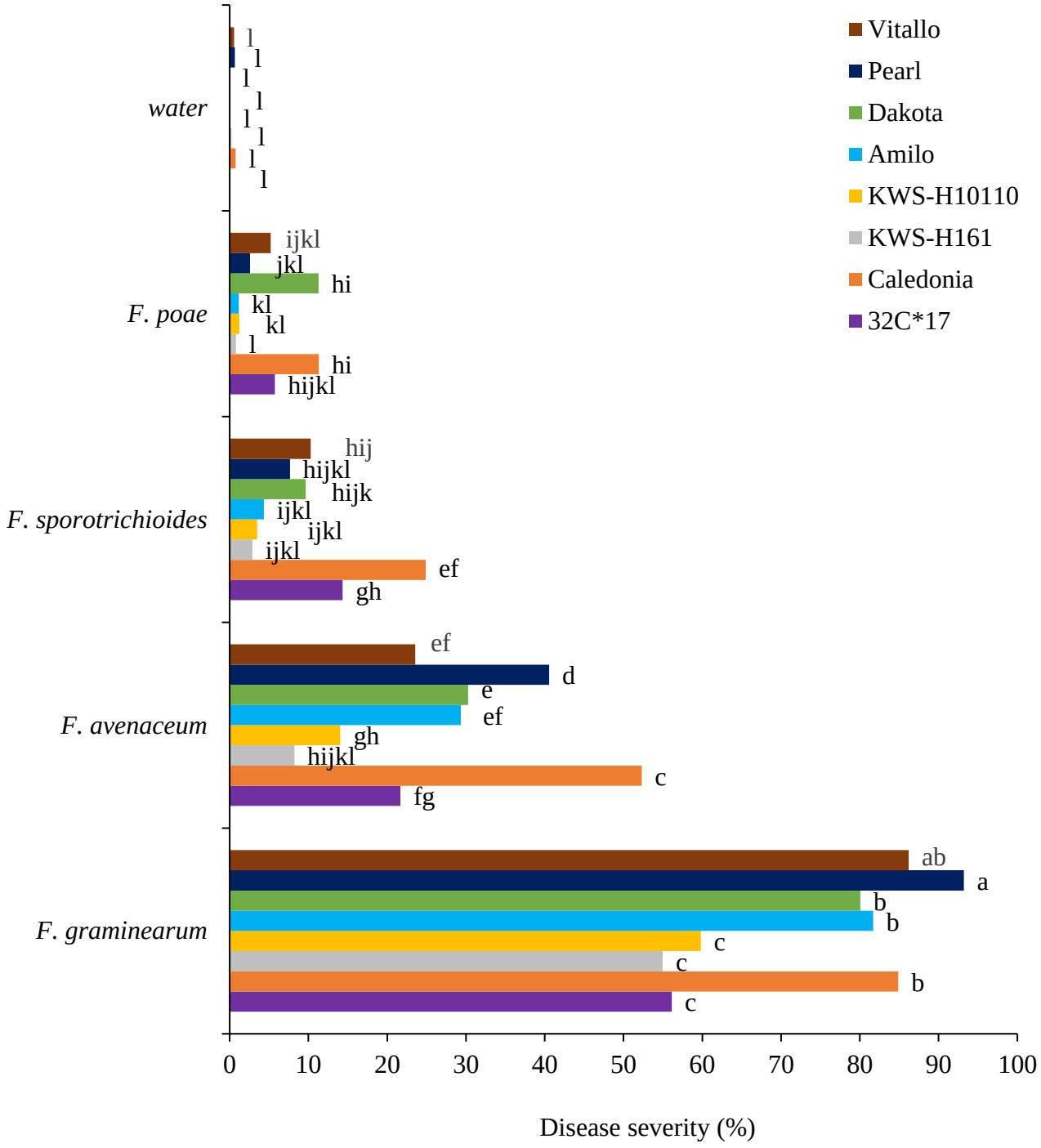

Figure 4.6 Mean disease severity (%) of six fall rye genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Vitallo, and Pearl) and two wheat genotypes (32C*17 and Caledonia) assessed 18 days after the first inoculation with *F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and distilled water. Genotype × treatment means with the same letter next to the columns are not significantly different at p=0.05 (LSD).

4.4.2 Disease progress of *Fusarium* species

The four *Fusarium* species differed in the rate of FHB symptom development on the six fall rye genotypes and two wheat genotypes (**Figure 4.7**). Disease caused by *F. graminearum* progressed much faster in the spikes than other three species. Except for resistant genotypes, spikes inoculated with *F. graminearum* were almost entirely bleached 18 days after the first inoculation. Therefore, disease severity of the spikes inoculated with *F. graminearum* was not recorded 23 or 27 days after first inoculation. Disease severity of the spikes inoculated with *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and distilled water was continually assessed until 27 days after the first inoculation, when plants were at maturity. As expected, there was almost no disease development on the spikes of all eight genotypes inoculated with distilled water. *Fusarium avenaceum* caused faster and more severe disease development on the susceptible genotypes than *F. sporotrichioides* and *F. poae*. The means of disease severity of the susceptible wheat check Caledonia and open-pollinated fall rye genotypes were over 50% in the last rating when inoculated with *F. avenaceum*. The mean disease severity of the susceptible wheat check Caledonia and open-pollinated fall rye genotypes inoculated with *F. sporotrichioides* was lower than 40% before ripening. At 27 days after the first inoculation of *F. poae*, only Caledonia had significant disease development with a mean disease severity close to 20%; other genotypes had almost no disease spread in spikes.

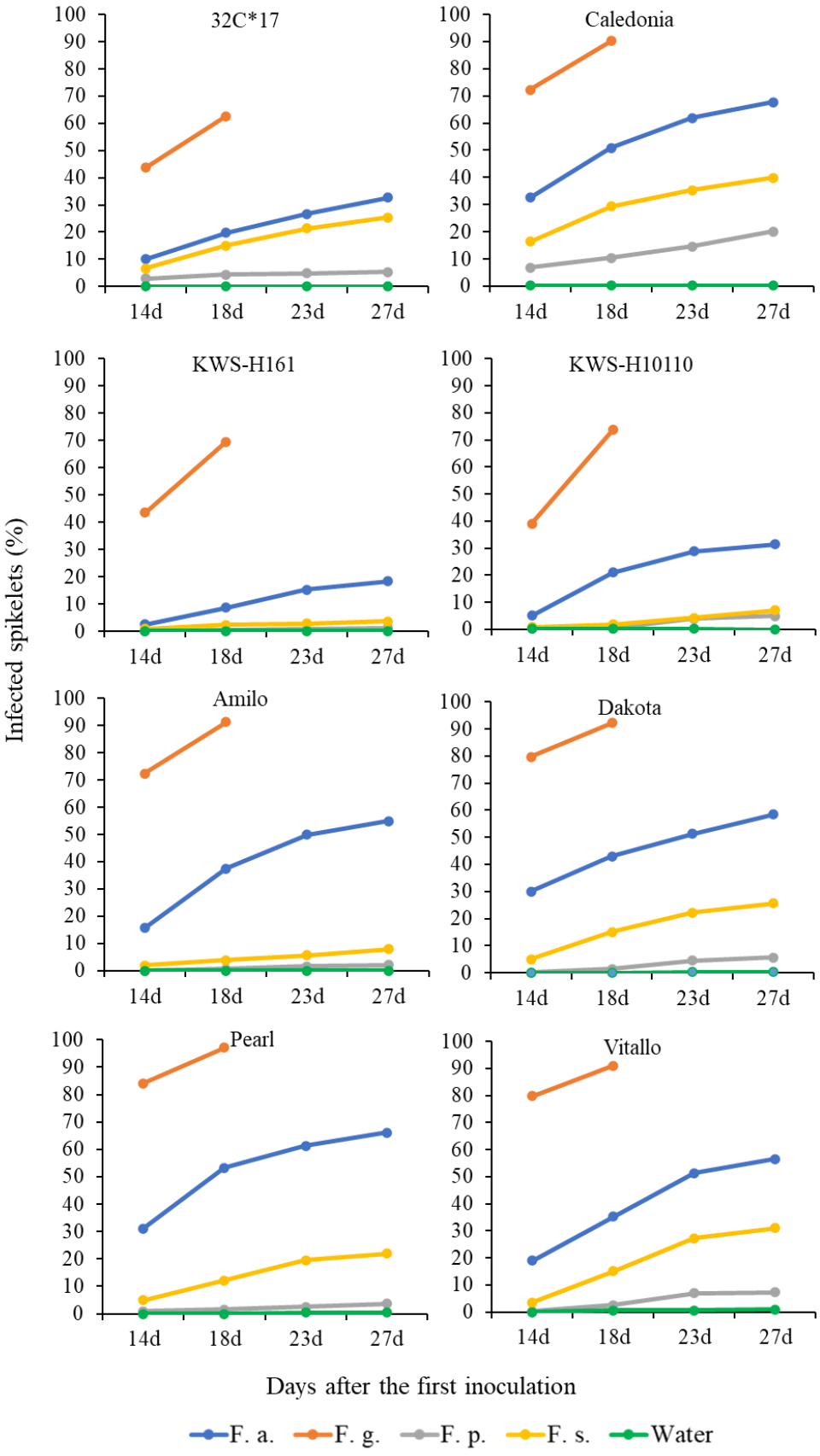
32C*17
Caledonia
KWS-H161
KWS-H10110
Amilo
Dakota
Pearl
Vitallo
Infected spikelets (%)
0
10
20
30
40
50
60
70
80
90
100
14d
18d
23d
27d
Days after the first inoculation
F. a.
F. g.
F. p.
F. s.
Water

Figure 4.7 Fusarium head blight disease progress curves for mean infected spikelets (%) of six fall rye genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Pearl, and Vitallo) and two wheat genotypes (32C*17 and Caledonia) assessed 14 and 18 days after the first inoculation with *F. graminearum* (F. g.), and 14, 18, 23, and 27 days after the first inoculation with *F. avenaceum* (F. a.), *F. sporotrichioides* (F. s.), *F. poae* (F. p.), and distilled water.

4.4.3 Quantification of *Fusarium* DNA in spikes

Analyses of variance of fungal biomass showed that the effect of treatment was significant, while genotype and the interaction between treatment and genotype had no significant effect on fungal biomass (**Table 4.3**). *Fusarium graminearum* resulted in significantly higher fungal biomass than the other three *Fusarium* species and distilled water (**Figure 4.8**). There was no significant difference in fungal biomass among the other three *Fusarium* species and distilled water. The amounts of *F. graminearum* DNA ranged from 41.63 (32C*17) to 202.83 (Caledonia) pg per ng plant DNA. The quantities of *F. avenaceum* DNA ranged from 4.52 (32C*17) to 49.42 (Pearl) pg per ng plant DNA. *Fusarium sporotrichioides* and *F. poae* were found in low amounts in all genotypes. The correlation between fungal biomass and disease severity by *F. graminearum* and *F. avenaceum* was significant, and the correlation coefficient was 0.70 and 0.64, respectively (**Figure 4.9**). Fungal biomass did not significantly associate with disease severity caused by *F. sporotrichioides* and *F. poae*.

Table 4.3 Combined analyses of variance for fungal biomass of two runs of greenhouse experiments conducted on six fall rye genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Pearl, and Vitallo) and two wheat genotypes (32C*17 and Caledonia) assessed 18 days after the first inoculation with *F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and distilled water

Source	DF	Mean Square	F Value	Pr > F
Trt	4	80101.00	42.45	0.001
Geno	7	3606.13	4.55	0.2632
Trt*Geno	28	2229.82	2.20	0.1027
Run	1	3424.00	2.09	0.2396
Run*Trt	4	1719.07	2.03	0.1173
Run*Geno	7	767.89	0.80	0.6261
Run*Trt*Geno	28	846.68	0.79	0.7354
Plant(Geno)	8	1205.07	0.89	0.5651
Run*Plant(Geno)	8	1179.94	1.10	0.3885
Trt*Plant(Geno)	32	1239.92	1.16	0.3417

Trt=treatment; Geno=genotype; DF=degree of freedom.
Note: the experiment was repeated four times, and each time was termed as one run. *Fusarium* DNA was quantified in the first two runs.

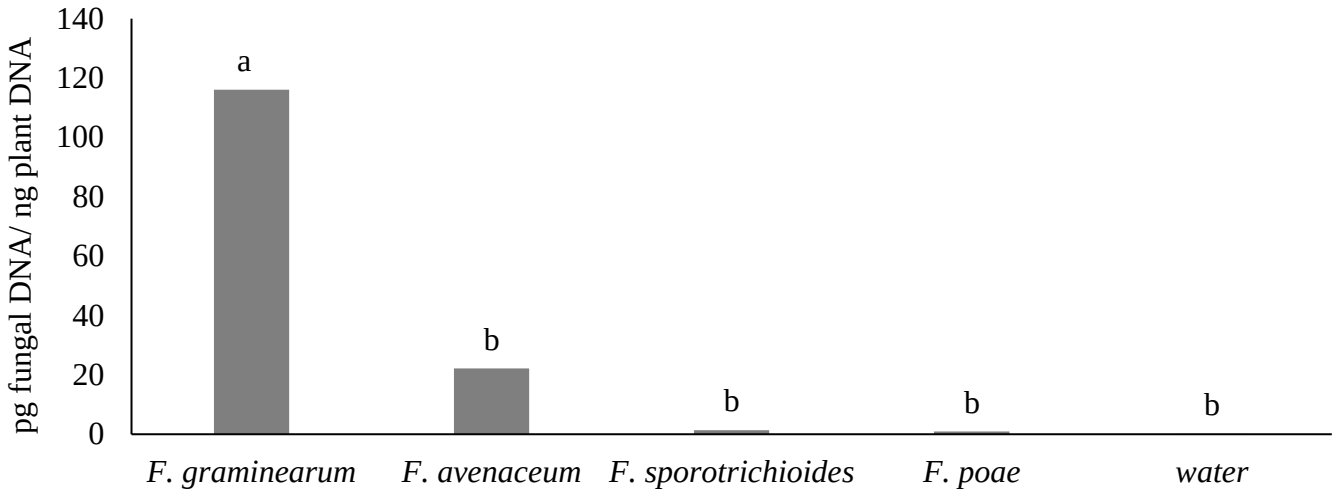

Figure 4.8 Mean fungal biomass of five treatments (*F. graminearum*, *F. avenaceum*, *F. sporotrichioides*, *F. poae*, and distilled water) across six fall rye genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Pearl, and Vitallo) and two wheat genotypes (32C*17 and Caledonia) assessed 18 days after the first inoculation. Treatments with the same letter above columns are not significantly different at p=0.05 (LSD).

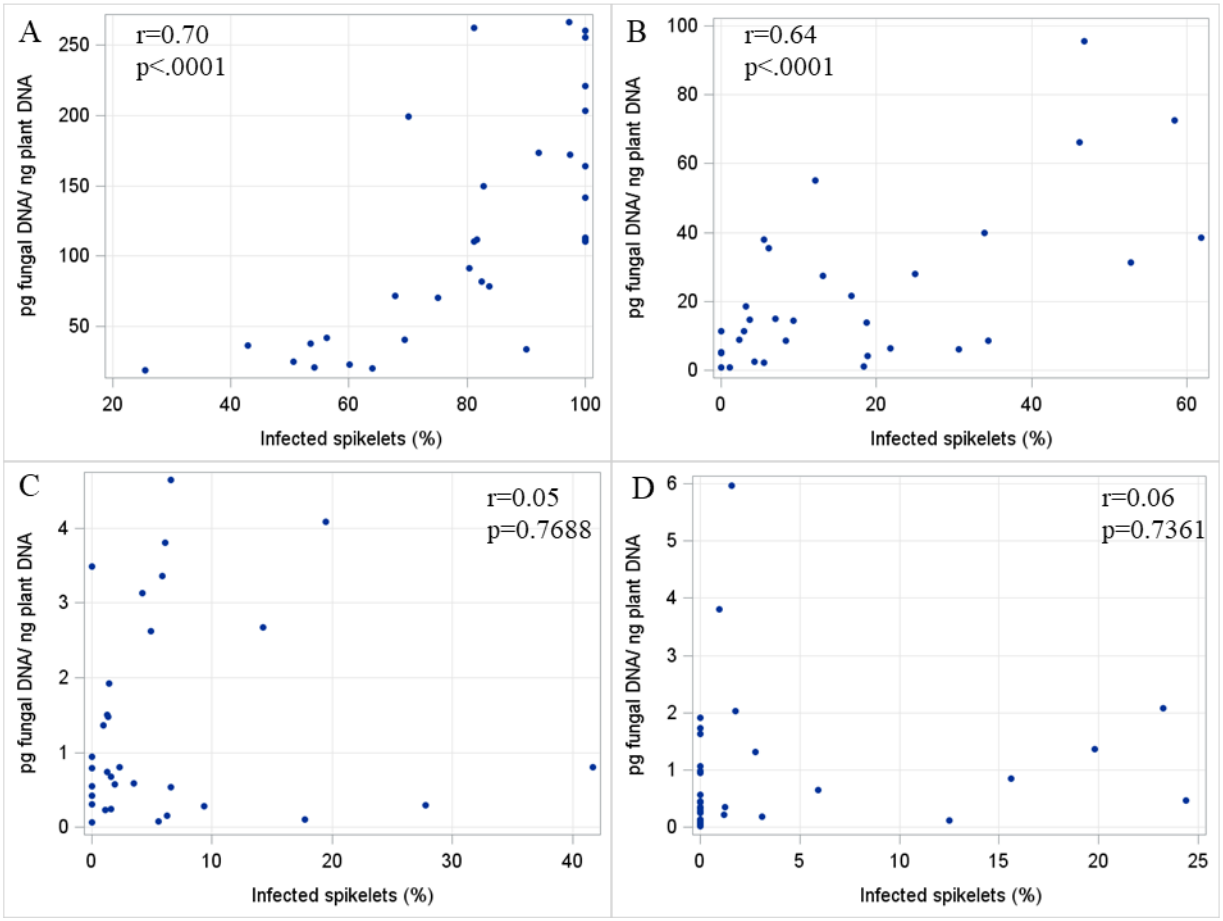

Figure 4.9 Scatter plot between infected spikelets (%) and fungal biomass (pg fungal DNA/ ng plant DNA) of six fall rye genotypes (KWS-H161, KWS-H10110, Amilo, Dakota, Pearl, and Vitallo) and two wheat genotypes (32C*17 and Caledonia) assessed 18 days after the first inoculation with (**A**) *F. graminearum*, (**B**) *F. avenaceum*, (**C**) *F. sporotrichioides*, and (**D**) *F. poae*. r=correlation coefficient, p=probability.

4.5 Discussion

As expected, *F. graminearum* was recognized to be highly pathogenic of FHB on rye. The results were consistent with variation in pathogenicity of *Fusarium* species on wheat (Stack and McMullen, 1985; Wong et al., 1995; Stack et al., 1997; Xue et al., 2004). Among the four *Fusarium* species, *F. avenaceum* was the second most pathogenic on rye in the experiment. However, on wheat, *F. sporotrichioides* had intermediate pathogenicity while *F. avenaceum* was weakly pathogenic in greenhouse and field tests with artificial spray inoculations (Wong et al.,

1995; Xue et al., 2004). This agrees with surveys conducted in western Canada, where *F. graminearum* was the predominant *Fusarium* species causing FHB in wheat followed by *F. sporotrichioides* (Gilbert and Tekauz, 2011). In rye, as described in Chapter 3, *F. avenaceum* was the second most frequently isolated species from Manitoba, after *F. graminearum.* The field survey agreed with the pathogenicity tests in the greenhouse.

Based on disease incidence, all four *Fusarium* species could cause infection in rye spikes. Compared to *F. graminearum* and *F. avenaceum*, almost no disease spread within spikes infected by *F. sporotrichioides* and *F. poae*. In rye, *F. graminearum* caused significantly higher disease incidence and disease severity than *F. avenaceum*, *F. sporotrichioides*, and *F. poae* under controlled conditions. In barley, *F. graminearum* was also more pathogenic than *F. avenaceum*, *F. sporotrichioides*, and *F. poae*, and it caused the greatest thousand kernel weight reduction and higher levels of seed infection in field trials with artificial inoculation (McCallum and Tekauz, 1999). *Fusarium graminearum* is the most common *Fusarium* species in North America (Gilbert and Tekauz, 2011). Rye growers need to be aware of FHB caused by *F. graminearum* due to its relative high pathogenicity in rye.

Overall, the two resistant hybrids had significantly lower disease incidence and disease severity than all open-pollinated rye genotypes, except Amilo for disease incidence. Although disease incidence of the resistant wheat check 32C*17 was not significantly different from all fall rye genotypes, it had a significantly lower disease severity than Dakota, Pearl, and Vitallo. The susceptible wheat check Caledonia had significantly higher disease incidence and disease severity than other genotypes. No significant difference of disease incidence was observed among all genotypes inoculated by *F. graminearum*. Differences in degree of type I resistance appear not to be detected under high disease pressure (Bai and Shaner, 2004). In general, the severity rankings of the six fall rye genotypes and two winter wheat genotypes were similar for all four *Fusarium* species. The resistant fall rye genotypes, KWS-H161 and KWS-H10110, and the resistant wheat check 32C*17 supported lower disease severity by *F. graminearum* and *F. avenaceum* than other genotypes, and almost none by the other two species. Scott and Benedikz (1986) reported that eight winter wheat cultivars had a similar order of resistance to *F. graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, and *F. nivale*. Similar results on resistant wheat cultivars suggested that genotypes displaying type II resistance to *F. graminearum* appear to be resistant to several other

Fusarium species (Stack et al., 1997). A similar reaction to *F. graminearum* and *F. culmorum* was observed in four winter wheat cultivars (Mesterhazy, 1987). Stack and McMullen (1985) also reported only minor differences between wheat cultivar reactions to *F. graminearum* and *F. culmorum*. An evaluation of a large number of spring wheat cultivars showed that cultivars resistant to FHB caused by *F. graminearum* were also resistant to *F. culmorum* (Snijders, 1993). Accordingly, breeding for resistance to *F. graminearum* may also increase resistance to other *Fusarium* species in wheat (Xue et al., 2004). In rye, the genetic basis of resistance to *F. graminearum* and *F. culmorum* was also the same (Miedaner et al., 1993). The similar ranks of genotypes in reaction to the four *Fusarium* species also indicated that resistance to *F. graminearum* may result in resistance to other *Fusarium* species. Therefore, the resistant rye genotypes identified by screening with *F. graminearum* could be used to manage FHB infection caused by other *Fusarium* species.

Fusarium graminearum infected and spread quickly in spikes of all genotypes and almost bleached out the whole spike of susceptible genotypes within 14 days after the first inoculation. The rapid disease spread caused by *F. graminearum* could cause kernel development failure and result in yield losses. Although FHB levels in Manitoba rye fields are very low as described in Chapter 3, the potential damage of FHB caused by *F. graminearum* in rye should be considered if environments are favorable for FHB development. Disease caused by *F. avenaceum* continued to progress after 14 days, and disease severity of intermediate and susceptible genotypes was over 50% 27 days after the first inoculation. The weakly pathogenic species *F. sporotrichioides* spread slowly in the spikes and only caused a low severity of FHB in susceptible genotypes before maturity. There was almost no disease established and developed by *F. poae*. Symptoms of FHB caused by *F. poae* on wheat are different from symptoms caused by other *Fusarium* species (Parry et al., 1995). The FHB symptoms were described as lesions with a bleached centre and dark brown margin on the glumes of winter wheat (Polley et al. 1991). In the current study, the glume spot symptoms caused by *F. poae* were only observed in the susceptible wheat check Caledonia. Based on observations in the greenhouse, the whole glume of infected spikelets in rye were bleached out when inoculated with *F. poae*. It was not distinct from symptoms of FHB caused by other *Fusarium* species.

The degree of fungal colonization was measured by ergosterol content in several studies (Miedaner and Perkowski, 1996; Gang et al., 1998; Miedaner et al., 2000). In the current study, fungal biomass was estimated using qPCR instead of ergosterol content. There was no significant difference for mean fungal biomass among genotypes. *Fusarium graminearum* produced a significantly higher fungal biomass than *F. avenaceum*, *F. sporotrichioides*, and *F. poae*. *Fusarium avenaceum* had a higher fungal biomass than *F. sporotrichioides* and *F. poae*, but the was not statistically significant. The rank of the fungal biomass of the four *Fusarium* species in infected spikes was consistent with that of proportion of infected spikelets. Deoxynivalenol content is usually highly correlated with fungal biomass (Snijders and Van Eeuwijk, 1991). Therefore, the high fungal biomass caused by *F. graminearum* may result in high DON accumulation in rye.

The relationship between the presence of *Fusarium* DNA and the percentage of infected spikelets was examined in the scatter plots. There was no apparent relationship between fungal biomass and infected spikelets by *F. sporotrichioides* and *F. poae* for rye. The lack of a relationship is not unexpected because the two species caused a very low disease in the spikes. The proportion of infected spikelets caused by *F. graminearum* and *F. avenaceum* were significantly correlated with fungal biomass. Increased levels of FHB disease correlated with increased fungal colonization when several isolates of *F. graminearum* and *F. culmorum* were evaluated in fall rye (Gang et al., 1998; Miedaner et al., 2000). However, visual disease severity could not accurately predict DNA accumulation of *F. sporotrichioides* and *F. poae* in infected spikes and vice versa. The high correlation between fungal biomass and infected spikelets by *F. graminearum* and *F. avenaceum* supports that quantification of *F. graminearum* and *F. avenaceum* DNA in infected spikes could be used for disease prediction.

In the current study, the pathogenicity of the four *Fusarium* species was evaluated through spray inoculation with two single isolates mixture for each species. Therefore, the aggressiveness of each isolate could not be compared in rye genotypes. Aggressiveness of isolates is the most important trait for breeding purposes (Mesterhazy, 2003). The aggressiveness of isolates for each *Fusarium* species can be further evaluated on rye genotypes. Aggressive isolates can be used by rye breeders for resistance tests in rye. Moreover, *Fusarium* isolates used in the current study were collected from naturally infected fall rye fields in Manitoba. Additional *Fusarium* isolates from other regions of Canada should be tested to confirm the pathogenicity of these species. Also, it is

essential to evaluate the effect of *Fusarium* species on toxin accumulation. Further research is required to assess the difference of pathogenicity between single and combined *Fusarium* species on rye. It will provide critical information for rye breeders to develop and improve FHB resistant genotypes.

Chapter 5

Evaluation of fall rye genotypes for fusarium head blight resistance in multi-environment replicated trials

5.1 Abstract

Fusarium head blight (FHB) is a common disease in cereal crops, but very little is known about FHB on rye from a Canadian perspective. Two sets of fall rye materials with seven wheat checks were assessed through artificial inoculation during multiple field seasons in Winnipeg and Carman, Manitoba. Set 1 included 54 open-pollinated genotypes and seven commercial hybrids; set 2 included 72 testcross hybrids and nine commercial hybrids. Field FHB disease symptoms, *Fusarium*-damaged kernels (FDK) in the grain, and deoxynivalenol (DON) concentrations produced by *Fusarium graminearum* were measured to evaluate fall rye genotypes for FHB resistance. In general, fall rye had much lower FHB severity and index than winter wheat checks. Results indicated that there is resistance to FHB disease spread within infected rye spikes. Moreover, fall rye had much lower FDK and DON than winter wheat checks. In Set 1, open-pollinated genotypes, such as Anna, Antelope, and SM4R, and hybrids, such as KWS Gatano, KWS Serafino, and KWS Trebiano, showed lower FHB disease levels and accumulated less DON than other genotypes. In Set 2, five commercial hybrids and seven testcross hybrids generated from the Carsten gene pool exhibited more FHB resistance than others. The FHB field traits of fall rye were moderately associated with FDK and DON. The correlations between FHB field traits and plant height on rye were inconsistent. Natural ergot infection was measured through the percentage of sclerotia in harvested seeds by weight. Hybrids registered in Canada tended to have much lower ergot severity than open-pollinated genotypes.

5.2 Introduction

As an economic crop, rye (*Secale cereal* L.) ranks eighth among the world's major cereal grain crops (Mcleod, 2010). The worldwide leading producers of rye are European countries and Russia (Mcleod, 2010). Although rye is a relatively minor cereal in Canada compared with wheat and barley, Canada is the world's largest exporter of rye (Small, 1999; Manitoba Agriculture). The United States and Japan are the most significant importers of rye from Canada (Manitoba

Agriculture). In Canada, as a cereal grain, the domestic use of rye is mainly for livestock feed followed by distilling and food (Small, 1999; Mcleod, 2010; Rye sector, MB, 2011). Rye acreage in Canada declined prior to 1990 and has fluctuated in the last 30 years (Statistics Canada).

Hybrid rye breeding started around 1970 in Germany (Geiger and Schnell, 1970). Combinations between Petkus and Carsten varieties usually exhibited the highest heterosis, and, therefore, the Petkus and Carsten gene pools were selected as starting material for the development of seed and pollinator lines, respectively (Geiger and Miedaner, 2009). In 2015, hybrid rye was introduced to western Canada on a trial basis from Germany and immediately succeeded (Barber, 2019). Six hybrids, including Brasetto, Guttino, KWS Bono, KWS Daniello, KWS Gatano, and KWS Serafino, were registered in Canada (Variety registration office). Hybrid rye has a higher yield and more uniform agronomic traits than open-pollinated rye (King, 2017; Wilde et al., 2017). Generally, hybrid rye has a 25 to 30% higher yield than non-hybrid rye (King, 2017; Barber, 2019).

Canadian production of rye has traditionally centered in the prairies, where outbreaks of fusarium head blight (FHB) have been reported since the 1990s (Stack, 1999; Rye sector, MB, 2011). Fusarium head blight caused by *Fusarium* species can reduce yield and grain quality in small grain cereals (Gaikpa et al., 2019). In Canada, FHB had been recorded on wheat, barley, oat, and rye, and *Fusarium graminearum*, *F. culmorum*, *F. avenaceum*, *F. poae*, and *F. sporotrichioides* were considered as the causal agents (Gordon, 1959; Parry et al., 1995). *Fusarium graminearum* is currently the most prevalent *Fusarium* species causing FHB in Canada and produces mycotoxins, such as deoxynivalenol (DON) in infected grain (Parry et al., 1995; Goswami and Kistler, 2004). In Canada, FHB has been a significant disease since 1986 for wheat and 1994 for barley (Tekauz et al., 1986, 1995, 2004). More frequent and more severe FHB epidemics posed a significant threat to food safety and security (Torres et al., 2019). The FHB epidemics of 1980 in eastern Canada and 1993 in Manitoba promoted research on wheat genetic resistance, an effective strategy of reducing FHB and mycotoxin contamination (Gilbert and Haber, 2013; Gaikpa et al., 2019).

Host resistance to FHB can be divided into active or passive resistance (Mesterhazy, 1995). Active resistance involves five types: type I resistance restricts initial infection; type II resistance limits the spread of infection within the infected spikes; type III resistance reduces mycotoxin

accumulation; type IV resistance prevents kernel infection; and type V resistance is tolerance to infection (Schroeder and Christensen, 1963; Miller et al., 1985; Mesterhazy, 1995; Mesterhazy et al., 1999). Type I and II resistances are the most evaluated resistance types in cereals (Burlakoti et al., 2010). Compared to type I resistance, type II resistance seems to be more stable and less influenced by non-genetic factors (Bai and Shaner, 1994). Therefore, type II resistance has been widely adopted to determine FHB resistance of germplasm and breeding lines in wheat (Bai and Shaner, 2004). Deoxynivalenol is important for the fungus to spread within the infected spikes (Proctor et al., 1995; Desjardins et al., 1996; Bai et al., 2001). Fusarium head blight resistance in wheat is a quantitative trait governed by multiple genes and highly influenced by environment (Becher et al., 2013). A few quantitative trait loci (QTLs), such as *Fhb1*, *Fhb5*, and *Fhb6*, obtained from Chinese wheat cultivars have a high effect on FHB resistance (Bai et al., 2018). Unfortunately, no studies have been conducted on QTLs for FHB resistance on rye (Gaikpa et al., 2019). However, some QTLs from rye have been identified in triticale, located on chromosomes 3R, 4R, 5R, and 7R (Kalih et al., 2015; Dhariwal et al., 2018; Galiano-Carneiro et al., 2019).

Passive resistance refers to the role of morphological traits of the crop, such as plant height, anther extrusion or retention, and spike morphology, which mainly affect disease infection (Mesterhazy, 1995; Buerstmayr et al, 2020). Under field conditions, taller plants tend to have less severe FHB symptoms possibly due to the reduced dispersal of ground inoculum to spikes (Jenkinson and Parry, 1994; Mesterhazy, 1995; Hilton et al., 1999). Additionally, plant height potentially affects microclimate such as relative humidity, as shorter plants are more influenced by soil moisture and a dense canopy structure may contribute to reduced air circulation (Hilton et al., 1999; Yan et al., 2011; Jones et al, 2018). High humidity promotes FHB infection and development, and may thus lead to increased disease pressure on shorter plants (Buerstmayr et al., 2020). Moreover, the presence of retained anthers provides a significant advantage for the onset and establishment of *Fusarium* pathogen and, therefore, leads to increased FHB susceptibility (Buerstmayr and Buerstmayr, 2015, 2016; He et al., 2016; Buerstmayr et al., 2020). Spike compactness and awnedness are also considered to contribute to FHB susceptibility (Mesterhazy, 1995).

Ergot caused by *Claviceps purpurea* is a common spike disease of rye (Pazoutova, 2002). Since the fungus, *C. purpurea*, cannot infect the ovary through intact glumes without the access

of conidia to the stigma, open-pollinated crops with open flowering are more severely affected than self-pollinating crops (Kirchhoff, 1929 cited by Miedaner et al., 2010b and Kodishch et al., 2020; Tudzynski et al., 1995; Mielke, 2000 cited by Kodishch et al., 2020). Once the ovary is infected, the fungus forms a purplish black sclerotium and replaces the floral tissue (Pazoutova, 2002). The threat from ergot disease includes yield losses and contamination of the grain with sclerotia containing alkaloids, which are highly toxic to humans and livestock (Shelby, 1999; Beuerle et al., 2012; Hulvova et al., 2013). In Canada, ergot limits in grain have been established, varying with the grade, from 0.01 to 0.33% for rye (Canadian Grain Commission, 2004). Grain exceeding the threshold limits is downgraded at the elevator, or rejected at the mill, resulting in severe economic damage (Miedaner et al., 2010a, 2010b; Gordon et al., 2019). The density of rye stands, weather conditions during flowering, duration of flowering, fertility of the ovary, and resistance of the ovary to ergot affect ergot reaction of a cultivar (Geiger and Bausback, 1979 cited by Miedaner et al., 2010b; Thakur and Williams, 1980; Betz and Mielke, 1996 cited by Miedaner et al., 2010b and Kodisch et al., 2020; Engelke, 2002 cited by Miedaner et al., 2010b; Mirdita, 2006 cited by Miedaner et al., 2010b). So far, resistance genes to ergot have not been found in rye, and only a few ergot resistance QTLs have been identified in wheat (Tudzynski and Scheffer, 2004; Gordon et al., 2020).

Little information is available about the FHB reaction of rye in Canada (King, 2018). With the introduction of new hybrid rye cultivars and the potential expansion of rye production in western Canada, it is essential to evaluate FHB reactions in multiple environments (Barber, 2019). The present research was undertaken to:

1) determine the response of open-pollinated rye genotypes and some hybrids to *F. graminearum* in southern Manitoba, based on disease levels in the field, *Fusarium*-damage kernels (FDK), and the concentration of DON in the grain
2) evaluate naturally infected ergot severity across the rye genotypes included in FHB nurseries
3) determine correlations among FHB incidence, FHB severity, FHB index, FDK, DON, ergot severity, plant height, and heading date.

5.3 Materials and methods

5.3.1 Plant materials

5.3.1.1 Set 1

Plant materials consisted of 54 open-pollinated fall rye genotypes and seven commercial hybrids (**Appendix 4**). The open-pollinated fall rye included genotypes from Canada, the United States, Russia, Germany, and other European countries. The hybrids were developed by KWS LOCHOW GMBH, Bergen, Germany. Seven winter wheat genotypes with known reaction to FHB were also included in the trials, to compare the relative levels of FHB in rye with those in wheat and to assess the overall disease levels in the field. The wheat genotypes included 32C*17 (Resistant (R)), Emerson (Moderately resistant (MR)), FHB148 (MR), 43I*18 (Intermediate (I)), Freedom (I), Caledonia (Susceptible (S)), and Hanover (S).

5.3.1.2 Set 2

Set 2 included nine commercial hybrids and 72 testcross hybrids generated by KWS LOCHOW GMBH, Bergen, Germany (**Appendix 12**). Twenty-eight testcross hybrids (Li1-Li42) were F_1 generations of crosses between a tester and inbred lines developed from the Petkus gene pool, and forty-four (Li102-Li169) were F_1 generations of crosses between a tester and inbred lines developed from the Carsten gene pool. The same seven winter wheat genotypes used in Set 1 were also included as checks.

5.3.2 Fungal inoculum

The *F. graminearum* inoculum used for the inoculations was a mixture of two isolates of the 15-acetyldeoxynivalenol (15-ADON) chemotype and two isolates of the 3-acetyldeoxynivalenol (3-ADON) chemotype. The 15-ADON chemotype isolates included HSW-15-27 and HSW-15-57, and the 3-ADON chemotype isolates included HSW-15-39 and HSW-15-87, provided by Dr. Maria Antonia Henriquez at the Morden Research and Development Centre, Agriculture and Agri-Food Canada (AAFC). All macroconidia suspensions were initially produced from single spore cultures of *F. graminearum* and cultured in spezieller nährstoffarmer agar (SNA) media (**Appendix 1**) plates under fluorescent light at room temperature (21 to 24 °C) for one week. Ten to fifteen SNA media were cut into small pieces and transferred into the flask

with aerated liquid carboxymethyl cellulose (CMC) media (**Appendix 1**). The flasks of CMC media were cultured under fluorescent light at room temperature (21 to 24 ℃) for another week. Subsequently, the macroconidia suspensions were collected from CMC media by filtering through two layers of sterile cheesecloth. The macroconidia suspensions were stored in sterile glass bottles at 4 ℃ for up to two weeks. The concentration of macroconidia was determined using a hemocytometer cell counter (Hausser Scientific, USA). Equal concentrations of each isolate were combined and diluted to 5×10^5 spores/mL using distilled water right before inoculation. The surfactant Tween™ 20 (VWR international LLC., USA) was added at a volume of 4 mL per two liters of inoculum to improve adherence of the inoculum to the plant.

5.3.3 Experimental design

The field experiments were carried out from 2017 to 2020 growing seasons for Set 1 and 2019 to 2020 for Set 2 at two sites. One site was the Ian N Morrison Research Farm, the University of Manitoba, Carman, Manitoba; another was the Point field research station, the University of Manitoba, Winnipeg, Manitoba. In Set 1, 2017 Winnipeg and 2018 Carman were lost due to winter kill. Each experiment was a randomized complete block design with three replicates for Set 1 and six replicates in 2019 and four replicates in 2020 for Set 2. The number of replicates was determined by seed availability. Each plot was sown in a one-meter row with seventy seeds. The space between each row was thirty centimeters. Each plot was spray inoculated at 30%, 50%, and 70% anthesis using a CO_2 powered backpack sprayer (BellSprayer Inc., USA) calibrated at 30 psi, at a rate of 50 mL per plot. Mist irrigation was operated for 10 minutes per hour for 10 hours daily after each inoculation using an overhead sprinkler system. Mist irrigation was continued for one week after all inoculations were completed.

5.3.4 Disease and agronomic traits evaluation

Fusarium head blight incidence and severity were assessed for each plot when FHB symptoms were well developed, generally 25 to 28 days after the first inoculation. Fusarium head blight incidence was recorded as the percentage of infected spikes in each plot, and FHB severity was assessed as the average percentage of infected spikelets in diseased spikes (Stack and McMullen, 1998). Fusarium head blight index was calculated using the formula: (FHB incidence

(%) × FHB severity (%))/100. Two people separately rated FHB incidence and severity on the same day, and the mean rating was used, except for the data from 2020. This was to reduce the chance of bias or error in the collection of the data.

Heading date for each plot was recorded when 50% of the spikes in the plot were fully emerged from the leaf sheath of the flag leaf using Julian Calendar. Plant height for each plot was determined by taking the mean height of three randomly selected stems measured from the soil to the tip of the spike (excluding awns) prior to harvest.

At maturity, each plot was separately hand harvested and carefully threshed using a stationary Wintersteiger Elite combine (Wintersteiger AG, Austria). The harvested seeds from each plot were stored in paper bags and placed on forced air-drying beds set at 30 ℃ for five to seven days. A blower (Bill's Welding LLC., USA) was used to remove straw and chaff from seed samples. Then clean seed samples were used to quantify FDK and DON. Individual plot samples were used for FDK and DON measurements in Set 1, while composite samples of replicates were used in Set 2. Samples of 2017 Carman and 2018 Winnipeg in Set 1 were tested for FDK and DON by SGS BioVision, Winnipeg, and all other trials were tested by Central Testing Laboratory Ltd., Winnipeg. The percentage of FDK was measured as the weight of *Fusarium* affected kernels in a 50 g sample randomly taken from the whole seed samples. The 50 g grain samples were then ground, and ten-gram of ground samples were used to undertake DON analysis using enzyme-linked immunosorbent assay (ELISA) with RIDASCREEN™FAST DON kit (R-Biopharm AG, Germany). Dilutions were done if DON exceeded the detection limits of the kit (personal communication with Yvan Bruneau from the Central Testing Laboratory Ltd., Canada).

Naturally infected ergot severity was evaluated as the percentage of sclerotia in grain of a whole plot by weight (Miedaner and Geiger, 2015).

5.3.5 Weather conditions

Daily high, low, and mean temperatures, and rainfall during the 30 days after the first inoculation in 2017, 2018, 2019, and 2020 were collected from the Environment Canada weather stations at Carman and Winnipeg.

5.3.6 Statistical analysis

Statistical analyses were performed using the SAS program (SAS version 9.4, SAS Institute Inc., USA). Analyses of variance (ANOVA) were conducted on all variables: FHB incidence, FHB severity, FHB index, FDK, DON, ergot severity, plant height, and heading date using the PROC MIXED procedure. Analyses of variance were performed on all variables for each site year and combined site years. Before analysis, FDK data in Set 1 were square-root-transformed, and ergot severity was log-transformed to satisfy the assumptions of ANOVA. The single site year data were analyzed with genotype as a fixed effect, and replicate treated as a random effect. Based on the evaluation of combined site years data using PROC UNIVARIATE, residuals conformed to a normal distribution, and the plot of residuals was symmetrically distributed. It suggested that the site year data could be pooled. Combined data were analyzed with genotype as a fixed effect, and environment, replicate, and all interactions treated as random effects.

An experiment-wise least significant difference (LSD) value was generated using the PROC GLM procedure as no options were available in the PROC MIXED procedure. The LSD value that SAS calculated using PROC GLM was the same LSD that was manually calculated from PROC MIXED. Pearson's correlation coefficients between response variables were accessed using the PROC CORR procedure.

5.4 Results

5.4.1 Weather conditions

In 2017, daily average temperatures were lower than 20 ℃ for most of the 30 days after the first inoculation in Carman, and the total precipitation was 67.1 mm with 11 precipitation events (**Figure 5.1**). In 2018, daily average temperatures mainly remained in the range between 20 to 25℃, and there were 18 precipitation events for a total accumulation of 79.4 mm distributed in the 30 days after the first inoculation in Winnipeg (**Figure 5.2**) (The precipitation information for June 21st is missing). In 2019, daily average temperature fluctuated during the 30 days in Winnipeg and Carman (**Figure 5.3**). Winnipeg usually had a higher daily temperature than Carman in 2019. Although total precipitation over the 30 days after the first inoculation was 91.8 mm in Winnipeg and 72.1 mm in Carman, there were only 5.5 mm in Winnipeg and 33 mm in Carman in

the first three weeks after the first inoculation. The high temperature in the third week further aggravated dry conditions in fields. In 2020, daily average temperature was lower than 20 ℃ in the first week after the first inoculation in Winnipeg, but it later increased and remained mainly in the range between 20 to 25℃ in both Winnipeg and Carman (**Figure 5.4**). Total precipitation was higher in Carman (84.7mm) than in Winnipeg (70.2mm).

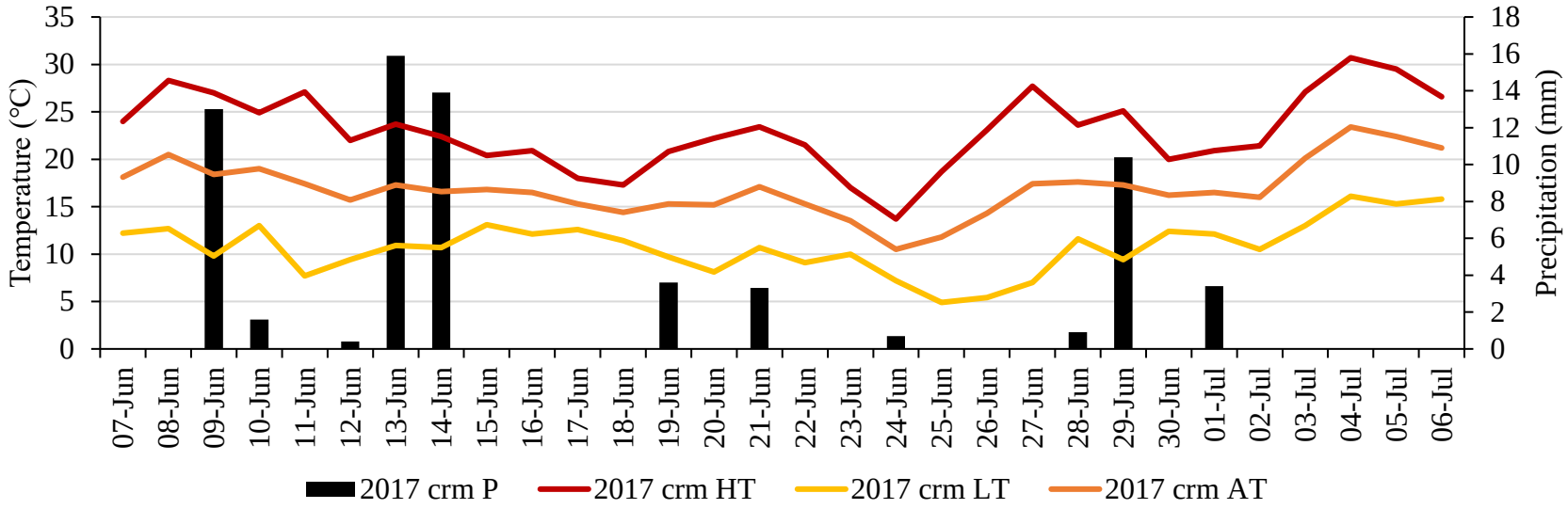

Figure 5.1 Daily high (HT), low (LT), and average temperatures (AT) (solid lines) and precipitation (P) (bars) during the 30 days after the first inoculation in 2017 Carman (crm)

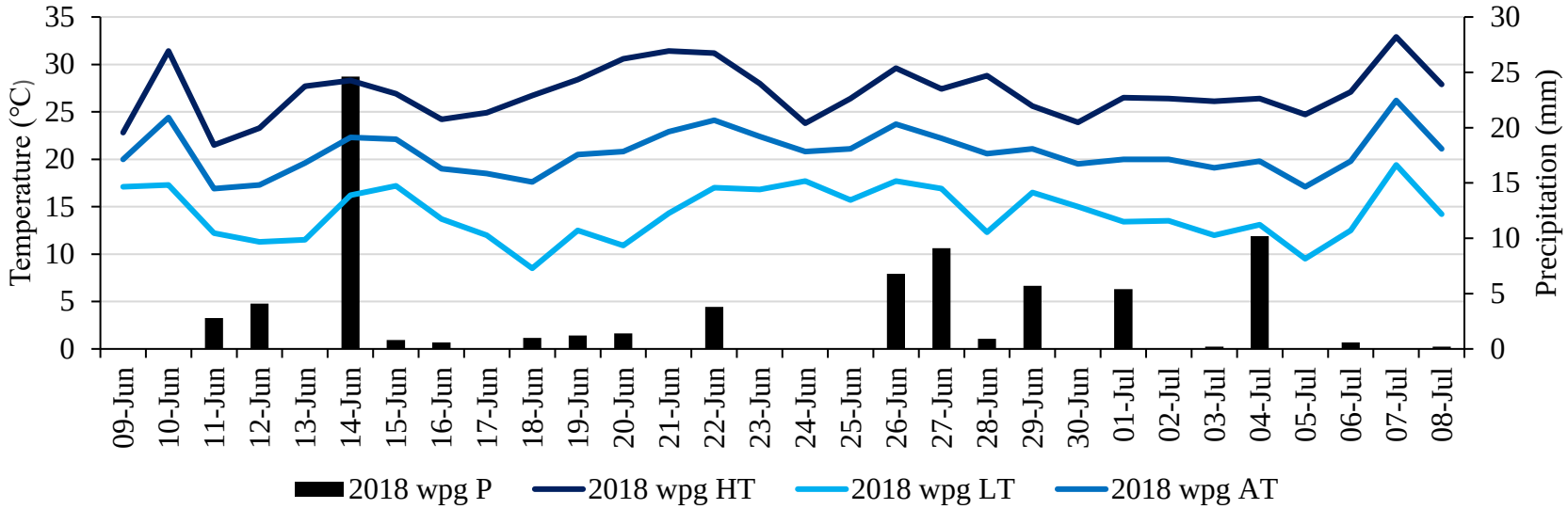

Figure 5.2 Daily high (HT), low (LT), and average temperatures (AT) (solid line) and precipitation (P) (bars) during the 30 days after the first inoculation in 2018 Winnipeg (wpg)

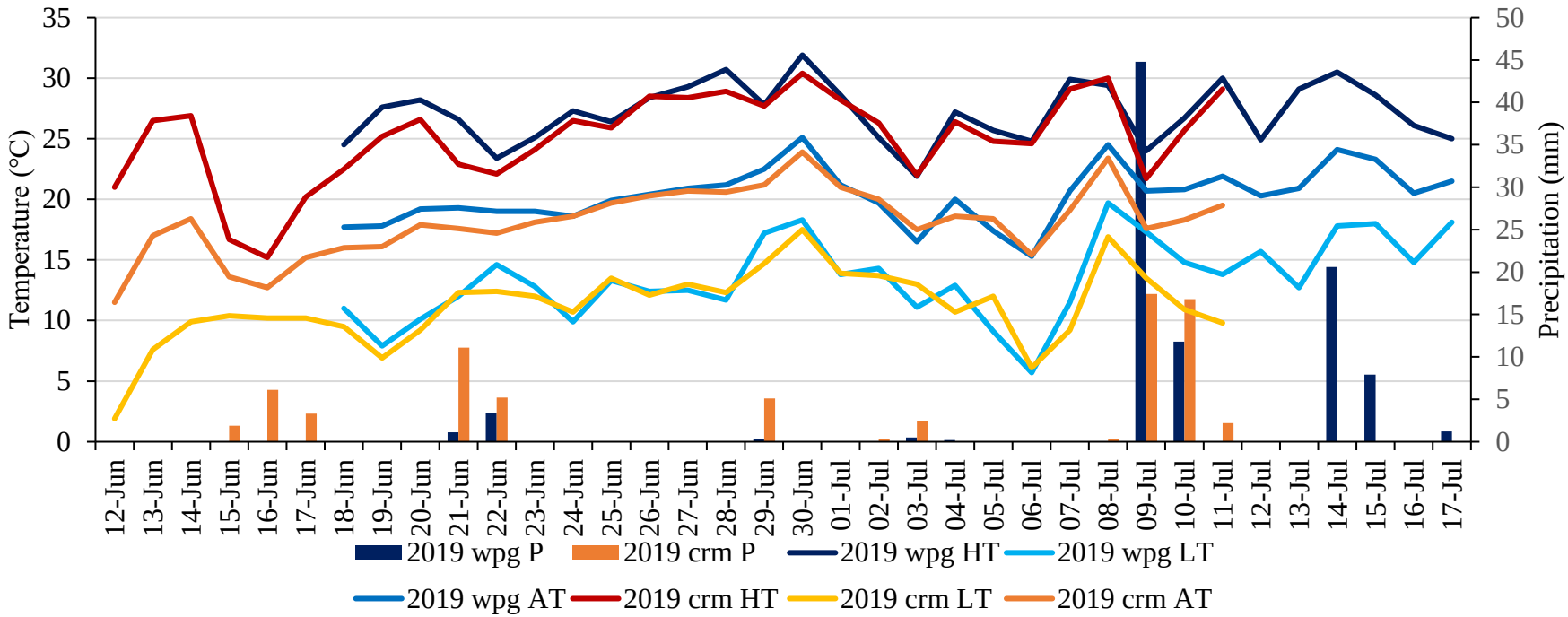

Figure 5.3 Daily high (HT), low (LT), and average temperatures (AT) (solid line) and precipitation (P) (bars) during the 30 days after the first inoculation in 2019 Winnipeg (wpg) and 2020 Carman (crm)

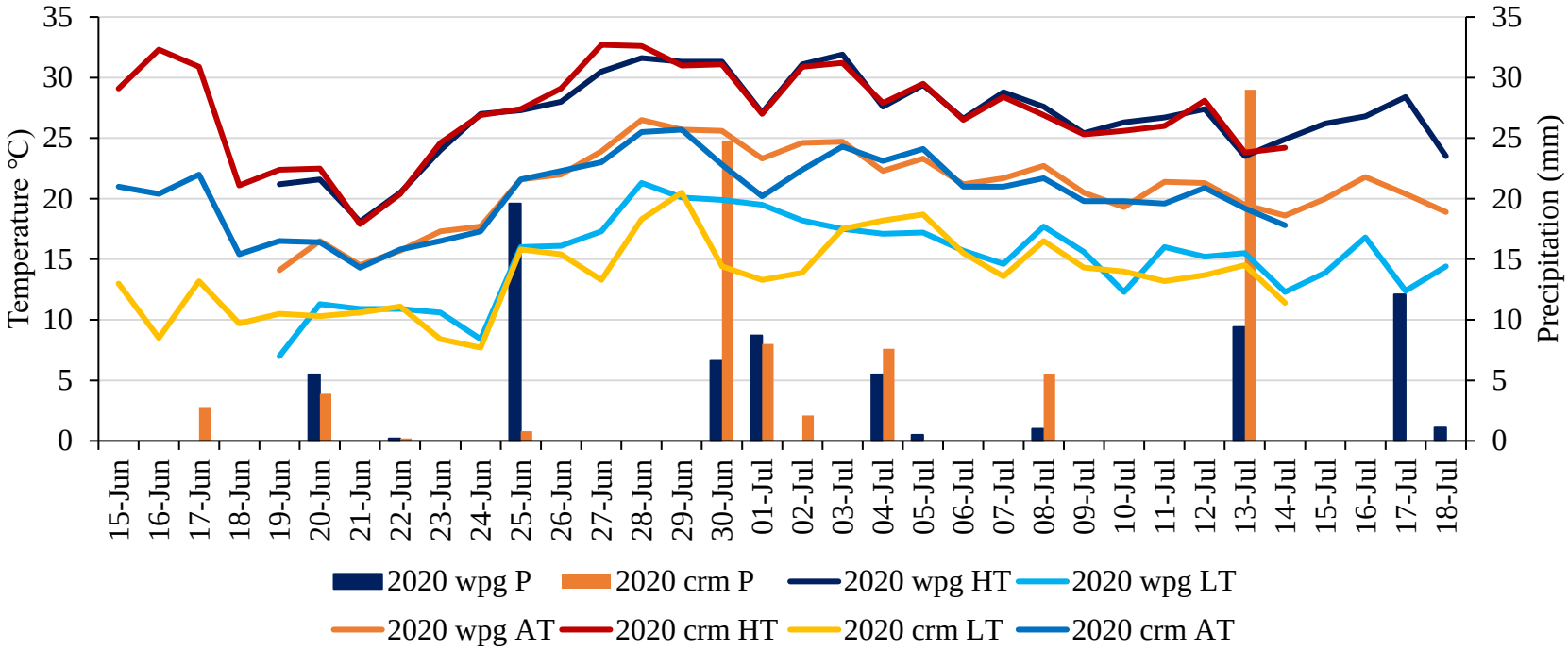

Figure 5.4 Daily high (HT), low (LT), and average temperatures (AT) (solid line) and precipitation (P) (bars) during the 30 days after the first inoculation in 2020 Winnipeg (wpg) and 2020 Carman (crm)

5.4.2 Results of Set 1

5.4.2.1 Analysis of variance for response variables

All sources of variation were highly significant ($p<.0001$) for all variables, including FHB incidence, FHB severity, FHB index, FDK, DON, ergot severity, plant height, and heading date (**Appendix 5**).

5.4.2.2 Reaction of fall rye to *Fusarium graminearum*

Differences for FHB reaction were observed among the 61 fall rye genotypes (**Table 5.1**). In general, the fall rye mean FHB incidence was similar to the mean of the wheat checks. Almost all fall rye genotypes had FHB incidence lower than the susceptible wheat checks Caledonia and Hanover, but higher than the resistant wheat check 32C*17. Fall rye genotypes had a lower mean FHB severity and mean FHB index than wheat checks. No fall rye genotypes had a higher FHB severity than the susceptible wheat checks Hanover and Caledonia, the intermediate checks Freedom and 43I*18, and the moderately resistant check Emerson. Twenty-six fall rye genotypes had a lower FHB severity than the resistant wheat check 32C*17. No fall rye genotypes had a higher FHB index than the susceptible wheat checks Hanover and Caledonia and the intermediate check Freedom. Fourteen fall rye genotypes had a lower FHB index than the resistant wheat check 32C*17. Meanwhile, fall rye genotypes had a much lower mean FDK than wheat checks. No fall rye genotypes had higher FDK than any of the seven wheat checks. Although fall rye genotypes also had a lower mean DON than wheat checks, the DON values of all fall rye genotypes were over the threshold limit of 2 ppm. No fall rye genotypes had DON content higher than the susceptible winter wheat checks Hanover and Caledonia and the intermediate checks Freedom and 43I*18. Overall, some fall rye genotypes from Canada, Germany, and Poland exhibited more resistance to FHB infection and DON accumulation than other genotypes. They included ACE-1, Anna, Antelope, Halo, KWS Daniello, KWS Gatano, KWS Serafino, KWS Trebiano, Kustro, Musketeer, Oklon, Prima, Protector, SM4R, Stoir, Syn 20L, Toivo, and Visa.

Natural ergot infection on rye constantly occurred in the FHB nurseries. Winter wheat genotypes had a much lower mean ergot severity than fall rye (**Table 5.1**). All fall rye genotypes had mean ergot severity higher than the threshold limit of 0.33%. Hybrids, including KWS Bono,

Brasetto, KWS Daniello, KWS Gatano, KWS Serafino, and KWS Trebiano, had lower ergot infection than most open-pollinated genotypes. Several open-pollinated genotypes, including AC Rifle, Anna, Kodiak, Othello, and Prima, had lower ergot severity than other genotypes.

Table 5.1 Mean and rank of fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernel (FDK), deoxynivalenol (DON), and ergot severity from FHB trials conducted on Set 1 genotypes in combined 2017 Carman, 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	Incidence (%)		Severity (%)		Index (%)		FDK (%)		DON (ppm)		Ergot (%)	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	38.41	2	27.06	27	11.61	15	2.89	63	12.24	61	0.12	3
2	Emerson	52.81	38	42.67	65	24.25	63	2.62	62	9.75	47	0.00	1
3	FHB 148	40.42	4	32.26	45	14.79	24	3.08	64	10.56	54	0.15	4
4	43I*18	45.56	11	41.94	64	20.17	53	5.40	65	16.73	65	0.09	2
5	Freedom	73.76	67	47.28	66	36.64	66	8.22	66	20.61	66	1.73	24
6	Caledonia	71.25	65	73.47	68	53.63	67	12.79	67	31.33	67	0.63	6
7	Hanover	81.11	68	73.19	67	60.18	68	15.63	68	38.86	68	1.31	16
8	AC Remington	55.28	48	27.08	28	17.05	37	1.43	45	9.14	42	1.46	19
9	AC Rifle	55.42	50	25.83	25	16.52	33	1.33	40	8.65	35	0.86	10
10	ACE-1	46.25	12	18.61	9	9.61	8	0.38	1	6.36	7	33.47	68
11	Amilo	56.11	55	29.31	34	16.75	34	1.78	55	10.06	52	2.12	34
12	Animo	49.58	21	32.36	46	18.02	41	1.20	31	8.65	36	2.22	37
13	Anna	47.36	17	17.92	8	9.32	6	0.59	2	8.10	31	1.27	14
14	Antelope	36.11	1	16.53	6	6.77	1	0.65	5	4.05	1	2.47	42
15	Balbo	52.78	37	25.00	22	15.71	30	1.28	37	10.41	53	3.53	54
16	Brasetto	55.14	45	19.86	13	13.30	22	1.96	60	11.85	60	1.21	13
17	Caribou	56.73	57	35.36	56	19.78	51	1.57	51	8.28	33	2.73	47
18	Carolkurz	51.06	29	33.43	51	19.54	49	1.35	41	11.65	58	3.64	55
19	Carsten	63.19	63	31.94	44	21.14	59	1.55	50	9.80	49	7.38	63
20	Cougar	54.72	44	25.56	24	15.88	31	1.22	35	6.61	13	2.65	45
21	Dakold	51.11	30	29.86	38	17.18	39	1.22	34	7.86	28	3.75	57
22	Dakota	73.06	66	36.39	61	28.52	65	2.11	61	14.62	64	1.94	29
23	Dankoskie Nowe	53.19	39	33.19	50	20.70	56	1.18	28	11.51	57	2.78	48
24	Dankoskie Seleke	49.86	24	35.14	55	20.33	55	1.82	56	13.62	63	3.22	53
25	Dankoskie Srebrne	53.75	41	27.50	29	14.96	26	1.59	52	9.76	48	2.79	49

26	Dominant	51.67	34	31.70	42	18.38	42	1.21	33	7.87	29	4.48	61
27	Florida 401	46.52	14	27.98	31	14.12	23	0.84	11	7.13	16	3.71	56
28	Frontier	46.67	15	37.08	63	19.57	50	1.15	26	7.57	24	1.49	20
29	Gator	58.33	59	36.39	61	21.79	61	1.20	30	7.32	19	9.06	65
30	GC-100	67.08	64	35.97	58	26.38	64	1.33	39	7.97	30	4.36	60
31	Guttino	54.44	43	14.17	3	8.98	5	1.39	43	9.83	50	1.82	28
32	Halo	48.61	19	24.17	21	12.35	18	0.79	9	7.52	22	1.71	22
33	Hazlet	62.92	62	32.78	49	20.89	57	1.46	48	12.79	62	2.32	38
34	Horton	60.56	61	33.61	52	21.72	60	1.47	49	7.27	18	1.76	25
35	Kharkivska 95	59.72	60	30.28	39	19.52	48	1.38	42	9.62	45	1.73	23
36	Kharkivska 98	55.28	48	32.36	46	18.53	43	1.71	53	11.73	59	2.11	33
37	Kodiak	55.83	53	31.81	43	19.10	45	0.72	6	7.09	15	1.21	12
38	Kustro	48.06	18	26.53	26	14.80	25	0.74	8	6.99	14	1.78	26
39	KWS Bono	52.64	35	19.03	11	12.40	19	0.99	16	9.31	44	1.29	15
40	KWS Daniello	49.76	23	15.05	5	10.24	10	1.71	54	11.19	56	0.63	6
41	KWS Gatano	44.58	8	13.06	1	8.01	2	0.92	12	8.82	37	0.54	5
42	KWS Serafino	44.58	8	13.75	2	8.79	4	1.06	21	8.85	39	0.74	9
43	KWS Trebiano	51.39	31	14.58	4	8.71	3	1.04	20	8.58	34	0.66	8
44	Maton	50.56	25	35.69	57	20.32	54	1.90	58	7.74	26	4.11	58
45	Motto	55.56	51	28.47	32	16.78	36	1.13	25	7.71	25	1.98	30
46	Musketeer	51.39	31	25.00	22	15.54	29	1.02	17	6.41	8	1.44	18
47	Oklon	38.75	3	21.81	18	10.05	9	1.04	19	6.46	9	2.33	39
48	Othello	53.19	39	36.25	60	21.90	62	0.93	13	6.50	11	1.43	17
49	Pearl	49.44	20	36.11	59	19.33	46	1.30	38	7.42	21	2.49	43
50	Petkus	52.77	36	29.33	35	17.13	38	1.86	57	8.20	32	10.33	66
51	Ponsi	50.56	25	29.72	37	16.76	35	1.12	24	7.34	20	2.05	32
52	Prima	46.94	16	19.31	12	10.30	11	0.96	14	6.47	10	0.92	11
53	Prolific Spring	51.67	33	32.64	48	19.86	52	1.93	59	8.91	40	3.17	52
54	Protector	40.56	5	22.78	20	11.14	14	1.10	22	4.82	2	1.66	21
55	Puma	55.83	53	30.97	40	19.35	47	1.19	29	7.20	17	3.00	50
56	R003-4	54.12	42	21.33	17	12.99	21	1.15	27	9.30	43	2.18	35
57	Reimann-Philipp	56.48	56	28.93	33	17.84	40	1.44	46	10.94	55	7.43	64

58	Saratovskava 4	55.69	52	34.17	53	21.01	58	1.24	36	7.53	23	4.25	59
59	Sellino	50.97	28	27.50	29	15.23	28	0.81	10	7.77	27	2.41	40
60	SM4R	46.25	12	18.75	10	9.39	7	0.64	4	5.64	4	1.81	27
61	Stoir	55.24	47	20.07	14	12.85	20	1.41	44	9.91	51	5.69	62
62	Syn 20L	44.31	7	22.22	19	10.84	13	1.12	23	6.25	6	2.67	46
63	Toivo	43.61	6	20.83	15	11.75	16	0.61	3	5.79	5	2.42	41
64	Visa	49.58	21	20.83	15	12.13	17	0.97	15	8.98	41	2.20	36
65	Vitallo	55.14	45	31.67	41	18.77	44	1.21	32	8.84	38	3.01	51
66	Voima	45.00	10	34.44	54	15.97	32	0.73	7	5.14	3	2.04	31
67	Wheeler	57.94	58	17.88	7	10.80	12	1.03	18	9.64	46	17.81	67
68	Wrens Abruzzi	50.56	25	29.58	36	15.21	27	1.46	47	6.54	12	2.56	44
	LSD (0.05)	7.72		6.60		5.20		0.77		2.70		2.32	
	Wheat means	57.62		48.27		31.61		7.23		20.01		0.58	
	Rye means	52.16		27.01		15.88		1.22		8.46		3.48	
	Rye range	36.11-73.06		13.06-37.08		6.77-28.52		0.38-2.11		4.05-14.62		0.54-33.47	

LSD=least significant difference.

Green highlight represents fall rye genotypes with better fusarium head blight resistance.

5.4.2.3 Correlation between the measured variables

Correlations among all FHB field traits (FHB incidence, severity, and index) of rye were significant and positive (**Table 5.2**). High correlation coefficients were observed between FHB severity and index (r=0.93). *Fusarium*-damaged kernels were significantly associated with all FHB field traits, but correlation coefficients were moderate (r=0.39-0.55). Deoxynivalenol was significantly associated with FHB incidence and index, but not with FHB severity. The correlation coefficients between DON and FHB incidence (r=0.58) or FHB index (r=0.35) were moderate. The correlation coefficients between FDK and DON (r=0.66) was higher than the correlations between FDK and DON and the other FHB field traits (r=0.14-0.58). Ergot severity was not associated with FHB field traits, FDK, or DON. Plant height was significantly correlated with FHB severity, FDK, DON, and ergot severity. The correlation was positive between plant height and FHB severity, but negative between plant height and FDK or DON. Heading date was significantly associated with FHB incidence and ergot severity.

Table 5.2 Pearson correlation coefficients for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), ergot severity, plant height (PH), and heading date (HD) from FHB trials conducted on Set 1 genotypes in combined 2017 Carman, 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

	Severity	Index	FDK	DON	Ergot	PH	HD
Incidence	0.46***	0.72****	0.55****	0.58****	0.05ns	-0.23ns	0.27*
Severity		0.93****	0.39**	0.14ns	-0.06ns	0.39**	-0.10ns
Index			0.52****	0.35**	-0.06ns	0.17ns	0.02ns
FDK				0.66****	-0.16ns	-0.27*	-0.17ns
DON					-0.05ns	-0.46***	0.14ns
Ergot						0.28*	0.57****
PH							-0.08ns

Note: genotype means across six site years were used for correlation analysis. n=61.
ns=not significant, * significant at p<0.05, ** significant at p<0.01, *** significant at p<0.001, and **** significant at p<0.0001.

5.4.3 Results of Set 2

5.4.3.1 Analysis of variance for response variables

Genotype and site year were highly significant ($p<0.01$-0.0001) for all variables, including FHB incidence, FHB severity, FHB index, FDK, DON, ergot severity, plant height, and heading date (**Appendix 13**). The interaction between genotype and site year also had a significant effect on FHB incidence, FHB severity, FHB index, ergot severity, plant height, and heading date.

5.4.3.2 Reaction of hybrid rye to *Fusarium graminearum*

Genotypes tested in Set 2 showed different reactions to *F. graminearum* (**Table 5.3**). Mean FHB incidence was similar to the mean of the wheat checks, but mean rye FHB severity and index was lower than the mean of the wheat checks. No hybrids had FHB incidence higher than the susceptible wheat checks, Hanover and Caledonia, and the intermediate check, Freedom. Only three genotypes (Exp.Variety1, Exp.Variety2, and Li125) had a lower FHB incidence than the resistant wheat check 32C*17. In contrast, all rye genotypes had FHB severity lower than the seven wheat checks. About half of the genotypes had a lower FHB index than the resistant wheat check 32C*17. No rye genotype had FHB index higher than the susceptible wheat checks Hanover and Caledonia, the intermediate checks Freedom and 43I*18, and the moderately resistant check Emerson. There was a wide range of distribution in FDK for the rye genotypes (0.38 to 2.59%). Similarly, rye genotypes had a lower mean FDK and DON than the mean of the wheat checks. Compared to the resistant winter wheat check 32C*17, 42 rye genotypes had a lower FDK, and 22 had a lower DON. No rye genotypes had higher FDK levels or DON content than the susceptible wheat checks, Hanover and Caledonia, and the intermediate checks, Freedom and 43I*18. Overall, Exp.Variety1, Exp.Variety2, Exp.Variety17, KWS-H10110, KWS-H144, Li20, Li122, Li125, Li129, Li135, Li137, Li142, and Li143 showed better resistance to FHB infection and DON contamination than other rye genotypes tested.

Rye genotypes tested had a much heavier ergot infection than the wheat checks (**Table 5.3**). No hybrid genotypes had ergot severity lower than 0.33%, the threshold limit for Canadian rye established by the Canadian Grain Commission. Nevertheless, Exp.Variety17, Exp.Variety31, KWS-H144, KWS-H161, and Li167 had lower ergot severity than other rye genotypes.

Table 5.3 Mean and rank of fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernel (FDK), deoxynivalenol (DON), and ergot severity from FHB trials conducted on Set 2 genotypes in combined 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	Incidence (%)		Severity (%)		Index (%)		FDK (%)		DON (ppm)		Ergot (%)	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	40.80	5	31.20	82	14.13	47	1.49	58	11.01	23	0.30	2
2	Emerson	60.48	76	40.45	84	27.03	84	1.70	68	12.03	41	0.24	1
3	FHB148	38.27	3	32.82	83	14.36	50	1.64	63	11.26	26	0.31	3
4	43I*18	56.56	56	45.70	85	27.61	85	4.25	85	19.69	85	0.53	4
5	Freedom	75.47	86	54.21	86	41.21	86	9.47	86	22.40	86	2.04	12
6	Caledonia	76.05	87	74.21	88	58.64	87	10.75	87	35.57	87	0.85	5
7	Hanover	80.24	88	73.41	87	59.79	88	19.09	88	39.85	88	3.05	30
8	Exp.Variety1	37.63	2	8.66	1	3.55	1	0.64	6	5.81	3	2.17	14
9	Exp.Variety2	37.10	1	9.48	2	4.00	2	0.38	1	5.65	1	2.31	16
10	Exp.Variety17	48.57	9	15.28	10	10.42	12	1.12	33	9.17	9	1.91	9
11	Exp.Variety31	46.97	7	20.55	54	13.45	37	1.49	57	10.50	18	1.60	6
12	KWS-H10110	50.49	20	16.41	19	9.58	9	0.91	21	8.10	6	3.23	35
13	KWS-H119	57.72	63	21.47	60	15.17	60	0.82	14	9.61	11	2.76	23
14	KWS-H144	49.85	16	11.06	3	6.85	3	0.86	17	6.86	4	1.60	7
15	KWS-H145	61.90	80	18.69	31	14.07	45	1.45	55	12.06	42	3.81	49
16	KWS-H161	52.46	34	18.96	37	13.99	44	1.02	29	12.86	53	1.79	8
17	Li1	51.81	26	19.30	41	12.97	30	1.18	38	11.22	25	4.48	63
18	Li2	48.66	10	23.55	75	16.88	74	1.90	78	15.23	77	5.28	75
19	Li3	51.35	22	19.29	40	13.55	39	0.77	12	11.45	28	5.02	71
20	Li4	55.49	49	22.81	70	15.89	65	0.79	13	18.32	83	4.57	65
21	Li5	51.69	24	18.92	36	12.44	29	0.94	23	12.38	43	4.41	60
22	Li6	60.15	73	24.43	80	17.63	79	0.95	24	12.80	52	5.91	85
23	Li7	59.11	69	28.46	81	19.72	83	0.75	11	13.78	65	5.83	83
24	Li8	56.59	57	21.52	61	13.58	40	0.86	16	13.89	66	5.14	73
25	Li9	55.62	51	19.49	42	14.40	52	0.87	18	11.62	37	4.23	56

26	Li10	60.75	77	23.58	76	17.79	80	1.09	31	13.24	58	5.39	78
27	Li12	55.31	48	18.97	38	13.44	36	1.46	56	14.24	69	5.33	77
28	Li14	53.87	38	20.66	55	15.51	64	0.93	22	13.74	62	4.03	53
29	Li15	59.36	70	22.96	71	16.60	69	0.88	19	11.81	39	3.93	50
30	Li16	58.45	66	22.61	68	15.96	66	1.42	54	11.60	35	5.83	84
31	Li17	49.82	15	18.74	32	11.63	24	1.13	34	13.11	57	5.70	82
32	Li18	57.52	61	18.14	27	13.30	34	0.90	20	13.48	60	4.54	64
33	Li19	52.04	29	19.23	39	11.51	20	1.41	53	10.80	22	4.72	68
34	Li20	53.72	37	15.28	9	8.85	6	0.65	8	11.54	33	4.71	67
35	Li21	53.54	36	19.91	47	14.34	49	0.99	25	18.80	84	5.42	79
36	Li22	57.83	64	22.48	65	16.43	68	1.24	42	15.66	79	5.32	76
37	Li23	54.52	43	23.66	77	16.87	73	0.61	5	16.81	82	6.09	87
38	Li24	54.98	45	18.47	30	13.24	33	0.57	3	12.50	46	4.74	69
39	Li25	58.43	65	22.44	64	17.62	78	0.68	9	14.31	70	4.85	70
40	Li26	59.37	71	20.13	50	15.32	61	0.65	7	12.73	50	4.70	66
41	Li27	55.49	49	18.11	26	13.98	43	0.52	2	14.19	68	4.48	62
42	Li40	62.79	82	24.05	79	17.88	81	1.00	27	14.78	75	6.12	88
43	Li41	54.24	41	22.49	67	15.35	62	1.01	28	13.04	55	5.03	72
44	Li42	54.45	42	19.83	45	14.48	53	1.67	65	11.71	38	5.92	86
45	Li102	49.18	12	17.39	22	12.02	25	1.41	52	11.46	29	3.31	39
46	Li103	50.41	19	21.05	58	14.08	46	1.71	69	9.16	8	2.00	11
47	Li104	56.28	54	20.71	56	15.12	58	1.73	70	10.54	19	2.16	13
48	Li105	48.28	8	17.83	25	10.98	16	2.03	82	10.49	17	4.05	54
49	Li106	60.24	75	23.78	78	16.70	72	2.59	84	15.34	78	4.02	52
50	Li107	58.83	68	19.85	46	14.80	55	1.51	59	13.59	61	3.29	37
51	Li111	49.24	13	17.54	23	12.12	26	1.76	72	14.71	73	3.18	34
52	Li113	62.90	84	22.22	63	16.99	75	1.77	74	10.43	15	3.00	28
53	Li114	61.76	79	20.49	53	14.99	56	1.34	48	9.83	13	3.09	32
54	Li116	52.39	33	17.67	24	11.63	23	1.76	73	10.46	16	2.71	22
55	Li117	51.94	27	19.59	43	13.71	41	1.64	64	12.44	44	3.23	36
56	Li119	56.02	53	18.81	34	12.36	27	1.59	62	12.75	51	3.76	48
57	Li120	57.54	62	23.25	73	16.65	70	1.94	80	16.36	81	4.02	51

58	Li122	52.72	35	12.50	5	7.98	5	1.16	35	7.42	5	3.57	46
59	Li123	55.26	46	21.22	59	14.63	54	2.47	83	14.65	71	2.60	20
60	Li125	39.48	4	12.10	4	6.95	4	0.82	15	5.81	2	3.73	47
61	Li126	50.23	18	19.72	44	13.81	42	1.52	61	11.84	40	4.36	58
62	Li127	53.99	39	18.36	29	13.12	31	1.28	44	11.59	34	2.31	15
63	Li128	56.79	59	20.02	48	15.10	57	1.87	77	12.44	45	2.66	21
64	Li129	50.19	17	14.78	8	9.45	8	0.70	10	8.36	7	3.34	41
65	Li131	46.69	6	16.06	16	11.10	17	1.30	47	10.79	21	2.58	19
66	Li132	62.35	81	18.75	33	14.40	51	1.93	79	13.07	56	2.85	25
67	Li133	56.74	58	16.17	17	10.61	14	1.68	66	13.77	64	3.07	31
68	Li134	55.30	47	17.23	21	12.43	28	1.22	41	12.62	49	3.50	44
69	Li135	51.52	23	16.38	18	10.20	11	1.11	32	11.17	24	2.54	17
70	Li136	57.36	60	15.94	14	11.58	22	1.28	45	11.50	31	5.21	74
71	Li137	51.97	28	15.31	11	10.76	15	0.60	4	9.74	12	4.32	57
72	Li138	54.74	44	13.63	6	9.74	10	1.21	39	10.32	14	4.39	59
73	Li139	51.10	21	15.55	13	10.58	13	1.09	30	13.32	59	5.58	80
74	Li140	54.03	40	20.27	51	14.28	48	1.28	43	11.61	36	3.33	40
75	Li141	49.73	14	15.34	12	11.17	19	1.36	49	13.95	67	3.04	29
76	Li142	49.14	11	14.51	7	9.02	7	0.99	26	9.33	10	3.55	45
77	Li143	52.15	30	16.05	15	11.11	18	1.21	40	11.49	30	5.59	81
78	Li144	55.85	52	21.02	57	15.35	63	1.38	50	12.54	48	2.79	24
79	Li145	52.31	31	18.31	28	13.21	32	1.52	60	10.63	20	3.49	43
80	Li146	58.52	67	16.72	20	11.56	21	1.73	71	11.30	27	3.29	38
81	Li147	56.56	55	20.35	52	15.12	59	1.70	67	12.99	54	4.14	55
82	Li148	60.05	72	23.05	72	16.33	67	1.99	81	14.89	76	4.42	61
83	Li164	63.69	85	22.49	66	17.07	76	1.80	75	15.92	80	2.55	18
84	Li165	62.88	83	23.47	74	17.14	77	1.39	51	14.68	72	2.99	27
85	Li166	52.34	32	18.90	35	13.32	35	1.16	36	12.53	47	3.47	42
86	Li167	61.23	78	22.64	69	18.09	82	1.29	46	13.77	63	1.94	10
87	Li168	60.16	74	21.86	62	16.69	71	1.83	76	14.75	74	2.97	26
88	Li169	51.69	24	20.11	49	13.53	38	1.17	37	11.50	32	3.17	33
	LSD (0.05)	7.29		4.20		3.61		1.53		5.30		0.89	

Wheat means	57.94	46.43	30.50	4.88	18.66	0.71
Rye means	54.34	19.12	13.40	1.26	12.19	3.85
Rye range	37.10-63.68	8.66-28.46	3.55-19.72	0.38-2.59	5.65-18.80	1.60-6.12

LSD=least significant difference.

Green highlight represents hybrid rye with better fusarium head blight resistance.

5.4.3.3 Correlation between the measured variables

Correlations among FHB field traits of hybrids were significant and positive (**Table 5.4**). Highly significant correlations were detected between FHB severity and index (r=0.97). Correlations between FDK and all FHB field traits were significant, but relatively low (r=0.27-0.28). In contrast, correlations between DON and all FHB field traits were significant and moderate (r=0.56-0.71). However, the correlation between FDK and DON was significant but low (r=0.25). Ergot severity was significantly associated with all FHB field traits, FDK, and DON, and significant coefficients were positive except the one between ergot severity and FDK. Plant height was significantly negatively correlated with all FHB field traits and DON. The correlation between heading date and FDK was positive and highly significant (r=0.46). Heading date was also significantly negatively correlated with ergot severity (r=-0.36) and plant height (r=-0.25).

Table 5.4 Pearson correlation coefficients for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), ergot severity, plant height (PH), and heading date (HD) from FHB trials conducted on Set 2 genotypes in combined 2019 Carman, 2019 Winnipeg, 2020 Carman, and 2020 Winnipeg

	Severity	Index	FDK	DON	Ergot	PH	HD
Incidence	0.69****	0.77****	0.27*	0.56****	0.22*	-0.36**	0.12ns
Severity		0.97****	0.27*	0.69****	0.34**	-0.33**	0.05ns
Index			0.28*	0.71****	0.30**	-0.43****	0.08ns
FDK				0.25*	-0.24*	-0.21ns	0.46****
DON					0.41***	-0.48****	0.04ns
Ergot						-0.10ns	-0.36**
PH							-0.25*

Note: genotype means across four site years were used for correlation analysis. n=81.
ns=not significant, * significant at p<0.05, ** significant at p<0.01, *** significant at p<0.001, and **** significant at p<0.0001.

5.5 Discussion

Based on mean FHB index, disease levels in FHB nurseries varied among locations and years (**Appendix 8** and **Appendix 16**). Mist irrigation system was applied regularly to facilitate FHB infection in each site year. However, humidity and temperature can be manipulated only to a

limited extent in fields (Miedaner, 1997). The optimum temperature for FHB infection and development was 25 ℃ while little or no FHB infection occurred at 15 ℃ (Andersen, 1948). Therefore, the relatively low daily average temperatures in 2017 were not conducive to FHB development. In 2018, daily average temperatures were in the range between 20 to 25 ℃, and there was adequate precipitation. Compared to 2017, weather conditions in 2018 were more favorable for FHB infection and development. Humidity in the first 48 h after inoculation played an important role on FHB infection (Andersen, 1948; Miedaner, 1997). Dry conditions in 2019 were undoubtedly unfavorable for FHB development. However, disease incidence increases as temperature raises from 20 to 30 ℃ (Andersen, 1948). Accordingly, high temperatures in 2019 and 2020 lead to higher mean FHB incidence, compared to 2017 and 2018. In 2020, FHB index in winter wheat fields was lower than the previous ten years according to FHB survey in Manitoba (Henriquez et al., 2021). Nevertheless, disease levels of FHB nurseries in Carman were much higher than other site years. The hot weather with precipitation within 48 h after inoculation was likely sufficient to stimulate FHB infection and spread. In contrast, inoculation in Winnipeg coincided with cool temperatures which may have resulted in lower FHB infection in Winnipeg compared to Carman.

Differences in FHB symptoms were observed among the rye genotypes tested. Infected spikelets were in a light-straw color, while healthy spikelets were green (**Figure 5.5** (A, B)). The infected section of the spike was usually narrower than healthy one due to lack of seed set associated with infection (**Figure 5.5** (A)). It was common to see a single bleached spikelet without spread to adjacent spikelets (**Figure 5.5** (B)), particularly for hybrid rye. Rye grains have variable colors, such as yellow, brown, or different shades of green (Schlegel, 2013a). In harvested rye grain samples, infected kernels were discolored and shriveled in appearance (**Figure 5.5** (C)). The so called “tombstone” kernels in wheat are shriveled up and characteristically smaller than normal, and the color is white to pale pink (Parry et al., 1995). However, distinctions between infected kernels and healthy kernels in rye were not as evident as in wheat. As a result, FDK assessment in rye is more difficult than it is in wheat (Perkowski et al., 1995).

Figure 5.5 (**A, B**) Fusarium head blight (FHB) infected spikes of fall rye in FHB nurseries; (**C**) Healthy kernels (top) and *Fusarium*-damaged kernels (bottom) of rye harvested from FHB nurseries.

Mid-anthesis is the most susceptible growth stage for FHB infection with the greatest yield losses occurring in both wheat and rye when infection occurs at this stage (Anderson, 1948; Miedaner, 1997). However, the flowering time of rye and wheat differed by 8 to 11 days (Gaikpa et al., 2019). To compensate for the longer flowering period of rye, three inoculations were conducted for each plot based on its respective flowering time. Both FHB incidence and severity were recorded to calculate FHB index, the percentage of infected spikelets per plot. However, FHB of rye investigated in several studies from Europe were only measured by FHB index directly (Miedaner et al., 1993; Perkowski et al., 1995; Miedaner and Perkowski, 1996; Miedaner et al., 2001, 2003; Gaikpa et al., 2019). Gaikpa et al. (2019) compared the reaction of rye, triticale, durum wheat, and bread wheat to FHB and reported that rye had very low mean FHB index compared to durum and bread wheat. In the current study, rye also had much lower mean FHB index than winter wheat checks in both Set 1 and Set 2.

Mean FHB incidence of fall rye was similar to FHB incidence for winter wheat checks and only a few genotypes showed higher FHB incidence than the susceptible winter wheat checks.

Relatively high FHB incidence among rye genotypes with few genotypes showing low incidence suggests that there is little resistance to initial infection among the genotypes tested. In 2020 Carman, type I resistance could not be detected among fall rye genotypes due to high FHB incidence (**Appendix 6**). Under high disease pressure, differences in degree of type I resistance may not be distinguishable among genotypes (Bai and Shaner, 2004). In contrast, rye had lower mean FHB severity than the mean of the winter wheat checks, especially in Set 2. It appeared that there is resistance to FHB disease spread within spikes on rye. Compared to wheat, barley had less FHB severity, but higher FHB incidence in field surveys (Gilbert et al., 1999; McCallum et al., 1999). Fusarium head blight symptoms in barley generally results in initial infection of spikelets, but there is no spread to adjacent spikelets (Bushnell et al., 2003). Therefore, type I resistance is more important for barley than type II resistance (Steffenson, 2003). Disease index may be a good measurement of type I resistance in barley (Bai and Shaner, 2004). Rye has good resistance to FHB spread. Fusarium head blight index was more closely associated with FHB severity than FHB incidence in rye. It indicates that differences in FHB severity are more important in determining the differences in FHB index in rye.

Differences in reaction to FHB existed among rye genotypes tested (**Table 5.1** and **Table 5.3**). Most of open-pollinated genotypes were resistant or moderately resistant. Fromme et al. (1993) also found a considerable range of genetic variation in FHB resistance among full sib families of the open-pollinated fall rye cultivar Halo, with many showing moderate resistance (Cited by Parry et al., 1995). In Set 2, testcross genotypes showed resistance or moderate resistance to FHB, with FHB index ranging from 7% to 20% (**Table 5.3**). Testcross genotypes, from the Carsten inbred, had better FHB resistance than testcross genotypes, from the Petkus inbred. Significant differences were also found in FHB index between rye genotypes, when F_1 and F_2 genotypes of crosses of six inbred lines derived from the Petkus gene pool were inoculated with *F. culmorum* and *F. graminearum* (Miedaner et al., 1993). The average FHB index was low to moderate, ranging from 3.1 to 5.2 (a 1-9 scale with 1=no symptoms visible and 9=100% of spikelets per plot infected) in a two-year field trial (Miedaner et al., 1993).

The FDK and DON contents also revealed differentiation among rye genotypes in the current study. The DON content ranged from 4.05 to 14.62 ppm in Set 1 and 5.65 to 18.80 ppm in Set 2. Twenty-nine single-cross hybrids inoculated with *F. culmorum* exhibited a wider range of

DON concentrations from 0.7 to 21.9 ppm (Perkowski et al., 1995). The different DON content levels among rye genotypes might be caused by a specific reaction that could prevent considerable accumulation of DON and degrade DON during infection (Miller et al., 1983; Miedaner and Perkowski, 1996). In several studies of rye and wheat, rye was more resistant to FHB infection with the lower amount of DON content in kernels (Miller et al., 1985; Miedaner et al., 2001, 2003; Gaikpa et al., 2019). Under inoculation with *F. graminearum*, six resistant rye landraces from Brazil had DON content ranging from 0.024 to 0.58 ppm, while DON concentrations in six resistant spring wheat cultivars ranged from 0.23 to 1.67 ppm (Miller et al., 1985). Mean kernel rating and DON content of twelve rye genotypes, consisting of hybrids and synthetics, were lower than eight winter wheat genotypes (Miedaner et al., 2001). In the current study, rye also had lower mean FDK levels and DON contents than the mean of the winter wheat checks, particularly in Set 1. Lower FDK and DON may be resulted from fewer infected kernels due to a higher level of resistance in rye compared to wheat. On the other hand, based on the observation in FHB nurseries, many infected florets in rye tended not to set seed compared to seed set still occurring in infected wheat florets (**Figure 5.5** (A)). As a result, compared to wheat, fewer infected kernels were available in the harvested rye grain to contribute to FDK and DON.

Additionally, previous studies reported different associations between FHB index, FDK, and DON on rye (Perkowski et al., 1995; Miedaner and Perkowski, 1996; Gaikpa et al., 2019). The correlations between FHB index, FDK, and DON were not significant in the study of 12 rye genotypes (Gaikpa et al., 2019). In the current study, the correlation between FHB index and FDK or DON was significant, although significant coefficients were moderate to low. The correlations could be significant at a low value due to the large number of genotypes. However, the small population in the study of Gaikpa et al. (2019) required a high correlation to be significant. Other authors also reported that correlation coefficients between FHB index and DON were in the medium range for rye (Miedaner and Perkowski, 1996; Miedaner et al., 2003). Miedaner et al. (2003) reported that the correlation between FHB index and DON content in fall rye was moderate (r=0.3-0.7) and varied with the environment. Several factors could cause a moderate correlation between FHB index and DON contents (Miedaner, 1997). Variation between environments might influence FHB symptom development and mycotoxin production (Miedaner and Perkowski, 1996; Miedaner, 1997). In a year when the disease levels are high, or many susceptible genotypes are

being evaluated, mycotoxin content may be underestimated due to the loss of infected kernels in the harvested grain (Miedaner, 1997). FHB infection can affect water and nutrient transport within the spike and result in bleaching of spikelets above the primary infection without colonization of the spikelets, leading to higher estimates of FHB infection and lower DON levels in the seed (Miedaner and Perkowski, 1996). As mentioned above, the absence of seed set in infected rye florets also reduce the correlation between visual assessment of FHB levels and DON contents. Measured DON contents could be lower than anticipated from visual clues, likely because in rye most FDK were not harvested.

A series of complicated physical, morphological, and genetic factors of crops regulate FHB infection (Miller et al., 1985). Several studies confirmed the negative association between plant height and FHB infection in wheat (Mesterhazy, 1995; Hilton et al., 1999; Buerstmayr et al., 2000; Somers et al., 2003). However, the correlation between FHB resistance and morphological characteristics, such as plant height, spikelet density, and awnedness was inconsistent (Lu et al., 1990; Yu, 1990; Zhou et al., 1988). The current study showed that the correlation between FHB traits and plant height on rye was just not consistent. In Set 2, FHB incidence, severity, and index were significantly negatively correlated with plant height, and the correlation coefficient was low. However, in Set 1, FHB incidence and index were not significantly associated with plant height, and FHB severity was significantly positively correlated with plant height. In the current study, plant height may have played a less significant role in FHB by inoculation directly on the spikes than the use of natural inoculum or corn spawn inoculum. Passive resistance mechanisms might play a larger role in the difference between wheat and rye than among rye genotypes (Gaikpa et al., 2019). Cultivated rye is, on average, 80 to 180 cm tall, which is much taller than wheat (Schlegel, 2013a; Gaikpa et al., 2019). This might contribute to the higher FHB resistance level of rye compared to wheat since taller plant height has a positive effect on FHB resistance (Mesterhazy, 1995; Gaikpa et al., 2019). In addition, anther characteristics may contribute to a higher FHB resistance on rye than wheat (Gaikpa et al., 2019). In wheat, retained anthers served as a base for invasion into the floret and promoted hyphal growth (Pugh et al., 1933; Buerstmayr and Buerstmayr, 2015). Disease severity of FHB infection is associated with the percentage of retained anthers (Pugh et al., 1933). As an open-pollinated crop, rye anthers are fully extruded at flowering

(Gaikpa et al., 2019). Accordingly, anthers were easily blown away from the spike in the field, and this might reduce the chance of initial infection.

Based on visual field symptoms, FHB did not affect rye as severely as wheat. However, the levels of DON measured on harvested rye seed from FHB nurseries indicated that considerable FHB damage had occurred, resulting in DON contents over official limits of 2 ppm for grain marketing. The potential expansion of rye use in food and feed markets makes it important to understand DON contamination in rye grain. In southern Manitoba, fall rye generally started to flower in early June when the environmental conditions were generally unfavorable for FHB infection. Current risks of natural FHB infection in fall rye are very low. Nevertheless, epidemics of FHB are strongly influenced by local environments, pathogen populations, and cultivars grown (Tekauz et al., 2000; Osborne and Stein, 2007; Popovski and Celar, 2013). Climate change could potentially lead to conditions that favour the disease earlier in the growing season, expand regions over which FHB occurs, and increase disease levels (Tekauz et al., 2000). Therefore, the potential of FHB epidemics in rye should not be overlooked. For instance, barley was not initially considered to be a main host for *Fusarium*, but FHB was found in most barley fields in Manitoba with FHB severity similar to wheat in 1998 (McCallum et al., 1999).

Rye genotypes also showed variable reactions to ergot infection (**Table 5.1** and **Table 5.3**). Some fall rye genotypes were very susceptible to ergot, such as ACE-1 and Wheeler. Although no artificial inoculation was supplied, mist irrigation systems used for FHB nurseries and low rye population density could be conducive to natural ergot infection. In general, KWS Daniello, KWS Gatano, KWS Trebiano, and KWS Serafino containing KWS Pollen Plus™ characteristics had lower ergot severity than others. Ergot severity was highly correlated with anther scores (Miedaner et al. 2021). Therefore, the characteristics leading to higher pollen shedding during flowering could reduce ergot infection (Miedaner and Wilde, 2019; Kodisch et al., 2020). Use of rye genotypes with low ergot severity is important for rye growers to lower ergot infection in fields and sclerotia contamination in grains. No economical chemicals are available to control ergot rather than prevention through cultural practices (Schumann and Uppala, 2000).

In conclusion, the study of open-pollinated rye and hybrids for FHB resistance revealed a wide range of variation for FHB field traits, FDK levels, and DON contents in the combined six

site years. Two open-pollinated rye genotypes (Hazlet and Prima) and six hybrids (KWS Bono, Brasetto, KWS Daniello, KWS Gatano, Guttino, and KWS Trebiano) are commercially grown in western Canada (**Appendix 4**). KWS Daniello, KWS Gatano, KWS Trebiano, and Prima exhibited better FHB resistance and had lower ergot severity than other genotypes. Knowing the level of FHB resistance present in current cultivars helps when attempting to manage the disease in FHB prone areas. Moreover, resistant genotypes could be used as parental lines to develop new rye genotypes with additional agronomic and disease resistance traits required for superior performance in prairies. Moderate correlations between FHB index and DON accumulations indicates that both traits are required to be considered for rye breeders during FHB resistance selection.

Chapter 6

Evaluation of the efficacy of fungicide with different application timings for mitigation of fusarium head blight in fall rye (*Secale cereale* L.)

6.1 Abstract

Fusarium head blight (FHB) is one of the most important diseases in small grain cereals. Several foliar fungicides have been used to manage FHB in North America. Field experiments were conducted in Winnipeg and Carman from 2018 to 2020. They evaluated the efficacy of different application times of the fungicide Prosaro™ (prothioconazole +tebuconazole) for FHB and deoxynivalenol (DON) control in fall rye. Treatments consisted of an inoculated-untreated check, an uninoculated-untreated check, and fungicide applications made at heading, 10% anthesis, 80% anthesis, and six days after 50% anthesis. Fusarium head blight incidence, FHB severity, FHB index, *Fusarium*-damaged kernels, DON, thousand kernel weight, test weight, grain yield, and ergot severity were measured. The efficacy of fungicide application timings was not consistent among locations and years. The highest mean percent control of FHB incidence, severity, and index was achieved with fungicide application at 10% anthesis, while fungicide applied at 80% anthesis resulted in the highest mean efficacy for reducing FDK and DON contents in fall rye. Grain yield was not affected by all fungicide treatments when the disease levels were low, while fungicide applications significantly increased yield in the site year with a high mean FHB index. Although the effect of genotype varied among locations and years, the most resistant genotype, KWS-H161, showed a consistently lower FHB index than the open-pollinated fall rye genotypes. As the first study of fungicide efficacy for FHB in fall rye in Canada, the results provide fall rye growers some basic information on fungicide use to manage FHB if the disease becomes an issue in some areas.

6.2 Introduction

Rye (*Secale cereale* L.) is a member of the grass family, the tribe “Gramineae” (Schlegel, 2013a). However, rye differs from wheat, barley, and oats in that it shows a high degree of self-incompatibility (Schlegel, 2013a). Rye grain can be used for distilling, bread making, and livestock feed (Gaurilcikiene et al., 2011). Fusarium head blight (FHB) of cereals is a fungal disease mainly

caused by *Fusarium graminearum* in North America (Parry et al., 1995; Goswami and Kistler, 2004). The flowering stage is considered to be the most susceptible period for FHB infection in cereals, particularly with prolonged wet weather, high humidity and temperatures between 15 and 30°C (Osborne and Stein, 2007; McMullen et al., 2012). Early infection results in grain yield loss because of floret sterility, or damage to kernels, and accumulation of mycotoxins, such as deoxynivalenol (DON) (McMullen et al., 2012). Likewise, late infection causes DON contamination in the grain, but may be less damaging to grain yield (Del Ponte et al., 2007; McMullen et al., 2012). The accumulation of mycotoxin is a concern for the milling and baking industries, and the animal feed market, as it adversely affects the health of humans and livestock (Desjardins, 2006).

The strategies for management of FHB generally include combinations of cultural practices, use of resistant cultivars, chemical control, biological control, and disease forecasting (Pirgozliev et al., 2003; Gilbert and Tekauz, 2011; Wegulo et al., 2015). Control of FHB aims to limit yield losses and reduce DON accumulation in commercial cereal grains (McMillen et al., 2012; Gilbert and Haber, 2013; Wegulo et al., 2015). Fungicide application is one of the management strategies for suppressing FHB and reducing DON levels (Wegulo et al., 2015). Several fungicides including carbendazim, hexaconazole, mancozeb, benomyl, prochloraz, propiconazole, tebuconazole, and triadimenol are useful for FHB management (Dweba et al., 2017). In Canada, some registered products, such as Caramba™ (metconazole), Folicur™ (tebuconazole), Proline™ (prothioconazole), and Prosaro™ (tebuconazole + prothioconazole), are currently available for FHB suppression on wheat (Gilbert and Tekauz, 2011; Guide to Crop Protection, 2021). However, some countries like South Africa have no fungicide registered to manage FHB on wheat and barley (Dweba et al., 2017).

Ideally, fungicides are expected to reduce FHB infection to less than 5% in fields, and *Fusarium*-damaged kernels (FDK) should be minimal or absent (Shah et al., 2018). However, fungicide efficiency is generally inconsistent and often insufficient for controlling FHB under field conditions (Pirgozliev et al., 2003; Lehoczki-Krsjak et al., 2010; Mesterhazy et al., 2011). Fungicide triazoles, such as tebuconazole, metconazole, and prothioconazole, are the most effective fungicides to suppress FHB symptoms and decrease mycotoxin contamination (Edwards et al., 2001; Pirgozliev et al., 2002; Paul et al., 2008, 2010). According to a quantitative analysis

of data from more than 100 wheat trials, tebuconazole + prothioconazole was the most effective fungicide for FHB index reduction, followed by metconazole, prothioconazole, tebuconazole, and propiconazole (Paul et al., 2008). For reduction of DON contents, metconazole was the most effective fungicide, followed by prothioconazole, tebuconazole + prothioconazole, tebuconazole, and propiconazole (Paul et al., 2008). Data collected from uniform fungicide trials across the United States showed that tebuconazole provided a 40% and 22% reduction of FHB index and DON, respectively (Paul et al., 2007). Tebuconazole + prothioconazole is more effective than tebuconazole for control of both FHB index and DON in wheat (Lehoczki-Krsjak et al., 2008; Paul et al., 2008; Mesterhazy et al., 2011; Haidukowski et al., 2012). The combination of fungicide application and resistant wheat cultivars resulted in more than 70% reduction of FHB and DON compared to the untreated susceptible check (Sip et al., 2010; Willyerd et al., 2012; Mesterhazy et al., 2018a). In susceptible wheat cultivars, fungicide failed to reduce DON accumulations low enough for grain acceptance, despite the significant reduction of FHB levels (Mesterhazy et al., 2012). Meanwhile, under a high disease pressure, a fungicide may not reduce disease in susceptible wheat cultivars adequately to produce marketable yields (Mesterhazy et al., 2003).

Fungicide effectiveness on FHB management is impacted by several factors, such as fungicide chemical class, the timing of fungicide application, application rate, and application techniques (Mesterhazy, 2003; Paul et al., 2007, 2008; Freije et al., 2015). It is commonly accepted that fungicide application at, or just before, anthesis is crucial for FHB control in wheat (Mesterhazy, 2003; Yoshida et al., 2012; Tateishi et al., 2014). These recommendations are based on wheat which is most susceptible to FHB during flowering, and initial infection of FHB usually occurs on extruded anthers (Sutton, 1982; Parry et al., 1995). However, several practical factors limit fungicide application to meet the optimal application timing (Freije et al., 2015). For example, temporal variation of tiller development in the same field contributes to variable time points of anthesis (Freije et al., 2015; Paul et al., 2018). As a result, the flowering period in the same field spreads over several days, depending on growing conditions and cultivar growth characteristics (Freije et al., 2015; Paul et al., 2018). Variability of anthesis within a field can influence the efficacy of fungicide application (Freije et al., 2015). Moreover, wet field condition during anthesis benefits the infection of FHB, but poses an obstacle for fungicide application when fungicides are most warranted (D'Angelo et al., 2014; Freije et al., 2015; Paul et al., 2018). These limitations

have led to questions about the benefit of pre-and post-anthesis fungicide application for FHB and DON management (D'Angelo et al., 2014; Freije et al., 2015; Paul et al., 2018; Feksa et al., 2019).

There is limited information on the efficacy of fungicide application and optimal application timing for FHB and DON management in fall rye. The label directions for the fungicide Prosaro™ recommend application at the early anthesis stage to suppress FHB in spring and winter wheat. The optimum time to spray Prosaro™ for the best wheat yield results, is between one and two days after heading (Bayer Crop Science). However, the timing of fungicide application in barley is slightly different from wheat (McMullen et al., 2012). The optimum fungicide application timing in barley is full head emergence rather than early anthesis because barley spikes start to flower at the boot stage (McMullen et al., 2012). Compared to winter wheat, the flowering period of rye lasts much longer, which may extend up to two weeks. It is unknown how the extended flowering period of fall rye affects the efficacy of fungicide application for FHB control. Previous studies have not evaluated the efficacy of the fungicide Prosaro™ for FHB control in fall rye. The objectives of this study were to:

1) evaluate the effect of fungicide application on FHB field traits, FDK levels, DON contents, thousand kernel weight (TKW), test weight (TWT), grain yield, and ergot severity
2) compare the efficacy of fungicide treatments with different application timings against FHB relative to the inoculated-untreated check
3) estimate the effect of genotypes on FHB field traits, FDK levels, DON contents, TKW, TWT, grain yield, and ergot severity
4) determine correlations among FHB incidence, FHB severity, FHB index, FDK, DON, TKW, TWT, grain yield, and ergot severity.

6.3 Materials and methods

6.3.1 Experimental design

Three fall rye genotypes were grown at two sites from 2018 to 2020. One site was the Ian N Morrison Research Farm, the University of Manitoba, Carman, Manitoba; another was the Point

field research station, the University of Manitoba, Winnipeg, Manitoba. The trial at Carman in 2018 was lost due to the winter kill.

The experimental design was a split-plot design with four replications. There were only three replicates in 2020 Winnipeg as one replicate was in standing water after seeding in fall and lost in the following spring. The main plot effect was four fungicide application timings and two checks. The fungicide application timings included heading, 10% anthesis, 80% anthesis, and six days after 50% anthesis, and the two checks were an inoculated-untreated control and an uninoculated-untreated control. Main plots were separated by buffer plots of the fall rye cultivar Prima. Prima is taller than the three fall rye genotypes used in the experiment and, as a result, reduced potential fungicide drift among main plots.

The subplot effect was three fall rye genotypes. In 2018 and 2019, the three fall rye genotypes used were KWS-H161 (Resistant (R)), Dakota (Moderately susceptible (MS)), and Vitallo (Intermediate (I)), which represented different levels of FHB resistance determined in previous studies. In 2020, Hazlet (I) replaced Vitallo due to insufficient Vitallo seed, and the other two genotypes were the same as the previous years. Plots were 3 m long and 1.5 m wide. Each plot contained six rows spaced 17 cm apart and was sown at a rate of 1200 seeds/plot.

Inoculated-untreated control plots were inoculated with *F. graminearum* and did not have fungicide application, while uninoculated-untreated control plots were not inoculated and had no fungicide application. Except for the uninoculated-untreated plots, all plots were inoculated with *F. graminearum* (5×10^5 spores/mL) three times at 30%, 50%, and 70% anthesis using an R&D Sprayer with a six-nozzle boom (BellSprayer Inc., USA). Air pressure for the sprayer was calibrated at 30 psi. The inoculation rate was 1 L of inoculum per plot. Different CO_2-powered backpack sprayers were used for inoculation and fungicide application. There was a two-day window between inoculation and fungicide application. Overhead mist irrigation was operated for 10 minutes per hour for 10 hours daily and continued for a week after inoculation.

6.3.2 Fungal inoculum

The *F. graminearum* inoculum used for the inoculation was a mixture of two isolates of the 15-acetyldeoxynivalenol (15-ADON) chemotype and two isolates of the 3-

acetyldeoxynivalenol (3-ADON) chemotype. The 15-ADON chemotype isolates included HSW-15-27 and HSW-15-57, and the 3-ADON chemotype isolates included HSW-15-39 and HSW-15-87. The isolates were provided by Dr. Maria Antonia Henriquez at the Morden Research and Development Centre, Agriculture and Agri-Food Canada (AAFC). All macroconidia suspensions were initially produced from single spore cultures of *F. graminearum* and first cultured in spezieller nährstoffarmer agar (SNA) media (**Appendix 1**) plates under fluorescent light at room temperature (21 to 24 ℃) for one week. Then cultures from ten to fifteen SNA media plates were transferred into the flask with aerated liquid carboxymethyl cellulose (CMC) media (**Appendix 1**). The CMC media were cultured under fluorescent light at room temperature (21 to 24 ℃) for another week. Subsequently, the macroconidia suspensions were collected from CMC media by filtering through two layers of the sterile cheesecloth. The macroconidia suspensions were stored in sterile glass bottles at 4 ℃ for up to two weeks. The concentration of the macroconidia was determined using a hemocytometer cell counter (Hausser Scientific, USA). Equal concentrations of each isolate were combined and diluted to 5×10^5 spores/mL using distilled water right before the inoculation. The surfactant Tween™ 20 (VWR international, USA) was added at a volume of 4 mL per two liters of inoculum.

6.3.3 Fungicide

A commercially available foliar fungicide Prosaro™ 250 EC (prothioconazole and tebuconazole) manufactured by Bayer Crop Science Inc. (Canada) was used in the experiment. Prosaro™ is used for suppression of FHB and control of rust, leaf blotch, tan spot, and powdery mildew (Prosaro™ 250 EC label). The application rate of Prosaro™ used was 800 mL/ha as recommended for wheat, barley, and oat.

6.3.4 Disease and agronomic traits evaluation

Plant stand in spring was estimated as the percentage of plant cover for each plot before seedlings started tillering (around mid-May). With the exception of Vitallo, plant stands of Dakota, Hazlet, and KWS-H161 generally ranged from 60% to 90% in Winnipeg from 2018 to 2020. In Carman, plant stands of all genotypes were close to, or over, 90% in 2019 and 2020. Due to the ability of fall rye to tiller, plots with plant stand over 60% were considered to be sufficient for the

expression of yield. Naturally infected ergot severity was evaluated as the percentage of sclerotia in the grain of a whole plot by weight (Miedaner and Geiger, 2015).

Fusarium head blight incidence and severity were assessed for each plot when FHB symptoms were well developed, which was generally 25 to 28 days after the first inoculation in fall rye. Fusarium head blight incidence was recorded as the percentage of infected spikes in each plot, and FHB severity was assessed as the average percentage of infected spikelets in diseased spikes (Stack and McMullen, 1998). Fusarium head blight index for each plot was calculated using the formula: (FHB incidence (%) × FHB severity (%))/100. Fusarium head blight incidence and severity of each plot were separately rated by two people on the same day, and the mean rating was used, except for the data from 2020. This was done to reduce the chance of bias or error in the collection of the data. Efficacy was calculated by the function for all traits y = [(inoculated-untreated control - treated)/ inoculated- untreated control] × 100 (Mesterhazy, 2003).

At maturity, each plot was mechanically harvested using a Wintersteiger Classic plot combine (Wintersteiger AG). The harvested seeds were dried on forced-air drying beds set at 30°C for 48 to 72 h. Then seed was cleaned by removing straw and chaff using a blower (Bill's Welding LLC., USA). Seed samples of 2018 Winnipeg were tested by SGS BioVision, Winnipeg, and all other seed samples were tested by Central Testing Laboratory Ltd., Winnipeg. The percentage of FDK of each plot was measured as the weight of *Fusarium* affected kernels in a fifty-gram sample randomly taken from the whole seed sample. Fifty-grams of grain were then ground, and ten-grams of each ground sample were used for DON analysis using enzyme-linked immunosorbent assay (ELISA) with RIDASCREEN™FAST DON kit (R-Biopharm AG, Germany). Dilutions were done if DON exceeded the detection limits of the kit (personal communication with Yvan Bruneau from the Central Testing Laboratory Ltd., Canada).

One thousand seeds from each plot were counted using a seed counter (Model U, International Marketing and Design Co., USA) and weighed to determine TKW. Test weight was measured as grams per half a liter for each plot according to the Official Grain Grading Guide of the Canadian Grain Commission. The actual plot size was determined by multiplying plot width by length. Due to variation of plot length, the two long sides of the plot were measured and averaged to calculate the plot size. Total grain weight from each plot was determined by weighting

the cleaned and dried samples. Then grain yield per area was calculated as y (t/ha) = [grain weight per plot (g)/plot area in square meters] × 0.01.

6.3.5 Weather conditions

Daily high, low, and average temperatures and precipitation for 30 days after the first inoculation in 2018, 2019, and 2020 were collected from the Environment Canada weather stations at Carman and Winnipeg.

6.3.6 Statistical analysis

Statistical analyses were performed using SAS programs (SAS version 9.4, SAS Institute, USA). Based on the evaluation of single site year data using PROC UNIVARIATE, residuals conformed to a normal distribution, and the plot of residuals was symmetrically distributed within site year. Levene's tests for homogeneity of variance indicated that residual variances were not homogeneous when data were combined over site years. Accordingly, data for each site year were analyzed separately, with treatment, variety, and their interaction as fixed effects, and replicate and the interaction between replicate and treatment as random effects in the analyses. Analyses of variance (ANOVA) for FHB incidence, FHB severity, FHB index, FDK, DON, TWT, TKW, grain yield, and ergot severity was performed using the PROC MIXED procedure in SAS. Eta squared was calculated as described by Brown (2008) to determine the percentage of variance associated with each of the main effects, interactions, and errors in an ANOVA analysis. The least significant difference (LSD, p=0.05) was used for comparisons of means for main effects and their interactions for each response variable at a 5% probability. Box plots were generated using the PROC SGPLOT procedure. Correlation coefficients between response variables were generated using the PROC CORR procedure.

6.4 Results

6.4.1 Weather conditions

In 2018, daily average temperatures mainly remained in the range of 15 to 25℃ and had a relatively small daily variation (**Figure 6.1**). There were 18 precipitation events for a total accumulation of 79.9 mm in the 30 days after the first inoculation in Winnipeg (the precipitation

information on June 21st was missing). In 2019, daily temperatures were highly variable during June and July in both Carman and Winnipeg (**Figure 6.2**). In the 30 days after the first inoculation, Winnipeg had 11 precipitation events for a total precipitation of 96.6 mm, and Carman had 12 precipitation events for a total accumulation of 70.1 mm. However, precipitation was concentrated in the last week of the 30 days. The first three weeks were rather dry, and the precipitation was only 11.5 and 32.9 mm in Winnipeg and Carman, respectively, and accompanied by hot weather. In 2020, daily average temperatures were above 20 ℃ in the first three days after the first inoculation in Carman (**Figure 6.3**). However, inoculation was accompanied by cool weather in Winnipeg, where daily average temperatures were lower than 20℃ in the first week after the first inoculation. Later, the weather was warm and even hot in both Carman and Winnipeg. In 2020, sometimes daily high temperatures were over 30℃ in Carman and Winnipeg. Precipitation in 2020 was relatively even during June and July compared to 2019, and Carman (84.7 mm) had more precipitation than Winnipeg (70.2 mm).

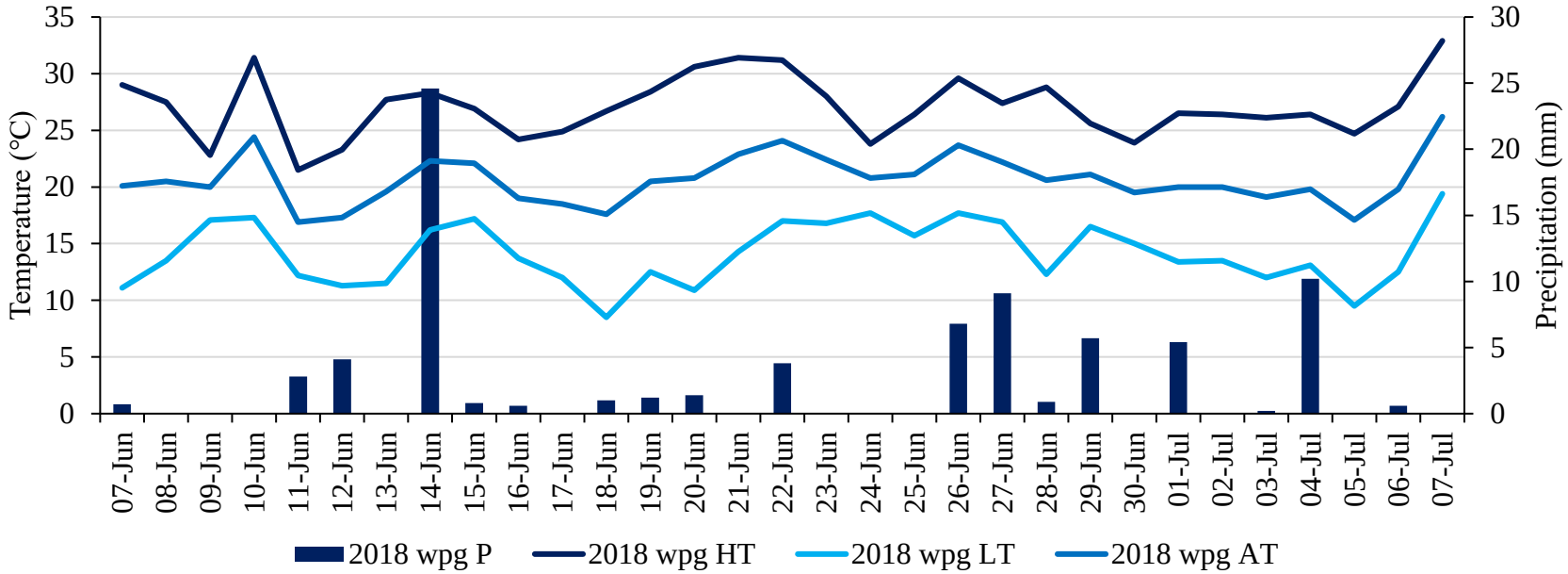

Figure 6.1 Daily high (HT), low (LT), and average temperatures (AT) (solid line) and precipitation (P) (bars) during the 30 days after the first inoculation in 2018 Winnipeg (wpg).

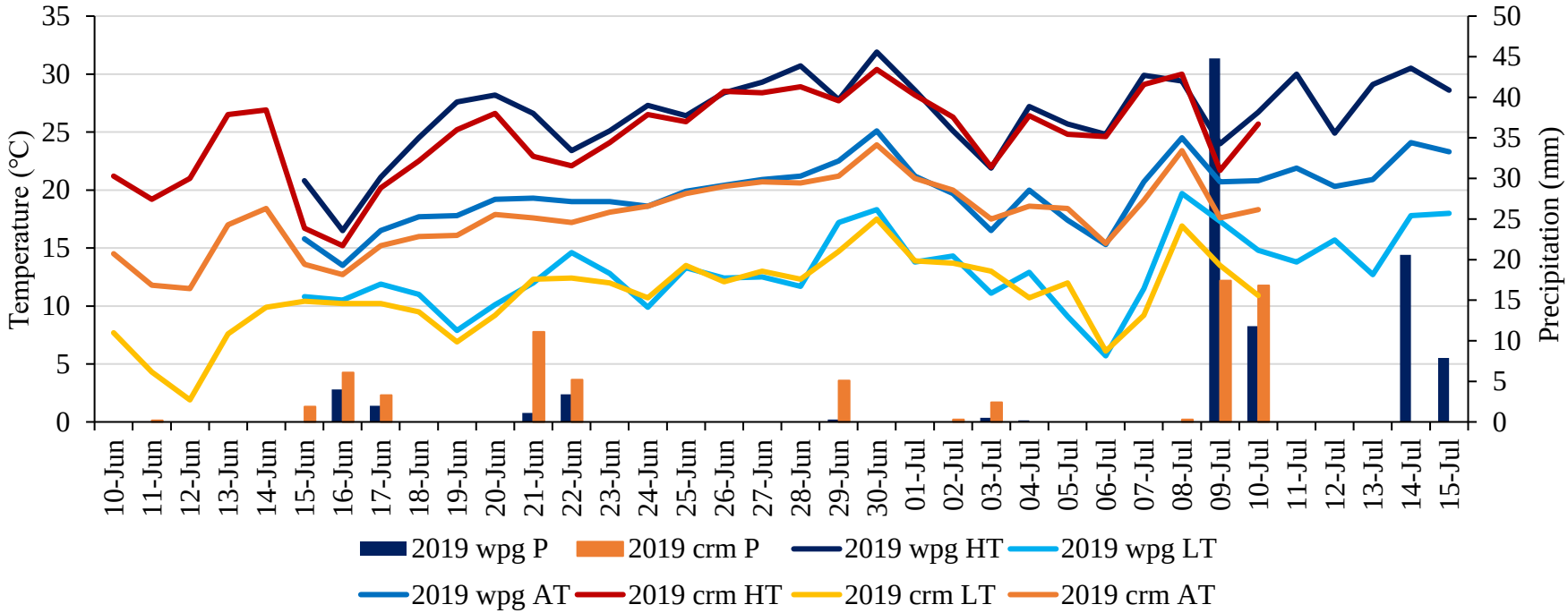

Figure 6.2 Daily high (HT), low (LT), and average temperatures (AT) (solid line) and precipitation (P) (bars) during the 30 days after the first inoculation in 2019 Winnipeg (wpg) and 2019 Carman (crm).

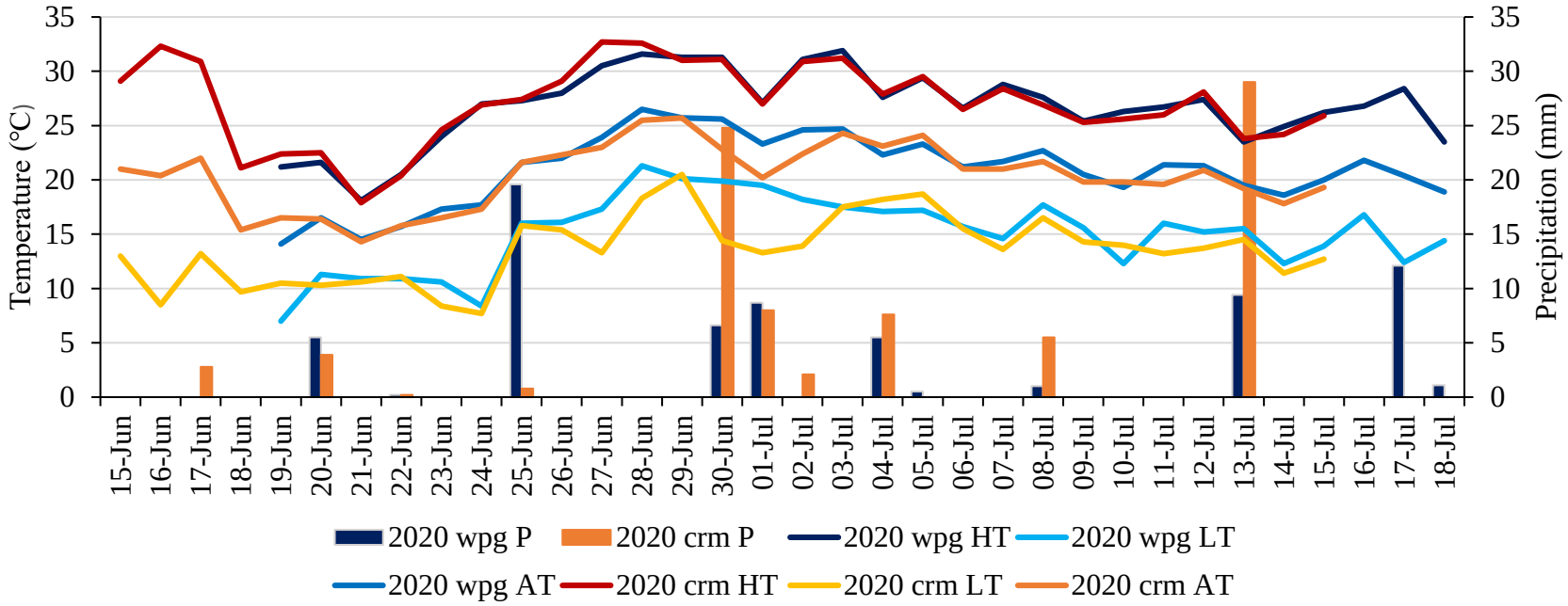

Figure 6.3 Daily high (HT), low (LT), and average temperatures (AT) (solid line) and precipitation (P) (bars) during the 30 days after the first inoculation in 2020 Winnipeg (wpg) and 2020 Carman (crm).

6.4.2 Analysis of variance for response variables

The effect of treatment on FHB field traits (FHB incidence, severity, and index) was highly significant and contributed to the highest proportion of variation in all five site years (**Table 6.1** and **Appendix 20**). The effect of genotype was also significant for these measured variables except FHB incidence in 2018 Winnipeg and 2019 Carman. The interaction between treatment and genotype was consistently significant for FHB severity, but was only statistically significant for FHB incidence and index in 2019 Winnipeg, 2020 Winnipeg, and 2020 Carman. The proportion of variance contributed by the two-way interaction between treatment and genotype were much smaller than the main effect of treatment for FHB field traits in all site years (**Appendix 20**). The significant treatment by genotype interaction was explained mainly by changes in magnitude between genotypes for the treatments for FHB field traits rather than changes in rank. The effect of treatment on FDK and DON was significant in all site years and generally accounted for the largest portion of the total variation (**Table 6.1** and **Appendix 20**). The genotype effect was significant for FDK and DON in all site years except for DON in 2020 Carman. The interaction between treatment and genotype only had a significant effect on FDK in 2020 Carman. However, the effect of the interaction between treatment and genotype was significant for DON in all site years except 2018 Winnipeg. In general, the contribution of the treatment by genotype interaction to the total variation was relatively small compared to the contribution of the treatment effect for FDK and DON (**Appendix 20**).

Table 6.1 Analyses of variance for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) of fungicide trials conducted in 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

Site year	Source of variance	DF	Incidence	Severity	Index	FDK	DON
2018 Winnipeg	Trt	5	****	****	****	***	****
	Geno	2	ns	****	****	**	**
	Trt*Geno	10	ns	*	ns	ns	ns
	Rep	3	ns	ns	ns	ns	ns
	Rep*Trt	15	ns	ns	ns	ns	ns
2019 Winnipeg	Trt	5	****	****	****	****	****
	Geno	2	****	****	****	**	****
	Trt*Geno	10	*	***	****	ns	**
	Rep	3	ns	*	*	ns	ns
	Rep*Trt	15	ns	ns	ns	ns	ns
2019 Carman	Trt	5	****	****	****	****	****
	Geno	2	ns	****	***	****	****
	Trt*Geno	10	ns	**	ns	ns	***
	Rep	3	**	ns	ns	*	*
	Rep*Trt	15	ns	ns	ns	ns	ns
2020 Winnipeg	Trt	5	****	****	***	*	**
	Geno	2	****	****	****	*	****
	Trt*Geno	10	*	*	****	ns	*
	Rep	2	ns	ns	ns	*	ns
	Rep*Trt	10	ns	ns	ns	ns	ns
2020 Carman	Trt	5	****	****	****	****	****
	Geno	2	***	****	****	**	ns
	Trt*Geno	10	**	***	***	*	*
	Rep	3	ns	ns	ns	ns	ns
	Rep*Trt	15	ns	ns	ns	ns	**

DF=degree of freedom; Trt=Treatment; Geno=Genotype; Rep=replicate.
ns=not significant, * significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$, and **** significant at $p<0.0001$.

The effect of treatment was statistically significant for TKW and TWT in all site years except 2019 Carman (**Table 6.2**). However, treatment was not statistically significant for yield in all site years except 2020 Carman. The effect of genotype on TKW, TWT, and yield was

significant in all site years and generally accounted for the largest portion of the total variation (**Appendix 20**). The interaction between treatment and genotype significantly affected TKW in 2019 Winnipeg, TWT in 2018 Winnipeg, 2019 Winnipeg, and 2020 Carman, and yield in 2020 Carman. The treatment effect was significant for ergot severity in 2019 Winnipeg and 2020 Carman (**Table 6.2**). The genotype effect on ergot severity was significant in all site years. The highest proportion of variation for ergot severity was attributed to treatment in 2020 Carman, and to genotype in all other site years. The interaction between treatment and genotype was significant for ergot severity in 2019 Winnipeg, 2019 Carman, and 2020 Carman.

Table 6.2 Analyses of variance for thousand kernel weight (TKW), test weight (TWT), grain yield, and ergot severity of fungicide trials conducted in 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

Site year	Source of variance	DF	TKW	TWT	Yield	Ergot
2018 Winnipeg	Trt	5	*	****	ns	ns
	Geno	2	***	****	****	****
	Trt*Geno	10	ns	*	ns	ns
	Rep	3	**	**	**	***
	Rep*Trt	15	ns	ns	ns	ns
2019 Winnipeg	Trt	5	***	****	ns	*
	Geno	2	****	****	****	****
	Trt*Geno	10	**	*	ns	**
	Rep	3	**	***	ns	ns
	Rep*Trt	15	ns	ns	****	ns
2019 Carman	Trt	5	ns	ns	ns	ns
	Geno	2	****	****	****	****
	Trt*Geno	10	ns	ns	ns	*
	Rep	3	**	*	*	ns
	Rep*Trt	15	***	***	**	ns
2020 Winnipeg	Trt	5	**	**	ns	ns
	Geno	2	****	****	****	****
	Trt*Geno	10	ns	ns	ns	ns
	Rep	2	ns	*	**	ns
	Rep*Trt	10	ns	ns	*	ns
2020 Carman	Trt	5	*	****	****	****
	Geno	2	****	****	****	****
	Trt*Geno	10	ns	*	***	**
	Rep	3	ns	ns	ns	**
	Rep*Trt	15	ns	*	**	ns

DF=degree of freedom; Trt=Treatment; Geno=Genotype; Rep=replicate.
ns=not significant, * significant at $p<0.05$, ** significant at $p<0.01$, *** significant at $p<0.001$, and **** significant at $p<0.0001$.

6.4.3 Mean fusarium head blight index

The distribution of FHB index was similar among site years except for 2020 Carman (**Figure 6.4**). Mean FHB index was much higher in 2020 Carman than the other four site years.

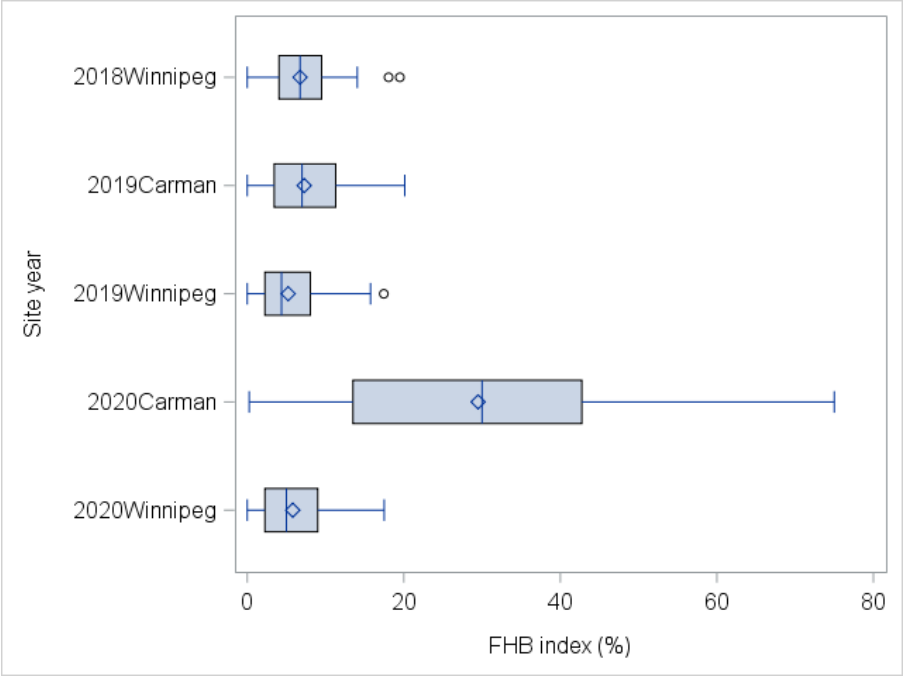

Figure 6.4 Box plots showing the distribution of fusarium head blight (FHB) index of fungicide trials conducted in 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman. The diamond and solid lines within the box represent the mean and median, respectively, whereas the top and bottom lines of the box represent the 75th and 25th percentiles of the data, respectively. Vertical bars represent the minimum and maximum, and circles indicate outliers.

6.4.4 Fusarium head blight (FHB) incidence, severity, index, FDK, and DON assessment

A comparison of fungicide treatment means across three fall rye genotypes showed that not all fungicide treatments significantly reduced FHB incidence, severity, and index in relation to the inoculated-untreated control in all site years except 2020 Carman (**Table 6.3**). In 2018 Winnipeg, FHB incidence and index were significantly reduced by the application of fungicide at 80% anthesis and six days after 50% anthesis, while FHB severity was only significantly reduced by the application of fungicide at 80% anthesis, compared to the inoculated-untreated control. There were no significant differences in FHB severity and index between fungicide treatments irrespective of their application timings in 2018 Winnipeg. In 2019 Winnipeg, fungicide applied at heading and 10% anthesis resulted in significantly lower FHB severity and index than fungicide

applied at 80% anthesis, six days after 50% anthesis, and the inoculated-untreated control. FHB incidence was significantly reduced by the application of fungicide at heading, compared to fungicide application at six days after 50% anthesis and the inoculated-untreated control. In 2020 Winnipeg, no significant differences in FHB incidence were observed between fungicide treatments and the inoculated-untreated control. FHB severity was significantly reduced by the application of fungicide at 10% anthesis, 80% anthesis, and six days after 50% anthesis, and FHB index was significantly decreased by the application of fungicide at 80% anthesis and six days after 50% anthesis compared to the inoculated-untreated control. However, the effect of fungicide treatment on FHB severity and index did not significantly vary with the application timings. In 2019 Carman, FHB incidence and index were significantly reduced by applying fungicide at 10% anthesis compared with the inoculated-untreated control and other fungicide treatments. However, FHB severity was not significantly reduced by any fungicide treatments. In 2020 Carman, all fungicide treatments resulted in significantly lower FHB severity and index than the inoculated-untreated control. Among the different fungicide treatments, FHB incidence, severity, and index were significantly lower when fungicide was applied at 10% anthesis.

The range of toxin levels varied among years and locations (**Table 6.3**). In the inoculated-untreated check, the lowest DON content was 1.07 ppm in 2018 Winnipeg, while the highest DON content was 46.30 ppm in 2020 Carman. In 2018 Winnipeg, fungicide applied at 80% anthesis and six days after 50% anthesis significantly lowered FDK and DON compared to the inoculated-untreated control and fungicide applied at heading and 10% anthesis. In 2019 Winnipeg, all fungicide treatments resulted in significantly lower FDK and DON than the inoculated-untreated control, except for fungicide application at 80% anthesis for FDK. In 2020 Winnipeg, no significant differences in FDK and DON were observed between fungicide treatments and the inoculated-untreated control. In 2019 Carman, all fungicide treatments significantly reduced DON, but did not significantly affect FDK compared to the inoculated-untreated control. In 2020 Carman, all fungicide treatments significantly reduced FDK and DON relative to the inoculated-untreated control. Among the fungicide treatments, fungicide applied at 10% anthesis resulted in significantly lower FDK. Also, fungicide applied at 10% anthesis resulted in significantly lower DON content than fungicide applied at heading and six days after 50% anthesis.

Table 6.3 Treatment means for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON), across genotypes in 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

Site year	Treatment	Incidence (%)	Severity (%)	Index (%)	FDK (%)	DON (ppm)
2018 Winnipeg	Uninoculated-untreated	3.14d	3.47c	0.17c	0.00c	0.01c
	Heading	36.67abc	21.25ab	8.03ab	0.15a	0.95a
	10% anthesis	39.79ab	20.42ab	8.31ab	0.15a	1.08a
	80% anthesis	34.38bc	18.54b	6.39b	0.09b	0.46b
	6 days after 50% anthesis	33.13c	21.88ab	7.28b	0.10b	0.52b
	Inoculated-untreated	41.67a	23.75a	9.73a	0.17a	1.07a
2019 Winnipeg	Uninoculated-untreated	1.04c	0.83c	0.03c	0.01c	0.08c
	Heading	37.08b	11.46b	4.40b	0.61b	6.37b
	10% anthesis	41.88ab	11.04b	4.86b	0.75b	7.11b
	80% anthesis	46.67ab	13.75a	7.03a	0.78ab	6.43b
	6 days after 50% anthesis	48.13a	15.00a	7.29a	0.64b	5.71b
	Inoculated-untreated	50.63a	14.58a	7.79a	1.02a	10.67a
2020 Winnipeg	Uninoculated-untreated	0.56b	0.56c	0.03c	0.15c	0.32c
	Heading	58.89a	12.22ab	7.56ab	0.83a	2.94a
	10% anthesis	53.33a	11.67b	6.72ab	0.58ab	2.17ab
	80% anthesis	51.11a	10.56b	5.78b	0.39bc	1.63b
	6 days after 50% anthesis	58.89a	11.11b	5.37b	0.49b	1.83b
	Inoculated-untreated	57.78a	15.00a	9.11a	0.64ab	2.38ab
2019 Carman	Uninoculated-untreated	3.13d	2.08b	0.13c	0.01b	0.07c
	Heading	46.67b	16.67a	8.11a	1.26a	11.95b
	10% anthesis	35.21c	13.54a	4.91b	1.38a	10.31b
	80% anthesis	58.33a	14.79a	8.94a	1.01a	10.22b
	6 days after 50% anthesis	62.71a	17.08a	10.78a	1.23a	12.75b
	Inoculated-untreated	63.75a	16.88a	10.73a	1.24a	18.00a
2020 Carman	Uninoculated-untreated	7.50d	5.00d	0.38d	0.14e	0.78e
	Heading	97.92a	34.17b	33.40b	8.31b	34.50b
	10% anthesis	89.58c	23.33c	21.46c	4.95d	22.73d
	80% anthesis	93.33b	31.25b	29.40b	6.47c	25.67cd
	6 days after 50% anthesis	94.17b	36.25b	34.42b	6.77c	28.47c
	Inoculated-untreated	100.00a	57.92a	57.92a	12.11a	46.30a

Within site years, means followed by the same letter within a column are not significant at $p<0.05$.

The efficacy of fungicide in FHB incidence, severity, index, FDK, and DON was not consistent across site years (**Table 6.4**). In 2018 Winnipeg, fungicide applied at 80% anthesis provided greater control of FHB severity, FHB index, FKD, and DON than other fungicide treatments, while the reduction of FHB incidence was higher with application of fungicide at six days after 50% anthesis. In 2019 Winnipeg, fungicide applied at heading resulted in the highest reduction of FHB incidence, FHB index, and FDK. In comparison, the reduction of FHB severity and DON was higher for fungicide application at 10% anthesis and six days after 50% anthesis, respectively. In 2020 Winnipeg, fungicide applied at 80% anthesis provided greater control of FHB incidence, FHB severity, FKD, and DON, while the reduction of FHB index was higher with application of fungicide at six days after 50% anthesis. In 2019 Carman, fungicide applied at 10% anthesis resulted in a higher reduction of FHB incidence, severity, and index, and fungicide applied at 80% anthesis resulted in a higher reduction of FDK and DON. In 2020 Carman, fungicide applied at 10% anthesis provided the highest reduction of all five FHB parameters. According to the combined mean, fungicide application at 10% anthesis had the highest mean efficacy for FHB incidence, severity, and index, and fungicide application at 80% anthesis had the highest mean efficacy for decreasing FDK and DON. Among site years, the best fungicide timing gave a reduction of 44.77%, 59.72%, 62.95%, 59.12%, and 57.01% for FHB incidence, FHB severity, FHB index, FDK, and DON, respectively. A negative efficacy in FHB incidence occurred with fungicide application at heading and six days after 50% anthesis in 2020 Winnipeg. Fungicide applied at six days after 50% anthesis resulted in negative efficacy in FHB severity in 2019 Winnipeg and 2019 Carman, and FHB index in 2019 Carman. Fungicide applied at heading and 10% anthesis in 2019 Carman and at heading in 2020 Winnipeg provided negative efficacy for reducing FDK. The efficacy for DON was negative when fungicide was applied at 10% anthesis in 2018 Winnipeg and at heading in 2020 Winnipeg.

Table 6.4 Efficacy (%) of fungicide treatment for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) across genotypes in 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

	Treatment	Incidence	Severity	Index	FDK	DON
2018 Winnipeg	Heading	12.00	10.53	17.47	11.76	11.21
	10% anthesis	4.51	14.02	14.59	11.76	-0.93
	80% anthesis	17.49	21.94	34.33	47.06	57.01
	6 days after 50% anthesis	20.49	7.87	25.18	41.18	51.40
2019 Winnipeg	Heading	26.76	21.40	43.52	40.20	40.30
	10% anthesis	17.28	24.28	37.61	26.47	33.36
	80% anthesis	7.82	5.69	9.76	23.53	39.74
	6 days after 50% anthesis	4.94	-2.88	6.42	37.25	46.49
2020 Winnipeg	Heading	-1.92	18.53	17.01	-29.69	-23.53
	10% anthesis	7.70	22.20	26.23	9.38	8.82
	80% anthesis	11.54	29.60	36.55	39.06	31.51
	6 days after 50% anthesis	-1.92	25.93	41.05	23.44	23.11
2019 Carman	Heading	26.79	1.24	24.42	-1.61	33.61
	10% anthesis	44.77	19.79	54.24	-11.29	42.72
	80% anthesis	8.50	12.38	16.68	18.55	43.22
	6 days after 50% anthesis	1.63	-1.18	-0.47	0.81	29.17
2020 Carman	Heading	2.08	41.00	42.33	31.38	25.49
	10% anthesis	10.42	59.72	62.95	59.12	50.91
	80% anthesis	6.67	46.05	49.24	46.57	44.56
	6 days after 50% anthesis	5.83	37.41	40.57	44.10	38.51
Means	Heading	13.14	18.54	28.95	10.41	17.42
	10% anthesis	16.94	28.00	39.13	19.09	26.98
	80% anthesis	10.41	23.13	29.31	34.95	43.21
	6 days after 50% anthesis	6.19	13.43	22.55	29.35	37.73

Efficacy was calculated by the function y = [(inoculated-untreated control - treated)/inoculated-untreated control] × 100

A comparison of genotype means across treatments showed that Vitallo had consistently lower FHB incidence than Dakota and KWS-H161, but they were not significantly different from each other in 2018 Winnipeg and 2019 Carman (**Table 6.5**). In 2020, KWS-H161 had consistently lower FHB incidence than Dakota and Hazlet in both locations. The hybrid KWS-H161 had significantly lower FHB severity and index than the open-pollinated genotypes in all site years.

The two open-pollinated genotypes showed significant differences, but their magnitude was associated with locations. Vitallo had lower FHB incidence, severity, and index than Dakota in Winnipeg, while Dakota was lower for all three FHB parameters than Vitallo in Carman. Similarly, Dakota had significantly lower FHB incidence, severity, and index than Hazlet in Winnipeg. However, Hazlet showed significantly lower FHB incidence, severity, and index than Dakota in Carman. The performance of genotypes for toxin levels differed from their FHB field symptoms. The hybrid KWS-H161 did not always have a significantly lower FDK and DON than the open-pollinated genotypes. Vitallo had consistently lower FDK and DON than Dakota, and the difference was significant in 2019, but not in 2018. Hazlet had lower DON than Dakota in 2020 Carman, but they were not significantly different. The rank of Hazlet and Dakota for FDK was not consistent across the two locations.

Comparison of fungicide treatments between resistant genotype KWS-H161 and intermediate genotype Vitallo, or moderately susceptible genotype Dakota, showed that FHB severity and index were lower in the resistant genotype than in the intermediate or moderately susceptible genotype in all site years (**Appendix 21**, **Appendix 22**, **Appendix 23**, **Appendix 24**, and **Appendix 25**). However, FHB incidence, FDK, and DON were not consistently lower in the resistant genotype than in the intermediate or moderately susceptible genotype in all site years.

Table 6.5 Genotype means for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), and deoxynivalenol (DON) across treatments in 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

Site year	Genotype	Incidence (%)	Severity (%)	Index (%)	FDK (%)	DON (ppm)
2018 Winnipeg	Dakota	33.54a	23.33a	8.89a	0.14a	0.87a
	Vitallo	28.96a	19.65b	6.66b	0.13a	0.72a
	KWS-H161	31.88a	11.67c	4.40c	0.06b	0.45b
2019 Winnipeg	Dakota	48.54a	13.44a	8.06a	0.81a	9.03a
	Vitallo	30.52b	11.98a	4.45b	0.60b	4.54b
	KWS-H161	33.65b	7.92b	3.19c	0.48b	4.61b
2019 Carman	Dakota	45.83a	14.48b	8.13a	1.34a	12.80a
	Vitallo	43.54a	17.08a	8.81a	0.92b	6.94b
	KWS-H161	45.52a	8.96c	4.85b	0.80b	11.90a
2020 Winnipeg	Dakota	50.83a	10.00b	5.51b	0.54ab	2.25a
	Hazlet	51.39a	15.00a	9.24a	0.64a	2.46a
	KWS-H161	38.06b	5.56c	2.54c	0.36b	0.94b
2020 Carman	Dakota	83.13a	37.92a	36.43a	6.72a	27.36a
	Hazlet	80.21b	31.25b	29.28b	5.65b	26.29a
	KWS-H161	77.92b	24.79c	22.77c	7.01a	25.57a

Withing site years, means followed by the same letter within a column are not significant at $p<0.05$.

6.4.5 Thousand kernel weight (TKW), TWT, grain yield, and ergot severity

In the three Winnipeg experiments, there were no significant differences for TKW among the fungicide treatments or differences between these treatments and the inoculated-untreated control (**Table 6.6**). In comparison with the inoculated-untreated control, fungicide treatments did not significantly improve yield for the three Winnipeg experiments. However, fungicide applied at 80% anthesis and six days after 50% anthesis resulted in significantly higher TWT in 2018 Winnipeg. All fungicide treatments in 2019 Winnipeg significantly increased TWT compared to the inoculated-untreated control. In 2019 Carman, fungicide applied at 80% anthesis resulted in a significantly higher TKW than the inoculated-untreated control, and fungicide applied at heading, 10% anthesis, and 80% anthesis resulted in a significantly higher TWT than the inoculated-

untreated control. In 2020 Carman, all fungicide treatments resulted in significantly higher TKW, TWT, and yield than the inoculated-untreated control.

Both inoculation and fungicide treatment affected ergot infection in the fungicide trials (**Table 6.6**). The inoculated-untreated check resulted in a significantly higher ergot severity than the uninoculated-untreated check in 2019 Winnipeg and 2020 Carman. Compared with the inoculated-untreated check, fungicide applied at 10% anthesis and 80% anthesis resulted in a significantly lower ergot severity in 2019 Winnipeg. However, fungicide applied at 80% anthesis resulted in significantly higher ergot severity in 2019 Carman. Ergot severity was significantly reduced by the application of fungicide at 80% anthesis in 2020 Winnipeg and 10% anthesis, 80% anthesis, and six days after 50% anthesis in 2020 Carman.

Table 6.6 Treatment means for thousand kernel weight (TKW), test weight (TWT), grain yield, and ergot severity across genotypes in 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

Site year	Treatment	TKW (g)	TWT (g/0.5 L)	Yield (t/ha)	Ergot severity (%)
2018 Winnipeg	Uninoculated-untreated	28.39a	357.81a	1.74b	0.26b
	Heading	26.29b	351.69cd	1.68b	0.18b
	10% anthesis	27.13b	N/A	1.71b	0.39a
	80% anthesis	26.88b	356.07ab	2.18a	0.24b
	6 days after 50% anthesis	26.32b	354.17bc	1.76b	0.25b
	Inoculated-untreated	26.58b	350.83d	1.90ab	0.28ab
2019 Winnipeg	Uninoculated-untreated	30.45a	362.28a	2.79a	0.38b
	Heading	28.26b	350.97b	2.42a	0.64a
	10% anthesis	27.63b	350.85b	2.23a	0.35b
	80% anthesis	28.24b	351.06b	2.63a	0.39b
	6 days after 50% anthesis	28.20b	350.72b	2.48a	0.65a
	Inoculated-untreated	27.32b	347.55c	2.39a	0.64a
2020 Winnipeg	Uninoculated-untreated	31.17a	350.77a	2.78a	1.09ab
	Heading	28.45b	340.95c	2.76a	1.13ab
	10% anthesis	29.15b	345.75b	2.80a	1.16ab
	80% anthesis	29.45b	345.00b	2.70a	0.83b
	6 days after 50% anthesis	28.56b	342.78bc	2.39a	1.16ab
	Inoculated-untreated	28.39b	342.81bc	2.43a	1.30a
2019 Carman	Uninoculated-untreated	21.13ab	341.48a	3.11a	0.05b
	Heading	21.45ab	340.88a	3.16a	0.06b
	10% anthesis	21.50ab	341.65a	3.19a	0.07b
	80% anthesis	22.14a	342.35a	3.15a	0.14a
	6 days after 50% anthesis	21.06ab	337.94ab	2.96a	0.09ab
	Inoculated-untreated	19.41b	328.54b	2.53a	0.05b
2020 Carman	Uninoculated-untreated	26.44a	346.99a	4.81a	0.64c
	Heading	25.38ab	328.79c	3.04c	1.66a
	10% anthesis	24.94b	334.45b	3.76b	0.97bc
	80% anthesis	24.97ab	332.93bc	3.32bc	1.06b
	6 days after 50% anthesis	25.15ab	332.28bc	3.32c	1.14b
	Inoculated-untreated	23.21c	319.45d	2.11d	1.83a

N/A means that data are not available due to small sample size.

Within site year, means followed by the same letter within a column are not significant at $p<0.05$.

The rank of KWS-H161, Dakota, and Vitallo for TKW and TWT was not consistent across site years (**Table 6.7**). Dakota, KWS-H161, and Vitallo had significantly higher TKW than the other two genotypes in 2018 Winnipeg, 2019 Winnipeg, and 2019 Carman, respectively. In 2018 and 2019, KWS-H161 had significantly higher TWT in Winnipeg, while Vitallo had significantly higher TWT in Carman. In contrast, the performance of KWS-H161, Dakota, and Hazlet was consistent for TKW and TWT across locations in 2020. Hazlet had significantly higher TKW and TWT than Dakota and KWS-H161 in both sites. Meanwhile, the yield results were consistent for all genotypes across locations and years. The hybrid KWS-H161 had a significantly higher grain yield than the open-pollinated genotypes. Dakota had a significantly higher grain yield than Vitallo and Hazlet. Vitallo and Hazlet had significantly higher ergot severity than Dakota and KWS-H161 for all site years. There was no significant difference in ergot severity between Dakota and KWS-H161 in all site years except 2020 Carman. In 2020 Carman, Dakota had significantly lower ergot severity than KWS-H161.

Table 6.7 Genotype means for thousand kernel weight (TKW), test weight (TWT), grain yield, and ergot severity across treatments in 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

Site year	Genotype	TKW (g)	TWT (g/0.5 L)	Yield (t/ha)	Ergot severity (%)
2018 Winnipeg	Dakota	27.95a	357.85a	2.21b	0.06b
	Vitallo	26.28b	N/A	N/A	0.61a
	KWS-H161	26.57b	356.92a	2.41a	0.13b
2019 Winnipeg	Dakota	28.53b	347.32c	2.85b	0.21b
	Vitallo	26.54c	350.31b	N/A	1.10a
	KWS-H161	29.98a	359.08a	3.68a	0.22b
2019 Carman	Dakota	20.74b	331.26c	2.93b	0.04b
	Vitallo	22.72a	350.26a	2.22c	0.16a
	KWS-H161	19.88c	334.89b	3.90a	0.02b
2020 Winnipeg	Dakota	28.24b	337.27c	2.52b	0.93b
	Hazlet	31.83a	349.60a	2.20c	1.49a
	KWS-H161	27.51b	347.16b	3.22a	0.93b
2020 Carman	Dakota	23.84b	323.37c	3.25b	0.90b
	Hazlet	28.01a	341.32a	2.77c	1.45a
	KWS-H161	23.20b	332.75b	4.16a	1.30a

N/A means that data are not available due to small sample size.
Within site years, means followed by the same letter within a column are not significant at $p<0.05$.

6.4.6 Correlation between the measured variables

All FHB traits, including FHB incidence, FHB severity, FHB index, FDK, and DON, were positively correlated, and the correlations among them were significant for all site years except the correlation between FHB severity and DON in 2019 Carman (**Table 6.8**). Moreover, FHB incidence, severity, and index were highly associated with each other. The highest correlation coefficients were observed between FHB severity and index, consistently over 90%. The correlations between TKW and FHB traits, TWT and FHB traits, and yield and FHB traits were not consistent and varied with site year. There were no significant correlations between TKW and all FHB traits in 2019 Carman, while TKW was significantly correlated with FHB severity, FHB index, and FDK in 2019 Winnipeg. Meanwhile, TWT was significantly correlated with all FHB

traits in 2018 Winnipeg, 2019 Winnipeg, and 2020 Carman. Moreover, TWT was significantly associated with TKW in all site years except 2018 Winnipeg. Significantly negative correlations were observed between grain yield and all FHB traits in 2020 Carman. No significant correlations were observed between grain yield and all FHB traits in 2018 Winnipeg and 2019 Winnipeg. Grain yield was significantly correlated with TKW in three of five site years, where the correlation was positive in 2019 Winnipeg, but negative in 2019 Carman and 2020 Winnipeg. The correlation between yield and TWT was significant only in 2020 Carman. Ergot severity did not significantly correlate with any FHB traits in 2018 Winnipeg, 2019 Winnipeg, and 2019 Carman. However, there were significant and positive correlations between ergot severity and all FHB traits in 2020 Carman. Ergot severity had a significantly negative correlation with yield in all site years.

Table 6.8 Pearson correlation coefficients for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), thousand kernel weight (TKW), test weight (TWT), grain yield, and ergot severity of fungicide trials conducted in 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

	Site year	Severity	Index	FDK	DON	TKW	TWT	Yield	Ergot severity
Incidence	2018 Winnipeg	0.74***	0.82****	0.70**	0.76***	-0.50*	-0.57*	0.17ns	-0.09ns
	2019 Winnipeg	0.88****	0.90****	0.89****	0.88****	-0.42ns	-0.76***	0.05ns	-0.11ns
	2019 Carman	0.73***	0.84****	0.69**	0.78***	-0.18ns	-0.28ns	-0.12ns	0.09ns
	2020 Winnipeg	0.82****	0.77***	0.73***	0.77***	-0.25ns	-0.52*	-0.41ns	0.18ns
	2020 Carman	0.75***	0.77***	0.81****	0.87****	-0.28ns	-0.65**	-0.70**	0.55*
Severity	2018 Winnipeg		0.97****	0.83****	0.78***	-0.24ns	-0.72**	-0.15ns	0.06ns
	2019 Winnipeg		0.92****	0.85****	0.79****	-0.68**	-0.89****	-0.30ns	0.29ns
	2019 Carman		0.94****	0.74***	0.42ns	0.22ns	0.10ns	-0.56*	0.27ns
	2020 Winnipeg		0.97****	0.79****	0.83****	0.17ns	-0.22ns	-0.68**	0.57*
	2020 Carman		1.00****	0.88****	0.92****	-0.31ns	-0.79****	-0.87****	0.59*
Index	2018 Winnipeg			0.86****	0.87****	-0.23ns	-0.76***	-0.03ns	-0.02ns
	2019 Winnipeg			0.86****	0.88****	-0.49*	-0.86****	-0.09ns	0.02ns
	2019 Carman			0.68**	0.51*	0.04ns	-0.09ns	-0.51*	0.19ns
	2020 Winnipeg			0.84****	0.86****	0.21ns	-0.17ns	-0.66**	0.56*
	2020 Carman			0.89****	0.93****	-0.32ns	-0.80****	-0.87****	0.60**
FDK	2018 Winnipeg				0.93****	-0.23ns	-0.81****	-0.29ns	0.19ns
	2019 Winnipeg				0.91****	-0.58*	-0.84****	-0.15ns	0.05ns
	2019 Carman				0.76***	-0.06ns	-0.29ns	-0.18ns	-0.07ns
	2020 Winnipeg				0.97****	-0.07ns	-0.37ns	-0.45ns	0.38ns
	2020 Carman				0.97****	-0.48*	-0.79****	-0.71***	0.69**
DON	2018 Winnipeg					-0.20ns	-0.78***	-0.13ns	0.07ns
	2019 Winnipeg					-0.45ns	-0.79****	0.02ns	-0.13ns
	2019 Carman					-0.48*	-0.64**	0.11ns	-0.27ns
	2020 Winnipeg					0.00ns	-0.44ns	-0.58*	0.38ns
	2020 Carman					-0.35ns	-0.76***	-0.82****	0.74***

TKW	2018 Winnipeg	-0.06ns	0.34ns	-0.43ns
	2019 Winnipeg	0.74***	0.83****	-0.69**
	2019 Carman	0.88****	-0.50*	0.77***
	2020 Winnipeg	0.61**	-0.57*	0.64**
	2020 Carman	0.75***	-0.12ns	0.01ns
TWT	2018 Winnipeg		0.03ns	0.23ns
	2019 Winnipeg		0.45ns	-0.30ns
	2019 Carman		-0.38ns	0.70**
	2020 Winnipeg		0.12ns	0.43ns
	2020 Carman		0.49*	-0.29ns
Yield	2018 Winnipeg			-0.87****
	2019 Winnipeg			-0.83****
	2019 Carman			-0.58*
	2020 Winnipeg			-0.64**
	2020 Carman			-0.67**

Note: genotype/treatment means were used for correlation analysis. n=18.
ns=no significant; *significant at p<0.05; **significant at p<0.01; ***significant at p<0.001; ****significant at p<0.0001.

6.5 Discussion

In fungicide trials, weather conditions played an important role on plant stands, the uniformity of the flowering period, the speed of maturity, and disease levels. Although the supplemental misting irrigation system was operated at both sites over the three years, weather conditions still affected FHB and ergot. Based on the uninoculated-untreated check, FHB index was generally higher in Carman than in Winnipeg. Higher precipitation in Carman could have contributed to a higher mean FHB index. In general, plant stands were better in Carman compared to Winnipeg, which may indicate that rye established or overwintered better in the sandy soils in Carman rather than clay soils in Winnipeg. In 2019, lower precipitation, accompanied by high temperature, accelerated drought in both Carman and Winnipeg. The dry conditions were not conducive to FHB infection and accelerated crop maturity. In 2020, high temperatures accompanied by precipitation after inoculation in Carman lead to a high mean FHB index. However, the low mean FHB index in Winnipeg may be attributed to the low temperatures coinciding with the inoculation.

To our knowledge, this is the first study to evaluate the efficacy of fungicide for FHB in fall rye in Canada. Fungicide treatments had significant effects on FHB incidence, severity, and index in fall rye. Although these effects varied between test locations and years, overall, fungicide treatments resulted in lower FHB disease than the inoculated-untreated check. The fungicide Prosaro™ was reported to give a mean reduction of FHB index of 52% in wheat through a multivariate meta-analysis of 100 uniform fungicide studies (Paul et al., 2008). Other studies by Mesterhazy et al. (2011, 2018a) indicated that Prosaro™ provided a significantly higher reduction of FHB disease than other fungicides in wheat, as the efficacies were seldom lower than 80% in small plot tests. Compared to the untreated control, up to 80% overall reduction in FHB symptoms was observed in hybrid rye by using Caramba and Miravis Ace fungicide (Minnesota Crop News, 2020). For the fungicide trials conducted in the current study, the highest fungicide efficacy for reducing FHB incidence, severity, and index was 44.77% in 2019 Carman, 59.72% in 2020 Carman, and 62.95% in 2020 Carman, respectively. Mesterhazy et al. (2018a) reported that the reduction of FHB severity in wheat was minor at high epidemic severities. In contrast, the highest fungicide efficacies for reducing FHB severity and index were observed in 2020 Carman with a high mean FHB index. The epidemic severity was considered to influence the effect of fungicide,

but an explicit correlation between FHB reduction and epidemic severity was not found by Mesterhazy et al. (2018a).

The efficacy of fungicide treatments with different application timings varied among site years. The largest reduction of FHB disease was observed when fungicide was applied at heading or 10% anthesis in 2019 Winnipeg, but 80% anthesis or six days after 50% anthesis in 2018 Winnipeg and 2020 Winnipeg. In 2019 Carman and 2020 Carman, fungicide applied at 10% anthesis resulted in the highest percent control for FHB incidence, severity, and index. These results agree with studies that evaluated the effect of Prosaro™ timings on FHB in wheat (D'Angelo et al., 2014; Paul et al., 2018; Bolanos-Carriel et al., 2020). Bolanos-Carriel et al. (2020) reported that Prosaro™ applied at early anthesis provided more effective reduction in FHB index compared to 6- and 12-days post-anthesis. It is challenging to accurately determine when anthesis occurs in a wheat spike population due to the variable flowering timing from different tillers (D'Angelo et al., 2004). As an open-pollinated crop, fall rye has a long flowering period, which may continue for two weeks. Several factors, such as plant stands and weather conditions, influence the uniformity of the flowering time between the main tiller and the late-produced tillers in the same plant, or from different plants in the same plot. Accordingly, it is difficult to determine the optimal time of fungicide application for FHB in fall rye. This could explain the inconsistent efficacy of fungicide treatments with the different application timings among site years, especially in Winnipeg, which had more variable plant stands than in Carman.

Application of fungicide is considered to be a preventive activity when fungicide application is done before infection, while it is regarded as a curative activity when fungicide is applied after infection (Ivic, 2010; Chen et al., 2012). In 2020 Carman, applications made before and after infection significantly reduced FHB severity and index, which indicated that the fungicide Prosaro™ had a suppressive effect at both timings. Although all fungicide treatments resulted in a significant reduction of FHB severity and index, the magnitude of treatment effects varied with fungicide application timings. The high mean FHB index in 2020 Carman appeared to provide for better differentiation of fungicide efficacy among fungicide treatments with different application timings. Fungicide applied at 10% anthesis resulted in significantly lower FHB incidence, severity, and index compared to the other application timings. Some studies showed that Prosaro™ applications made up to six days following anthesis achieved comparable efficacy

to that achieved by the anthesis application in wheat (D'Angelo et al., 2014; Bolanos-Carriel et al., 2020). D'Angelo et al. (2014) explained that fungicide application after anthesis appeared to protect more spikes because the secondary tillers were at the critical flowering period based on primary tillers. The different lengths of the flowering periods between fall rye and winter wheat may influence the efficacy of fungicide treatments with different application timings. Nevertheless, fungicide applied at 80% anthesis and six days after 50% anthesis also significantly reduced FHB levels compared to the inoculated-untreated control in three site years. The extensive application window provides farmers with more options if it is difficult for them to spray at the beginning of anthesis due to unsatisfactory weather conditions or uneven flowering across a field (Freije et al., 2015).

The efficacy of fungicide for reducing DON is imperative as the amount of DON is important in considering food and feed safety (Mesterhazy et al., 2011). In the fungicide trials of fall rye, all fungicide treatments significantly reduced DON in three of five site years. In 2019, there was no significant difference in DON levels among fungicide application timings. Some studies showed that DON levels in an application made up to six days following anthesis were generally not significantly different from those in anthesis applications of Prosaro™ in wheat (D'Angelo et al., 2014; Bolanos-Carriel et al., 2020). These results suggest that fungicide application can be postponed by up to six days after 50% anthesis and still achieve an effective reduction of DON concentrations. Meta-analysis of data from 19 years of fungicide trials showed that Prosaro™ applied at heading resulted in less efficacious DON reduction than applications at anthesis or five to seven days later (Paul et al., 2018). Fungicide application at anthesis resulted in lower DON concentrations than applications five to seven days later (Paul et al., 2018). Likewise, fungicide applied at 10% anthesis achieved the best efficacy for DON reduction, followed by 80% anthesis, six days after 50% anthesis, and heading in 2020 Carman. Although fungicide application significantly reduced DON levels in the current study, mean DON levels in site years with high FHB levels exceeded the official limit of 2 ppm. The fungicide application could not reduce DON contamination below 2 ppm if the inoculated-untreated control exceeded the threshold limit by more than 1-2 ppm. The application of Prosaro™ resulted in a 42% reduction of DON in wheat according to a multivariate meta-analysis of 100 uniform fungicide studies (Paul et al., 2008). The best fungicide efficacy from D'Angelo et al. (2014) was a 54% reduction of DON in soft red winter

wheat. Mesterhazy et al. (2018a) reported that the decrease in DON in winter wheat across four years was 75% by applying fungicide Prosaro™. The average of the best efficacy for reducing DON from each site year was 45.83% in the current study. This means that the fungicide would effectively reduce DON levels to meet food standards if naturally occurring DON contamination is lower than 3.7 ppm. In comparison, larger DON contents cannot be reduced below the official limit of 2 ppm. Fungicide use may effectively reduce DON content during a weak epidemic, but would not be enough to prevent economic loss when the FHB epidemic is strong (Mesterhazy et al., 2011; Freije et al., 2015).

Additionally, negative fungicide efficacy in the current study was primarily associated with FDK and DON. Fungicide applied at heading in 2020 Winnipeg resulted in increased DON by 25.53% compared to the inoculated-untreated check. Higher DON content in harvested grain may result from more infected kernels as fungicide was able to suppress disease sufficiently, and infected kernels were not light enough to be blown out of the combine. Feksa et al. (2019) reported that pre-infection application of azoxystrobin increased DON contents by 42% in wheat compared with the untreated control. A mixture of tebuconazole and azoxystrobin was reported to increase DON contents relative to the untreated controls in wet conditions (Blandino et al., 2006). An increase in DON concentrations was observed with the application of azoxystrobin and carbendazim on a susceptible wheat cultivar (Mesterhazy et al., 2003). The role of fungicides in increasing DON concentrations in grains requires additional research (Mesterhazy et al., 2003).

In the current study, fungicide applied at 80% anthesis resulted in the largest mean reduction of FDK and DON, whereas the highest mean percent control of FHB incidence, severity, and index was obtained by fungicide application at 10% anthesis. It appeared that the optimal application timing for reducing FHB symptoms and mycotoxin accumulations were not always consistent. Tateishi et al. (2014) reported that the mid-flowering stage was the optimal application timing of metconazole for suppressing FHB symptom development, while the milk stage was optimal for reducing mycotoxin concentrations in wheat. Thiophanate-methyl applied at 20 days after anthesis reduced DON and nivalenol concentrations in wheat grain without reducing FHB severity (Yoshida et al., 2012). This indicates that fungicide application timing differentially influences FHB disease and mycotoxin accumulations (Yoshida et al., 2012). Nevertheless, in 2020 Carman, fungicide applied at 10% anthesis resulted in the largest reduction of FHB incidence,

FHB severity, FHB index, FDK, and DON. Bolanos-Carriel et al. (2020) also showed that Prosaro™ applied at early anthesis was the most effective fungicide treatment in reducing FHB index, FDK, and DON in winter wheat.

In the current study, all FHB traits, including FHB incidence, FHB severity, FHB index, FDK, and DON, were positively correlated, which is consistent with some fungicide studies in wheat (Menniti et al., 2003; Paul et al., 2005; Wegulo et al., 2011; Freije et al., 2015). The associations were consistently significant among site years, but varied from moderate to strong. Several factors, including technical problems, infection levels, and fungicide coverage, may play an important role in the discrepancies among locations and years (Mesterhazy et al., 2011). For instance, harvest with a combine may blow out light diseased grains, resulting in a loss of 30%-40% of the FDKs (Mesterhazy et al., 2011). Wegulo et al. (2011) reported a consistently strong relationship between FHB index and DON in wheat fungicide trials with artificial inoculation. In the study of fall rye, the relationship between FHB index and DON was moderate to strong (r=0.51-0.93). It is not surprising that visual FHB index does not consistently produce a high correlation with FDK or DON, as spikes can develop FDK and accumulate DON without visible FHB symptoms in fields (Andersen, 1948; Cowger and Arellano, 2013). Moreover, fungicidal suppression of FHB symptoms may not always be associated with a reduction of mycotoxin accumulations (Parry et al., 1995). Propiconazole provided an effective reduction of FHB disease, but DON contents were not significantly reduced in treated plots (Martin and Johnston, 1982). Thiabendazole did not affect FHB disease, while it reduced DON concentration by 83% in wheat (Boyacioglu et al., 1992). Accordingly, it may not be accurate to use FHB index to estimate the impact of FHB on grain quality and DON content within fungicide treatments. Paul et al. (2005) found comparatively higher correlations between measures of disease and DON variables at lower disease intensity when they used meta-analysis to analyze 163 studies evaluating correlations between DON and FHB incidence, FHB severity, FHB index, and FDK in harvested wheat grain from FHB screening nurseries and FHB fungicide trials. In contrast, higher associations between FHB index and DON or FDK were observed in 2020 Carman with a higher mean FHB index in fall rye. Disease intensity between fall rye and wheat could not be accurately comparable here. Additionally, a strong association was observed between DON and FDK in all site years, ranging from 0.76 to 0.97. Even if FDK is more strongly correlated with DON content than other traits,

measurement of FDK still involves a time-consuming process (Mesterhazy et al., 2011). Furthermore, FDK assessment in fall rye was more difficult than wheat as infected rye kernels were less discolored and less shriveled because of the narrower shape and smaller size (Perkowski et al., 1995).

The most resistant genotype, KWS-H161, had consistently lower FHB severity and index than the open-pollinated rye genotypes. It indicated that more resistant genotypes showed more stable performance. Several studies have evaluated the effect of moderately resistant cultivars on DON contents, as no wheat cultivars exhibit complete resistance to DON accumulation (Freije et al., 2015). In the current study, the effect of resistant rye genotype could not reduce DON levels below the official limit of 2 ppm in site years with a relatively high mean FHB index. The resistance level of wheat cultivars significantly influenced the fungicide effect (Mesterhazy et al., 2018a, 2018b). The more resistant cultivars could be better protected from DON contamination through the same fungicide treatment (Blandino et al., 2006). Some studies showed that moderately resistant cultivars provided higher fungicide efficacy than susceptible wheat cultivars (Wegulo et al., 2011; Mesterhazy et al., 2003, 2011, 2018a). In contrast, Bolanos-Carriel et al. (2020) reported that the effect of fungicide application timing was most apparent in a susceptible wheat cultivar. In the study of fall rye, the effects of genotype on fungicide treatments varied among locations and years (**Appendix 21**, **Appendix 22**, **Appendix 23**, **Appendix 24**, and **Appendix 25**). Although the resistant genotype had the consistently lower FHB severity and index among fungicide treatments than intermediate and moderately susceptible genotypes, it did not always have the highest fungicide efficacy. Even if the combined effect of fungicides and wheat cultivar resistance is larger than either independently, it is not always sufficient to reduce DON below the limit (Sip et al., 2010; Mesterhazy et al., 2011, 2018a). In the current study, the combination of fungicides and resistant rye genotypes could not consistently reduce DON levels below the threshold.

As an open-pollinated crop, the flowering period of fall rye is more extended than a self-pollinating crop, and fungicide spraying during anthesis may impact the seed set (King, 2018). In the current study, all fungicide treatments did not significantly reduce yield relative to the inoculated-untreated check in all site years. It indicated that Prosaro™ applied at heading, 10% anthesis, 80% anthesis, and six days after 50% anthesis did not adversely affect seed set in fall rye.

A study at the University of Kentucky also showed that an application of Caramba and Miravis Ace at Feekes 10.5 (fully headed), Feekes 10.5.1 (beginning of flowering), and five days after the beginning of flowering in hybrid rye did not impact pollination or seed set (Minnesota Crop News, 2020). Comparison of uninoculated-untreated and inoculated-untreated checks showed no significant difference for grain yield in all site years except 2020 Carman. It indicated that yield losses caused by *F. graminearum* in rye were very low under a relatively low mean FHB index. Meanwhile, fungicide treatments had no significant effect on grain yield compared to the inoculated-untreated check in all site years except 2020 Carman. However, yield loss of 56% was observed in 2020 Carman. Compared to the inoculated-untreated check, all fungicide treatments significantly improved yield, and fungicide applied at 10% anthesis provided the best yield improvement of 78%. Several studies showed significant yield increases following fungicide application in wheat (Haidukowski et al., 2012; Caldwell et al., 2017; Mesterhazy et al., 2018a).

The study of the fungicide Prosaro™ in wheat showed that yield was negatively correlated with FHB index, FDK, and DON (Wegulo et al., 2011). In the current study, the negatively significant correlations between yield and FHB traits were inconsistent among locations and years. Under a high mean FHB index, yield was significantly associated with all FHB traits. The significant correlation between FHB traits and TWT or TKW was negative and highly variable. It could be explained by the damaged kernels associated with FHB development, which are shriveled and lightweight and directly affect test weight (Salgado et al., 2015; Paul et al., 2018). Jevtic et al. (2022) reported that the FHB index was more related to yield, while FDK had closer relation to TKW in wheat FHB nurseries. However, the same tendency was not observed in the study of fall rye.

Genotypes had a more significant effect on TKW, TWT, and grain yield than treatments. Hazlet has a lower grain yield, but higher TWT and kernel weight, than Dakota (McLeod and Gan, 2008). In the fungicide trials, the ranks between Hazlet and Dakota for TKW, TWT, and yield were consistent with the results of McLeod and Gan (2008). The hybrid KWS-H161 had a consistently higher yield than the open-pollinated genotypes. High yield is the advantage of hybrid rye per se. On the other hand, KWS-H161 showed better resistance to FHB. Test weight is one of the factors related to grain quality (Paul et al., 2008). All genotypes had TWT over the minimum

requirements for No. 3 grades (304 g/0.5 L) of the Canada rye class (Canadian Grain Commission, 2021).

Studies in Europe showed that fungicide application for FHB control during anthesis increased ergot infection (Hybrid fall rye production guide, FP Genetics). In the current study, the effect of *Fusarium* inoculum spray on ergot was not consistent across site years, but there was a slight trend towards higher ergot with spray inoculation. The impact of fungicide application on ergot could not be separated from the impact of *Fusarium* inoculum spray. In comparison with the inoculated-untreated controls, fungicide treatments in the current study reduced ergot infection in most cases. It indicates that fungicide application may not interfere with the pollen shed on rye. Moreover, ergot severity was consistently associated with yield, and correlations were significant and negative. Accordingly, yield increase in fungicide trials was not entirely due to the efficacy of FHB control, but also control of ergot infection.

Overall, application of Prosaro™ at 10% anthesis provided the highest percent control for FHB incidence, severity, and index, while application at 80% anthesis had the highest efficacy for reducing FDK and DON. This indicates that rye growers have a window of fungicide application between 10% anthesis and 80% anthesis to manage FHB and reduce DON levels in fall rye. On the other hand, fungicide application did not adversely affect pollination and seed set that related to yield and ergot infection. In contrast, fungicide treatments could significantly increase yield under a high mean FHB index. In addition, ergot infection was decreased by using the fungicide Prosaro™. However, fungicide applications failed to reduce DON levels low enough for grain acceptance under a relatively high mean FHB index, despite the significant reduction of FHB symptoms. Deoxynivalenol concentrations were reduced to the acceptable levels under conditions where FHB did not significantly decrease yield. It seems to be important to consider the reduction of DON contamination rather than yield return when using a fungicide to manage FHB in fall rye. The more resistant genotypes appeared to have a more stable reduction of FHB disease. Use of resistant cultivars is highly recommended to rye growers, particularly when fungicide cannot provide satisfactory control.

Chapter 7

Evaluation of adult plant resistance to leaf rust in fall rye

7.1 Abstract

Leaf rust is common in rye growing areas in Europe, but little is known about the reaction of fall rye to leaf rust in western Canada. Leaf rust pustules were collected from naturally infected fall rye leaves from Winnipeg and Carman, Manitoba and increased to generate inoculum for field trials. Two sets of fall rye lines were evaluated through artificial inoculation across multiple years in Winnipeg. One set contained a mixture of germplasm including open-pollinated and hybrid genotypes, and the second set contained commercial hybrids and testcross hybrids derived from the Petkus and Carsten gene pools. Both disease severity (DS) and infection types (IT) were assessed on the penultimate leaf of ten randomly chosen plants per plot. Infection type data were converted to a numerical scale, and the coefficient of infection was calculated using the formula: IT numeric value × DS (%). Genotypes had significant effects on all variables, while environment effects were not significant. Correlations between DS and IT were significantly positive. Most open-pollinated genotypes were moderately susceptible or susceptible to leaf rust. Only a few genotypes, including ACE-1, Florida 401, Gator, Reimann-Philipp, and Sellino, had lower disease severity than other genotypes. Most hybrids in the first set were moderately resistant or intermediate to leaf rust with low disease severity. Among them, KWS-H187, KWS Serafino, KWS Tayo, and KWS Trebiano showed more resistance. In the second set, testcross hybrids generated from the Petkus gene pool exhibited better resistance than those from the Carsten gene pool. The results provide practical information for rye growers in western Canada. The information will assist farmers to choose commercial rye cultivars with leaf rust resistance when rye is grown in rust prone areas. Meanwhile, breeders are able to use the resistant genotypes as parents to develop new resistant genotypes against leaf rust.

7.2 Introduction

Rye (*Secale cereale* L.) is a multiple use crop and can be used as a cereal grain, forage, pasture, or cover crop (Manitoba Agriculture; FAO, 2018). It is an important cereal crop in Europe, with the highest production in Germany and Poland (Miedaner et al., 2012; Bankina et al.,

2013). Hybrids account for approximately 50% of the total rye acreage in Germany (Miedaner et al., 2012). In Canada, rye is a minor cereal crop, and acreage has fluctuated over the last twenty years (FAOSTAT). Since 2015, six hybrid cultivars, including Brasetto, Guttino, KWS Bono, KWS Daniello, KWS Gatano, and KWS Serafino, have been introduced from Germany into Canada (Variety registration office). The introduction of hybrids increased rye acreage across the prairies (Melchior, 2020). Currently, about 250,000 acres of fall rye are seeded in western Canada, and only a small percentage of those acres are open-pollinated varieties (Barber, 2019; Melchior, 2020).

Rust diseases are highly specialized to infect specific hosts (Fetch et al., 2011). The classification of the pathogens causing leaf rust on cultivated wheat, wild wheat, and rye has undergone several revisions (Peksa and Bankina, 2019). The causal agent of leaf rust in wheat and rye is considered as a single broad species of *Puccinia recondita*, while some scientists use *P. recondita* for rye leaf rust and *P. triticina* for wheat leaf rust (Peksa and Bankina, 2019). The *P. recondita* complex collected from cultivated wheat, wild wheat, and rye were separated into two major groups based on differences in pycnial-aecial and uredinial-telial host range, interfertility, teliospore dimensions, and nuclear DNA content (Anikster et al., 1997). Group I included a broad uredinial-telial host range of cultivated wheat, wild wheat, rye, and wild barley (Anikster et al., 1997). Group II was separated into four types, and Type D was restricted to rye (Anikster et al., 1997). Savile (1984) used the name *P. triticina* of the cereal rust for Group I described in Anikster et al. (1997), and designated leaf rust of rye as *P. recondita*.

Leaf rust, caused by *P. recondita* Rob. ex Desm. f. sp. *secalis*, is one of the most important leaf diseases of rye (Miedaner and Sperling, 1995; Wehling et al., 2003; Roux et al., 2004; Tupits and Soovali, 2010; Miedaner et al., 2012). In western Canada, leaf rust of rye is not very destructive because fall rye generally matures before the rust has had time to produce severe infection (McDonald, 1967). In contrast, leaf rust is widely distributed and prevalent in all rye growing regions of Europe (Miedaner and Sperling, 1995; Wehling et al., 2003). Heading and grain filling stages are the most susceptible periods for leaf rust infection on rye (Tupits and Soovali, 2010). Leaf rust epidemics have the potential to cause yield losses (Wehling et al., 2003). Under continental climates, natural infection of rye leaf rust could result in yield losses up to 40% (Kobylanski and Solodukhina, 1983 cited by Miedaner and Sperling, 1995 and Wehling et al.,

2003). In some studies, lower severity of leaf rust only reduced kernel weight, and severe infection significantly decreased kernel number per spike in fall rye (Frauenstein, 1985 cited by Miedaner and Sperling, 1995, Miedaner et al., 2002, and Wehling et al., 2003). The reduction of thousand kernel weight ranged from 11 to 27% when artificial inoculation was conducted in multiple environments (Miedaner and Sperling, 1995).

Leaf rust resistance demonstrates both qualitative and quantitative inheritance (Mains, 1926; Reichel, 1981 cited by Miedaner and Sperling, 1995; Musa et al., 1984; Miedaner et al., 2002). Race-specific resistance has been found in various European rye germplasm (Miedaner et al., 2002; Wehling et al., 2003; Roux et al., 2004;). Race-specific resistance genes were detected on all rye chromosomes except for 5R (Zeller and Hsam, 1983; Singh and McIntosh, 1990; McIntosh et al., 1995; Wehling et al., 2003; Roux et al., 2004). Five dominant leaf rust resistance genes, *Pr1*, *Pr2*, *Pr3*, *Pr4*, and *Pr5*, were identified in the chromosomes 6RL, 7RL, 1RS, 1RL, and 1RL of rye, respectively (Wehling et al., 2003; Roux et al., 2004). Several leaf rust resistance genes from rye have been widely used in wheat breeding, even before the genetic analysis of resistance on rye (Zeller and Hsam, 1983; Roelfs et al., 1992; Sawhney and Sharma, 1999; Wehling et al., 2003). The leaf rust resistance gene *Lr25* from rye cultivar Rosen was detected in a T4BS·4BL-5RL translocation wheat line (Friebe et al., 1994). Two leaf rust resistance genes, Lr26 and Lr45, were derived from the rye cultivar Petkus and were found in a T1BL·1RS and T2AS-2RS·2RL translocation wheat line, respectively (Mettin et al., 1973; Zeller, 1973; Zeller and Hsam, 1983; McIntosh et al., 1995). The genes derived from rye provided resistance to leaf rust of wheat, but no information is available on whether they are effective against leaf rust of rye (Welhling et al., 2003).

Quantitatively inherited leaf rust resistance was also observed on rye, although qualitative resistance governed by single genes seems to be predominant (Welhling et al., 2003). Under field conditions, quantitative resistance contributes to slow disease development (Frauenstein and Reichel, 1978 cited by Miedaner and Sperling, 1995; Reichel, 1981 cited by Miedaner and Sperling, 1995 and Miedaner et al., 2002;). Rye varieties with quantitative resistance have a susceptible infection type, but the degree of host resistance affects the size of uredial pustules, the latent period of infection, sporulation, and germination rate of uredospores (Reichel, 1981 cited by Miedaner and Sperling, 1995 and Miedaner et al., 2002; Parlevliet, 1988).

Based on European studies, leaf rust resistance is not common in rye (Miedaner et al., 2002; Miedaner, 2007 cited by Tupits and Soovali, 2010). The resistance level to leaf rust is low within the Petkus and Carsten gene pools, which are used for hybrid rye breeding (Miedaner et al., 2002). Leaf rust resistance has been found mainly in rye varieties from Poland, Russia, and the United States (Rollwitz, 1985 cited by Wilde et al., 2006; Anonymous, 2004 cited by Wilde et al., 2006). Compared to major cereals such as wheat and barley, there is little information available on the reaction of fall rye to leaf rust in Canada. The current study was undertaken to assess adult plant resistance to leaf rust in a mixture of open-pollinated, hybrid, and testcross germplasm tested across multiple years in Winnipeg, Manitoba.

7.3 Materials and methods

7.3.1 Plant materials

7.3.1.1 Set 1

Plant materials consisted of fifty-four open-pollinated fall rye genotypes and thirteen hybrids (**Table 7.3**). The open-pollinated fall rye genotypes included lines from Canada, the United States, Russia, Germany, and other European countries, provided by the rye breeding program at the Lethbridge Research and Development Center, Agriculture and Agri-Food Canada (AAFC), Alberta. The hybrids were developed by KWS LOCHOW GMBH, Bergen, Germany. Three winter wheat cultivars, Moats (Resistant (R)), Ptarmigan (Susceptible (S)), and Readymade (S), with known resistance levels to wheat leaf rust (*P. triticina*), were included as checks.

7.3.1.2 Set 2

There were nine commercial hybrids and seventy-two testcross hybrids generated by KWS LOCHOW GMBH, Bergen, Germany (**Table 7.6**). Twenty-eight testcross hybrids (Li1-Li42) were F_1 generations of crosses between a tester and inbred lines developed from the Petkus gene pool, and forty-four (Li102-Li169) were F_1 generations of crosses between a tester and inbred lines developed from the Carsten gene pool. The same three winter wheat cultivars used in Set 1 were included as checks.

7.3.2 Inoculum production

Infected fall rye leaves with sporulating rust pustules were collected from naturally infected fall rye rust and fusarium head blight (FHB) trials grown at the Point field research station, the University of Manitoba, Winnipeg, and fall rye FHB trials grown at the Ian N Morrison Research Farm, the University of Manitoba, Carman in late July 2017. The leaves were air-dried at room temperature (24 ℃) for 12 to 24 h and stored at 4 ℃ for no more than one week. Urediniospores were collected from the leaf samples using a vacuum-powered cyclone spore collector (custom-made) attached with a 00 gelatin capsule. Then rust urediniospores were poured from capsules into labeled glass tubes. The tubes were sealed after vacuuming dry overnight according to the protocol provided by Elsa Reimer (personal communications), and stored at 4 ℃ for later inoculum production.

Inoculum was produced according to the methods used for wheat leaf rust (McCallum and Seto-Goh, 2003; McCallum et al., 2018). Fall rye genotypes, including AC Rifle, Dankowskie Nowe, Hazlet, Motto, and Prima, were used for rust inoculum production due to their seedling susceptibility to leaf rust based on preliminary tests. Each cultivar was sown in three 4.4-inch diameter pots using a mixture of 50/50 soil/compost mix and Sunshine™ soil mix #5 (Sungro™ Horticulture Ltd., USA) in a ratio of 2:1, and each pot included 20 to 30 seeds. The soil was treated with 50 ml maleic hydrazide solution (0.36 g/L concentration) (Sigma-Aldrich, USA) approximately four days after seeding to slow the emergence of secondary leaves. They were grown in the greenhouse for seven days after planting and then used for inoculation. Supplemental light in the greenhouse was set for 16 h daylight, the temperature ranged from 18 to 24 ℃. Before inoculation, glass tubes with urediniospores were opened to rehydrate for three hours at room temperature. After rehydration, the glass tubes were heat-shocked at 40 ℃ for six minutes in water. Then a very small amount of urediniospores was transferred from glass tubes into capsules, and 0.75 mL light mineral oil, Soltrol 170 (Chevron Philips Chemical Company LLC., USA), was added into the capsule. A compressed air sprayer was used to spray urediniospores onto the leaves of seedlings. After inoculation, plants were left at room temperature for about one hour to dry the leaves and then covered with a plastic cone about 25 cm in height with an open top over each pot. They were placed into a dew chamber (Model I-60D, Percival Scientific Inc., USA) with nearly 100% relative humidity for approximately 17 h and moved back into the greenhouse. Seven days

after inoculation, leaves with separated single pustules were trimmed before pustules sporulated to limit cross-contamination. Leaves with a single pustule on the top half were chosen where possible. In that case, a single uredinium could remain on the trimmed upper edge of the leaf, and more than half of the leaf could be left after trimming. Ideally, about ten leaves with single pustules were left in each pot. The shape of single pustules was used to ensure that only leaf rust (oval) and not stem rust (diamond) was selected. At 14 days after inoculation, urediniospores were collected from ten single isolated pustules using a custom-made collector.

The urediniospores were increased on the five fall rye genotypes following the procedure as described above without trimming. Urediniospores were collected using a wooden stake to tap infected leaves over a cellophane sheet 14 days after inoculation. Then rust urediniospores were poured from the cellophane into labeled glass tubes. The tubes were sealed after vacuum drying overnight and stored at 4 ℃ for field inoculation.

7.3.3 Field experiments

Field experiments with artificial inoculations were conducted from 2018 to 2020 for Set 1 and 2019 to 2020 for Set 2 at the Point field research station, the University of Manitoba, Winnipeg, Manitoba. Separate trials were conducted for each set of lines. Lines within each set were sown as a randomized complete block design (RCBD) with two replicates. Each plot was a single three-meter row. To prepare for inoculation, glass tubes with urediniospores were opened to rehydrate for three hours at room temperature. After rehydration, the glass tubes were heat-shocked at 40 ℃ for six minutes in water. One gram of urediniospores was mixed with 1.5 L mineral oil, Soltrol 170 (Chevron Philips Chemical Company LLC., USA) to inoculate approximately 320 plots. All plants were spray inoculated using a hand-held Herbi 4 sprayer (Micron Group, UK). The first inoculation was conducted when the air temperature in the spring reached at least 10 ℃ at night. The second inoculation was carried out three to seven days after the first inoculation. A mist irrigation system was operated for ten minutes per hour for 16 hours after each inoculation.

7.3.4 Disease assessment

Leaf rust reactions were evaluated using the modified Cobbs scale when symptoms were noted on the penultimate leaf (Peterson et al., 1948). Disease severity (DS) was rated as the percentage of leaf area infected. The percentage scale was 0 to 100%, with an interval of 5%. Infection types (IT) were recorded as R (resistant, no visible infection or necrotic area without pustules), MR (moderately resistant, small pustules surrounded by either necrosis or chlorosis), I (intermediate, variable sized pustules with necrosis or chlorosis), MS (moderately susceptible, medium-sized pustules surrounded by chlorosis), and S (susceptible, large pustules with little or no chlorosis and no necrosis) (Rust Scoring Guide). Infection type data were converted to a numerical scale using 0 (R), 0.25 (MR), 0.5 (I), 0.75 (MS), and 1 (S). The coefficient of infection (CI) was calculated using the formula: IT numeric value × DS (%).

7.3.5 Statistical analysis

Statistical analyses were performed using the SAS program (SAS version 9.4, SAS Institute Inc., USA). Analyses of variance (ANOVA) for all variables for an individual year and combined data across years were generated using the PROC MIXED procedure. Before analysis, DS and CI in Set 2 were square-root-transformed to satisfy the assumptions of analyses of variance. Data were evaluated using the PROC UNIVARIATE procedure. Results showed that residuals conformed to a normal distribution, and the plot of residuals was symmetrically distributed. It suggested that data from different site years could be pooled. In the PROC MIXED procedure, no options were available for an experiment-wise least significant difference (LSD) value. The LSD value that SAS calculated using PROC GLM was the same LSD that was manually calculated from PROC MIXED. Therefore, LSD was generated using the PROC GLM procedure. Pearson's correlation coefficients between response variables were evaluated using the PROC CORR procedure.

7.4 Results

7.4.1 Results of Set 1

Effects of genotype and the interaction between genotype and year were statistically significant for DS, IT, and CI, while year did not have a significant effect on all variables (**Table**

7.1). Correlations between DS, IT, and CI were all highly significant and positive (**Table 7.2**). Leaf rust DS strongly correlated with CI (r=1.00).

Table 7.1 Combined analyses of variance for leaf rust disease severity (DS), infection types (IT), and coefficient of infection (CI) values measured in field trials conducted on Set 1 genotypes in 2018, 2019, and 2020 in Winnipeg

Source	DF	DS		IT		CI	
		MS	Pr > F	MS	Pr > F	MS	Pr > F
Genotype	69	830.98	<.0001	0.24	<.0001	1016.64	<.0001
Year	2	3145.20	0.1198	0.03	0.867	2897.80	0.176
Replicate (Year)	3	723.38	0.0035	0.20	<.0001	920.66	0.0009
Year*Genotype	130	224.38	0.009	0.02	0.0103	238.30	0.0065
Residual	199	154.67		0.02		161.22	

DF=degree of freedom; MS=mean squares.

Table 7.2 Pearson correlation coefficients for leaf rust disease severity (DS), infection types (IT), and coefficient of infection (CI) values from trials conducted on Set 1 genotypes in combined trials conducted in 2018, 2019, and 2020 in Winnipeg

	IT	CI
DS	0.81****	1.00****
IT		0.85****

Note: genotype means across three years were used for correlation analysis. n=67.
****significant at p<0.0001.

In Set 1, sixty-seven fall rye genotypes showed a wide range of responses to leaf rust, from resistant or moderately resistant to susceptible (**Table 7.3**). The mean DS was variable, ranging from 7.16 to 53.33%. The mean CI was closely associated with DS. Most open-pollinated genotypes were moderately susceptible or susceptible to leaf rust with a mean DS for open-pollinated genotypes of 53.33%. A few open-pollinated genotypes had lower DS than others, such as ACE-1, Florida 401, Gator, Reimann-Philipp, and Sellino, although they were intermediate or moderately susceptible to leaf rust. Generally, hybrids in Set 1 had better leaf rust resistance than the open-pollinated genotypes. Some hybrids were moderately susceptible or susceptible to leaf rust, but most had very low DS. Overall, KWS Daniello, KWS Fratello, KWS Serafino, KWS

Tayo, KWS Trebiano, and KWS-H187 showed moderate resistance or resistance to leaf rust with extremely low DS. Almost no visible disease symptoms were found on the resistant wheat cultivar Moats. Susceptible symptoms were observed on the two susceptible wheat cultivars, with mean DS ranging from 26.67 to 36.67%. In comparison, fall rye generally had higher mean DS, IT, and CI than the mean of the wheat checks.

Table 7.3 Mean leaf rust disease severity (DS), infection types (IT), and coefficient of infection (CI) values of 67 fall rye genotypes and three winter wheat cultivars from trials conducted on Set 1 genotypes in combined trials conducted in 2018, 2019, and 2020 in Winnipeg

No.	Genotype	Source	DS (%)		IT (0-1)		CI (%)	
			Mean	Rank	Mean	Rank	Mean	Rank
1	Moats	N/A	2.50	1	0.00	1	0.00	1
2	Ptarmigan	N/A	36.67	51	1.00	57	36.67	54
3	Readymade	N/A	26.67	29	1.00	57	26.67	33
4	AC Remington	AAFC-Swift Current	43.33	64	1.00	57	43.33	64
5	AC Rifle	AAFC-Swift Current	40.00	56	1.00	57	40.00	58
6	ACE-1	AAFC-Lethbridge	8.33	4	0.63	13	5.21	7
7	Amilo	Poland	26.67	29	0.92	29	24.58	29
8	Animo	Netherlands	15.83	15	0.88	24	15.00	17
9	Anna	Finland	53.33	70	1.00	57	53.33	70
10	Antelope	U of Saskatchewan	31.67	44	1.00	57	31.67	47
11	Balbo	Italy	30.00	40	0.92	29	28.33	39
12	Brasetto	KWS Lochow (Germany)	24.17	27	0.96	42	23.96	28
13	Caribou	U of Saskatchewan/U of Minnesota	30.00	40	0.92	29	28.33	39
14	Carolkurz	Germany	28.33	34	1.00	57	28.33	39
15	Carsten	Germany	38.33	55	1.00	57	38.33	55
16	Cougar	U of Manitoba	30.00	40	0.92	29	29.17	43
17	Dakold	North Dakota	20.00	18	0.92	29	19.17	23
18	Dakota	Canada	41.67	60	1.00	57	41.67	62
19	Dankowskie Nowe	N/A	28.33	34	0.96	42	27.92	38
20	Dankowskie Selekc	Poland	40.00	56	1.00	57	40.00	58
21	Dankowskie Srebrne	Poland	26.67	29	0.92	29	25.00	30
22	Dominant	Holland	35.00	50	0.96	42	33.33	49
23	Florida 401	Florida	10.83	9	0.58	9	7.29	10
24	Frontier	AAFC- Swift Current	20.00	18	0.92	29	19.17	23

25	Gator	University of Florida	16.67	16	0.67	15	11.25	15
26	GC-100	Russia	50.83	69	0.92	29	50.42	69
27	Guttino	KWS Lochow (Germany)	28.33	34	0.92	29	26.25	32
28	Halo	Germany	33.33	47	0.83	20	30.42	44
29	Hazlet	AAFC- Swift Current	30.83	43	0.96	42	30.63	45
30	Horton	Canada	46.67	66	0.96	42	45.00	66
31	Kharkivska 95	Ukraine	36.67	51	0.96	42	36.25	53
32	Kharkivska 98	Ukraine	33.33	47	0.96	42	32.50	48
33	Kodiak	U of Alberta	46.67	66	1.00	57	46.67	68
34	Kustro	AAFC-Kentville	31.67	44	0.96	42	31.25	46
35	KWS Bono	KWS Lochow (Germany)	20.00	18	0.75	17	16.25	18
36	KWS Daniello	KWS Lochow (Germany)	10.00	8	0.58	9	6.25	9
37	KWS Fratello	KWS Lochow (Germany)	8.41	5	0.50	6	3.98	6
38	KWS Gatano	KWS Lochow (Germany)	11.67	11	0.71	16	8.75	11
39	KWS Loretto	KWS Lochow (Germany)	14.91	13	0.62	11	11.06	14
40	KWS Serafino	KWS Lochow (Germany)	9.17	6	0.38	5	2.71	5
41	KWS Tayo	KWS Lochow (Germany)	7.16	2	0.31	3	1.79	2
42	KWS Trebiano	KWS Lochow (Germany)	9.17	6	0.29	2	2.50	4
43	KWS-H101	KWS Lochow (Germany)	13.66	12	0.62	11	9.19	12
44	KWS-H187	KWS Lochow (Germany)	7.16	2	0.37	4	2.41	3
45	KWS-H192	KWS Lochow (Germany)	10.91	10	0.56	8	5.85	8
46	Maton	Oklahoma	21.67	25	0.88	24	19.58	25
47	Motto	Poland	28.33	34	0.92	29	27.08	34
48	Musketeer	AAFC-Swift Current	20.00	18	0.79	18	16.67	19
49	Oklon	Oklahoma	28.33	34	0.92	29	27.08	34
50	Othello	Sweden	36.67	51	0.96	42	35.00	51
51	Pearl	Denmark	23.33	26	0.83	20	20.00	26
52	Petkus	Germany	42.93	63	0.94	41	40.59	61
53	Ponsi	Sweden	28.33	34	0.83	20	25.42	31

54	Prima	AAFC-Swift Current	20.83	24	0.88	24	18.96	22
55	Prolific Spring	Canada	41.67	60	0.96	42	40.00	58
56	Protector	Germany	24.17	27	0.83	20	21.88	27
57	Puma	U of Manitoba	31.67	44	0.88	24	28.75	42
58	R003-4	Canada	20.00	18	0.79	18	16.67	19
59	Reimann-Philipp	Germany (U of Hohenheim)	16.67	16	0.54	7	11.88	16
60	Saratovskava 4	Russia	36.67	51	0.96	42	35.00	51
61	Sellino	Germany	15.00	14	0.63	13	10.42	13
62	SM4R	AAFC-Saskatoon Research Centre	41.67	60	1.00	57	41.67	62
63	Stoir	Ukraine	40.00	56	0.96	42	38.33	55
64	Syn 20L	Germany	20.00	18	0.88	24	18.75	21
65	Toivo	Finland via U of Alberta	43.33	64	1.00	57	43.33	64
66	Visa	Finland	46.67	66	0.96	42	45.00	66
67	Vitallo	Germany	40.00	56	0.96	42	38.33	55
68	Voima	Finland	33.33	47	1.00	57	33.33	49
69	Wheeler	Michigan State University	27.41	32	1.00	56	27.31	37
70	Wrens Abruzzzi	U of Georgia	27.50	33	0.92	29	27.08	34
	LSD (0.05)		14.56		0.14		14.86	
	Wheat means		21.94		0.67		21.11	
	Rye means		27.70		0.84		25.71	
	Rye range		7.16-53.33		0.29-1.00		1.79-53.33	

LSD=least significant difference.
N/A means that data are not available.
Green highlight represents fall rye genotypes with better leaf rust resistance, and underline represents the cultivars listed in prairie seed guides.

7.4.2 Results of Set 2

The genotype effect was significant for DS, IT, and CI. Effects of year and the interaction between genotype and year were not significant for all measured variables (**Table 7.4**). Correlations between DS, IT, and CI were all significant and positive (**Table 7.5**). A high correlation coefficient was observed between DS and CI (r=0.98).

Table 7.4 Combine analyses of variance for leaf rust disease severity (DS), infection types (IT), and coefficient of infection (CI) values measured in field trials conducted on Set 2 genotypes in 2019 and 2020 in Winnipeg

Source	DF	DS		IT		CI	
		MS	Pr > F	MS	Pr > F	MS	Pr > F
Genotype	83	2.869045	<.0001	0.191356	<.0001	4.111563	<.0001
Year	1	0.167213	0.522	1.252125	0.0716	7.9299	0.3902
Replicate (year)	2	0.250484	0.6164	0.06328	0.2259	0.088891	0.8865
Year*Genotype	83	0.570242	0.292	0.033858	0.8676	0.709887	0.5697
Residual	166	0.516101		0.042159		0.737061	

DF=degree of freedom; MS=mean squares.

Table 7.5 Pearson correlation coefficients for leaf rust disease severity (DS), infection types (IT), and coefficient of infection (CI) values from combined trials conducted on Set 2 genotypes in 2019 and 2020 in Winnipeg

	IT	CI
DS	0.60****	0.98****
IT		0.72****

Note: genotype means across three years were used for correlation analysis. n=81.
****significant at p<0.0001.

In Set 2, rye genotypes showed very low mean DS, ranging from 3.75 to 20%, although there was a wide range of responses to leaf rust, from resistant to susceptible (**Table 7.6**). The mean CI was highly variable, ranging from 0.94 to 18.75. Among nine commercial hybrids, only KWS-H145 showed moderate resistance with mean DS of 5%. All other hybrids were more susceptible than KWS-H145. Half of the testcross hybrids generated from the Petkus gene pool

produced resistant to intermediate reactions to leaf rust with mean DS as low as 3.75%. They included Li2, Li3, Li4, Li6, Li7, Li9, Li10, Li14, Li17, Li23, Li24, Li25, Li26, and Li41. In contrast, most testcross hybrids developed from the Carsten gene pool were moderately susceptible or susceptible to leaf rust, with mean DS ranging from 3.75 to 20%. No disease symptoms were observed on leaves of the resistant wheat cultivar Moats, while both susceptible wheat checks Ptarmigan and Readymade showed susceptible reactions. Overall, fall rye genotypes in Set 2 had much lower mean DS and CI, but the same mean IT compared to the mean of the wheat checks.

Table 7.6 Mean leaf rust disease severity (DS), infection types (IT), and coefficient of infection (CI) values of 81 fall rye genotypes and three winter wheat cultivars from combined trials conducted on Set 2 genotypes in 2019 and 2020 in Winnipeg

No.	Genotype	Type	Gene pool	Tester	DS (%)		IT (0-1)		CI (%)	
					Mean	Rank	Mean	Rank	Mean	Rank
1	Moats	Winter wheat	N/A	N/A	0.00	1	0.00	1	0.00	1
2	Ptarmigan	Winter wheat	N/A	N/A	45.00	84	1.00	83	45.00	84
3	Readymade	Winter wheat	N/A	N/A	40.00	83	1.00	83	40.00	83
4	Exp.Variety1	KWS-Hybrid	N/A	None	10.00	66	0.75	37	8.44	67
5	Exp.Variety2	KWS-Hybrid	N/A	None	7.50	51	0.81	51	6.25	54
6	Exp.Variety17	KWS-Hybrid	N/A	None	6.25	34	0.75	37	5.00	42
7	Exp.Variety31	KWS-Hybrid	N/A	None	6.25	34	0.81	51	5.00	42
8	KWS-H10110	KWS-Hybrid	N/A	None	8.75	57	0.75	37	7.50	60
9	KWS-H119	KWS-Hybrid	N/A	None	11.25	72	0.88	64	10.31	72
10	KWS-H144	KWS-Hybrid	N/A	None	5.00	7	0.81	51	4.06	33
11	KWS-H145	KWS-Hybrid	N/A	None	5.00	7	0.38	9	1.88	9
12	KWS-H161	KWS-Hybrid	N/A	None	6.25	34	0.69	31	4.69	38
13	Li1	Testcross	Petkus	T517	5.00	7	0.63	25	3.13	24
14	Li2	Testcross	Petkus	T517	5.00	7	0.31	7	1.56	7
15	Li3	Testcross	Petkus	T517	5.00	7	0.25	3	1.25	3
16	Li4	Testcross	Petkus	T517	5.00	7	0.25	3	1.25	3
17	Li5	Testcross	Petkus	T517	6.25	34	0.75	37	4.69	38
18	Li6	Testcross	Petkus	T517	3.75	2	0.38	9	1.88	9
19	Li7	Testcross	Petkus	T517	5.00	7	0.25	3	1.25	3
20	Li8	Testcross	Petkus	T517	5.00	7	0.75	37	3.75	28
21	Li9	Testcross	Petkus	T517	5.00	7	0.38	9	1.88	9
22	Li10	Testcross	Petkus	T517	5.00	7	0.44	16	2.19	15
23	Li12	Testcross	Petkus	T517	5.00	7	0.75	37	3.75	28
24	Li14	Testcross	Petkus	T517	3.75	2	0.25	3	1.25	3
25	Li15	Testcross	Petkus	T517	5.00	7	0.50	18	2.50	17
26	Li16	Testcross	Petkus	T517	6.25	34	0.63	25	3.75	28

27	Li17	Testcross	Petkus	T517	5.00	7	0.44	16	2.19	15
28	Li18	Testcross	Petkus	T517	7.50	51	0.75	37	5.94	53
29	Li19	Testcross	Petkus	T517	6.25	34	0.69	31	4.38	35
30	Li20	Testcross	Petkus	T517	5.00	7	0.81	51	4.06	33
31	Li21	Testcross	Petkus	T517	5.00	7	0.50	18	2.50	17
32	Li22	Testcross	Petkus	T517	8.75	57	0.38	9	5.63	50
33	Li23	Testcross	Petkus	T517	5.00	7	0.38	9	1.88	9
34	Li24	Testcross	Petkus	T517	5.00	7	0.38	9	1.88	9
35	Li25	Testcross	Petkus	T517	5.00	7	0.31	7	1.56	7
36	Li26	Testcross	Petkus	T517	3.75	2	0.19	2	0.94	2
37	Li27	Testcross	Petkus	T517	5.00	7	0.56	23	2.81	22
38	Li40	Testcross	Petkus	T517	8.75	57	0.69	31	7.19	58
39	Li41	Testcross	Petkus	T517	5.00	7	0.38	9	1.88	9
40	Li42	Testcross	Petkus	T517	5.00	7	0.50	18	2.50	17
41	Li102	Testcross	Carsten	T217	20.00	82	0.88	64	18.75	82
42	Li103	Testcross	Carsten	T217	18.75	81	0.94	81	18.44	81
43	Li104	Testcross	Carsten	T217	10.00	66	0.75	37	8.75	68
44	Li105	Testcross	Carsten	T217	7.50	51	0.88	64	6.25	54
45	Li106	Testcross	Carsten	T217	6.25	34	0.75	37	5.00	42
46	Li107	Testcross	Carsten	T217	15.00	77	0.88	64	13.75	77
47	Li111	Testcross	Carsten	T217	10.00	66	0.88	64	8.75	68
48	Li113	Testcross	Carsten	T217	5.00	7	0.56	23	2.81	22
49	Li114	Testcross	Carsten	T217	7.50	51	0.63	25	5.31	49
50	Li116	Testcross	Carsten	T217	8.75	57	0.81	51	7.19	58
51	Li117	Testcross	Carsten	T217	5.00	7	0.69	31	3.44	26
52	Li119	Testcross	Carsten	T217	8.75	57	0.81	51	7.50	60
53	Li120	Testcross	Carsten	T217	8.75	57	0.81	51	7.81	64
54	Li122	Testcross	Carsten	T217	12.50	74	0.88	64	10.63	74
55	Li123	Testcross	Carsten	T217	17.50	80	0.88	64	16.88	80
56	Li125	Testcross	Carsten	T217	5.00	7	0.75	37	3.75	28
57	Li126	Testcross	Carsten	T217	6.25	34	0.69	31	4.38	35
58	Li127	Testcross	Carsten	T217	6.25	34	0.81	51	5.00	42

59	Li128	Testcross	Carsten	T217	11.25	72	0.88	64	10.31	72
60	Li129	Testcross	Carsten	T217	10.00	66	0.63	25	7.81	64
61	Li131	Testcross	Carsten	T217	15.00	77	0.94	81	14.38	78
62	Li132	Testcross	Carsten	T217	5.00	7	0.50	18	2.50	17
63	Li133	Testcross	Carsten	T217	15.00	77	0.88	64	14.38	78
64	Li134	Testcross	Carsten	T217	10.00	66	0.88	64	9.06	70
65	Li135	Testcross	Carsten	T217	8.75	57	0.88	64	7.50	60
66	Li136	Testcross	Carsten	T217	5.00	7	0.75	37	3.75	28
67	Li137	Testcross	Carsten	T217	6.25	34	0.88	64	5.63	50
68	Li138	Testcross	Carsten	T217	6.25	34	0.75	37	4.69	38
69	Li139	Testcross	Carsten	T217	7.50	51	0.88	64	6.56	56
70	Li140	Testcross	Carsten	T217	6.25	34	0.81	51	5.00	42
71	Li141	Testcross	Carsten	T217	8.75	57	0.81	51	7.81	64
72	Li142	Testcross	Carsten	T217	6.25	34	0.88	64	5.63	50
73	Li143	Testcross	Carsten	T217	13.75	76	0.88	64	12.81	76
74	Li144	Testcross	Carsten	T217	7.50	51	0.88	64	6.56	56
75	Li145	Testcross	Carsten	T217	5.00	7	0.69	31	3.44	26
76	Li146	Testcross	Carsten	T217	6.25	34	0.81	51	5.00	42
77	Li147	Testcross	Carsten	T217	6.25	34	0.63	25	4.38	35
78	Li148	Testcross	Carsten	T217	6.25	34	0.75	37	4.69	38
79	Li164	Testcross	Carsten	T217	8.75	57	0.81	51	7.50	60
80	Li165	Testcross	Carsten	T217	10.00	66	0.88	64	9.38	71
81	Li166	Testcross	Carsten	T217	3.75	2	0.63	25	3.13	24
82	Li167	Testcross	Carsten	T217	3.75	2	0.50	18	2.50	17
83	Li168	Testcross	Carsten	T217	6.25	34	0.75	37	5.00	42
84	Li169	Testcross	Carsten	T217	12.50	74	0.81	51	10.63	74
	LSD (0.05)				7.44		0.29		7.89	
	Wheat means				28.33		0.67		28.33	
	Rye means				7.48		0.67		5.76	
	Rye range				3.75-20.00		0.19-0.94		0.94-18.75	

LSD=least significant difference.
N/A means that data are not available.

Green highlight represents hybrids with better leaf rust resistance.

7.5 Discussion

In the current study, almost no pustules were observed on the flag leaves before senescence, even though spray inoculations were conducted every year. Therefore, it was not feasible to determine plant reactions to leaf rust based on the symptoms on the flag leaf. Differences in pustules or uredinia on the penultimate leaf were observed. Consequently, rye reaction to leaf rust was assessed based on symptoms on the penultimate leaf. This provided good differentiation among the genotypes tested for reaction to leaf rust in the field. Compared to winter wheat, the flag leaf of fall rye is usually much smaller, and therefore, difficult to evaluate for leaf rust by visual rating (Miedaner and Sperling, 1995; Schlegel,2013b). Miedaner and Sperling (1995) suggested that leaf rust reactions should not be done exclusively on the flag leaf when assessing quantitative resistance due to the highly significant interaction between genotype and environment on the flag leaf rating. Multiple leaf ratings were generally used to assess leaf rust reaction of fall rye (Miedaner and Sperling, 1995; Miedaner et al., 2002; Wehling et al., 2003; Roux et al., 2004; Bankina et al., 2013). For example, Miedaner and Sperling (1995) used the flag leaf and the first and second leaves below the flag leaf to assess quantitative resistance to leaf rust on rye. The high correlation coefficients among the three leave ratings indicated that quantitative adult plant resistance did not depend on leaf position (Miedaner and Sperling, 1995).

The pathogen *P. recondita* causing rye leaf rust has been differentiated from the causal agent of wheat leaf rust, *P. triticina*, due to differences in spore morphology and failure to intercross (Anikster et al., 1997). Nevertheless, rye was the host for both *P. triticina* and *P. recondita*, but wheat was the host only for *P. triticina* (Anikster et al., 1997). Urediniospores used for inoculations in the current study were collected from fall rye fields under natural rust infection. Limited information is available for the pathogen of leaf rust on rye in western Canada. According to the host ranges of *P. triticina* and *P. recondita* described by Anikster et al. (1997), leaf rust collected from rye could belong to *P. triticina* or *P. recondita*, or a mixture of both. The susceptible winter wheat checks, Ptarmigan and Readymade, showed medium to large-sized uredia with little or no chlorosis in Set 1 and Set 2. It indicated that *P. triticina* may be included in the collected leaf rust samples to cause infection on wheat checks. On the other hand, winter wheat cultivars could be infected by *P. triticina* from natural epidemics or the spread of inoculum from inoculated winter wheat rust nurseries at the research station.

Genotype effect was significant for all variables in both Set 1 and Set 2, while years had no significant effect on all measured variables. The interaction between genotype and year had a significant effect on all variables in Set 1, but not in Set 2. The significant interaction between year and genotypes in Set 1 was mainly derived from relatively minor changes in rank between genotypes across years. In several European studies, genotype × environment interaction is considerable under natural leaf rust infection (Wilde et al., 2006). Pathogen populations of rye leaf rust in Europe have much higher diversity than wheat leaf rust (Miedaner et al., 2012). In the current study, ten single pustules were mixed and increased on five rye cultivars. The mixtures may include several races of the pathogen. There is no information about the pathogen population of rye leaf rust in western Canada. If the diversity of the pathogen population in Canada is as high as in Europe, rye genotype materials included in the current study can be further tested by inoculum collected from multiple locations and years. The same location may provide different virulence patterns in different years (Miedaner et al., 2012).

Previous studies indicated that leaf rust resistance is not common on rye (Loiveke, 1997 cited by Bankina et al., 2013; Miedaner et al., 2002). Loiveke (1997) found that all fall rye lines tested were susceptible to leaf rust in Estonia. In a study conducted in Finland, differences in leaf rust resistance of fall rye lines were not consistently expressed across environments (Serenius et al., 2005). In Germany, all widely grown open-pollinated varieties and hybrids are rated as 5 or 6 on a 1-9 scale with 1=healthy under natural infections (Miedaner et al., 2002). In the current study, fall rye genotypes differed in reactions to leaf rust. Most open-pollinated genotypes were moderately susceptible or susceptible to leaf rust, and a few genotypes had lower DS than other genotypes. In addition, most hybrids in Set 1 and testcross hybrids developed from the Petkus gene pool in Set 2 showed moderate resistance or intermediate IT to leaf rust with very low DS. Compared to wheat checks, open-pollinated rye and hybrids in Set 1 had higher mean DS, IT, and CI, while hybrids in Set 2 had much lower mean DS and CI. It indicated that hybrids in the study were more resistant than open-pollinated genotypes. However, in previous studies, widely grown hybrids have been reported to show a rather high level of susceptibility and be more susceptible than open-pollinated cultivars, such as Amilo (Anonymous, 2004 cited by Wilde et al., 2006; Bankina et al., 2013). Most previously released hybrids were susceptible to leaf rust due to the

lack of resistance in the source population used for hybrid breeding (Rollwitz, 1985 cited by Wilde et al., 2006; Miedaner and Sperling, 1995).

Resistance sources have been found from German, Polish, Russian, and North American varieties (Rollwitz, 1985 cited by Wilde et al., 2006; Solodukhina, 1997). Some open-pollinated varieties, including Amilo, Born, Danko, and Motto, were reported as moderately resistant (score 3-4) in Germany (Anonymous, 1998 cited by Miedaner et al., 2012). In the southern United States, rye variety Gator has resistance to leaf rust (Martin and Leonard, 1967; Morey, 1973). In the current study, although Gator exhibited intermediate or moderate susceptibility to leaf rust, it showed mean DS lower than 20%. Results indicated that Gator had better leaf rust resistance than other open-pollinated genotypes. However, in Set 1, Amilo and Motto were moderately susceptible or susceptible to leaf rust with mean DS of 26.67% and 28.33%, respectively. Different scoring methods, inoculum sources, and experimental environments may contribute to the inconsistent reports of leaf rust response of the same varieties in different studies. Miedaner et al. (2012) indicated that rye genotypes that showed resistance to leaf rust in some sites might be confronted with virulent isolates in other areas. The composition of pathogen populations plays an important role in leaf rust resistance selection (Miedaner et al., 2012). In the German leaf rust population, the high diversity and complexity of host-specific virulence resulted in decreasing levels of race-specific resistance (McDonald and Linde, 2002; Wilde et al., 2006). Quantitative resistance was recommended to achieve durable resistance to leaf rust in fall rye (Wilde et al., 2006; Miedaner et al., 2012).

Leaf rust infection of rye occurs after flowering in different European countries (Miedaner and Sperling, 1995; Loiveke, 1997 cited by Bankina et al., 2013; Bankina et al., 2013). In Germany, natural leaf rust epidemics typically start at ripening (Miedaner and Sperling, 1995). However, the first infection of leaf rust could be observed as early as flowering (Miedaner and Sperling, 1995). In southern Manitoba, natural leaf rust infection generally started to appear on susceptible fall rye cultivars in late July, based on our observation in fields. Fall rye may escape leaf rust infection because it matures before epidemics can develop in most years. Nevertheless, cereal rust occurrence in southern Manitoba is a reflection of its development in the south-central United States. Rusts overwinter there and move northward into Canada by wind through the Puccinia pathway (Aboukhaddour et al., 2020). If the rust season starts earlier than usual in the

United States, rye may have some rust issues in the rust prone areas involving southern Manitoba and eastern Saskatchewan.

The use of resistant cultivars is the most economically and environmentally friendly strategy against leaf rust, although leaf rust in rye may be controlled by fungicide applications (Roux et al., 2004; Miedaner et al., 2012). The current study included open-pollinated genotypes from different countries and all hybrids registered in Canada. The information about the leaf rust reaction of current cultivars and germplasms was not available to producers prior to the study. It will assist rye growers in determining which cultivars to grow in the rust regions of western Canada. Breeders will also be able to use the information to develop resistant rye cultivars to leaf rust, and the information can also be used in breeding resistant lines of wheat and triticale. In addition to adult plant resistance, seedling resistance can be assessed in future studies. It is important to have seedling resistance to leaf rust if rye is used for pasture or silage. Agronomic traits can be evaluated to determine the impact of leaf rust on yield and quality of rye biomass for forage.

Chapter 8

General discussion and conclusion

The current study provides fundamental information on fall rye resistance to fusarium head blight (FHB), leaf rust, and ergot, and the potential for use of the fungicide Prosaro™ for FHB control on rye. Overall, the study:

- filled gaps in our understanding of the prevalence of *Fusarium* species causing FHB on rye in Manitoba
- compared pathogenicity of different *Fusarium* species on six rye genotypes in the greenhouse
- evaluated FHB and ergot resistance on open-pollinated and hybrid rye in multi-environment replicated trials
- assessed the efficacy of fungicide with different application timings for mitigation of FHB on rye
- evaluated leaf rust resistance on a mixture of fall rye germplasm in fields.

The first study assessed *Fusarium* species isolated from rye spike samples collected in Manitoba from 2016 to 2018. Twenty-four percent of 960 kernel samples were infected with one or more of five *Fusarium* species, including *Fusarium graminearum, F. avenaceum, F. poae, F. sporotrichioides*, and *F. equiseti*. Sixty-seven percent of the *Fusarium* species isolates were *F. graminearum*, and twenty percent were *F. avenaceum*. Other species, including *F. poae*, *F. sporotrichioides*, and *F. equiseti*, were identified at low frequencies. It showed that, in Manitoba, *F. graminearum* was the most prevalent *Fusarium* species causing FHB on rye. Analyses of rye spike samples collected in 2010 also reported that *F. graminearum* accounted for 56% of isolated *Fusarium* species from Manitoba (Grafenhan et al., 2013). In comparison, *F. avenaceum* was the most frequently isolated species from rye samples collected from Alberta and Saskatchewan, accounting for 100% and 82% of isolated species, respectively (Grafenhan et al., 2013). Overall, the information provided by the current study indicates that rye can be infected by several *Fusarium* species. Although natural infections of FHB occurred at low frequencies in rye fields in southern Manitoba, producers need to monitor weather conditions during flowering to determine

the potential risk of FHB infection. In Manitoba, *F. graminearum* was the predominant species causing FHB in wheat and rye. The finding suggests that in Manitoba, the pathogen of concern and FHB management on rye will be similar to wheat.

The second study evaluated the pathogenicity of *F. graminearum, F. avenaceum, F. sporotrichioides*, and *F. poae* in six fall rye genotypes and two winter wheat checks under controlled environment conditions. *Fusarium graminearum* caused significantly higher FHB incidence and severity than the other three species, and as a result, it was the most pathogenic species causing FHB on rye. The high pathogenicity of *F. graminearum* was also reported in wheat and barley (Wong et al., 1995; Stack et al., 1997; McCallum and Tekauz, 1999; Xue et al., 2004). Moreover, the disease spread quickly in infected spikes inoculated with *F. graminearum* isolates, and the whole spike of susceptible genotypes of rye and wheat was almost bleached out within 18 days after the first inoculation. The rapid disease spread of *F. graminearum* on rye is a concern for rye growers, as it has the potential to cause severe FHB infection when conditions favor the disease. The fungal biomass in spikes infected by *F. graminearum* was significantly higher than the other three species. The fungal biomass of *F. graminearum* (r=0.70) and *F. avenaceum* (r=0.64) was significantly correlated with infected spikelets. The high correlation indicates that visual assessment of infected spikelets in fields may be used for the prediction of fungal biomass. Compared to *F. graminearum*, *F. avenaceum* caused less disease and resulted in a relatively lower percentage of infected spikelets, particularly in resistant genotypes. As the second most pathogenic *Fusarium* species, *F. avenaceum* produces primarily moniliformin (MON) which is one of the emerging *Fusarium* toxins of concern in small grain cereals (Grafenhan et al., 2013). Although *F. sporotrichioides* and *F. poae* could infect rye genotypes, there was almost no disease spread, particularly for *F. poae*. The fungal biomass of *F. sporotrichioides* and *F. poae* was very low and had no relationship with infected spikelets. Accordingly, when infection levels are low, visual FHB severity cannot be a prediction of the fungal biomass of *F. sporotrichioides* and *F. poae*.

The third study evaluated the reaction of a mixture of germplasm, including open-pollinated and hybrid genotypes, to *F. graminearum* with artificial inoculation from 2017 to 2020 in southern Manitoba. Generally, fall rye had lower FHB disease levels than winter wheat, which was consistent with the results of previous studies (Arseniuk et al., 1993, 1999; Miedaner et al., 2001; Gaikpa et al., 2019). The relatively high FHB incidence, but low FHB severity in rye

suggested that rye had better resistance to disease spread within infected spikes than resistance to initial infection. Meanwhile, fall rye had much lower *Fusarium*-damaged kernels (FDK) and deoxynivalenol (DON) than winter wheat. Gaikpa et al. (2019) also reported that fall rye had lower FDK and DON than winter wheat and triticale. More resistance to FHB disease spread in rye may result in less infection and DON accumulations in kernels. In addition, it was observed that the infected fall rye florets often did not set seed in the field, which may have led to fewer FDK and lower DON in harvested grain samples. This differs from wheat, which generally still sets seed even when infected with *Fusarium*. Among fall rye genotypes, there was a wide range of reactions to *F. graminearum*. Some open-pollinated genotypes from Canada, Finland, and Germany, hybrids registered in Canada, and testcross hybrids derived from the Carsten gene pool showed better FHB resistance than other genotypes. The results from this work indicate that FHB resistant germplasm is available for breeders to develop new FHB resistant cultivars. This would provide farmers with access to more resistant cultivars in the future. The study also provided critical and practical information for rye growers in western Canada about FHB rection of commercial rye cultivars. The reaction of commercial rye cultivars to FHB is novel information for producers and will help them make informed decisions when choosing rye cultivars to grow in regions that are prone to FHB epidemics. Additionally, the correlation between FHB field traits and FDK or DON was moderate in the current study. This could be caused by the lack of seed set in rye as described above, or infected seed losses during harvest using a combine. The moderate associations reveal that rye breeders should evaluate FHB field traits, FDK, and DON in harvested seeds to determine FHB resistance on rye.

Fall rye genotypes showed variable reactions to natural ergot infection. Some fall rye genotypes were very susceptible to ergot, but several hybrids had lower ergot severity than other genotypes. Use thses hyrbids could be an important strategy for ergot reduction in rye.

The fourth study determined the optimal application timing of the fungicide Prosaro™ for FHB management in fall rye. The efficacy of different application timings was variable across site years. Several factors, such as plant stands and weather conditions, influenced the uniformity of flowering periods and made it difficult to assess the growth stage of each plot. The accuracy of the plant growth stage would directly affect the evaluation of the optimal application timing. The overall results suggest that a window between 10% to 80% anthesis is recommended for rye

growers to apply fungicide to suppress FHB infection and reduce DON accumulation. In wheat, the recommended fungicide application timing for FHB suppression was early flowering and up to six days after flowering (D'Angelo et al., 2014). However, rye has a longer flowering time (up to two weeks) than wheat. This means that rye growers have a wider window for fungicide application and may be able to schedule fungicide application around precipitation events. For thousand kernel weight (TKW), test weight (TWT), and grain yield, the genotype effect was generally more significant than the treatment effect. Fungicide applications only significantly reduced yield losses under a high mean FHB index. However, under a relatively high mean FHB index, the fungicide Prosaro™ could not reduce DON concentrations below the official threshold of 2 ppm, despite significant suppression of FHB symptoms. Overall, DON concentrations were decreased to the acceptable level by fungicide application under the conditions where grain yield was neither significantly reduced by the disease, nor improved by fungicide treatments. Therefore, the main benefit of fungicide applications would be to prevent grain rejection from elevators. Risk assessment tools are expected to help farmers make better decisions regarding fungicide use for FHB and DON management on rye. As the first study to evaluate the fungicide Prosaro™ for FHB on rye, the results also reveal that fungicide treatments may not adversely affect pollination and seed set, which agrees with the study of the fungicides Caramba and Miravis Ace on rye (Minnesota Crop News, 2020).

The most resistant fall rye genotype used in the fungicide trials exhibited more stable performance than the other genotypes. This agrees with previous studies that reported that moderately resistant wheat cultivars provided higher fungicide efficacy than susceptible cultivars (Wegulo et al., 2011; Mesterhazy et al., 2003, 2011; 2018a). However, the advantage of a combination of resistant genotypes and fungicide application was not consistent in the current study. Therefore, the use of resistant genotypes is highly recommended as the first step for FHB management on rye. Fungicide application should be determined based on FHB disease forecasting models and crop stage.

The correlations between FHB index and DON were moderate to strong in the fungicide trials, but moderate in the FHB nurseries of fall rye. Paul et al. (2005) reported that fungicide evaluation studies had significantly higher correlations between FHB index and DON than genotype evaluation studies in wheat. As explained by Paul et al. (2005), treatments rather than

genotypes mainly contributed to the variation in FHB index and DON in the fungicide trials. In the current study, a range of genotypes with different levels of FHB resistance was used in the FHB nurseries, which potentially resulted in a range of DON contents for a similar level of disease, or a range of FHB index for a similar level of DON. As a result, a weaker correlation between FHB index and DON was found in the FHB nurseries than in the fungicide trials.

The fifth study assessed the reaction of open-pollinated genotypes and hybrids to leaf rust in Winnipeg across multiple years. The practical experiences demonstrate that the short average leaf age and small leaf area of the flag leaf of rye make it more difficult to evaluate leaf rust on the flag leaf compared to wheat. The flag leaf was not suggested to be the only leaf used for assessing leaf rust resistance in rye due to the highly significant genotype × environment interaction (Miedaner and sperling, 1995). In the current study, the penultimate leaf exhibited differentiation among plots in the rust nurseries and was used to distinguish the reaction of rye germplasm to leaf rust. This information is useful for optimizing methodologies to assess leaf rust in fall rye. Although leaf rust resistance is not common in rye based on several studies in Europe, resistance has been mainly found in varieties from Finland, Russia, and the United States and rarely in old German varieties (Rollwitz, 1985 cited by Wilde et al., 2006; Loiveke, 1997 cited by Bankina et al., 2013; Solodukhina, 1997; Miedaner et al., 2002). In the current study, several open-pollinated genotypes from Canada, the United States, and Germany exhibited better leaf rust resistance with low disease severity. Hybrids have been reported to be highly susceptible to leaf rust in Europe (Anonymous, 2004 cited by Wilde et al., 2006; Bankina et al., 2013). However, in this study, most commercial hybrids and some testcross hybrids derived from the Petkus gene pool showed resistance or moderate resistance to leaf rust with very low disease severity. The difference could be due to a different composition of leaf rust races than what is present in Europe. Previous studies have shown that virulence patterns were variable in different regions and years (Miedaner et al., 2012). The diversity of the pathogen population is much higher in rye leaf rust than in wheat leaf rust (Wilde et al., 2006). The current study provides leaf rust rating of current rye cultivars which will help producers in rust prone regions choose the best cultivars for their region. Although leaf rust is not destructive on rye in western Canada in most years, the use of resistant cultivars is an important strategy to control leaf rust if leaf rust becomes a problem in years when conditions

favor the disease. Meanwhile, breeders will also be able to use the information to develop rust resistant cultivars.

Together, hybrids KWS Daniello, KWS Gatano, KWS Serafino, and KWS Trebiano provided better resistance to all three pathogens (FHB, leaf rust and ergot) than other genotypes tested. Among them, KWS Daniello, KWS Gatano, and KWS Trebiano are currently available genotypes listed in the prairie seed guides. The open-pollinated genotype ACE-1 exhibited resistance to FHB and leaf rust. Hybrids Exp.Variety17 and KWS-H144, and the open-pollinated genotype Anna showed FHB resistance and low ergot severity. These genotypes would be the most useful use in breeding programs.

In conclusion, the current studies provided comprehensive information about the reaction of a mixture of fall rye germplasm to FHB, leaf rust, and ergot. The study of the causal agent of FHB suggests that the pathogen causing FHB on rye is similar to wheat. In consideration of fungicide efficacy for FHB management in fall rye, the optimal fungicide application timing is recommended as a window between 10% to 80% anthesis. Hybrids registered in Canada are desirable for producers due to their excellent agronomic traits, such as high yield and lodging tolerance. The current studies confirm the superiority of hybrid rye in terms of resistance, or tolerance, to three major diseases, FHB, leaf rust and ergot. The resistance to FHB and leaf rust assessed in currently available commercial rye cultivars will assist rye growers to choose resistant cultivars for the management of FHB and leaf rust. The information derived from the fungicide trials will help producers with decision making when the risk of FHB infection is high. Meanwhile, screening of a mixture of germplasm, including open-pollinated genotypes and hybrids, provides useful information for rye breeders developing new resistant genotypes to FHB and leaf rust. Together, the current studies provide baseline information for breeders to support the future development of resistant cultivars and practical information for rye growers to control FHB and leaf rust in western Canada and perhaps other regions of the country.

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Appendices

Appendix 1. Medium preparation

Water agar (WA)
Components:
20 g agar, 0.25 g streptomycin sulfate, and 1 L distilled water.
Procedure:
Melt agar into heated distilled water (approximately 80 ℃) in a KIMBLE™ KIMAX™ Media bottle (Sigma-Aldrich, USA). Autoclave at 121℃ for 15 mins to sterility. Remove from autoclave and add streptomycin sulfate. Allow medium to cool to 55-60 ℃ before pouring.

Spezieller nährstoffarmer agar (SNA)
Components:
1 g potassium phosphate, 1 g potassium nitrate, 0.5 g magnesium sulphate, 0.5 g potassium chloride, 0.2 g glucose, 0.2 g sucrose, 20 g agar, 0.25 g streptomycin sulfate, and 1 L distilled water.
Procedure:
Mix distilled water and all the ingredients except streptomycin sulfate into a 1 L KIMBLE™ KIMAX™ Media bottle (Sigma-Aldrich, USA). Autoclave at 121℃ for 25 mins to sterility. Remove from autoclave and add streptomycin sulfate. Allow medium to cool to 55-60 ℃ before pouring.

Potato dextrose agar (PDA)
Components:
39 g PDA, 0.25 g streptomycin sulfate, and 1 L distilled water.
Procedure:
Mix the PDA into boiled distilled water into a 1 L KIMBLE™ KIMAX™ Media bottle (Sigma-Aldrich, USA). Autoclave at 121℃ for 15 mins. After autoclaving, add streptomycin sulfate into the solution. Allow medium to cool to 55-60 ℃ before pouring.

Carboxymethyl cellulose (CMC) medium
Components:
1.5 g ammonium nitrate, 1.5 g potassium phosphate, 075 g magnesium sulphate, 1.5 g yeast extract, 22.5 g CMC, 0.375 g streptomycin sulfate, and 1.5 L distilled water.
Procedure:
Mix 750 mL of distilled water and all the ingredients except the CMC and streptomycin sulfate in a plastic beaker using a hand blender. Once mixed, slowly add the CMC to the mixture and mix again. Pour the mixture into a 2 L Erlenmeyer flask. Add the remaining 750 mL of distilled water to the beaker and mix until the blender and beaker is rinsed. Pour the mixture into the flask. Cover the flask with foil and autoclave at 121 ℃ for 30 mins. After autoclaving, allow the solution to cool to room temperature and add streptomycin sulfate.

Appendix 2. Sequencing information (personal communication with Dr. Maria Antonia Henriquez)

Final concentration of PCR reagents for IGS primer:

Dream Taq Buffer	1×
$MgCl_2$	1.2mM
dNTP	0.2mM
BSA	0.8mg/mL
CNL12	0.16µM
CNS1B	0.16µM
Dream Taq	1U
Genomic DNA	40ng

Final concentration of PCR for ITS primer:

Dream Taq Buffer	1×
$MgCl_2$	1.2mM
dNTP	0.2mM
BSA	0.8mg/mL
ITS4	0.16µM
ITS5	0.16µM
Dream Taq	1U
Genomic DNA	40ng

PCR Conditions:

Step 1: 95 ℃	3 minutes
Step 2: 95 ℃	30 seconds
Step 3: 55 ℃	30 seconds
Step 4: 72 ℃	1 minute
Go to Step 2 35× cycle	
Step 5: 72 ℃	5 minute
Step 6: 4 ℃	forever

The sequencef of the primers were:

CNL12	CTG AAC GCC TCT AAG TCA G
CNS1b	GAG ACA AGC ATA TGA CTA CTG
ITS4	TCC TCC GCT TAT TGA TAT GC
ITS5	GGA AGT AAA AGT CGT AAC AAG G

The company that provided the sequencing is Genome Quebec.

Appendix 3. 2016 fall rye fusarium head blight (FHB) survey in Manitoba (personal communication with Dr. Maria Antonia Henriquez)

Field Code	Date surveyed	1		2		3		Disease incidence (%)
		Healthy heads	Diseased heads	Healthy heads	Diseased heads	Healthy heads	Diseased heads	
FR-16-01	30-Jun	16	1	18	0	15	0	1.96
FR-16-02	06-Jul	43	1	N/A	N/A	N/A	N/A	2.27
FR-16-03	30-Jun	16	1	16	1	13	3	10.17
FR-16-04	30-Jun	17	0	14	4	17	0	7.41
FR-16-05	29-Jun	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FR-16-06	06-Jul	50	3	N/A	N/A	N/A	N/A	5.66
FR-16-07	06-Jul	66	16	N/A	N/A	N/A	N/A	19.51
FR-16-08	04-Jul	17	0	16	1	15	1	4.04
FR-16-09	06-Jul	85	15	N/A	N/A	N/A	N/A	15.00
FR-16-10	30-Jun	14	3	17	2	14	2	13.56
FR-16-11	30-Jun	17	1	16	0	19	0	1.85
FR-16-12	30-Jun	17	0	18	0	15	0	0.00
FR-16-13	30-Jun	14	2	17	0	18	0	4.17
FR-16-14	30-Jun	16	1	16	0	16	1	3.92
FR-16-15	04-Jul	17	0	13	3	16	0	6.25
FR-16-16	04-Jul	15	2	16	1	16	2	9.59
FR-16-17	04-Jul	16	0	18	0	17	1	1.85

N/A=not available.

Appendix 4. Source, type, and plant height (PH) of fall rye genotypes in Set 1 (personal communication with Dr. Jamie Larsen)

No.	Genotype	Source	Type	Plant height (cm)
1	AC Remington	AAFC-Swift Current	OP	95.31
2	AC Rifle	AAFC-Swift Current	OP	84.46
3	ACE-1	AAFC-Lethbridge	OP	103.02
4	Amilo	Poland	OP	89.07
5	Animo	Netherlands	OP	95.91
6	Anna	Finland	OP	97.43
7	Antelope	U of Saskatchewan	OP	99.22
8	Balbo	Italy	OP	98.69
9	Brasetto	KWS Lochow (Germany)	Hybrid	79.50
10	Caribou	U of Saskatchewan/U of Minnesota	OP	96.21
11	Carolkurz	Germany	OP	90.72

12	Carsten	Germany	OP	97.69
13	Cougar	U of Manitoba	OP	97.15
14	Dakold	North Dakota	OP	97.91
15	Dakota	Canada	OP	83.67
16	Dankoskie Nowe	N/A	OP	98.35
17	Dankoskie Selekc	Poland	OP	93.98
18	Dankoskie Srebrne	Poland	OP	97.13
19	Dominant	Holland	OP	92.94
20	Florida 401	Florida	OP	100.29
21	Frontier	AAFC- Swift Current	OP	100.13
22	Gator	University of Florida	OP	98.48
23	GC-100	Russia	OP	84.41
24	Guttino	KWS Lochow (Germany)	Hybrid	76.65
25	Halo	Germany	OP	88.72
26	Hazlet	AAFC- Swift Current	OP	88.63
27	Horton	Canada	OP	100.17
28	Kharkivska 95	Ukraine	OP	87.43
29	Kharkivska 98	Ukraine	OP	86.85
30	Kodiak	U of Alberta	OP	101.46
31	Kustro	AAFC-Kentville	OP	98.46
32	KWS Bono	KWS Lochow (Germany)	Hybrid	76.09
33	KWS Daniello	KWS Lochow (Germany)	Hybrid	79.03
34	KWS Gatano	KWS Lochow (Germany)	Hybrid	79.65
35	KWS Serafino	KWS Lochow (Germany)	Hybrid	78.91
36	KWS Trebiano	KWS Lochow (Germany)	Hybrid	80.37
37	Maton	Oklahoma	OP	100.37
38	Motto	Poland	OP	86.83
39	Musketeer	AAFC-Swift Current	OP	90.52
40	Oklon	Oklahoma	OP	96.56
41	Othello	Sweden	OP	101.11
42	Pearl	Denmark	OP	93.00
43	Petkus	Germany	OP	95.23
44	Ponsi	Sweden	OP	100.59
45	Prima	AAFC-Swift Current	OP	97.19
46	Prolific Spring	Canada	OP	98.83
47	Protector	Germany	OP	97.76
48	Puma	U of Manitoba	OP	96.59
49	R003-4	Canada	OP	98.03
50	Reimann-Philipp	Germany (U of Hohenheim)	OP	87.93
51	Saratovskaya 4	Russia	OP	92.81
52	Sellino	Germany	OP	90.50
53	SM4R	AAFC-Saskatoon Research Centre	OP	92.59
54	Stoir	Ukraine	OP	81.73
55	Syn 20L	Germany	OP	94.69

56	Toivo	Finland via U of Alberta	OP	94.54
57	Visa	Finland	OP	96.41
58	Vitallo	Germany	OP	89.26
59	Voima	Finland	OP	103.67
60	Wheeler	Michigan State University	OP	99.44
61	Wrens Abruzzi	U of Georgia	OP	95.31

AAFC=Agriculture and Agri-Food Canada; OP=open-pollinated genotypes; U=University; N/A=not available.
Yellow highlight represents the cultivars listed in prairie seed guides.

Appendix 5. Mean squares and significance for the combined analyses of variance of fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), ergot severity, plant height (PH), and heading date (HD) from trials conducted on Set 1 genotypes in 2017 Carman, 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

Source of variance	Incidence	Severity	Index	FDK	DON	Ergot	PH	HD
Genotype	1139.66****	2061.76****	1340.77****	4.32****	411.85****	47.45****	1663.03****	101.77****
SY	75094.00****	16683.00****	29973.00****	55.33****	8965.13****	756.85****	38519.00****	4499.25****
Rep(SY)	630.87****	344.22****	296.81****	0.35****	116.62****	15.91****	202.37****	9.00****
SY*Genotype	259.83****	228.68****	141.54****	0.21****	42.82****	7.00****	68.15****	4.96****

SY=site year; Rep=replicate.
****significant at $p<0.0001$.

Appendix 6. Mean (%) and rank of fusarium head blight (FHB) incidence from trials conducted on Set 1 genotypes in 2017 Carman, 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2017 Carman		2018 Winnipeg		2019 Carman		2019 Winnipeg		2020 Carman		2020 Winnipeg	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	32.50	15	21.81	6	21.67	4	39.17	20	63.33	1	51.67	19
2	Emerson	45.00	43	27.50	12	17.06	2	55.83	58	86.67	19	80.00	65
3	FHB 148	45.00	43	25.00	10	11.46	1	32.50	10	71.67	3	48.33	9
4	43I*18	45.00	43	20.83	3	37.50	24	40.00	21	80.00	6	50.00	14
5	Freedom	90.83	66	43.33	46	59.56	65	78.33	67	93.33	43	72.69	58
6	Caledonia	90.00	65	55.83	61	58.33	64	56.67	60	95.00	53	71.67	56
7	Hanover	87.50	64	66.67	68	69.17	67	81.67	68	100.00	68	81.67	66
8	AC Remington	42.50	38	49.17	57	32.50	14	42.50	28	98.33	64	66.67	46
9	AC Rifle	37.50	27	33.33	22	50.00	54	55.00	56	88.33	22	68.33	51
10	ACE-1	44.17	42	21.67	4	52.50	62	42.50	28	71.67	3	45.00	7
11	Amilo	50.83	50	55.83	61	40.83	35	42.50	28	85.00	13	61.67	37

12	Animo	31.67	13	47.50	55	40.00	32	36.67	17	88.33	22	53.33	20
13	Anna	35.83	20	30.00	18	47.50	48	42.50	28	88.33	22	40.00	3
14	Antelope	36.67	24	29.17	15	18.33	3	20.83	1	70.00	2	41.67	4
15	Balbo	28.33	7	41.67	40	33.33	15	40.00	21	96.67	61	76.67	62
16	Brasetto	43.33	41	42.50	43	46.67	47	46.67	45	95.00	53	56.67	29
17	Caribou	54.17	53	40.73	38	50.83	56	44.17	39	86.67	19	63.33	41
18	Carolkurz	30.00	9	38.88	34	45.83	43	40.83	25	95.00	53	56.67	29
19	Carsten	54.17	53	60.00	65	49.17	52	64.17	64	93.33	43	58.33	34
20	Cougar	36.67	24	45.83	54	45.83	43	56.67	60	81.67	7	61.67	37
21	Dakold	30.83	10	29.17	15	42.50	39	44.17	39	93.33	43	66.67	46
22	Dakota	40.00	32	61.67	67	75.83	68	75.83	66	98.33	64	86.67	67
23	Dankoskie Nowe	33.33	16	43.33	46	35.00	19	50.83	52	91.67	38	65.00	43
24	Dankoskie Selekc	40.83	33	21.67	4	43.33	40	43.33	34	95.00	53	55.00	24
25	Dankoskie Srebrne	41.67	35	28.33	14	55.00	63	50.83	52	85.00	13	61.67	37
26	Dominant	41.67	35	33.33	22	51.67	58	43.33	34	93.33	43	43.66	6
27	Florida 401	25.00	4	31.67	19	50.00	54	56.08	59	85.00	13	35.00	2
28	Frontier	31.67	13	42.50	43	36.67	22	29.17	4	91.67	38	48.33	9
29	Gator	42.50	38	50.83	60	51.67	58	60.00	62	90.00	31	55.00	24
30	GC-100	81.67	63	50.00	59	43.33	40	44.17	39	96.67	61	86.67	67
31	Guttino	50.83	50	48.33	56	37.50	24	33.33	12	88.33	22	68.33	51
32	Halo	28.33	7	45.00	50	29.17	10	35.83	15	93.33	43	60.00	35
33	Hazlet	55.83	56	36.67	29	51.67	58	61.67	63	98.33	64	73.33	60
34	Horton	54.17	53	59.17	64	45.83	43	42.50	28	90.00	31	71.67	56
35	Kharkivska 95	56.67	58	45.00	50	49.17	52	44.17	39	93.33	43	70.00	55
36	Kharkivska 98	50.83	50	44.17	49	39.17	31	42.50	28	88.33	22	66.67	46
37	Kodiak	35.83	20	60.00	65	50.83	56	38.33	19	81.67	7	68.33	51
38	Kustro	25.83	5	43.33	46	34.17	17	43.33	34	85.00	13	56.67	29
39	KWS Bono	30.83	10	34.17	26	40.83	35	55.00	56	95.00	53	60.00	35
40	KWS Daniello	N/A	N/A	34.17	26	28.33	9	34.17	13	95.00	53	66.67	46
41	KWS Gatano	25.83	5	26.67	11	48.33	50	30.00	6	95.00	53	41.67	4
42	KWS Serafino	22.50	3	37.50	32	27.50	7	35.00	14	98.33	64	46.67	8
43	KWS Trebiano	39.17	30	41.67	40	38.33	27	40.83	25	93.33	43	55.00	24

44	Maton	37.50	27	35.00	28	41.67	37	27.50	2	93.33	43	68.33	51
45	Motto	39.17	30	42.50	43	35.83	21	49.17	50	93.33	43	73.33	60
46	Musketeer	42.50	38	45.00	50	34.17	17	43.33	34	93.33	43	50.00	14
47	Oklon	35.83	20	13.33	2	22.50	5	30.83	7	81.67	7	48.33	9
48	Othello	40.83	33	40.83	39	40.00	32	44.17	39	96.67	61	56.67	29
49	Pearl	41.67	35	41.67	40	47.50	48	35.83	15	81.67	7	48.33	9
50	Petkus	N/A	N/A	31.67	19	38.67	30	46.14	44	91.67	38	65.00	43
51	Ponsi	45.83	46	45.00	50	31.67	13	37.50	18	90.00	31	53.33	20
52	Prima	47.50	49	24.17	7	38.33	27	40.00	21	81.67	7	50.00	14
53	Prolific Spring	38.33	29	33.33	22	41.67	37	48.33	47	95.00	53	53.33	20
54	Protector	35.83	20	33.33	22	23.33	6	32.50	10	88.33	22	30.00	1
55	Puma	57.50	60	56.67	63	27.50	7	50.00	51	88.33	22	55.00	24
56	R003-4	45.83	46	40.00	37	33.33	15	43.33	34	91.67	38	72.92	59
57	Reimann-Philipp	56.67	58	29.17	15	64.17	66	41.67	27	81.67	7	67.69	50
58	Saratovskaya 4	55.83	56	37.50	32	45.83	43	48.33	47	90.00	31	56.67	29
59	Sellino	36.67	24	39.17	35	30.00	12	46.67	45	88.33	22	65.00	43
60	SM4R	30.83	10	39.17	35	29.17	10	40.00	21	85.00	13	53.33	20
61	Stoir	34.17	18	9.17	1	48.33	50	75.00	65	90.00	31	77.92	63
62	Syn 20L	21.67	2	24.17	7	35.00	19	31.67	8	90.00	31	63.33	41
63	Toivo	20.00	1	27.50	12	44.17	42	31.67	8	88.33	22	50.00	14
64	Visa	45.83	46	24.17	7	37.50	24	48.33	47	86.67	19	55.00	24
65	Vitallo	61.67	61	36.67	29	40.00	32	50.83	52	91.67	38	50.00	14
66	Voima	35.00	19	36.67	29	36.67	22	28.33	3	85.00	13	48.33	9
67	Wheeler	63.33	62	32.50	21	38.33	27	51.67	55	90.00	31	79.16	64
68	Wrens Abruzzi	33.33	16	49.17	57	51.67	58	29.17	4	78.33	5	61.67	37
	LSD (0.05)	17.00		19.17		23.37		18.25		12.98		23.14	
	Wheat means	62.26		37.28		39.25		54.88		84.29		65.15	
	Rye means	40.93		38.56		41.25		43.63		89.59		59.20	
	Rye range	20.00-91.67		9.17-61.67		18.33-75.83		20.83-75.83		70.00-98.33		30.00-86.67	

LSD=least significant difference.

N/A means that data were not available.

Appendix 7. Mean (%) and rank of fusarium head blight (FHB) severity from trials conducted on Set 1 genotypes in 2017 Carman, 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2017 Carman		2018 Winnipeg		2019 Carman		2019 Winnipeg		2020 Carman		2020 Winnipeg	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	17.50	26	35.27	45	19.17	18	20.00	26	43.33	26	28.33	47
2	Emerson	23.33	41	52.50	62	37.45	58	24.17	39	43.33	26	75.00	65
3	FHB 148	25.83	47	29.17	27	19.91	22	20.83	30	48.33	41	45.00	62
4	43I*18	46.67	63	37.50	51	33.33	48	24.17	39	48.33	41	61.67	64
5	Freedom	47.50	64	33.33	39	44.95	63	29.17	51	66.67	66	79.82	66
6	Caledonia	71.67	66	70.83	68	61.67	68	58.33	67	85.00	67	93.33	67
7	Hanover	65.00	65	66.67	66	60.83	67	63.33	68	88.33	68	95.00	68
8	AC Remington	17.50	26	29.17	27	29.17	42	20.00	26	55.00	52	11.67	14
9	AC Rifle	10.00	6	20.83	16	19.17	18	23.33	37	53.33	49	28.33	47
10	ACE-1	9.17	5	11.67	1	20.83	24	15.00	12	28.33	4	26.67	42
11	Amilo	31.67	58	36.67	48	31.67	45	29.17	51	35.00	13	11.67	14
12	Animo	22.50	39	31.67	37	24.17	29	34.17	61	58.33	57	23.33	36
13	Anna	11.67	9	20.00	13	26.67	36	17.50	21	26.67	3	5.00	2
14	Antelope	17.50	26	18.33	12	16.67	13	13.33	8	25.00	2	8.33	10
15	Balbo	10.83	7	29.17	27	16.67	13	21.67	32	50.00	44	21.67	32
16	Brasetto	12.50	10	27.50	25	11.67	4	9.17	5	50.00	44	8.33	10
17	Caribou	30.00	55	61.96	65	40.83	61	28.33	48	38.33	18	16.67	26
18	Carolkurz	14.17	14	38.21	54	35.00	51	25.00	42	58.33	57	30.00	52
19	Carsten	25.83	47	30.83	35	36.67	55	28.33	48	43.33	26	26.67	42
20	Cougar	14.17	14	30.83	35	18.33	17	16.67	18	46.67	34	26.67	42
21	Dakold	16.67	23	14.17	4	48.33	64	25.00	42	46.67	34	28.33	47
22	Dakota	18.33	33	46.67	61	31.67	45	21.67	32	63.33	63	36.67	60
23	Dankoskie Nowe	15.83	18	37.50	51	28.33	39	32.50	56	53.33	49	31.67	54
24	Dankoskie Selekc	29.17	53	22.50	19	36.67	55	30.83	54	63.33	63	28.33	47
25	Dankoskie Srebrne	24.17	43	22.50	19	38.33	60	35.00	63	31.67	9	13.33	20
26	Dominant	20.00	37	30.00	30	37.50	59	32.50	56	46.67	34	22.59	35

27	Florida 401	29.17	53	21.67	17	55.00	66	17.61	22	33.33	10	10.00	12
28	Frontier	31.67	58	36.67	50	26.67	36	32.50	56	60.00	60	35.00	59
29	Gator	26.67	50	67.50	67	19.17	18	25.00	42	48.33	41	31.67	54
30	GC-100	22.50	39	56.67	64	15.00	10	20.00	26	61.67	61	40.00	61
31	Guttino	13.33	13	15.83	5	10.00	3	5.83	2	33.33	10	6.67	6
32	Halo	19.17	35	28.33	26	25.83	31	25.00	42	33.33	10	13.33	20
33	Hazlet	28.33	52	43.33	58	36.67	55	20.00	26	45.00	31	23.33	36
34	Horton	30.00	55	40.00	55	34.17	49	25.83	46	56.67	54	15.00	24
35	Kharkivska 95	17.50	26	32.50	38	32.50	47	27.50	47	56.67	54	15.00	24
36	Kharkivska 98	30.00	55	33.33	39	34.17	49	33.33	60	46.67	34	16.67	26
37	Kodiak	20.83	38	44.17	60	28.33	39	34.17	61	36.67	15	26.67	42
38	Kustro	10.83	7	33.33	39	25.83	31	24.17	39	45.00	31	20.00	30
39	KWS Bono	14.17	14	17.50	11	11.67	4	12.50	7	46.67	34	11.67	14
40	KWS Daniello	N/A	N/A	15.83	5	7.50	1	7.50	4	46.67	34	5.00	2
41	KWS Gatano	7.50	3	11.67	1	12.50	6	6.67	3	35.00	13	5.00	2
42	KWS Serafino	6.67	1	20.00	13	7.50	1	5.00	1	38.33	18	5.00	2
43	KWS Trebiano	7.50	3	15.83	5	12.50	6	15.00	12	30.00	7	6.67	6
44	Maton	36.67	61	25.00	22	35.00	51	22.50	35	61.67	61	33.33	56
45	Motto	15.00	17	30.00	30	30.00	44	30.83	54	38.33	18	26.67	42
46	Musketeer	16.67	23	26.67	24	17.50	16	19.17	25	56.67	54	13.33	20
47	Oklon	26.67	50	20.00	13	14.17	8	13.33	8	36.67	15	20.00	30
48	Othello	19.17	35	52.50	62	35.83	54	18.33	23	58.33	57	33.33	56
49	Pearl	23.33	41	35.83	46	40.83	61	40.00	65	43.33	26	33.33	56
50	Petkus	N/A	N/A	30.00	30	26.26	35	16.19	17	51.67	47	28.33	47
51	Ponsi	25.00	44	41.67	56	25.83	31	15.83	15	53.33	49	16.67	26
52	Prima	12.50	10	16.67	9	27.50	38	10.83	6	36.67	15	11.67	14
53	Prolific Spring	17.50	26	34.17	43	22.50	28	35.00	63	63.33	63	23.33	36
54	Protector	17.50	26	30.00	30	19.17	18	18.33	23	40.00	22	11.67	14
55	Puma	36.67	61	42.50	57	20.00	23	28.33	48	51.67	47	6.67	6
56	R003-4	16.67	23	22.50	19	15.00	10	16.67	18	38.33	18	19.54	29
57	Reimann-Philipp	17.50	26	34.17	43	20.83	24	16.67	18	46.67	34	49.82	63
58	Saratovskaya 4	25.00	44	36.67	48	25.83	31	32.50	56	55.00	52	30.00	52

59	Sellino	15.83	18	33.33	39	28.33	39	20.83	30	45.00	31	21.67	32
60	SM4R	15.83	18	25.00	22	14.17	8	14.17	11	21.67	1	21.67	32
61	Stoir	15.83	18	12.50	3	20.83	24	23.33	37	41.67	23	4.54	1
62	Syn 20L	18.33	33	30.00	30	15.83	12	15.83	15	30.00	7	23.33	36
63	Toivo	6.67	1	21.67	17	16.67	13	15.00	12	41.67	23	23.33	36
64	Visa	12.50	10	15.83	5	25.00	30	21.67	32	43.33	26	6.67	6
65	Vitallo	31.67	58	43.33	58	29.17	42	22.50	35	50.00	44	13.33	20
66	Voima	25.00	44	37.50	51	50.00	65	40.83	66	41.67	23	11.67	14
67	Wheeler	15.83	18	16.67	9	20.83	24	13.33	8	28.33	4	10.91	13
68	Wrens Abruzzi	25.83	47	35.83	46	35.00	51	29.17	51	28.33	4	23.33	36
	LSD (0.05)	14.60		15.48		17.98		13.70		19.01		16.82	
	Wheat means	42.50		46.47		39.61		34.29		60.48		68.31	
	Rye means	19.60		30.24		25.83		22.10		44.75		19.85	
	Rye range	6.67-36.67		11.67-67.50		7.50-55.00		5.00-40.83		21.67-66.67		4.54-49.82	

LSD=least significant difference.
N/A means that data were not available.

Appendix 8. Mean (%) and rank of fusarium head blight (FHB) index from trials conducted on Set 1 genotypes in 2017 Carman, 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2017 Carman		2018 Winnipeg		2019 Carman		2019 Winnipeg		2020 Carman		2020 Winnipeg	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	5.75	21	8.04	22	5.00	8	8.13	25	28.00	9	14.92	40
2	Emerson	10.83	47	14.79	44	5.80	18	13.44	52	37.92	28	60.50	66
3	FHB 148	11.54	49	7.35	20	1.76	1	6.58	16	36.50	24	21.58	58
4	43I*18	19.75	62	8.04	23	12.08	40	9.75	34	39.08	32	32.33	62
5	Freedom	43.21	64	14.88	46	27.17	66	23.23	66	62.83	66	59.41	65
6	Caledonia	64.58	66	39.90	67	36.17	67	33.13	67	80.92	67	67.08	67

7	Hanover	56.98	65	44.21	68	42.08	68	51.88	68	88.33	68	77.58	68
8	AC Remington	7.50	30	14.69	43	9.67	30	8.44	27	54.25	57	7.75	21
9	AC Rifle	3.92	8	7.00	17	9.25	27	12.63	46	47.08	47	19.25	54
10	ACE-1	4.17	9	3.21	4	11.04	38	6.83	17	20.58	3	11.83	32
11	Amilo	16.21	57	20.69	58	12.35	44	13.90	54	29.92	14	7.42	20
12	Animo	6.65	27	15.44	49	10.58	36	11.96	44	51.33	53	12.17	34
13	Anna	4.21	10	6.88	16	12.27	41	6.96	19	23.58	5	2.00	1
14	Antelope	6.29	23	5.83	11	4.67	7	2.73	5	17.50	1	3.58	7
15	Balbo	3.23	6	12.23	34	5.71	16	7.92	24	48.25	49	16.92	44
16	Brasetto	5.58	20	11.63	33	6.04	19	4.27	7	47.50	48	4.75	12
17	Caribou	16.29	58	25.57	62	20.63	61	12.92	49	34.08	19	10.83	29
18	Carolkurz	4.27	11	15.10	48	15.73	52	9.96	36	55.42	59	16.58	43
19	Carsten	15.06	55	18.17	55	16.58	54	18.92	65	40.58	36	17.50	48
20	Cougar	4.92	13	15.02	47	9.67	30	9.98	37	37.67	27	18.00	50
21	Dakold	5.25	16	4.44	5	19.71	58	11.33	41	43.33	38	19.00	53
22	Dakota	7.54	31	28.58	64	24.13	64	16.29	60	62.33	65	32.25	61
23	Dankoskie Nowe	5.19	15	18.48	56	12.29	42	17.65	64	48.67	50	21.92	59
24	Dankoskie Selekc	11.94	51	4.88	7	15.00	51	12.90	47	60.33	63	16.92	44
25	Dankoskie Srebrne	10.48	45	6.38	14	21.00	62	17.48	63	26.25	6	8.17	22
26	Dominant	8.38	37	10.50	30	22.31	63	14.21	56	43.67	40	9.79	25
27	Florida 401	7.54	31	8.75	24	26.50	65	9.94	35	28.33	11	4.00	10
28	Frontier	10.06	42	16.17	51	9.94	33	8.98	31	55.17	58	17.08	46
29	Gator	11.67	50	33.69	66	10.04	34	14.60	57	43.50	39	17.25	47
30	GC-100	18.54	60	29.50	65	6.50	20	8.48	28	59.83	62	35.42	64
31	Guttino	6.75	28	7.56	21	3.25	4	1.96	2	29.50	12	4.83	13
32	Halo	5.40	17	12.63	36	7.46	22	9.10	32	30.92	16	8.58	23
33	Hazlet	15.88	56	16.00	50	19.00	56	12.90	47	44.08	41	17.50	48
34	Horton	17.08	59	24.10	60	15.92	53	11.02	40	51.58	54	10.58	28
35	Kharkivska 95	10.27	44	14.81	45	16.73	55	12.25	45	52.75	56	10.33	26

36	Kharkivska 98	14.83	54	14.63	41	13.31	48	15.33	59	42.00	37	11.08	30
37	Kodiak	8.15	35	27.67	63	14.88	50	13.69	53	31.75	17	18.50	52
38	Kustro	2.94	4	14.65	42	8.88	26	10.33	39	39.33	33	12.67	35
39	KWS Bono	4.46	12	5.98	12	5.17	11	7.21	21	44.42	43	7.17	17
40	KWS Daniello	N/A	N/A	5.65	10	2.13	2	2.56	4	44.25	42	3.33	4
41	KWS Gatano	1.94	3	3.08	3	5.75	17	2.10	3	33.08	18	2.08	2
42	KWS Serafino	1.63	2	7.21	19	2.25	3	1.75	1	37.58	25	2.33	3
43	KWS Trebiano	3.00	5	6.29	13	5.42	14	5.98	13	28.08	10	3.50	6
44	Maton	13.54	52	9.25	27	13.00	46	6.04	14	57.67	61	22.42	60
45	Motto	6.48	25	12.88	37	12.63	45	13.04	51	36.17	23	19.50	55
46	Musketeer	7.08	29	12.58	35	5.35	13	8.54	30	52.67	55	7.00	16
47	Oklon	9.10	40	2.96	2	3.33	5	4.13	6	30.42	15	10.33	26
48	Othello	8.17	36	23.96	59	14.81	49	8.13	25	56.33	60	20.00	57
49	Pearl	10.71	46	16.67	53	19.81	59	13.96	55	38.33	30	16.50	41
50	Petkus	N/A	N/A	8.88	25	10.27	35	7.50	23	46.92	46	18.08	51
51	Ponsi	11.33	48	19.00	57	7.65	23	5.75	12	49.50	51	7.33	18
52	Prima	6.31	24	4.58	6	10.96	37	4.42	8	29.83	13	5.67	14
53	Prolific Spring	7.81	34	10.33	29	9.54	29	17.00	61	60.33	63	14.17	36
54	Protector	6.48	25	9.96	28	5.08	9	6.13	15	35.33	22	3.83	8
55	Puma	23.50	63	24.63	61	5.23	12	12.94	50	45.92	44	3.92	9
56	R003-4	7.60	33	9.00	26	5.08	9	7.42	22	35.17	20	14.39	37
57	Reimann-Philipp	10.13	43	10.67	31	13.27	47	6.90	18	40.33	35	34.41	63
58	Saratovskaya 4	14.79	53	14.33	39	12.31	43	15.29	58	49.75	52	19.58	56
59	Sellino	5.83	22	13.02	38	8.00	25	9.50	33	40.25	34	14.75	38
60	SM4R	5.06	14	11.17	32	4.19	6	5.50	11	18.75	2	11.67	31
61	Stoir	5.52	18	2.23	1	9.88	32	17.33	62	37.58	25	3.39	5
62	Syn 20L	3.73	7	7.08	18	5.46	15	5.25	10	27.00	8	16.50	41
63	Toivo	1.35	1	6.46	15	7.96	24	4.92	9	37.92	28	11.92	33
64	Visa	5.52	18	5.04	9	9.25	27	10.29	38	38.67	31	4.00	10

65	Vitallo	19.48	61	16.38	52	11.69	39	11.58	42	46.17	45	7.33	18
66	Voima	8.60	38	14.35	40	20.00	60	11.60	43	35.25	21	6.00	15
67	Wheeler	9.94	41	4.98	8	7.42	21	7.08	20	26.25	6	8.73	24
68	Wrens Abruzzi	8.79	39	17.52	54	19.25	57	8.52	29	22.42	4	14.75	38
	LSD (0.05)	8.81		10.75		11.06		8.23		19.81		14.67	
	Wheat means	30.38		19.60		18.58		20.88		53.37		47.63	
	Rye means	8.54		12.80		11.20		9.74		40.71		12.44	
	Rye range	1.35-23.50		2.23-33.69		2.13-26.50		1.75-18.92		17.50-62.33		2.00-35.42	

LSD=least significant difference.
N/A means that data were not available.

Appendix 9. Mean (%) and rank of *Fusarium*-damaged kernels (FDK) from trials conducted on Set 1 genotypes in 2017 Carman, 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2017 Carman		2018 Winnipeg		2019 Carman		2019 Winnipeg		2020 Carman		2020 Winnipeg	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	6.55	59	0.69	52	0.72	40	3.49	64	3.18	29	3.09	62
2	Emerson	4.87	57	1.25	56	1.43	61	1.55	62	2.80	25	3.77	63
3	FHB 148	8.49	60	0.41	36	0.53	24	2.48	63	4.07	49	2.50	61
4	43I*18	12.19	61	1.85	57	1.81	64	4.01	65	8.00	65	5.03	64
5	Freedom	15.90	62	4.30	58	7.21	66	4.67	66	10.93	66	5.53	65
6	Caledonia	35.53	64	8.11	60	7.97	67	5.58	67	12.41	67	10.85	66
7	Hanover	27.33	63	7.57	59	12.62	68	13.41	68	19.50	68	13.21	67
8	AC Remington	2.36	31	0.56	48	0.79	43	0.32	36	4.33	54	0.20	23
9	AC Rifle	2.23	27	0.43	39	0.86	51	0.43	48	3.57	40	0.47	53
10	ACE-1	0.08	1	N/A	N/A	0.80	45	0.10	3	0.78	2	0.71	59
11	Amilo	4.44	52	0.52	47	0.52	23	0.41	45	4.57	57	0.28	43
12	Animo	2.21	26	0.51	46	0.51	22	0.33	37	3.33	33	0.39	49

13	Anna	0.97	4	0.20	23	0.54	26	0.21	16	1.57	5	0.05	1
14	Antelope	0.82	3	0.08	9	0.43	13	0.14	6	2.30	11	0.21	27
15	Balbo	1.65	16	0.34	32	0.59	30	0.19	15	4.62	58	0.35	47
16	Brasetto	4.70	54	0.46	44	0.70	36	0.27	27	5.34	63	0.30	44
17	Caribou	6.16	58	N/A	N/A	0.34	6	0.23	19	2.63	22	0.07	5
18	Carolkurz	3.25	43	0.14	14	0.41	10	0.15	8	3.85	46	0.27	36
19	Carsten	N/A	N/A	0.16	15	0.46	14	0.57	54	4.12	51	0.37	48
20	Cougar	2.50	32	0.11	11	0.80	46	0.70	57	2.93	28	0.28	40
21	Dakold	2.02	23	0.04	7	1.09	55	0.40	43	3.33	34	0.28	42
22	Dakota	3.67	48	0.81	54	1.62	63	0.67	55	5.07	60	0.78	60
23	Dankoskie Nowe	1.18	8	0.59	49	0.80	47	0.38	40	3.76	45	0.50	55
24	Dankoskie Selekc	4.43	51	0.22	26	0.84	50	0.48	50	4.45	55	0.48	54
25	Dankoskie Srebrne	4.57	53	0.04	6	0.89	53	0.40	44	3.59	42	0.24	31
26	Dominant	3.00	39	0.08	8	0.82	48	0.52	51	2.57	20	0.28	41
27	Florida 401	3.05	41	0.08	10	1.31	59	0.31	30	0.73	1	0.06	4
28	Frontier	1.37	14	0.37	33	0.70	37	0.25	23	3.88	48	0.39	50
29	Gator	3.06	42	N/A	N/A	0.78	42	0.70	56	2.49	17	0.27	37
30	GC-100	3.87	49	0.42	37	0.49	21	0.26	25	2.40	13	0.53	56
31	Guttino	3.55	46	0.38	35	0.40	9	0.27	26	3.33	32	0.40	51
32	Halo	1.02	5	0.49	45	0.38	8	0.38	41	2.43	14	0.08	7
33	Hazlet	2.06	24	0.84	55	0.99	54	0.48	49	4.11	50	0.56	57
34	Horton	2.58	35	0.21	24	0.46	15	0.28	29	5.14	62	0.14	16
35	Kharkivska 95	3.04	40	0.17	17	0.46	16	0.32	35	4.14	52	0.21	24
36	Kharkivska 98	3.62	47	0.64	51	1.28	58	0.32	33	4.15	53	0.25	33
37	Kodiak	1.30	12	0.34	31	0.66	34	0.31	32	1.61	6	0.11	12
38	Kustro	1.20	9	0.00	2	0.36	7	0.25	22	2.46	16	0.17	22
39	KWS Bono	1.66	17	0.17	16	0.20	1	0.23	18	3.43	36	0.25	32
40	KWS Daniello	N/A	N/A	0.17	18	0.54	25	0.14	5	5.52	64	0.23	30
41	KWS Gatano	1.15	6	0.20	22	0.26	2	0.07	1	3.62	44	0.25	34

42	KWS Serafino	0.54	2	0.14	12	0.28	4	0.15	9	5.08	61	0.17	21
43	KWS Trebiano	1.72	18	0.17	18	0.49	20	0.18	12	3.49	38	0.21	26
44	Maton	4.72	55	0.03	5	1.34	60	0.15	7	4.93	59	0.15	18
45	Motto	2.52	33	0.70	53	0.47	17	0.56	52	2.37	12	0.27	39
46	Musketeer	2.29	29	0.45	42	0.41	11	0.37	39	2.43	15	0.23	29
47	Oklon	2.68	36	0.03	4	0.62	32	0.26	24	2.59	21	0.10	10
48	Othello	1.95	22	N/A	N/A	0.71	38	0.28	28	2.72	23	0.27	38
49	Pearl	1.93	21	0.61	50	0.83	49	0.32	34	3.87	47	0.32	45
50	Petkus	N/A	N/A	N/A	N/A	2.71	65	0.74	58	3.50	39	0.13	15
51	Ponsi	2.06	25	0.46	43	0.60	31	0.12	4	3.22	31	0.21	25
52	Prima	1.15	6	0.21	25	0.80	44	0.08	2	3.42	35	0.10	9
53	Prolific Spring	4.85	56	N/A	N/A	0.55	27	0.77	59	4.48	56	0.25	35
54	Protector	N/A	N/A	0.00	1	0.33	5	0.16	10	2.90	27	0.15	19
55	Puma	2.25	28	0.44	40	0.70	35	0.24	20	3.18	30	0.11	11
56	R003-4	2.81	37	0.45	41	0.48	19	0.24	21	2.85	26	0.16	20
57	Reimann-Philipp	3.40	44	0.42	38	1.19	57	0.42	47	2.76	24	0.45	52
58	Saratovskaya 4	2.34	30	0.19	20	0.88	52	0.36	38	3.59	41	0.15	17
59	Sellino	1.28	11	0.28	27	0.71	39	0.31	31	2.26	9	0.11	13
60	SM4R	1.53	15	0.30	28	0.26	3	0.17	11	1.38	4	0.22	28
61	Stoir	2.57	34	N/A	N/A	1.13	56	0.81	60	3.60	43	0.05	2
62	Syn 20L	1.74	19	0.19	20	0.42	12	0.41	46	3.43	37	0.57	58
63	Toivo	1.22	10	0.33	30	0.58	29	0.18	13	1.31	3	0.09	8
64	Visa	2.96	38	0.14	13	0.63	33	0.19	14	2.05	7	0.07	6
65	Vitallo	3.47	45	0.38	34	0.55	28	0.38	42	2.13	8	0.35	46
66	Voima	1.35	13	0.03	3	0.47	18	0.23	17	2.27	10	0.06	3
67	Wheeler	1.78	20	N/A	N/A	0.72	41	0.81	61	2.57	19	N/A	N/A
68	Wrens Abruzzi	3.96	50	0.32	29	1.52	62	0.57	53	2.49	18	0.13	14
	LSD (0.05)	2.84		1.15		1.44		0.94		2.33		1.69	
	Wheat means	15.84		3.45		4.61		5.03		8.70		6.28	

	Rye means	2.49	0.30	0.71	0.34	3.23	0.26
	Rye range	0.08-6.16	0.00-0.84	0.20-2.71	0.07-0.81	0.73-5.52	0.05-0.78

LSD=least significant difference.
N/A means that data were not available.

Appendix 10. Mean (ppm) and rank of deoxynivalenol (DON) from trials conducted on Set 1 genotypes in 2017 Carman, 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2017 Carman		2018 Winnipeg		2019 Carman		2019 Winnipeg		2020 Carman		2020 Winnipeg	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	20.03	60	1.06	19	4.93	5	26.15	65	15.19	11	11.36	65
2	Emerson	13.50	54	1.10	22	8.61	29	12.05	57	16.20	13	7.42	62
3	FHB 148	17.17	58	1.17	25	7.96	24	14.80	63	16.89	19	6.04	60
4	43I*18	14.89	57	3.57	55	12.18	51	16.96	64	43.74	66	8.75	63
5	Freedom	34.00	62	7.30	59	18.46	65	36.19	66	23.81	45	10.28	64
6	Caledonia	55.19	63	6.93	58	22.12	66	36.83	67	50.11	67	26.86	66
7	Hanover	58.40	64	16.70	60	36.52	68	41.74	68	52.31	68	27.96	67
8	AC Remington	9.47	38	1.33	28	9.40	34	8.38	45	24.50	50	1.76	28
9	AC Rifle	8.17	24	1.13	24	7.17	17	10.81	53	22.37	36	2.23	39
10	ACE-1	10.22	44	N/A	N/A	10.61	39	7.71	32	8.93	1	2.33	40
11	Amilo	10.80	46	2.93	51	11.53	48	8.20	39	25.56	54	1.50	17
12	Animo	7.90	21	1.68	37	12.28	52	5.75	19	23.03	42	1.42	15
13	Anna	8.40	26	0.97	18	12.43	53	10.06	48	15.66	12	1.12	8
14	Antelope	4.17	2	0.48	9	2.74	1	3.29	2	13.72	6	0.53	2
15	Balbo	6.97	18	1.98	46	7.84	22	7.52	30	34.56	65	3.54	51
16	Brasetto	12.30	51	2.93	52	10.70	40	13.28	58	30.06	61	1.81	30
17	Caribou	9.69	40	N/A	N/A	13.06	57	8.93	46	16.22	14	1.56	20
18	Carolkurz	8.57	29	1.49	32	10.80	41	10.37	49	33.91	63	3.94	53

19	Carsten	N/A	N/A	2.09	47	9.90	37	9.62	47	22.23	35	3.98	54
20	Cougar	4.33	3	1.49	32	8.14	27	7.89	34	16.43	16	2.52	43
21	Dakold	9.16	33	0.31	5	8.52	28	10.49	50	16.39	15	2.37	42
22	Dakota	12.57	53	3.47	54	23.51	67	11.90	56	29.69	60	6.27	61
23	Dankoskie Nowe	10.73	45	2.79	50	12.76	56	10.58	51	27.49	57	4.44	59
24	Dankoskie Selekc	17.50	59	1.50	34	13.61	61	13.43	60	31.60	62	4.08	56
25	Dankoskie Srebrne	5.50	8	1.79	41	12.10	50	14.14	62	21.22	32	1.63	23
26	Dominant	9.73	42	0.83	15	9.81	36	7.91	35	17.64	21	1.60	21
27	Florida 401	9.39	35	0.12	3	12.67	54	13.31	59	9.02	2	0.50	1
28	Frontier	10.80	46	1.11	23	5.13	7	4.07	4	22.60	38	1.91	32
29	Gator	8.69	30	N/A	N/A	11.52	47	8.09	36	14.81	10	1.53	18
30	GC-100	12.43	52	1.70	38	7.93	23	5.38	16	16.65	18	4.06	55
31	Guttino	13.70	55	1.77	40	9.57	35	6.61	23	24.65	52	2.67	46
32	Halo	5.37	5	2.31	49	6.00	10	8.35	44	22.03	34	1.47	16
33	Hazlet	14.83	56	3.68	56	14.44	63	10.78	52	28.38	58	4.21	57
34	Horton	8.10	23	0.73	13	4.96	6	3.77	3	24.54	51	1.77	29
35	Kharkivska 95	9.23	34	1.08	20	13.58	60	8.12	38	24.24	48	1.28	12
36	Kharkivska 98	11.70	50	1.50	34	13.44	59	13.48	61	28.54	59	1.71	25
37	Kodiak	9.47	38	1.73	39	15.91	64	5.24	13	9.14	3	1.05	7
38	Kustro	3.40	1	0.71	12	7.10	16	4.48	8	23.71	44	2.79	47
39	KWS Bono	7.93	22	1.83	43	12.75	55	7.40	29	23.83	46	2.09	37
40	KWS Daniello	N/A	N/A	1.50	34	11.39	45	5.39	17	34.02	64	2.35	41
41	KWS Gatano	6.23	13	0.67	10	13.07	58	8.24	41	23.43	43	1.26	11
42	KWS Serafino	5.67	9	1.17	25	10.94	42	7.09	27	27.25	56	0.97	6
43	KWS Trebiano	6.63	14	1.33	28	10.05	38	6.24	21	25.50	53	1.73	27
44	Maton	10.83	48	0.18	4	6.95	14	4.35	6	22.72	40	0.93	5
45	Motto	5.93	11	1.83	42	8.13	26	6.92	26	20.00	28	3.75	52
46	Musketeer	7.77	20	0.68	11	7.01	15	5.69	18	16.44	17	1.22	10
47	Oklon	6.97	18	0.01	2	5.83	9	4.09	5	20.67	29	1.39	14

48	Othello	6.07	12	N/A	N/A	7.77	20	6.06	20	18.01	24	2.61	45
49	Pearl	9.13	32	1.38	30	7.77	21	4.54	9	18.86	26	3.12	49
50	Petkus	N/A	N/A	N/A	N/A	11.08	43	6.75	25	20.98	30	1.55	19
51	Ponsi	6.73	16	1.93	45	6.23	12	5.10	12	22.59	37	1.86	31
52	Prima	4.67	4	0.47	8	9.40	33	4.47	7	18.19	25	1.62	22
53	Prolific Spring	11.37	49	N/A	N/A	8.02	25	8.30	43	22.95	41	1.93	33
54	Protector	N/A	N/A	0.39	7	3.99	2	5.37	15	13.77	7	0.74	4
55	Puma	10.20	43	1.91	44	6.18	11	5.31	14	19.28	27	1.34	13
56	R003-4	6.73	16	2.23	48	11.16	44	6.50	22	26.44	55	2.85	48
57	Reimann-Philipp	9.43	36	3.39	53	14.21	62	10.99	54	24.34	49	2.58	44
58	Saratovskaya 4	9.70	41	0.78	14	7.43	18	7.82	33	17.66	22	1.97	34
59	Sellino	8.40	26	3.78	57	8.69	30	6.64	24	17.98	23	1.71	25
60	SM4R	5.37	5	0.93	17	7.48	19	4.59	10	13.46	5	2.03	36
61	Stoir	8.23	25	N/A	N/A	11.51	46	11.23	55	23.88	47	1.98	35
62	Syn 20L	5.67	9	0.38	6	4.06	3	2.30	1	21.66	33	3.28	50
63	Toivo	5.40	7	1.21	27	5.32	8	7.69	31	13.93	8	1.64	24
64	Visa	8.77	31	0.00	1	11.82	49	8.11	37	22.71	39	2.16	38
65	Vitallo	8.50	28	1.47	31	9.36	32	8.22	40	21.15	31	4.34	58
66	Voima	6.67	15	0.90	16	4.56	4	4.99	11	13.15	4	0.60	3
67	Wheeler	22.82	61	N/A	N/A	9.25	31	8.27	42	17.03	20	N/A	N/A
68	Wrens Abruzzi	9.44	37	1.08	20	6.66	13	7.36	28	14.81	9	1.21	9
	LSD (0.05)	7.34		4.14		6.02		7.41		8.33		3.88	
	Wheat means	30.45		5.40		15.82		26.39		31.18		14.10	
	Rye means	8.85		1.45		9.63		7.67		21.25		2.17	
	Rye range	3.40-22.82		0.00-3.78		2.74-23.51		2.30-14.14		8.93-34.56		0.50-6.27	

LSD=least significant difference.
N/A means that data were not available.

Appendix 11. Mean (%) and rank of ergot severity from trials conducted on Set 1 genotypes in 2017 Carman, 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2017 Carman		2018 Winnipeg		2019 Carman		2019 Winnipeg		2020 Carman		2020 Winnipeg	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	0.00	3	0.06	7	0.00	1	0.00	2	0.34	2	0.37	4
2	Emerson	0.00	1	0.00	3	0.00	5	0.00	3	0.07	1	0.14	1
3	FHB 148	0.00	1	0.00	3	0.00	5	0.00	3	0.88	4	0.22	2
4	43I*18	0.00	3	0.00	1	0.00	5	0.00	3	0.57	3	0.23	3
5	Freedom	0.00	3	0.00	3	0.00	1	0.00	3	3.44	39	6.98	49
6	Caledonia	0.05	7	0.00	1	0.00	5	0.00	3	2.60	27	1.35	5
7	Hanover	0.00	3	0.00	6	0.00	5	0.00	3	3.56	40	4.39	28
8	AC Remington	0.66	19	0.82	22	0.76	47	0.63	34	1.84	19	4.07	24
9	AC Rifle	0.16	8	0.96	25	0.00	5	0.26	17	1.63	15	2.11	11
10	ACE-1	19.47	66	40.11	67	19.17	68	12.83	68	58.67	68	50.57	68
11	Amilo	0.85	25	5.65	61	0.39	41	0.39	22	2.13	22	3.30	17
12	Animo	0.43	13	2.88	55	0.11	27	0.02	9	3.18	35	6.68	47
13	Anna	1.05	31	1.74	46	0.00	5	0.29	18	1.58	14	2.98	14
14	Antelope	1.25	38	3.49	56	0.00	5	0.76	36	2.30	23	7.04	50
15	Balbo	0.96	28	4.12	58	0.43	43	1.11	50	6.56	59	7.99	54
16	Brasetto	1.28	39	0.20	13	0.00	5	0.79	39	2.73	28	2.29	12
17	Caribou	1.13	32	4.28	59	0.00	5	1.07	47	5.76	54	4.41	30
18	Carolkurz	0.99	30	5.00	60	0.16	29	1.79	57	4.61	49	9.31	59
19	Carsten	9.26	63	17.65	65	0.11	28	0.80	40	5.10	52	11.34	61
20	Cougar	1.23	37	1.70	42	0.00	5	0.54	28	6.65	61	5.82	42
21	Dakold	3.39	58	1.49	37	1.12	54	2.03	58	3.70	43	10.77	60
22	Dakota	1.17	33	1.83	47	0.67	46	0.79	37	2.79	30	4.35	26
23	Dankoskie Nowe	3.51	59	1.28	30	1.38	57	0.79	38	4.06	46	5.67	41
24	Dankoskie Selekc	1.22	36	1.31	32	1.30	56	2.43	63	5.19	53	7.89	53

25	Dankoskie Srebrne	4.36	61	1.28	29	1.02	52	2.69	64	2.97	33	4.40	29
26	Dominant	0.49	15	1.71	44	0.00	5	0.00	1	7.43	63	17.23	64
27	Florida 401	5.19	62	2.44	51	2.14	58	1.75	55	4.76	50	5.95	43
28	Frontier	1.84	46	0.37	15	0.18	31	0.49	25	1.53	11	4.53	31
29	Gator	9.93	64	23.59	66	3.99	64	2.06	61	8.42	65	6.36	46
30	GC-100	0.78	22	6.11	62	1.04	53	0.56	30	5.91	55	11.78	62
31	Guttino	0.73	21	0.77	21	0.17	30	0.35	20	3.40	37	5.48	40
32	Halo	0.31	10	1.71	43	0.94	51	0.06	11	2.35	24	4.92	37
33	Hazlet	2.83	57	1.18	28	0.78	48	0.58	31	3.61	42	4.91	36
34	Horton	1.71	45	0.88	23	0.84	49	2.04	59	1.94	21	3.17	16
35	Kharkivska 95	0.84	23	0.11	9	0.43	42	0.74	35	3.36	36	4.90	35
36	Kharkivska 98	1.20	35	1.15	27	3.29	63	0.52	27	1.83	17	4.67	33
37	Kodiak	0.44	14	0.92	24	0.44	44	0.18	16	1.43	9	3.84	22
38	Kustro	0.37	11	0.33	14	0.20	33	0.08	13	3.02	34	6.69	48
39	KWS Bono	0.69	20	0.40	16	0.00	5	0.88	43	2.42	25	3.38	19
40	KWS Daniello	N/A	N/A	0.40	17	0.00	5	0.60	32	1.49	10	1.71	9
41	KWS Gatano	0.28	9	0.18	11	0.00	5	0.11	14	1.21	6	1.46	6
42	KWS Serafino	0.62	18	0.09	8	0.06	22	0.14	15	1.93	20	1.62	7
43	KWS Trebiano	0.37	11	0.12	10	0.06	23	0.07	12	1.65	16	1.70	8
44	Maton	2.35	53	1.63	41	2.39	59	4.79	65	4.46	48	9.06	58
45	Motto	2.09	50	1.37	34	0.00	1	0.80	41	2.42	26	5.22	39
46	Musketeer	1.35	41	0.20	12	0.08	24	1.47	53	1.54	13	3.98	23
47	Oklon	2.75	54	0.53	19	2.61	60	1.65	54	2.83	31	3.60	20
48	Othello	0.57	16	1.73	45	0.09	25	0.99	45	1.54	12	3.63	21
49	Pearl	1.48	43	2.17	49	0.31	39	1.11	49	6.52	57	3.32	18
50	Petkus	N/A	N/A	8.66	64	13.03	67	8.98	67	9.45	67	13.28	63
51	Ponsi	1.18	34	1.56	39	0.30	38	0.36	21	2.77	29	6.11	44
52	Prima	0.91	26	1.40	36	0.00	5	0.06	10	1.40	7	1.76	10
53	Prolific Spring	2.80	55	4.09	57	2.86	61	1.29	52	4.85	51	3.12	15

54	Protector	2.27	52	1.30	31	1.15	55	0.88	42	1.41	8	2.94	13
55	Puma	1.93	48	1.55	38	4.66	65	0.63	33	1.20	5	8.03	55
56	R003-4	1.36	42	1.01	26	0.91	50	1.01	46	3.74	44	5.10	38
57	Reimann-Philipp	3.78	60	6.29	63	0.53	45	1.76	56	9.41	66	22.84	66
58	Saratovskaya 4	2.22	51	2.80	53	3.11	62	2.35	62	7.23	62	7.79	52
59	Sellino	1.71	44	1.63	40	0.10	26	0.49	24	2.85	32	7.70	51
60	SM4R	0.94	27	1.35	33	0.27	36	2.05	60	1.84	18	4.38	27
61	Stoir	1.31	40	1.85	48	0.33	40	0.56	29	6.59	60	25.80	67
62	Syn 20L	2.06	49	2.83	54	0.19	32	1.20	51	3.58	41	6.14	45
63	Toivo	0.61	17	1.39	35	0.00	1	0.51	26	3.79	45	8.22	56
64	Visa	0.97	29	0.48	18	0.03	21	0.91	44	6.54	58	4.27	25
65	Vitallo	1.85	47	2.38	50	0.20	34	1.10	48	4.19	47	8.38	57
66	Voima	0.84	23	2.74	52	0.25	35	0.45	23	3.43	38	4.56	32
67	Wheeler	11.60	65	51.29	68	7.91	66	7.46	66	8.01	64	20.07	65
68	Wrens Abruzzi	2.81	56	0.76	20	0.28	37	0.31	19	6.49	56	4.70	34
	LSD (0.05)	2.37		8.54		3.46		2.88		3.40		9.41	
	Wheat means	0.01		0.01		0.00		0.00		1.64		1.95	
	Rye means	2.25		3.95		1.36		1.38		4.71		7.20	
	Rye range	0.16-19.47		0.09-51.29		0.00-19.17		0.00-12.83		1.20-58.67		1.46-50.57	

LSD=least significant difference.
N/A means that data were not available.

Appendix 12. Type, gene pool, tester, and plant height (PH) of fall rye genotypes in Set 2 (personal communication with Dr. Jakob Eifler)

No.	Genotype	Type	Gene pool	Tester	PH (cm)
1	Exp.Variety1	KWS-Hybrid	N/A	None	81.40
2	Exp.Variety2	KWS-Hybrid	N/A	None	81.59
3	Exp.Variety17	KWS-Hybrid	N/A	None	77.28
4	Exp.Variety31	KWS-Hybrid	N/A	None	72.90
5	KWS-H101	KWS-Hybrid	N/A	None	91.98
6	KWS-H119	KWS-Hybrid	N/A	None	73.53
7	KWS-H144	KWS-Hybrid	N/A	None	74.19
8	KWS-H145	KWS-Hybrid	N/A	None	72.19
9	KWS-H161	KWS-Hybrid	N/A	None	76.15
10	Li1	Testcross	Petkus	T517	77.07
11	Li2	Testcross	Petkus	T517	70.90
12	Li3	Testcross	Petkus	T517	72.79
13	Li4	Testcross	Petkus	T517	71.87
14	Li5	Testcross	Petkus	T517	73.77
15	Li6	Testcross	Petkus	T517	77.58
16	Li7	Testcross	Petkus	T517	79.74
17	Li8	Testcross	Petkus	T517	76.48
18	Li9	Testcross	Petkus	T517	72.79
19	Li10	Testcross	Petkus	T517	75.64
20	Li12	Testcross	Petkus	T517	75.24
21	Li14	Testcross	Petkus	T517	74.45
22	Li15	Testcross	Petkus	T517	73.43
23	Li16	Testcross	Petkus	T517	74.44
24	Li17	Testcross	Petkus	T517	78.29
25	Li18	Testcross	Petkus	T517	79.52
26	Li19	Testcross	Petkus	T517	79.31
27	Li20	Testcross	Petkus	T517	79.04
28	Li21	Testcross	Petkus	T517	74.56
29	Li22	Testcross	Petkus	T517	71.49
30	Li23	Testcross	Petkus	T517	70.90
31	Li24	Testcross	Petkus	T517	72.82
32	Li25	Testcross	Petkus	T517	73.33
33	Li26	Testcross	Petkus	T517	72.92
34	Li27	Testcross	Petkus	T517	70.50
35	Li40	Testcross	Petkus	T517	71.55
36	Li41	Testcross	Petkus	T517	74.10
37	Li42	Testcross	Petkus	T517	76.50
38	Li102	Testcross	Carsten	T217	73.34
39	Li103	Testcross	Carsten	T217	72.15
40	Li104	Testcross	Carsten	T217	77.32

41	Li105	Testcross	Carsten	T217	76.90
42	Li106	Testcross	Carsten	T217	75.44
43	Li107	Testcross	Carsten	T217	75.10
44	Li111	Testcross	Carsten	T217	78.24
45	Li113	Testcross	Carsten	T217	73.71
46	Li114	Testcross	Carsten	T217	74.65
47	Li116	Testcross	Carsten	T217	76.97
48	Li117	Testcross	Carsten	T217	72.90
49	Li119	Testcross	Carsten	T217	76.04
50	Li120	Testcross	Carsten	T217	71.82
51	Li122	Testcross	Carsten	T217	80.50
52	Li123	Testcross	Carsten	T217	74.79
53	Li125	Testcross	Carsten	T217	75.71
54	Li126	Testcross	Carsten	T217	73.86
55	Li127	Testcross	Carsten	T217	76.27
56	Li128	Testcross	Carsten	T217	76.04
57	Li129	Testcross	Carsten	T217	78.24
58	Li131	Testcross	Carsten	T217	73.16
59	Li132	Testcross	Carsten	T217	71.86
60	Li133	Testcross	Carsten	T217	71.53
61	Li134	Testcross	Carsten	T217	71.59
62	Li135	Testcross	Carsten	T217	76.78
63	Li136	Testcross	Carsten	T217	74.13
64	Li137	Testcross	Carsten	T217	77.52
65	Li138	Testcross	Carsten	T217	73.45
66	Li139	Testcross	Carsten	T217	70.42
67	Li140	Testcross	Carsten	T217	75.28
68	Li141	Testcross	Carsten	T217	73.62
69	Li142	Testcross	Carsten	T217	76.41
70	Li143	Testcross	Carsten	T217	72.29
71	Li144	Testcross	Carsten	T217	75.82
72	Li145	Testcross	Carsten	T217	73.37
73	Li146	Testcross	Carsten	T217	72.98
74	Li147	Testcross	Carsten	T217	77.09
75	Li148	Testcross	Carsten	T217	72.89
76	Li164	Testcross	Carsten	T217	70.83
77	Li165	Testcross	Carsten	T217	71.50
78	Li166	Testcross	Carsten	T217	72.05
79	Li167	Testcross	Carsten	T217	75.07
80	Li168	Testcross	Carsten	T217	68.22
81	Li169	Testcross	Carsten	T217	74.83

Appendix 13. Mean squares and significance for the combined analyses of variance of fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), ergot severity, plant height (PH), and heading date (HD) from trials conducted on Set 2 genotypes in 2019 Carman, 2019 Winnipeg, 2020 Carman, and 2020 Winnipeg

Source of variance	Incidence	Severity	Index	FDK	DON	Ergot	PH	HD
Genotype	880.82****	1891.22****	1231.19****	22.21****	89.73****	37.78****	355.86****	90.67****
SY	237736.00****	71488.00****	98809.00****	311.43****	10003.00****	3093.54****	2990.94****	6914.73****
Rep(SY)	3244.41****	235.69****	204.77****	N/A	N/A	17.62****	301.58****	6.82****
SY*Genotype	201.22****	181.81****	139.25****	N/A	N/A	8.07****	25.06**	3.30****

SY=site year; Rep=replicate.

****significant at $p<0.0001$; **significant at $p<0.01$.

N/A means that data were not available due to analysis on composite samples.

Appendix 14. Mean (%) and rank of fusarium head blight (FHB) incidence from trials conducted on Set 2 genotypes in 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2019 Carman		2019 Winnipeg		2020 Carman		2020 Winnipeg	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	23.33	5	35.00	11	71.25	2	32.50	7
2	Emerson	27.50	8	55.42	77	91.25	31	71.25	85
3	FHB148	18.16	1	26.25	3	70.00	1	40.00	33
4	43I*18	38.75	56	47.08	51	88.75	13	55.22	75
5	Freedom	62.50	87	70.42	87	95.00	61	72.48	86
6	Caledonia	62.08	86	69.17	86	92.50	42	87.28	88
7	Hanover	69.17	88	72.92	88	96.25	70	80.00	87
8	KWS-H10110	30.42	15	42.50	29	91.25	31	37.48	23
9	KWS-H119	40.83	64	53.33	74	90.00	21	45.00	48
10	KWS-H144	37.08	45	37.92	18	88.75	13	35.00	15
11	KWS-H145	50.42	82	54.17	75	95.00	61	44.09	46
12	KWS-H161	42.08	67	36.25	14	96.25	70	29.78	5
13	Li1	27.92	9	45.42	44	95.00	61	40.00	33
14	Li2	30.42	15	30.83	5	96.25	70	40.00	33
15	Li3	37.08	45	41.25	25	90.00	21	32.38	6
16	Li4	35.42	38	52.50	73	95.00	61	36.00	19
17	Li5	36.67	44	36.25	14	86.25	6	48.75	59
18	Li6	42.50	69	50.00	69	96.25	70	52.48	67
19	Li7	33.33	32	60.00	85	92.50	42	50.00	62
20	Li8	42.08	67	43.33	36	87.50	7	53.75	71
21	Li9	34.17	34	46.25	48	97.50	82	45.75	52
22	Li10	38.75	56	57.50	81	97.50	82	48.75	59
23	Li12	39.58	61	44.17	40	98.75	86	38.75	27
24	Li14	32.92	27	42.92	31	93.75	48	47.50	55
25	Li15	41.67	66	48.75	61	96.25	70	51.25	63
26	Li16	37.50	48	55.42	77	91.25	31	48.75	59
27	Li17	32.08	22	37.08	16	88.75	13	42.50	40
28	Li18	45.00	76	47.50	54	96.25	70	40.00	33
29	Li19	39.17	59	48.33	59	88.75	13	28.75	4
30	Li20	34.58	36	48.33	59	88.75	13	42.50	40
31	Li21	29.58	11	41.25	25	93.75	48	52.50	68
32	Li22	37.50	48	56.25	79	95.00	61	39.33	32
33	Li23	32.08	22	47.92	56	93.75	48	45.00	48
34	Li24	35.42	38	48.75	61	88.75	13	47.64	57
35	Li25	37.92	51	50.83	70	97.50	82	47.66	58
36	Li26	37.50	48	46.25	48	96.25	70	60.00	79
37	Li27	35.00	37	47.50	54	100.00	88	40.00	33
38	Li40	46.67	78	49.17	64	95.00	61	61.25	81

39	Li41	35.42	38	49.17	64	91.25	31	39.15	31
40	Li42	32.92	27	48.75	61	98.75	86	37.50	24
41	Li102	30.42	15	41.25	25	91.25	31	33.75	10
42	Li103	32.08	22	45.83	46	83.75	3	38.75	27
43	Li104	43.75	70	43.75	38	93.75	48	42.48	39
44	Li105	30.83	18	39.58	22	87.50	7	35.00	15
45	Li106	39.17	59	49.58	67	95.00	61	58.75	78
46	Li107	50.83	83	49.17	64	93.75	48	38.75	27
47	Li111	30.00	12	34.58	9	96.25	70	37.36	21
48	Li113	45.42	77	54.17	75	92.50	42	60.75	80
49	Li114	46.67	78	52.08	72	91.25	31	57.43	77
50	Li116	24.58	6	47.92	56	93.75	48	45.00	48
51	Li117	39.58	61	41.25	25	90.00	21	34.15	14
52	Li119	32.50	25	47.08	51	93.75	48	52.50	68
53	Li120	43.75	70	45.00	42	90.00	21	51.25	63
54	Li122	33.33	32	44.17	40	90.00	21	43.75	44
55	Li123	41.25	65	47.08	51	96.25	70	35.00	15
56	Li125	19.58	2	26.25	3	90.00	21	22.66	2
57	Li126	31.25	19	34.58	9	95.00	61	42.50	40
58	Li127	43.75	70	45.83	46	90.00	21	33.75	10
59	Li128	44.17	74	42.92	31	95.00	61	45.00	48
60	Li129	32.92	27	40.42	24	91.25	31	36.25	20
61	Li131	25.00	7	37.92	18	87.50	7	37.50	24
62	Li132	43.75	70	57.08	80	93.75	48	54.15	73
63	Li133	48.33	80	43.33	36	87.50	7	46.25	53
64	Li134	49.17	81	35.83	13	91.25	31	44.33	47
65	Li135	38.33	53	38.75	21	90.00	21	38.75	27
66	Li136	51.67	84	47.92	56	92.50	42	33.75	10
67	Li137	38.75	56	37.50	17	96.25	70	34.09	13
68	Li138	35.42	38	42.50	29	91.25	31	51.25	63
69	Li139	38.33	53	32.50	6	87.50	7	47.50	55
70	Li140	28.75	10	42.92	31	92.50	42	57.42	76
71	Li141	37.08	45	33.33	8	91.25	31	37.43	22
72	Li142	32.92	27	39.58	22	88.75	13	35.00	15
73	Li143	38.33	53	46.67	50	88.75	13	32.50	7
74	Li144	34.17	34	49.58	67	96.25	70	43.75	44
75	Li145	31.25	19	42.92	31	93.75	48	42.43	38
76	Li146	39.58	61	45.00	42	90.00	21	61.25	81
77	Li147	35.42	38	50.83	70	93.75	48	46.25	53
78	Li148	37.92	51	58.75	83	87.50	7	55.00	74
79	Li164	58.33	85	45.42	44	96.25	70	53.75	71
80	Li165	32.92	27	57.50	81	93.75	48	70.00	84
81	Li166	32.50	25	37.92	18	90.00	21	51.25	63
82	Li167	44.17	74	43.75	38	97.50	82	64.33	83

83	Li168	35.42	38	58.75	83	93.75	48	52.50	68
84	Li169	30.00	12	42.92	31	92.50	42	42.50	40
85	Exp.Variety1	21.25	3	21.25	1	83.75	3	24.78	3
86	Exp.Variety2	21.25	3	22.50	2	83.75	3	20.75	1
87	Exp.Variety17	31.25	19	35.42	12	91.25	31	37.50	24
88	Exp.Variety31	30.00	12	32.92	7	93.75	48	32.50	7
	LSD (0.05)	14.83		13.11		9.42		19.85	
	Wheat means	43.07		53.75		86.43		62.68	
	Rye means	36.77		44.44		92.40		43.74	
	Rye range	19.58-58.33		21.25-60.00		83.75-100.00		20.75-70.00	

LSD=least significant difference.

Appendix 15. Mean (%) and rank of fusarium head blight (FHB) severity from trials conducted on Set 2 genotypes in 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2019 Carman		2019 Winnipeg		2020 Carman		2020 Winnipeg	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	31.67	84	20.42	81	45.00	45	27.50	82
2	Emerson	29.58	83	23.75	84	46.25	55	63.75	84
3	FHB148	27.54	82	21.67	83	42.50	37	40.00	83
4	43I*18	38.75	85	26.67	85	59.75	85	65.06	85
5	Freedom	59.17	86	32.08	86	61.25	86	66.57	86
6	Caledonia	68.75	88	57.92	87	82.50	87	94.94	88
7	Hanover	61.67	87	59.58	88	85.00	88	88.75	87
8	KWS-H10110	14.17	55	17.08	70	25.00	7	8.23	36
9	KWS-H119	12.08	37	18.75	77	43.75	39	11.25	58
10	KWS-H144	9.17	5	9.17	2	20.00	3	5.00	3
11	KWS-H145	14.17	55	13.75	45	40.00	30	6.11	13
12	KWS-H161	10.42	15	9.58	7	46.25	55	9.94	47
13	Li1	12.92	45	14.17	48	40.00	30	10.00	48
14	Li2	10.83	20	12.08	27	53.75	78	18.75	79
15	Li3	10.00	12	12.50	30	46.25	55	8.12	35
16	Li4	14.17	55	16.25	63	47.50	63	13.47	74
17	Li5	14.17	55	13.75	45	40.00	30	7.50	28
18	Li6	16.25	73	17.92	75	48.75	69	14.90	77
19	Li7	17.50	77	19.58	80	56.25	83	21.25	80
20	Li8	13.75	52	15.00	56	33.75	15	23.75	81
21	Li9	11.25	29	13.75	45	45.00	45	7.78	34
22	Li10	15.00	65	17.08	70	51.25	75	11.25	58
23	Li12	11.67	34	14.17	48	38.75	26	11.25	58
24	Li14	9.58	7	12.50	30	53.75	78	7.50	28
25	Li15	14.58	62	16.25	63	47.50	63	13.75	75

26	Li16	14.17	55	18.75	77	48.75	69	8.75	42
27	Li17	13.33	47	15.00	56	35.00	19	11.25	58
28	Li18	13.75	52	14.58	52	37.50	25	6.25	16
29	Li19	17.92	79	14.58	52	31.25	12	12.50	66
30	Li20	16.67	74	12.08	27	21.25	4	10.00	48
31	Li21	11.25	29	12.50	30	45.00	45	11.25	58
32	Li22	17.08	75	16.25	63	47.50	63	8.47	38
33	Li23	15.00	65	16.25	63	52.50	77	11.25	58
34	Li24	10.83	20	9.58	7	45.00	45	8.48	41
35	Li25	10.83	20	14.17	48	55.00	80	10.14	57
36	Li26	10.00	12	14.58	52	46.25	55	10.00	48
37	Li27	8.75	4	10.42	9	43.75	39	10.00	48
38	Li40	17.50	77	17.50	72	51.25	75	10.00	48
39	Li41	15.83	70	17.50	72	46.25	55	9.90	46
40	Li42	10.42	15	14.17	48	43.75	39	11.25	58
41	Li102	10.83	20	11.25	19	41.25	34	6.25	16
42	Li103	13.33	47	12.50	30	48.75	69	10.00	48
43	Li104	12.50	41	11.67	22	47.50	63	11.57	65
44	Li105	10.83	20	12.92	37	35.00	19	12.50	66
45	Li106	17.08	75	19.17	79	46.25	55	12.50	66
46	Li107	15.83	70	13.33	41	43.75	39	6.25	16
47	Li111	11.25	29	11.67	22	40.00	30	6.52	25
48	Li113	15.00	65	12.50	30	55.00	80	6.11	13
49	Li114	15.83	70	14.58	52	43.75	39	7.18	27
50	Li116	12.50	41	12.92	37	36.25	23	8.75	42
51	Li117	13.33	47	11.67	22	45.00	45	8.23	36
52	Li119	10.83	20	16.67	68	33.75	15	13.75	75
53	Li120	14.58	62	16.25	63	50.00	74	12.50	66
54	Li122	11.25	29	9.17	2	22.50	5	6.25	16
55	Li123	19.17	81	17.50	72	41.25	34	6.25	16
56	Li125	9.58	7	10.42	9	22.50	5	5.14	10
57	Li126	9.17	5	15.00	56	46.25	55	8.75	42
58	Li127	9.58	7	11.67	22	43.75	39	8.75	42
59	Li128	12.92	45	11.25	19	48.75	69	7.50	28
60	Li129	12.08	37	12.50	30	28.75	9	5.00	3
61	Li131	9.58	7	10.83	16	38.75	26	5.00	3
62	Li132	11.67	34	15.00	56	41.25	34	6.57	26
63	Li133	13.33	47	15.42	61	30.00	10	5.00	3
64	Li134	10.83	20	10.83	16	38.75	26	8.47	38
65	Li135	14.58	62	10.42	9	30.00	10	10.00	48
66	Li136	12.50	41	10.83	16	35.00	19	5.00	3
67	Li137	14.17	55	10.42	9	31.25	12	4.45	1
68	Li138	7.50	1	10.42	9	31.25	12	5.00	3
69	Li139	10.83	20	8.75	1	36.25	23	6.25	16

70	Li140	10.42	15	13.33	41	45.00	45	12.78	73
71	Li141	7.92	2	9.17	2	38.75	26	5.52	11
72	Li142	10.83	20	15.00	56	25.00	7	6.25	16
73	Li143	11.67	34	12.08	27	35.00	19	5.00	3
74	Li144	12.08	37	12.50	30	47.50	63	12.50	66
75	Li145	11.25	29	11.25	19	45.00	45	5.52	11
76	Li146	9.58	7	13.33	41	33.75	15	10.00	48
77	Li147	12.50	41	12.92	37	48.75	69	7.50	28
78	Li148	13.75	52	20.83	82	45.00	45	12.50	66
79	Li164	17.92	79	17.92	75	46.25	55	7.50	28
80	Li165	15.00	65	16.67	68	47.50	63	15.00	78
81	Li166	8.33	3	10.42	9	45.00	45	12.50	66
82	Li167	12.08	37	11.67	22	58.75	84	8.47	38
83	Li168	13.33	47	13.33	41	55.00	80	6.25	16
84	Li169	15.00	65	12.92	37	42.50	37	10.00	48
85	Exp.Variety1	10.00	12	9.17	2	10.00	1	4.94	2
86	Exp.Variety2	10.42	15	9.17	2	11.25	2	6.11	13
87	Exp.Variety17	10.42	15	10.42	9	33.75	15	6.25	16
88	Exp.Variety31	14.17	55	15.42	61	45.00	45	7.50	28
	LSD (0.05)	6.90		5.42		14.33		8.42	
	Wheat means	45.30		34.58		60.32		63.80	
	Rye means	12.64		13.53		40.97		9.22	
	Rye range	7.50-19.17		8.75-20.83		10.00-58.75		4.45-23.75	

LSD=least significant difference.

Appendix 16. Mean (%) and rank of fusarium head blight (FHB) index from trials conducted on Set 2 genotypes in 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2019 Carman		2019 Winnipeg		2020 Carman		2020 Winnipeg	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	7.58	78	7.20	59	32.38	24	9.06	78
2	Emerson	8.10	82	13.33	85	42.25	53	46.06	85
3	FHB148	4.85	45	5.75	40	30.50	17	16.50	83
4	43I*18	13.81	85	12.29	84	53.01	83	35.85	84
5	Freedom	37.77	86	22.76	86	58.19	86	47.66	86
6	Caledonia	42.88	87	40.46	87	76.88	87	83.27	88
7	Hanover	43.36	88	44.05	88	81.94	88	71.38	87
8	KWS-H101	4.83	44	6.68	54	23.06	8	2.75	24
9	KWS-H119	5.09	47	9.98	79	39.81	40	5.69	65
10	KWS-H144	3.32	12	3.46	6	17.94	3	1.75	6
11	KWS-H145	7.17	75	7.48	65	38.31	34	3.07	30
12	KWS-H161	4.36	35	3.50	7	44.69	63	3.40	35

13	Li1	4.03	28	6.49	48	38.00	33	3.25	33
14	Li2	3.85	23	3.75	8	51.81	81	9.06	78
15	Li3	3.66	20	5.22	30	41.94	49	2.40	18
16	Li4	5.49	51	8.08	68	45.13	67	4.82	59
17	Li5	5.93	61	4.93	22	34.81	27	3.88	45
18	Li6	7.75	80	8.85	75	46.94	74	6.75	73
19	Li7	6.02	64	11.77	82	52.31	82	9.31	80
20	Li8	6.21	65	6.77	55	29.56	14	11.56	82
21	Li9	3.95	25	6.53	51	43.88	58	3.49	38
22	Li10	5.92	60	10.02	81	50.19	77	5.38	63
23	Li12	4.63	40	6.55	52	38.31	34	4.19	50
24	Li14	3.08	9	5.39	36	50.44	78	3.75	42
25	Li15	5.95	62	8.22	71	45.56	69	6.94	75
26	Li16	5.38	50	10.00	80	44.38	61	4.13	47
27	Li17	4.55	39	5.69	39	31.13	19	4.81	58
28	Li18	6.76	71	7.30	62	36.31	29	2.44	19
29	Li19	7.02	74	6.97	57	27.50	12	3.81	44
30	Li20	5.76	57	5.96	43	18.94	4	3.69	40
31	Li21	3.77	21	5.25	33	42.44	54	6.25	68
32	Li22	7.44	76	8.94	76	45.50	68	3.49	37
33	Li23	5.89	59	7.25	61	49.44	76	5.31	61
34	Li24	3.96	26	4.83	20	40.06	42	3.77	43
35	Li25	4.17	30	7.23	60	53.81	84	5.74	66
36	Li26	4.04	29	7.18	58	44.75	65	5.63	64
37	Li27	3.43	15	4.71	19	43.75	57	4.38	55
38	Li40	7.94	81	8.78	74	48.94	75	6.13	67
39	Li41	5.57	53	8.21	70	43.00	55	4.16	49
40	Li42	3.77	21	6.46	47	43.25	56	4.69	57
41	Li102	3.57	19	4.63	18	37.69	32	2.13	12
42	Li103	4.63	40	5.76	41	41.75	48	4.31	54
43	Li104	5.81	58	5.11	28	44.81	66	4.58	56
44	Li105	3.33	13	5.07	25	31.00	18	4.25	52
45	Li106	6.85	72	9.33	77	43.94	59	6.75	74
46	Li107	8.63	83	6.61	53	41.44	47	2.31	15
47	Li111	3.45	16	3.84	9	38.50	36	2.48	21
48	Li113	6.41	67	6.88	56	50.75	79	4.24	51
49	Li114	7.46	77	7.51	66	40.50	44	3.94	46
50	Li116	3.25	11	6.02	44	34.00	26	3.00	27
51	Li117	5.72	55	5.15	29	40.69	46	2.83	25
52	Li119	3.47	17	7.46	63	31.88	21	6.38	71
53	Li120	6.57	68	7.46	63	46.25	72	6.63	72
54	Li122	3.89	24	4.19	16	20.31	5	2.69	23
55	Li123	7.60	79	8.20	69	40.25	43	2.19	13
56	Li125	2.74	4	2.82	4	20.44	6	1.24	1

57	Li126	2.95	7	5.24	31	43.94	59	3.44	36
58	Li127	4.39	36	5.52	37	39.56	39	3.00	27
59	Li128	6.34	66	4.86	21	46.31	73	3.13	31
60	Li129	4.22	31	5.09	26	26.00	9	1.81	7
61	Li131	2.50	3	4.02	13	35.94	28	1.88	10
62	Li132	5.72	55	8.51	73	39.38	37	3.33	34
63	Li133	7.01	73	6.03	45	26.25	10	2.31	15
64	Li134	5.61	54	4.00	12	36.31	29	3.66	39
65	Li135	5.96	63	4.06	14	27.00	11	3.19	32
66	Li136	6.63	70	5.24	31	32.25	23	1.69	5
67	Li137	6.58	69	4.09	15	30.19	16	1.82	8
68	Li138	2.82	6	4.40	17	28.75	13	2.56	22
69	Li139	4.30	34	2.66	3	32.19	22	2.94	26
70	Li140	3.13	10	5.61	38	42.00	50	7.07	76
71	Li141	2.98	8	3.17	5	36.38	31	1.86	9
72	Li142	4.52	37	6.09	46	22.38	7	2.19	13
73	Li143	4.52	37	5.38	35	32.50	25	1.63	4
74	Li144	4.25	32	6.50	50	45.81	70	5.19	60
75	Li145	3.42	14	5.06	24	42.00	50	2.11	11
76	Li146	3.96	26	5.92	42	29.81	15	6.25	68
77	Li147	4.63	40	6.49	48	45.94	71	3.69	40
78	Li148	5.19	48	12.27	83	40.00	41	7.75	77
79	Li164	10.96	84	8.31	72	44.63	62	4.25	52
80	Li165	4.77	43	9.34	78	44.69	63	10.13	81
81	Li166	2.78	5	3.96	11	40.56	45	6.31	70
82	Li167	5.50	52	4.95	23	57.19	85	5.32	62
83	Li168	4.88	46	7.80	67	51.44	80	3.06	29
84	Li169	5.34	49	5.29	34	39.38	37	4.13	47
85	Exp.Variety1	2.35	2	2.11	1	8.38	1	1.40	2
86	Exp.Variety2	2.32	1	2.16	2	9.56	2	1.41	3
87	Exp.Variety17	3.54	18	3.92	10	31.50	20	2.44	19
88	Exp.Variety31	4.26	33	5.09	27	42.19	52	2.38	17
	LSD (0.05)	4.35		3.65		15.15		5.60	
	Wheat means	22.62		20.83		53.59		44.26	
	Rye means	4.97		6.14		38.28		4.11	
	Rye range	2.32-10.96		2.11-12.27		8.37-57.19		1.24-11.56	

LSD=least significant difference.

Appendix 17. Mean (%) and rank of *Fusarium*-damaged kernels (FDK) from trials conducted on Set 2 genotypes in 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2019 Carman	2019 Winnipeg	2020 Carman	2020 Winnipeg

		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	0.95	80	0.70	81	1.07	82	3.25	32
2	Emerson	1.63	85	0.49	68	1.80	84	2.90	28
3	FHB 148	1.21	82	0.98	84	1.70	83	2.65	21
4	43I*18	1.56	84	1.87	85	5.74	85	7.83	83
5	Freedom	10.74	87	8.42	86	7.09	86	11.62	86
6	Caledonia	7.81	86	12.04	87	7.39	87	15.75	87
7	Hanover	23.91	88	15.75	88	11.61	88	25.07	88
8	KWS-H10110	0.89	78	0.66	77	0.04	7	2.05	11
9	KWS-H119	0.43	42	0.18	25	0.13	21	2.55	17
10	KWS-H144	0.26	13	0.46	66	0.06	11	2.67	22
11	KWS-H145	0.55	63	0.43	63	0.29	51	4.53	54
12	KWS-H161	0.40	39	0.30	46	0.02	2	3.35	33
13	Li1	0.37	35	0.18	29	0.55	78	3.61	38
14	Li2	0.35	32	0.12	19	0.43	72	6.69	80
15	Li3	0.21	11	0.05	9	0.08	14	2.72	24
16	Li4	0.17	4	0.13	20	0.22	42	2.62	19
17	Li5	0.33	28	0.10	16	0.22	40	3.09	30
18	Li6	0.44	44	0.31	49	0.20	38	2.86	27
19	Li7	0.84	75	0.09	14	0.34	60	1.72	6
20	Li8	0.74	70	0.20	31	0.20	37	2.30	12
21	Li9	0.49	53	0.27	42	0.33	59	2.40	15
22	Li10	0.46	48	0.24	38	0.46	75	3.22	31
23	Li12	0.82	73	0.14	22	0.25	46	4.64	57
24	Li14	0.53	59	0.09	13	0.40	69	2.71	23
25	Li15	0.73	69	0.06	10	0.43	73	2.31	13
26	Li16	0.84	74	0.11	18	0.26	47	4.46	50
27	Li17	0.42	40	0.22	34	0.30	52	3.57	36
28	Li18	0.85	76	0.07	11	0.13	22	2.56	18
29	Li19	0.50	54	0.26	40	0.22	41	4.68	58
30	Li20	0.44	45	0.04	6	0.14	27	1.99	9
31	Li21	0.56	66	0.35	54	0.29	50	2.75	25
32	Li22	0.55	64	0.10	17	0.31	58	3.97	45
33	Li23	0.27	18	0.33	50	0.31	57	1.52	4
34	Li24	0.46	47	0.05	8	0.30	53	1.47	3
35	Li25	0.35	30	0.04	7	0.35	62	1.97	8
36	Li26	0.32	24	0.20	30	0.35	61	1.72	5
37	Li27	0.28	19	0.23	36	0.18	34	1.37	2
38	Li40	0.30	22	0.50	69	0.64	81	2.54	16
39	Li41	0.48	52	0.67	78	0.24	44	2.65	20
40	Li42	0.56	65	0.83	83	0.38	66	4.89	62
41	Li102	0.21	10	0.67	80	0.14	29	4.61	55
42	Li103	0.34	29	0.61	74	0.24	45	5.66	68
43	Li104	0.55	62	0.23	35	0.14	25	6.00	73

44	Li105	0.33	26	0.42	62	0.10	17	7.26	82
45	Li106	0.17	3	0.51	70	0.52	77	9.14	85
46	Li107	0.52	58	0.60	73	0.20	36	4.70	59
47	Li111	0.19	9	0.18	26	0.08	13	6.58	79
48	Li113	0.55	61	0.40	60	0.09	16	6.02	74
49	Li114	0.78	72	0.36	57	0.17	33	4.06	46
50	Li116	0.29	20	0.45	65	0.03	4	6.26	76
51	Li117	0.36	33	0.30	45	0.05	9	5.86	71
52	Li119	0.36	34	0.26	39	0.59	80	5.16	65
53	Li120	0.47	49	0.35	55	0.44	74	6.51	77
54	Li122	0.35	30	0.58	72	0.05	10	3.67	40
55	Li123	0.40	38	0.61	75	0.14	28	8.74	84
56	Li125	0.26	15	0.23	37	0.02	1	2.78	26
57	Li126	0.52	56	0.21	33	0.31	55	5.06	64
58	Li127	0.19	8	0.29	44	0.13	23	4.53	52
59	Li128	0.50	55	0.36	58	0.09	15	6.55	78
60	Li129	0.22	12	0.14	23	0.11	18	2.31	14
61	Li131	0.47	50	0.18	28	0.29	49	4.26	48
62	Li132	0.54	60	0.08	12	0.38	64	6.71	81
63	Li133	1.21	83	0.43	64	0.26	48	4.80	60
64	Li134	0.26	14	0.31	47	0.42	71	3.92	42
65	Li135	0.72	68	0.63	76	0.05	8	3.04	29
66	Li136	0.48	51	0.04	4	0.11	19	4.51	51
67	Li137	0.33	27	0.03	2	0.03	5	2.01	10
68	Li138	0.52	57	0.33	51	0.07	12	3.93	44
69	Li139	0.46	46	0.09	15	0.19	35	3.61	37
70	Li140	0.31	23	0.03	1	0.16	31	4.63	56
71	Li141	0.18	6	0.04	5	0.30	54	4.93	63
72	Li142	0.39	36	0.04	3	0.14	26	3.41	34
73	Li143	0.60	67	0.18	27	0.16	30	3.92	43
74	Li144	0.17	2	0.27	43	0.23	43	4.87	61
75	Li145	0.44	43	0.20	32	0.21	39	5.23	66
76	Li146	0.75	71	0.67	79	0.12	20	5.38	67
77	Li147	0.32	25	0.33	52	0.38	65	5.75	70
78	Li148	1.05	81	0.41	61	0.56	79	5.95	72
79	Li164	0.85	77	0.26	41	0.39	67	5.72	69
80	Li165	0.39	37	0.14	24	0.50	76	4.53	53
81	Li166	0.26	16	0.54	71	0.35	63	3.49	35
82	Li167	0.42	41	0.14	21	0.31	56	4.30	49
83	Li168	0.17	5	0.46	67	0.40	70	6.26	75
84	Li169	0.27	17	0.37	59	0.39	68	3.67	39
85	Exp.Variety1	0.30	21	0.31	48	0.02	3	1.92	7
86	Exp.Variety2	0.18	7	0.34	53	0.04	6	0.95	1
87	Exp.Variety17	0.12	1	0.36	56	0.16	32	3.85	41

88	Exp.Variety31	0.90	79	0.78	82	0.14	24	4.14	47
	Wheat means	6.83		5.75		5.20		9.87	
	Rye means	0.45		0.29		0.24		4.07	
	Rye range	0.16-1.21		0.03-0.83		0.02-0.64		0.95-9.14	

Appendix 18. Mean (ppm) and rank of deoxynivalenol (DON) from trials conducted on Set 2 genotypes in 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2019 Carman		2019 Winnipeg		2020 Carman		2020 Winnipeg	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	7.53	24	6.54	5	2.79	73	27.16	46
2	Emerson	8.78	36	7.28	7	4.28	84	27.77	47
3	FHB 148	12.16	73	8.35	16	3.44	80	21.11	16
4	43I*18	18.38	84	12.80	69	11.21	85	36.38	80
5	Freedom	29.54	87	18.73	86	11.42	86	29.92	62
6	Caledonia	26.62	85	29.27	87	23.77	88	62.60	87
7	Hanover	41.57	88	32.70	88	19.88	87	65.24	88
8	KWS-H10110	5.96	12	7.77	9	0.55	7	18.09	7
9	KWS-H119	7.10	20	8.87	24	0.98	17	21.50	17
10	KWS-H144	5.95	11	7.33	8	0.75	10	13.40	1
11	KWS-H145	9.27	41	11.00	50	0.95	16	27.01	44
12	KWS-H161	5.46	7	9.64	31	0.69	8	35.66	78
13	Li1	7.23	21	8.74	22	1.89	43	27.03	45
14	Li2	10.17	55	10.08	39	2.69	69	37.96	83
15	Li3	12.19	74	8.48	19	1.49	32	23.62	26
16	Li4	27.92	86	14.30	80	2.43	59	28.63	53
17	Li5	10.91	63	8.37	17	1.81	39	28.44	49
18	Li6	12.43	75	13.17	73	1.70	38	23.90	30
19	Li7	11.46	66	10.00	38	2.77	71	30.88	66
20	Li8	10.48	59	11.23	56	2.53	63	31.32	68
21	Li9	10.00	53	8.31	14	1.66	37	26.49	39
22	Li10	11.05	64	10.86	48	2.61	66	28.44	49
23	Li12	11.73	70	10.86	48	1.42	29	32.96	74
24	Li14	13.54	78	12.43	65	1.99	45	27.00	43
25	Li15	14.76	81	8.08	10	2.78	72	21.60	18
26	Li16	11.52	67	10.77	46	2.30	55	21.83	20
27	Li17	11.57	68	9.62	29	2.24	53	29.02	55
28	Li18	7.92	29	11.86	62	2.12	49	32.01	69
29	Li19	5.92	10	9.88	37	0.93	14	26.49	39
30	Li20	5.79	8	11.15	53	0.78	11	28.44	49
31	Li21	7.82	25	9.25	25	2.33	57	55.81	86
32	Li22	10.68	60	11.36	58	3.87	82	36.74	81

33	Li23	11.76	72	12.87	70	3.04	77	39.60	85
34	Li24	8.79	37	9.72	33	2.27	54	29.22	58
35	Li25	9.74	51	12.61	67	2.88	74	32.01	69
36	Li26	4.92	5	9.76	34	2.52	62	33.71	76
37	Li27	6.25	13	11.31	57	1.86	42	37.34	82
38	Li40	9.69	47	10.71	45	2.54	65	36.16	79
39	Li41	8.86	38	11.05	51	2.21	51	30.03	63
40	Li42	7.33	22	8.54	20	2.15	50	28.82	54
41	Li102	7.06	19	13.20	74	1.31	26	24.29	31
42	Li103	5.85	9	9.52	28	0.95	15	20.32	10
43	Li104	8.07	30	12.74	68	0.70	9	20.65	13
44	Li105	6.69	15	10.53	42	0.89	13	23.85	29
45	Li106	11.34	65	13.61	76	2.91	75	33.48	75
46	Li107	10.41	57	16.09	85	1.05	20	26.80	42
47	Li111	7.91	28	10.17	40	1.20	23	39.54	84
48	Li113	9.30	42	8.23	11	1.47	31	22.73	22
49	Li114	8.48	33	9.79	35	0.13	1	20.90	15
50	Li116	6.86	17	9.72	32	0.86	12	24.40	32
51	Li117	9.69	47	10.64	43	1.41	28	28.02	48
52	Li119	9.78	52	9.35	26	2.22	52	29.66	60
53	Li120	14.15	79	14.15	79	2.68	68	34.48	77
54	Li122	4.37	4	7.25	6	0.34	3	17.73	6
55	Li123	13.23	77	11.10	52	1.92	44	32.36	71
56	Li125	3.94	3	5.42	2	0.27	2	13.62	2
57	Li126	7.33	22	10.25	41	1.20	23	28.60	52
58	Li127	9.39	43	14.32	81	1.05	20	21.60	19
59	Li128	10.46	58	11.21	54	1.43	30	26.67	41
60	Li129	5.01	6	11.21	54	0.39	4	16.81	5
61	Li131	7.88	27	14.75	84	1.30	25	19.24	9
62	Li132	15.64	83	9.85	36	2.06	48	24.73	33
63	Li133	14.93	82	11.72	59	3.24	79	25.20	36
64	Li134	9.72	50	13.99	77	1.83	41	24.96	35
65	Li135	8.67	34	12.87	71	2.30	56	20.82	14
66	Li136	10.22	56	9.51	27	2.53	63	23.75	27
67	Li137	8.41	31	8.74	21	1.41	27	20.41	11
68	Li138	10.81	62	8.37	17	1.53	34	20.57	12
69	Li139	8.87	39	12.27	64	3.10	78	29.04	56
70	Li140	8.74	35	9.63	30	1.66	36	26.42	38
71	Li141	11.73	70	11.78	61	2.62	67	29.66	60
72	Li142	9.68	46	8.30	12	1.13	22	18.19	8
73	Li143	9.72	49	10.69	44	1.82	40	23.75	27
74	Li144	9.39	43	8.30	12	2.01	46	30.46	64
75	Li145	7.83	26	8.33	15	1.51	33	24.85	34
76	Li146	7.00	18	12.60	66	2.37	58	23.23	23

77	Li147	9.67	45	10.79	47	2.45	60	29.04	56
78	Li148	12.80	76	11.72	59	2.49	61	32.54	73
79	Li164	14.75	80	13.99	77	4.14	83	30.79	65
80	Li165	11.60	69	12.21	63	3.62	81	31.30	67
81	Li166	10.07	54	13.60	75	3.01	76	23.43	25
82	Li167	8.43	32	14.40	83	2.74	70	29.50	59
83	Li168	10.68	60	14.32	81	1.65	35	32.36	71
84	Li169	8.89	40	12.94	72	2.04	47	22.16	21
85	Exp.Variety1	2.64	1	5.50	3	0.48	5	14.62	4
86	Exp.Variety2	2.89	2	5.35	1	0.49	6	13.88	3
87	Exp.Variety17	6.28	14	6.04	4	1.02	19	23.34	24
88	Exp.Variety31	6.71	16	8.84	23	1.01	18	25.46	37
	Wheat means	20.65		16.52		10.97		38.60	
	Rye means	9.38		10.62		1.82		26.93	
	Rye range	2.64-27.92		5.35-16.09		0.13-4.14		13.40-55.81	

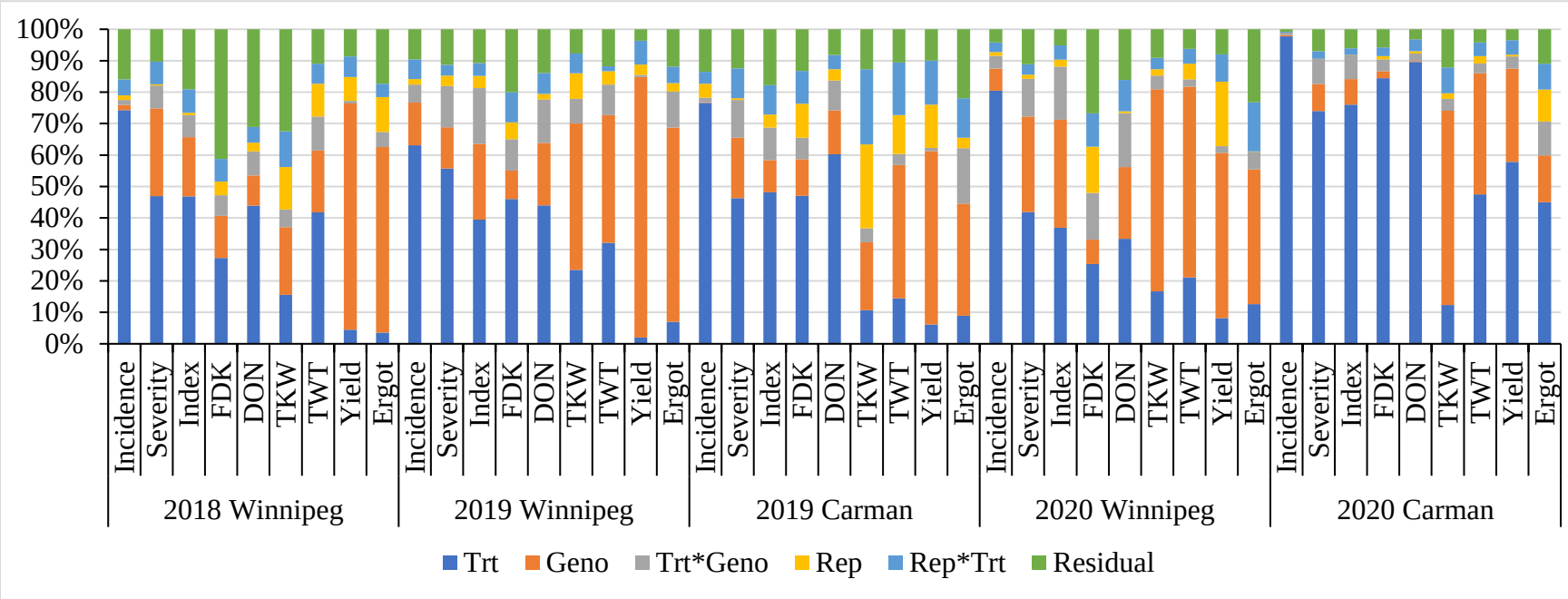
Appendix 19. Mean (%) and rank of ergot severity from trials conducted on Set 2 genotypes in 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman

No.	Genotype	2019 Carman		2019 Winnipeg		2020 Carman		2020 Winnipeg	
		Mean	Rank	Mean	Rank	Mean	Rank	Mean	Rank
1	32C*17	0.00	1	0.34	5	0.23	2	0.36	2
2	Emerson	0.00	1	0.34	5	0.28	3	0.06	1
3	FHB148	0.00	1	0.20	2	0.04	1	0.65	4
4	43I*18	0.00	1	0.34	5	0.44	4	0.40	3
5	Freedom	0.00	8	0.00	1	2.45	10	5.46	25
6	Caledonia	0.00	1	0.27	4	0.96	5	2.06	5
7	Hanover	0.00	1	0.20	2	4.32	35	7.78	65
8	KWS-H10110	0.59	76	2.99	50	3.96	28	5.14	19
9	KWS-H119	0.33	57	2.24	40	3.50	23	4.84	18
10	KWS-H144	0.00	1	0.74	11	2.13	9	3.35	10
11	KWS-H145	0.21	39	2.65	45	6.41	59	5.98	34
12	KWS-H161	0.03	13	1.12	17	2.67	12	2.74	6
13	Li1	0.53	72	4.03	76	6.58	63	6.78	51
14	Li2	0.41	63	3.80	72	8.33	81	8.80	72
15	Li3	0.33	56	4.03	75	7.93	77	7.95	66
16	Li4	0.55	75	3.43	61	7.66	75	6.61	47
17	Li5	0.23	46	3.60	64	6.89	66	7.01	56
18	Li6	0.77	79	4.78	82	8.54	82	9.83	82
19	Li7	0.54	73	3.61	65	9.68	87	9.82	81
20	Li8	0.50	69	5.30	87	7.41	71	7.38	60
21	Li9	0.07	23	3.73	70	6.93	67	6.15	38

22	Li10	0.53	71	3.80	73	8.28	80	9.19	77
23	Li12	0.33	58	5.25	86	9.61	86	6.22	39
24	Li14	0.42	65	3.38	60	6.78	64	5.55	26
25	Li15	0.51	70	2.88	49	6.51	61	5.82	30
26	Li16	1.09	85	4.88	83	9.20	84	8.30	69
27	Li17	0.82	81	4.06	77	6.57	62	11.57	86
28	Li18	0.81	80	3.24	58	5.10	47	9.09	76
29	Li19	0.15	33	3.61	66	6.07	57	9.19	78
30	Li20	1.04	84	5.34	88	4.56	41	7.77	64
31	Li21	0.94	82	3.80	71	7.19	69	9.92	83
32	Li22	0.46	67	4.92	84	7.65	74	8.34	70
33	Li23	2.36	88	3.53	63	9.93	88	8.76	71
34	Li24	0.67	78	3.43	62	8.00	79	6.73	50
35	Li25	0.44	66	4.00	74	8.61	83	6.31	43
36	Li26	0.94	83	3.62	67	7.42	72	6.87	53
37	Li27	0.16	34	3.30	59	7.94	78	6.62	48
38	Li40	0.55	74	4.07	78	7.87	76	12.31	87
39	Li41	1.19	86	3.07	54	7.05	68	9.06	75
40	Li42	1.22	87	4.28	81	7.64	73	10.74	85
41	Li102	0.07	22	1.76	32	4.86	44	6.59	46
42	Li103	0.10	29	0.77	12	3.71	25	3.30	9
43	Li104	0.11	30	0.52	10	1.97	8	6.07	37
44	Li105	0.40	61	2.35	42	5.95	54	7.63	63
45	Li106	0.35	60	2.61	44	4.22	31	8.98	73
46	Li107	0.20	37	1.80	33	4.30	34	6.88	54
47	Li111	0.08	24	3.10	55	5.19	48	3.74	11
48	Li113	0.33	55	1.00	14	5.31	49	5.36	24
49	Li114	0.21	41	1.71	31	4.52	39	5.91	33
50	Li116	0.17	36	2.05	36	3.17	19	5.32	23
51	Li117	0.10	28	2.23	39	3.84	26	6.81	52
52	Li119	0.60	77	3.12	56	4.87	45	6.40	44
53	Li120	0.25	49	2.72	46	6.45	60	6.73	49
54	Li122	0.27	51	2.09	37	3.98	29	7.97	67
55	Li123	0.42	64	1.41	22	3.31	21	5.18	20
56	Li125	0.25	48	4.14	80	4.44	37	5.89	32
57	Li126	0.34	59	3.68	69	6.00	56	7.45	61
58	Li127	0.23	43	1.09	16	2.99	15	4.82	17
59	Li128	0.27	50	1.68	28	3.00	16	5.62	27
60	Li129	0.06	19	2.22	38	3.95	27	7.15	57
61	Li131	0.01	9	1.43	23	4.46	38	4.36	12
62	Li132	0.32	53	1.70	29	4.60	42	4.63	14
63	Li133	0.14	32	2.83	47	3.50	22	5.69	29
64	Li134	0.22	42	2.28	41	4.23	32	7.32	58
65	Li135	0.03	14	0.93	13	3.16	18	6.02	35

66	Li136	0.24	47	4.08	79	7.34	70	9.36	79
67	Li137	0.17	35	3.01	51	4.90	46	9.52	80
68	Li138	0.40	62	3.02	52	5.99	55	8.27	68
69	Li139	0.13	31	2.46	43	9.55	85	10.63	84
70	Li140	0.02	10	1.61	26	4.29	33	7.62	62
71	Li141	0.10	26	1.65	27	3.21	20	7.33	59
72	Li142	0.21	40	3.67	68	4.52	40	5.67	28
73	Li143	0.10	27	2.84	48	6.82	65	13.01	88
74	Li144	0.04	15	1.26	20	3.58	24	6.27	41
75	Li145	0.23	45	3.02	53	4.08	30	6.56	45
76	Li146	0.33	54	1.84	35	4.71	43	6.29	42
77	Li147	0.29	52	1.82	34	5.66	53	8.99	74
78	Li148	0.23	44	4.95	85	5.49	51	6.95	55
79	Li164	0.06	18	1.70	30	3.07	17	5.25	22
80	Li165	0.04	16	1.26	19	5.52	52	5.20	21
81	Li166	0.07	20	1.28	21	6.41	58	6.25	40
82	Li167	0.20	38	1.13	18	2.84	14	3.24	8
83	Li168	0.04	17	1.56	25	5.49	50	4.80	16
84	Li169	0.47	68	3.18	57	4.33	36	4.53	13
85	Exp.Variety1	0.07	21	1.04	15	1.60	6	6.07	36
86	Exp.Variety2	0.09	25	1.48	24	1.75	7	5.87	31
87	Exp.Variety17	0.02	12	0.39	8	2.47	11	4.64	15
88	Exp.Variety31	0.02	11	0.47	9	2.70	13	3.04	7
	LSD (0.05)	0.68		1.40		2.19		3.51	
	Wheat means	0.00		0.23		1.25		2.39	
	Rye means	0.36		2.72		5.47		6.89	
	Rye range	0.00-2.36		0.39-5.34		1.60-9.93		2.74-13.01	

LSD=least significant difference.



Appendix 20. Percentage of variation in the analysis of variance sums of squares (Brown, 2008) associated with treatment (Trt), genotype (Geno), treatment × genotype (Trt*Geno), replicate (Rep), replicate × treatment (Rep*Trt), and residual for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), thousand kernel weight (TKW), test weight (TWT), grain yield, and ergot severity of fungicide trials conducted in 2018 Winnipeg, 2019 Winnipeg, 2019 Carman, 2020 Winnipeg, and 2020 Carman.

Appendix 21. Means by treatment and genotype for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), test weight (TWT), thousand kernel weight (TKW), yield, and ergot severity of the fungicide trial conducted in 2018 Winnipeg

Treatment	Genotype	Incidence (%)	Severity (%)	Index (%)	FDK (%)	DON (ppm)	TWT (g/0.5 L)	TKW (g)	Yield (t/ha)	Ergot severity (%)
Uninoculated-untreated	Dakota	5.00d	5.63hi	0.31h	0.01f	0.00g	352.03gh	29.30a	1.99c	0.02e
Uninoculated-untreated	KWS-H161	1.88d	1.88i	0.08h	0.00f	0.00g	360.91a	28.52ab	2.36abc	0.08e
Uninoculated-untreated	Vitallo	2.54d	2.92i	0.13h	0.00f	0.03fg	360.47ab	27.34abcde	0.85d	0.67ab
Heading	Dakota	40.00ab	29.38ab	11.64ab	0.21ab	1.23ab	348.61ij	27.19bcde	2.06bc	0.05e
Heading	KWS-H161	35.63abc	12.50g	4.89g	0.11bcdef	0.73bcde	354.59efg	26.05def	2.17bc	0.08e
Heading	Vitallo	34.38abc	21.88cde	7.55cdefg	0.15abcd	0.90bcd	351.85efghij	25.65ef	0.80d	0.40cd
10% anthesis	Dakota	45.00a	23.13bcd	10.89abc	0.21ab	1.65a	347.40j	27.99abc	2.16bc	0.10e
10% anthesis	KWS-H161	39.38ab	13.75g	5.41fg	0.07cdef	0.58cdef	358.19abcd	27.02bcde	2.27bc	0.13e
10% anthesis	Vitallo	35.00abc	24.38bcd	8.63bcdef	0.17abc	1.00abc	-	26.40cdef	0.70d	0.95a
80% anthesis	Dakota	34.38abc	20.63def	7.02defg	0.14abcde	0.58cdef	352.84fgh	28.08abc	2.56ab	0.06e
80% anthesis	KWS-H161	35.00abc	11.88gh	4.20g	0.01ef	0.28efg	359.47abc	26.66cdef	2.91a	0.14e
80% anthesis	Vitallo	33.75bc	23.13bcd	7.94bcdef	0.12abcde	0.53cdefg	355.91def	25.91ef	1.07d	0.52bc
6 days after 50% anthesis	Dakota	36.25abc	28.13abc	10.30abcd	0.10bcdef	0.50bcdeg	350.69hij	27.39bcde	2.14bc	0.04e
6 days after 50% anthesis	KWS-H161	35.00abc	15.63efg	5.47fg	0.04def	0.40defg	356.43bcde	25.05f	2.33bc	0.13e
6 days after 50% anthesis	Vitallo	28.13c	21.88d	6.06efg	0.15abcd	0.65cde	355.38cdefg	26.53cdef	0.81d	0.57bc
Inoculated-untreated	Dakota	40.63ab	33.13a	13.20a	0.16abcd	1.28ab	350.88hi	27.74abcd	2.36bc	0.07e
Inoculated-untreated	KWS-H161	44.38ab	14.38fg	6.33efg	0.10abcde	0.70bcde	351.90ghi	26.14def	2.43abc	0.22de
Inoculated-untreated	Vitallo	40.00ab	23.75bcd	9.66abcde	0.23a	1.23ab	349.71ghij	25.87def	0.92d	0.57bc

Means followed by the same letter within a column are not significant at $p<0.05$.

Appendix 22. Means by treatment and genotype for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), test weight (TWT), thousand kernel weight (TKW), yield, and ergot severity of the fungicide trial conducted in 2019 Winnipeg

Treatment	Genotype	Incidence (%)	Severity (%)	Index (%)	FDK (%)	DON (ppm)	TWT (g/0.5 L)	TKW (g)	Yield (t/ha)	Ergot severity (%)
Uninoculated-untreated	Dakota	1.25e	0.63g	0.03g	0.01e	0.05g	358.36bcd	31.2ab	3.31bcdef	0.13d
Uninoculated-untreated	KWS-H161	0.63e	0.63g	0.02g	0.01e	0.04g	366.40a	31.66a	3.87a	0.27cd
Uninoculated-untreated	Vitallo	1.25e	1.25g	0.03g	0.00e	0.14g	362.08ab	28.48ef	1.18g	0.74d
Heading	Dakota	42.50cd	12.50def	5.47def	0.61cd	8.1cd	350.54efg	28.72ef	2.88def	0.18d
Heading	KWS-H161	35.00d	8.13f	2.95f	0.51d	5.89cdef	358.27bcd	29.37cde	3.54abc	0.24d
Heading	Vitallo	33.75d	13.75de	4.77def	0.71bcd	5.11def	344.1hi	26.69gh	0.85g	1.51a
10% anthesis	Dakota	50.63bc	11.88def	6.48cd	0.93abc	9.45bc	347.82fgh	27.72fg	2.62f	0.24d
10% anthesis	KWS-H161	38.75cd	9.38f	3.69ef	0.48d	5.68def	357.32bcd	30.07bcd	3.43abcde	0.18d
10% anthesis	Vitallo	36.25d	11.88def	4.42def	0.83bcd	6.19cdef	347.42fgh	25.09i	0.65g	0.62bc
80% anthesis	Dakota	64.38ab	21.25a	13.55a	1.29a	12.17b	340.86i	27.94fg	2.93cdef	0.21d
80% anthesis	KWS-H161	43.75cd	9.38f	4.20def	0.49d	4.05ef	359.62b	29.22cde	3.95ab	0.17d
80% anthesis	Vitallo	31.88d	10.63ef	3.34ef	0.55cd	3.09fg	352.71def	27.57fg	1.00g	0.78b
6 days after 50% anthesis	Dakota	63.75ab	15.63bcd	9.86b	0.77bcd	6.96cde	346.51gh	28.86def	2.68ef	0.24d
6 days after 50% anthesis	KWS-H161	39.38cd	9.38f	3.75ef	0.66bcd	4.75def	358.51bc	30.21bc	3.82ab	0.21d
6 days after 50% anthesis	Vitallo	41.25cd	20.00ab	8.25bc	0.49d	5.41def	347.15fgh	25.52hi	0.93g	1.51a
Inoculated-untreated	Dakota	68.75a	18.75abc	12.98a	1.28a	17.45a	339.88i	26.77gh	2.69ef	0.26cd
Inoculated-untreated	KWS-H161	44.38cd	10.63ef	4.52def	0.74bcd	7.25cde	354.38cde	29.32cde	3.48abcd	0.25d
Inoculated-untreated	Vitallo	38.75cd	14.38cde	5.86cde	1.04ab	7.32cde	348.38efgh	25.86hi	1.00g	1.42a

Means followed by the same letter within a column are not significant at $p<0.05$.

Appendix 23. Means by treatment and genotype for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), test weight (TWT), thousand kernel weight (TKW), yield, and ergot severity of the fungicide trial conducted in 2019 Carman

Treatment	Genotype	Incidence (%)	Severity (%)	Index (%)	FDK (%)	DON (ppm)	TWT (g/0.5 L)	TKW (g)	Yield (t/ha)	Ergot severity (%)
Uninoculated-untreated	Dakota	2.50g	1.25h	0.13h	0.00h	0.10h	335.63fg	21.14bcd	3.02defg	0.01d
Uninoculated-untreated	KWS-H161	3.75g	2.50gh	0.14h	0.01h	0.06h	338.83cefg	19.75de	3.96abc	0.01d
Uninoculated-untreated	Vitallo	3.13g	2.50gh	0.11h	0.01h	0.05h	349.96abd	22.51abc	2.35hijk	0.12bc
Heading	Dakota	49.38bcdef	16.88bcd	8.89cde	1.50abcd	12.91de	332.47fgh	20.15cde	3.00defh	0.03cd
Heading	KWS-H161	45.00def	8.75f	4.06fgh	1.05cdefg	14.42cd	340.12defg	21.53bcd	4.17a	0.04cd
Heading	Vitallo	45.63cdef	24.38a	11.38abcd	1.24abcdef	8.51fg	350.04abc	22.67ab	2.29gijk	0.10bcd
10% anthesis	Dakota	35.00ef	15.63cde	5.38efg	1.44abcde	9.85efg	336.27efg	21.49bcd	3.23cde	0.06cd
10% anthesis	KWS-H161	32.50f	8.13fg	2.66gh	1.16bcdefg	11.89def	333.25fgh	19.66de	3.98ab	0.02cd
10% anthesis	Vitallo	38.13ef	16.88bcd	6.70defg	1.54abcd	9.18efg	355.43a	23.33ab	2.37fghij	0.12bc
80% anthesis	Dakota	65.63ab	20.63abc	13.95ab	1.59abc	12.04def	331.11ghi	21.04bcd	2.91defghi	0.04cd
80% anthesis	KWS-H161	57.50abcd	10.00ef	5.81efg	0.76fg	12.22def	340.09bcdef	21.03bcd	4.16a	0.02cd
80% anthesis	Vitallo	51.88abcde	13.75def	7.05defg	0.67g	6.42g	355.84a	24.34a	2.39fghij	0.36a
6 days after 50% anthesis	Dakota	61.25abcd	16.88bcd	10.66abcd	1.80a	17.90bc	332.05fgh	20.92bcd	2.84defgh	0.07cd
6 days after 50% anthesis	KWS-H161	66.88a	12.50def	8.47def	0.89efg	13.04de	334.41fg	19.66de	3.89abc	0.01d
6 days after 50% anthesis	Vitallo	60.00abcd	21.88ab	13.22abc	1.00defg	7.30g	347.36abcde	22.6abc	2.14ij	0.19b
Inoculated-untreated	Dakota	61.25abcd	15.63cde	9.78bcde	1.71ab	24.04a	320.04i	19.71d	2.57efghi	0.03cd
Inoculated-untreated	KWS-H161	67.50a	11.88def	7.98def	0.92efg	19.78b	322.66hi	17.66e	3.23bcd	0.02cd
Inoculated-untreated	Vitallo	62.50abc	23.13a	14.42a	1.08cdefg	10.17efg	342.92bcdef	20.88bcd	1.79j	0.10bcd

Means followed by the same letter within a column are not significant at $p<0.05$.

Appendix 24. Means by treatment and genotype for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), test weight (TWT), thousand kernel weight (TKW), yield, and ergot severity of the fungicide trial conducted in 2020 Winnipeg

Treatment	Genotype	Incidence (%)	Severity (%)	Index (%)	FDK (%)	DON (ppm)	TWT (g/0.5 L)	TKW (g)	Yield (t/ha)	Ergot severity (%)
Uninoculated-untreated	Dakota	0.00d	0.00h	0.00h	0.01e	0.04g	344.86de	30.52bc	2.74cde	0.97defg
Uninoculated-untreated	Hazlet	1.67d	1.67gh	0.08h	0.04e	0.03g	355.13a	34.58a	2.33efgh	1.31abcde
Uninoculated-untreated	KWS-H161	0.00d	0.00h	0.00h	0.40cde	0.90fg	352.31ab	28.40d	3.28ab	1.00cdefg
Heading	Dakota	65.00a	11.67cde	7.58cde	0.95ab	3.79a	333.21f	27.70de	2.58cdef	0.92efg
Heading	Hazlet	63.33a	20.00ab	12.67b	1.04a	3.88a	345.61de	31.29bc	2.31efgh	1.52abc
Heading	KWS-H161	48.33bc	5.00fgh	2.42gh	0.49bcde	1.16defg	344.02e	26.35e	3.37ab	0.96defg
10% anthesis	Dakota	61.67a	11.67cde	7.50de	0.80abcd	3.18abc	337.10f	27.78de	2.52cdefg	0.89efg
10% anthesis	Hazlet	61.67a	16.67bc	10.33bc	0.67abcd	2.57abcde	350.77abc	31.99bc	2.38defgh	1.65ab
10% anthesis	KWS-H161	36.67c	6.67efg	2.33gh	0.27de	0.77fg	349.37bcd	27.69de	3.51a	0.96defg
80% anthesis	Dakota	60.00ab	11.67cde	7.17cdef	0.49bcde	2.06bcdef	335.52f	28.13d	2.57cdef	0.48g
80% anthesis	Hazlet	56.67ab	13.33cd	7.67cd	0.39cde	1.96bcdef	351.63abc	32.20b	2.24efgh	1.32abcde
80% anthesis	KWS-H161	36.67c	6.67efg	2.50gh	0.30de	0.89fg	347.83bcde	28.00de	3.30ab	0.69fg
6 days after 50% anthesis	Dakota	60.00ab	13.33cd	4.12efg	0.39cde	1.76cdef	335.83f	27.93de	2.19fgh	1.18abcde
6 days after 50% anthesis	Hazlet	58.33ab	13.33cd	8.00cd	0.77abcd	2.97abc	347.11cde	30.29c	2.01gh	1.47abcd
6 days after 50% anthesis	KWS-H161	58.33ab	6.67efg	4.00fg	0.30de	0.75fg	345.41de	27.47de	2.98bc	0.84efg
Inoculated-untreated	Dakota	58.33ab	11.67cde	6.67def	0.61abcd	2.65abcd	337.08f	27.39de	2.51cdefg	1.12bcdef
Inoculated-untreated	Hazlet	66.67a	25.00a	16.67a	0.92abc	3.32ab	347.31cde	30.62bc	1.91h	1.66a
Inoculated-untreated	KWS-H161	48.33bc	8.33def	4.00fg	0.38cde	1.15efg	344.03e	27.17de	2.88bcd	1.13bcdef

Means followed by the same letter within a column are not significant at $p<0.05$.

Appendix 25. Means by treatment and genotype for fusarium head blight (FHB) incidence, severity, index, *Fusarium*-damaged kernels (FDK), deoxynivalenol (DON), test weight (TWT), thousand kernel weight (TKW), yield, and ergot severity of the fungicide trial conducted in 2020 Carman

Treatment	Genotype	Incidence (%)	Severity (%)	Index (%)	FDK (%)	DON (ppm)	TWT (g/0.5 L)	TKW (g)	Yield (t/ha)	Ergot severity (%)
Uninoculated-untreated	Dakota	11.25f	5.00j	0.56i	0.17g	0.85i	337.00cdef	25.07cd	4.55b	0.60g
Uninoculated-untreated	Hazlet	5.00g	5.00j	0.25i	0.08g	0.72i	359.48a	30.49a	4.46b	0.58g
Uninoculated-untreated	KWS-H161	6.25fg	5.00j	0.31i	0.17g	0.76i	344.49b	23.77def	5.42a	0.75fg
Heading	Dakota	96.25abc	31.25efg	29.94ef	6.67cde	30.49def	324.26ijk	24.46de	3.39cd	1.06def
Heading	Hazlet	97.50ab	42.50cd	41.50cd	7.62cd	35.66cd	334.33fg	28.21b	2.16gh	2.13ab
Heading	KWS-H161	100.00a	28.75fgh	28.75efg	10.62b	37.34bc	327.78hi	23.46def	3.58c	1.78bc
10% anthesis	Dakota	97.50ab	33.75def	33.00de	5.24ef	23.67gh	325.75hij	24.10de	3.54c	0.77fg
10% anthesis	Hazlet	91.25cd	21.25hi	19.37gh	3.97f	23.03gh	342.20bc	26.86bc	3.03def	1.18def
10% anthesis	KWS-H161	80.00e	15.00i	12.00h	5.64ef	21.48h	335.41defg	23.86de	4.71b	0.97efg
80% anthesis	Dakota	95.00abcd	40.00de	38.12de	6.93cde	26.91fgh	322.92ijk	24.23de	3.15cde	0.88fg
80% anthesis	Hazlet	95.00abcd	33.75def	32.06e	5.72def	26.34fgh	340.97bcd	27.87b	2.56fg	1.30de
80% anthesis	KWS-H161	90.00d	20.00hi	18.00h	6.77cde	23.75gh	334.91efg	22.83efg	4.26b	0.99efg
6 days after 50% anthesis	Dakota	98.75a	50.00bc	49.44bc	7.94c	33.68cde	321.61jk	23.91de	2.94def	0.88efg
6 days after 50% anthesis	Hazlet	92.50bcd	35.00def	32.50de	5.63ef	28.46efg	340.48bcde	27.99b	2.70ef	1.52cd
6 days after 50% anthesis	KWS-H161	91.25cd	23.75ghi	21.31fgh	6.73cde	23.25gh	334.77fg	23.55def	4.32b	1.02efg
Inoculated-untreated	Dakota	100.00a	67.50a	67.50a	13.35a	48.54a	308.71l	21.27g	1.94h	1.18def
Inoculated-untreated	Hazlet	100.00a	50.00bc	50.00bc	10.85b	43.55ab	330.48gh	26.64bc	1.71h	1.99ab
Inoculated-untreated	KWS-H161	100.00a	56.25b	56.25b	12.12ab	46.81a	319.17k	21.71fg	2.69ef	2.32a

Means followed by the same letter within a column are not significant at $p<0.05$.

Appendix 26. List of abbreviations

15-ADON	15-acetyldeoxynivalenol
3-ADON	3-acetyldeoxynivalenol
AAFC	Agriculture and Agri-Food Canada
ANOVA	Analysis of variance
BCAs	Biological control agents
CI	Coefficient of infection
CIMMYT	International Maize and Wheat Improvement Center
CMC	Carboxymethyl cellulose
Ct	Cycle threshold
DMI	Demethylation inhibitor
DNA	Deoxyribonucleic Acid
DON	Deoxynivalenol
DS	Disease severity
EF1α	Elongation factor 1α
ELISA	Enzyme-linked immunosorbent assay
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FDK	*Fusarium*-damaged kernels
FHB	Fusarium head blight
I	Intermediate
IT	Infection types
LSD	Least significant difference
MON	Moniliformin
MR	Moderately resistant
MS	Moderate susceptible
NIV	Nivalenol
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
QoI	Quinone outside inhibitor
qPCR	Real-time quantitative polymerase chain reaction
QTLs	Quantitative trait loci
R	Resistant
S	Susceptible
SNA	Spezieller nährstoffarmer agar
TKW	Thousand kernel weight
TWT	Test weight
WA	Water agar
ZEA	Zearalenone