The Value of Early Fungicide Applications in Wheat

by

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Abstract

Leaf spot diseases and, to a lesser extent, fusarium head blight (FHB), are a serious threat to wheat production in Alberta. To manage leaf spots, some growers apply fungicides tank-mixed with herbicides or plant growth regulators (PGR) at early growth stages. These practices, however, conflict with previous research suggesting later fungicide applications to be more effective. In fact, over time these practices may increase the risk of fungicide resistance, making current fungicide tools ineffective. Twelve fungicide treatments were tested in 2018 and 2019 across eight site-years in Alberta, to determine the yield and quality benefits of fungicide rates, multiple fungicide applications (single, dual and triple), and the performance of single and multiple fungicide modes of action (MOA) at four different growth stages: BBCH 22-23 (herbicide timing), BBCH 30-32 (PGR timing), BBCH 39-45 (flag leaf), BBCH 61-63 (head timing). Treatments were applied to two Canadian Western Red Spring cultivars, AAC Brandon and AAC Viewfield, and compared with a non-treated control. Both cultivars have 'intermediate' resistance to leaf spots and different genetic resistance to FHB according to the Alberta seed guide 2020. These commonly grown cultivars are recently registered and have improved genetic disease resistance. Four of eight site-years showed significant yield responses to fungicide treatments. Overall, earlier fungicide applications (BBCH 22-23 and BBCH 30-32) had lower yield and quality versus the later fungicide applications (BBCH 39-45 and BBCH 61-63). Foliar and flag leaf disease levels, at the end of the growing season, were also higher when fungicides were applied at early growth stages versus when fungicide treatments were applied at later growth stages. This is due to the fungicide applications at later growth stages (BBCH 39-45 and BBCH 61-63) protecting the top yielding leaves in the upper canopy. Generally, single fungicide applications had 3% (0.2 t ha^{-1}) lower yields and 45% higher foliar disease compared with dual or triple fungicide applications. At most sites, there were no significant differences between AAC Viewfield and AAC Brandon with respect to leaf spot severity, fusarium damaged kernels, or mycotoxin levels. There was no difference among fungicides rates (recommended versus 1.5x label rate) and single versus multiple fungicide MOA, likely due to the limited disease pressure observed at earlier growth stages. Based on this study, single fungicide applications at BBCH 39-45 (flag leaf) and BBCH 61-63 (head timing), or dual fungicide applications at these same growth stages, are recommended to maintain productivity and profitability. However, results were highly dependent on whether or not the environmental conditions were conducive for disease development. Four site years in this study had conducive conditions for disease development and fungicide applications were economically justified. In contrast, at the other four sites, conditions were not favorable and fungicide applications were unnecessary.

Preface

This is an original work by me, Ms. Mahnoor Asif, who was responsible for data collection in the field trials with the assistance of technical staff at Alberta Agricultural and Forestry (AAF) and Syngenta. The candidate also was responsible for the compilation of all data, statistical analysis, and writing of the thesis with the editorial assistance of co-supervisors Dr. Sheri Strydhorst and Dr. Stephen Strelkov. Dr. Rong-Cai provided statistical assistance throughout. Dr. Sheri Strydhorst was responsible for the experimental design, acquiring funding, project management and financial management of the research program.

Field-site technical support, including seeding, treatment application, data collection and harvest was provided by Syngenta technical staff (Red Deer), Doon Pauly and AAF technical staff (Lethbridge), as well as the cereal agronomy technical staff at AAF (Bon Accord and Barrhead). Laboratory technical support was provided by Dr. Jie Feng (AAF) and technical staff at the Alberta Plant Health Lab, Edmonton, and Dr. Michael Harding (AAF) and technical staff at the Crop Diversification Center South, Brooks.

Dedication

This is dedicated to my family and friends.

My loving parents, Asif and Shahzadi, for always supporting me and for sacrificing their own careers so that their children could have a better life. For my brother, Shahzeb, who from day one, has taught me to be independent and strong, even if it came with me being an annoying little sister.

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Abbreviations

ABC	ATB-binding cassette
ACIS	Alberta climate information system
AOX	Alternate oxidase
ATP	Adenine triphosphate
BBCH	Biologische Bundesanstalt, Bundessortenamt and Chemical industry
CWRS	Canadian Western Red Spring
CYTB	Cytochrome *b*-gene
DH	Double haploid
DMI	Demethylation Inhibitors
DON	Deoxynivalenol
DTM	Days to maturity
ELISA	Enzyme-linked immunosorbent assay
FDK	Fusarium damaged kernels
FHB	Fusarium head blight
FRAC	Fungicide resistance action committee
GS	Growth stage
HPLC	High Performance Liquid Chromatography
IUPAC	International Union of Pure and Applied Chemistry
LC/MS/MS	Liquid Chromatography with tandem mass spectrometry
LTA	Long term average
MBC	Methyl benzimidazole carbamates
MFS	Major facilitator superfamily

MT	Metric tonnes
NDVI	Normalized difference vegetation index
PTR	*Pyrenophora tritici-repentis*
QoI	Quinone outside inhibitors
QTL	Quantitative trait loci
RH	Relative humidity
SBI	Sterol biosynthesis inhibitors
SCRDC	Swift Current Research and Development Centre
SDH	Succinate dehydrogenase
SDHI	Succinate dehydrogenase inhibitors
SNB	Septoria nodorum blotch
SQR	Succinate ubiquinone oxidoreductase
STB	Septoria tritici blotch
TKW	Thousand kernel weight

Chapter One: Introduction

1.1. Background

1.1.1. Wheat production in western Canada

Wheat (*Triticum aestivum* L.) is one of the leading cereal grains produced worldwide. It is a member of the Poaceae family and is usually grown for its seed, which provides over 20% of the calories for the global population (Bushuk and Rasper 1994; Oleson 1994; Pesticide Risk Reduction Program Canada 2019). Since the 1920s, wheat has played a major role in western Canada's financial and cultural development. The Prairie Provinces, Manitoba, Saskatchewan and Alberta, are responsible for nearly all of Canada's spring wheat production, as they have productive, arable land that is well suited to this crop (Campbell et al. 2002; McCallum and DePauw 2008). In 2018, about 53% of the harvested acres of wheat were in Saskatchewan, 30% in Alberta and 12% in Manitoba, with the remaining 5% in the eastern Canadian provinces (Statistics Canada 2018). There are three main types of wheat produced in Canada, hexaploid spring wheat (*T. aestivum* L.), hexaploid winter wheat (*T. aestivum* L.) and allotetraploid durum wheat (*T. turgidum* L. ssp. *durum* (Desf.) Husn.). The 'spring' types are sown in the spring and harvested in the fall, whereas 'winter' types are seeded in the fall and harvested the following summer or early fall (McCallum and DePauw 2008; Pesticide Risk Reduction Program Canada 2019). Of these three, spring bread wheat, a sub-class of spring wheat, is the most produced with an average of 22,568,007 metric tonnes (MT) over five years (2015 – 2019); durum wheat is second with about 5,766,900 MT of production, and winter wheat comes third with 2,607,800 MT from 2015 to 2019 (Statistics Canada 2019).

The popularity of spring wheat is attributed to its capacity to meet domestic and export market needs. Under this category, the Canadian Western Red Spring (CWRS) cultivars are the most common, as they have a wide range of adaptation qualities and good flour milling characteristics and can be used to make a variety of baked goods. Therefore, CWRS cultivars are sought out and priced at a premium on world markets. Thus, CWRS cultivars represent good economic choices for western Canadian wheat farmers (McCallum and DePauw 2008). In 2017, Canada was among the top 10 countries for wheat production and third for wheat exportation (about 22,061,500 MT) (FAOSTAT 2017a; FAOSTAT 2017b; FAOSTAT 2017c).

1.1.2. Wheat diseases in western Canada and associated yield losses

1.1.2.a Foliar diseases

Survey data collected in 2014, 2015 and 2016 indicated that the main foliar wheat diseases of concern in the Canadian prairies are spot blotch (*Bipolaris sorokiniana* (Sacc.) Shoemaker), tan spot (*Pyrenophora tritici-repentis* (Died.) Drechsler), stripe rust (*Puccinia striiformis* Westend), leaf rust (*Puccinia triticina* Erikss) and the multiple pathogens associated with the septoria leaf spot complex. These include *Zymoseptoria tritici* (syn. *Mycosphaerella graminicola* (Fuckel) J. Schröt.), which is a causal agent of Septoria tritici blotch (STB), and *Parastagonospora nodorum* (Berk.) Quaedvlieg, Verkley, & Crous, which is the causal agent of stagonospora nodorum blotch (SNB) (MacLean et al. 2018; Murray et al. 2015; Pesticide Risk Reduction Program Canada 2019).The incidence and severity of each disease varies across the Prairies, but all of the diseases are widespread and occur annually. For example, in Manitoba, tan spot, the septoria leaf complex, leaf and stripe rust have moderate incidence and severity. In comparison, spot blotch, tan spot and stripe rust are of concern in Alberta, and the septoria leaf complex occurs yearly but with generally low incidence and severity. In Saskatchewan, the septoria leaf complex and tan spot are the main diseases of wheat (Bailey et al. 2000; Pesticide Risk Reduction Program Canada 2019).

Under suitable environmental conditions, these diseases can increase the risk of yield loss. Field trials in the US and Australia reported that tan spot may lead to a 50-70% yield loss and in severe cases there can be a 9-20% reduction in thousand kernel weight (TKW) (MacLean et al. 2018; Shabeer and Bockus 1988). Septoria tritici blotch (STB) and SNB are responsible for a range of yield losses from 31-53%. Specifically, SNB infections tend to cause minor yield loss of about 10-15% but this is highly variable. For both diseases, the worldwide loss in 1982 was estimated to be 9 million MT with a value of over U.S. $1 billion. In severe epidemics, wheat kernels of susceptible cultivars may shrivel up and cannot be milled (Eyal et al. 1987).

Yield losses associated with the leaf spots depend on the year, location, environment and cultural practices (Eyal et al. 1987; MacLean et al. 2018). When there is ample rainfall or dew combined with high humidity, moisture on the leaf surface creates a perfect environment for pathogens to thrive, resulting in major losses in susceptible and lesser losses in resistant cultivars (Hosford and Busch 1974). For example, Evans et al. (1999) found that inoculated plots saw a 15 to 17% reduction in yield in Oklahoma due to leaf spots; similar findings were reported by Shabeer and Bockus (1988), who reported a 17% yield loss from infections early in the season on winter wheat in Kansas. Wheat yield losses were almost 50% in Australia in untreated plots (Rees et al. 1982; MacLean et al. 2018).

1.1.2.b Fusarium damaged kernels (FDK) and deoxynivalenol (DON) levels and *Fusarium* risk

Fusarium head blight (FHB), caused by *Fusarium graminearum* Schwabe *sensu lato* (syn. *Gibberella zeae* (Schwein.) Petch), is another major disease of cereals common on the Canadian Prairies. The disease causes pre-mature senescence of the whole spike or parts thereof, ultimately resulting in yield losses (MacLean et al. 2018). Additionally, infected heads are known to contain *Fusarium* mycotoxins that are associated with chronic or acute mycotoxicosis in livestock and humans (Bottalico and Perrone 2002). Currently, *F. graminearum* is established in Manitoba where it is widespread and causing high FHB severity. The disease has been a problem since its first appearance in 1923 on corn stubble, where it overwinters and produces spores in favorable conditions. Since then, FHB has been increasing in incidence. In 1991, fusarium damaged kernels (FDK) were identified from 21.9% of Manitoba's bread wheat acreage. As the years have passed, *F. graminearum* has become increasingly common, with the Manitoba government creating a fusarium risk report to predict the future likelihood of fusarium infections (Canadian Grain Commission 2019a). Over the period July 13-17, 2019, Manitoba saw an extreme risk of FHB and most of the southern region was forecast to be at a high FHB risk (Manitoba Agriculture 2019).

In the mid to late 1980s, FHB was first observed in Alberta's irrigated areas. In 1996, *F. graminearum* was detected at extremely low levels in irrigated areas and in a few wheat fields in southern Alberta, near Edmonton, and in northwestern Saskatchewan. Since then, Saskatchewan and Alberta have seen generally low, but increasing, levels of FHB. In 1993 and 1994, southern Saskatchewan and crop districts bordering Manitoba began reporting *F. graminearum* in durum wheat, barley, and oats. In Alberta in 2009, over 10% of the CWRS and durum wheat was downgraded due to FDK and associated mycotoxins levels were above accepted levels. Nonetheless, apart from southern Alberta, there are few reported cases of FHB in the rest of the province (Canadian Grain Commission 2019a). A comprehensive disease management program will aid growers in limiting exposure of their crops to FHB and the associated yield and quality losses.

1.1.3. Wheat disease management in western Canada

1.1.3.a Crop rotations in western Canada

Crop rotations are defined as growing a planned sequence of crops on the same land in reoccurring succession (Bullock 1992). It requires an integration of management practices and diverse plant genotypes to produce crops and achieve environmental benefits (Bullock 1992; Cook 2006; Kutcher et al. 2013). Ideally, a sustainable cropping system will also help to control soil erosion and maintain soil health (Kutcher et al. 2011). A diverse mix of crops will increase biological diversity while maximizing profits.

Crop rotations are a fundamental strategy to mitigate the impact of residue borne plant pathogens. These pathogens use dead host tissues to reproduce and disseminate spores to infect incoming host crops (Cook 2006). The risk is greater when crop resides are left unburied on the soil surface, which is common with no-tillage farming. Crop rotations provide a gap between susceptible host crops, allowing more time for soil or residue borne pathogens to die out. Even a 1-year break, in some cases, can provide significant relief in pest pressure (Cook 2006). Nonetheless, short crop rotations may not provide sufficient control for some pathogens, so it is ultimately recommended to include a complex diversity of host crops in a 3-year or longer rotation (Bullock 1992; Cook 2006).

In western Canada, due to current competitive global commodity markets, farmers opt to primarily grow two crops, canola (*Brassica napus* L.) and wheat (*T. aestivum* L.). These two are the most economically profitable crops and in the last 10-12 years, canola is grown more frequently than once in four years, making cereal-canola rotations and canola monocultures more common (Kutcher et al. 2013). According to Statistics Canada (2017), in 2016 canola was the most widely grown field crop in Canada with 20.6 million acres. The second was wheat, which was seeded on 15.7 million acres.

In Alberta in 2019, 7.4 million acres were seeded to all wheat varieties (spring, durum, and winter wheat) with 7.2 million acres harvested. Total wheat production was 10.3 MT, representing about 43% of the total crop production in Alberta. In comparison, 5.9 million acres of canola were seeded in Alberta 2019, of which 5.8 million acres were harvested. Canola production was 5.3 MT, representing 22% of the total Alberta crop production in 2019 (Government of Alberta 2019; Statistics Canada 2019). From 2016 to 2020, an average of 6.3 million acres of canola and 7.4 million acres of wheat (spring, durum, and winter) were seeded in the province.

A 12-year (2008 – 2020) rotational study conducted by Harker et al. (2015), documented an increase in pest problems. The highest disease levels occurred in the continuous canola rotations. The two-year wheat-canola rotations did not have adverse effects on wheat or canola yield in the short term (Barker 2018). However, over time, short rotations result in yield reductions and increased production/management costs as diseases build up (Kutcher et al. 2013). Another concerning factor is that higher disease pressure leads growers to rely more heavily on fungicides for disease control. As fungicide applications increase, the risk of fungicide resistance also increases.

As these problems surface, growers need to effectively use fungicides for disease management while maintaining them as viable tools for the long term. This requires managing fungicide timings, fungicide modes of action (MOA) and frequency of use. For example, to manage most foliar diseases, fungicide application at growth stage BBCH 39 (flag leaf emergence) is recommended to protect the green of the leaf, as the top three leaves in wheat are responsible for grain filling (Turkington et al. 2015). The optimal time for the application of fungicides for FHB control is at BBCH 60 (beginning of anthesis) (Wiersma and Motteberg 2005). There are also studies that have found that multiple applications of fungicide with different MOA provides improved control. Wiersma and Motteberg (2005) reported that applying propiconazole plus trifloxystrobin at BBCH 15 followed by a tebuconazole at BBCH 60 provided the best leaf spot disease control, resulting in a 11-31% yield increase in hard red spring wheat.

Avoiding sub-lethal fungicide rates is key to reducing the risk of fungicide resistance. Unfortunately, many cereal growers apply a half rate of Tilt 250E (62.5g propiconazole ha^{-1}) tank-mixed with herbicides at early growth stages (BBCH 13-23). This is usually an attractive option to famers due to lower fungicide cost and convenience, as one pass over the field may provide both weed and disease control. However, many previous studies have found that this practice has little, or no yield benefits attributed to disease control. A study conducted with spring wheat in Saskatchewan suggested that fungicide applications at BBCH 41-75 were optimal compared with earlier timings (Duczek and Jones-Flory 1994).

1.2. Research objectives

The overall objective of the project is to identify the optimum fungicide application practices for achieving yield, quality, and profitability in CWRS wheat.

My specific objectives were:

1.2.1. To determine the yield, quality and economic advantages of single fungicide applications at BBCH 22-23 (2-3 tillers) [herbicide timing], BBCH 30-32 (stem elongation) [plant growth regulator (PGR) timing], BBCH 39-45 (flag leaf), and BBCH 61-63 (10-30% of anthers are mature) on two CWRS cultivars, AAC Brandon and AAC Viewfield.

1.2.2. To determine the effects of single fungicide applications timings at BBCH 22-23 [2-3 tillers (herbicide timing)], BBCH 30-32 [stem elongation (plant growth regulator (PGR) timing)], BBCH 39-45 (flag leaf), and BBCH 61-63 [10-30% of anthers are mature (head timing)] on disease incidence and severity on AAC Brandon and AAC Viewfield.

1.2.3. To determine the yield, quality, and economic benefits associated with the application of multiple fungicide MOAs, higher than label rates, and multiple fungicide applications on AAC Brandon and AAC Viewfield.

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Chapter Two: Literature Review

2.1. Fungicide groups used in Alberta

2.1.1. What are fungicides?

Fungicides are chemical agents that kill or inhibit the growth of fungi or fungi-like organisms (Mueller and Bradley 2008). Fungicides come in many different forms such as foliar fungicides, seed treatments, fungigations, and in-furrow treatments. Foliar fungicides are commonly used in field crops to manage surface lesions on aboveground plant tissues. Seed treatments provide protection from soil or seed borne fungi that can cause fusarium head blight (FHB), rotting, damping off and/or seedling blight. Fungicide seed treatments may help the seed to establish in an unfavorable environment such as cold, wet weather or if the seed is of poor quality (Mueller and Bradley 2008). Fungigation is an application method where fungicides are applied through a sprinkler irrigation system, while in-furrow treatments are when the soil is drenched with the fungicide through drip irrigation at planting (McGrath 2004; Mueller and Bradley 2008).

Fungicides have a long history going back to the 1800s, when lime sulphur sprays were used to control plant pathogens. The discovery of the Bordeaux mixture in 1885 and the use of copper compounds on downy mildew of grapes accelerated fungicide-related research and the discovery of more novel ways of controlling pathogens (Hollomon 2015). As the years progressed, more specific modes of action (MOA) were discovered such as the benzimidazoles, carboxamides and primitive sterol biosynthesis inhibitors (SBIs). Eventually, fungicides with specific MOA and systemic properties were developed, representing a true progression in fungicide technology.

As more fungicides were developed, they have been classified according to six categories: mobility in the host, role in plant protection, metabolic activity, MOA, chemical group, and the Fungicide Resistance Action Committee (FRAC) group (Mueller and Bradley 2008).

2.1.2. Fungicide classification

2.1.2.a Mobility

There are two different types of fungicide mobility. First are the contact or protectant fungicides that remain on the surface of the plant where they were applied and do not penetrate the leaf. Repeated applications of the fungicide are required to provide continuous protection, especially if there is the chance of fungicide degradation through sunlight, rain, or irrigation. These types of fungicides do not offer any protection after infection.

The second are systemic fungicides that penetrate or are absorbed by the plant tissue and may offer some after infection protection. These fungicides can move upwards in the plant via the xylem tissue (Mueller and Bradley 2008) and when applied to the root zone they are absorbed by the roots and move upward through the plant with the transpiration stream. When applied to the foliage, the fungicide moves through the leaves where it was deposited and affords those leaves protection. Leaves that do not have fungicide deposition will not be protected. If there are fungicide droplets on the stem, then the fungicide will be able to move upwards in the plant and protect new leaves. However, no fungicide will move towards the roots so they are left unprotected (McGrath 2004). Phloem mobile systemics are “true” or amphibole systemics as they have bi-directional mobility where fungicide actives move towards the roots or the leaves regardless of where they were deposited. Locally systemic or translaminar fungicides only move a small distance from the deposition point to other parts of the leaf (McGrath 2004; Mueller and Bradley 2008).

2.1.2.b Role in protection

There are three categories of fungicide protection activity: preventive, early-infection, or anti-sporulant. Preventive activity is when the fungicide acts as a protective barrier against plant pathogens. These fungicides shield the plant from potential pathogens that arrive before infection takes hold (Mueller and Bradley 2008). Contact fungicides are an example of a preventive fungicide because they come into contact with the leaf surface to provide protection (McGrath 2004). Some fungicide in this category include Bravo 500, Bravo ZN or Dithane DG 75 (Alberta Agriculture and Forestry 2020a).

Early-infection activity is when the fungicide penetrates the plant and stops the pathogen in the plant tissues. These fungicides are usually systemic and effective 24-72 h after infection. They also have preventive properties that protect the plant before infection happens (Mueller and Bradley 2008). Some examples of this type of mobility include Quilt, Caramba and Blanket AP (Alberta Agriculture and Forestry 2020a).

Anti-sporulant activity is when the fungicides prevent spore production. These types of fungicides are effective in preventing further disease development or the lesions from getting larger. Preventing spore production limits inoculum at the time of infection, thus reducing the risk of future infections (Mueller and Bradley 2008). Some of these fungicide are Quadris and Quilt (Alberta Agriculture and Forestry 2020a).

2.1.2.c Metabolic activity

Metabolic activity refers to whether a fungicide is single site or multisite. Single site fungicides tend to be systemic and are active against a single critical enzyme or protein. Multisite fungicides affect a number of different metabolic sites within a fungus or pathogen (Mueller and Bradley 2008).

2.1.2.d Mode of action (MOA)

Mode of action refers to the mechanism by which a fungicide kills or suppresses fungi, either by damaging cell membranes, inactivating critical enzymes or proteins, or by interfering with essential biochemical pathways such as respiration (Mueller and Bradley 2008). Each fungicide has its own unique MOA which targets specific pathogens (FRAC 2019).

Currently, there are 56 specific fungicide MOA that are available for use and classified under the FRAC codes (Hermann and Stenzel 2019). However, there are only a few fungicides that are appropriate for each crop region and are restricted in others due to small market size or the fungicide not being registered (Hermann and Stenzel 2019). Of the 56 MOA, a few dominate the market. For example, SBIs represent over 30% of the market, 94% of which are demethylation inhibitor (DMIs) fungicides. The quinone outside inhibitors (QoIs) hold over 20% of the global market and the succinate dehydrogenase inhibitors (SDHIs) have quickly become popular, representing over 8% of the market in 2015 which accounts to almost 1 billion (€) (Hermann and Stenzel 2019).

Additionally, knowledge of MOA can help in developing a diverse disease management program (McGrath 2004). FRAC uses these MOA to classify fungicides, along with their target sites, group names based on chemical structure, chemical groups according to the International Union of Pure and Applied Chemistry (IUPAC), and their common name and risk of resistance (FRAC 2019).

2.1.2.e Chemical group

Chemical grouping is based on the chemical nature of the compound. The nomenclature is according to the IUPAC and Chemical abstract name (FRAC 2019). Each group can include one or more chemical subgroups. For example, the group SDHIs is divided into eleven subgroups including the phenyl-benzamides, pyrazole-4carboxamides, pyridine carboxamides and so on.

2.1.2.f FRAC group/code

Numbers and letters are assigned to fungicide groups depending to their cross-resistance behavior (FRAC 2019). This is the code used to define the group number on product labels. It is usually assigned according to the time of product introduction to the market. Some of the letters used are ‘P’ (host plant defense inducers), ‘M’ (chemical multi-site inhibitors), ‘U’ (unknown MOA and risk of resistance), and ‘BM’ (biologicals with multiple MOA). When the products in the ‘U’ group are defined, they are reclassified into a new code. For example, the SDHIs are assigned the letter ‘C’ and number ‘7’. The letter ‘C’ identifies them under the category of fungicides that target cell respiration and can be shared with other fungicide groups but the number ‘7’ uniquely identifies them to the SDHI group.

2.1.3. Most common fungicide MOA: Demethylation inhibitors (DMI), quinone outside inhibitors (QoIs) and the succinate dehydrogenase inhibitors (SDHI)

2.1.3.a Demethylation inhibitors (DMI)

The DMIs are the largest sub-group within the SBI fungicides. They are categorized as group 3 under the FRAC code. These fungicides are low cost and effective against a broad range of fungi (Mueller and Bradley 2008; Price et al. 2015). They were first introduced in the 1970s as imazalil and triadimefon (Price et al. 2015). DMIs inhibit the sterol 14α-demethylase cytochrome P450 (CYP51) in fungi, which is a regulatory enzyme involved in the ergosterol biosynthetic pathway (FRAC 2018a; Mueller and Bradley 2008; Price et al. 2015). This results in abnormal fungal growth and ultimately cell death.

However, each DMI fungicide acts slightly different on biochemical sterol production to achieve cell death. For example, triazoles (or azoles), part of the largest chemical group under the DMIs, have no effect on spore germination because the spores contain enough sterol to produce germ tubes, and some even have enough to produce infection structures (Mueller and Bradley 2008). Some triazoles have anti-sporulant properties where they can inhibit spore production and slow disease development, but if the fungus begins to produce spores then the triazoles will not have any effect. DMI fungicides may be used as preventive fungicides when applied early in the season or before early fungal infections. Nevertheless, they are usually locally systemic and readily taken up by the plant, having a residual period of about 14 days (Mueller and Bradley 2008). Some common DMI fungicides used in Alberta include Quilt, Tilt 250E, Trivapro and Pivot 418 EC (Alberta Agriculture and Forestry 2020a).

2.1.3.b Quinone outside inhibitors (QoIs)

Categorized as group 11 under the FRAC coding system, these broad-spectrum fungicides include three families, the strobilurins, fenamidone and famoxadone. The strobilurins are an important sub-group of fungicides in agriculture and include a variety of fungicides registered for use on field crops such as azoxystrobin, fluoxastrobin, pyraclostrobin, and trifloxystrobin (Mueller and Bradley 2008). These were developed from a naturally occurring fungal derivative, β-methoxyacrylic acid, which is found in a range of Basidiomycete wood rotting fungi. In contrast, fenamidone and famoxadone are synthetic fungicides (Bartlett et al. 2002; Mueller and Bradley 2008).

These fungicides inhibit mitochondrial cell respiration by binding to the Q_o site of cytochrome b located in the inner mitochondrial membrane (Bartlett et al. 2002). The fungicide blocks electron transfer to cytochrome *c* which disrupts the energy cycle and halts adenosine triphosphate (ATP) production (Bartlett et al. 2002). Cell functions dependent on respiration, such as spore germination and mycelial growth, are very sensitive to strobilurins and will not proceed normally (Gewehr and Sauter 2019).

Group 11 fungicides should be applied as early as possible to prevent infections since they prevent spore germination. They will not have any effect when the fungus is growing inside the plant tissue. These fungicides are usually locally systemic, although some can move through the xylem. They have a residual period of about 7-21 days (Mueller and Bradley 2008). Some common QoI fungicides in Alberta include Trivapro, Elatus, Evito, Acapela and Twinline (Alberta Agriculture and Forestry 2020a).

2.1.3.c Succinate dehydrogenase inhibitors (SDHI)

Succinate dehydrogenase inhibitors (SDHI), classified as group 7 under the FRAC system, are one the fastest growing classes in terms of new products launched in the market (Sierotzki and Scalliet 2013). Their popularity is attributed to their high level of activity and diverse chemical structures. Additionally, they are a great alternative to DMIs and QoIs as part of an integrated pest management program because they are at a lower risk of fungicide resistance (Sierotzki and Scalliet 2013). Early SDHIs (generation I) included carboxin and oxycarboxin and were introduced in the 1960s. They are highly effective against *Rhizoctonia* sp., rusts and other basidiomycete pathogens (Avenot and Michailides 2010; McKay et al. 2011). Newer SDHIs (generation II), that include novel compounds (adepidyn, benodanil, benzovindiflupyr, bixafen, boscalid, carboxin, fenfuram, fluindapyr, fluopyram, flutolanil, fluxapyroxad, furametpyr, inpyrfluxam, isofetamid, isopyrazam, mepronil, oxycarboxin, penflufen, penthiopyrad, pydiflumetofen, sedaxane, thifluzamide) are categorized as broad spectrum for controlling fungal activity on a variety of crops (Avenot and Michailides 2010; FRAC 2018b; McKay et al. 2011).

The SDHI fungicides target succinate dehydrogenase (SDH) in the mitochondrial electron transport chain. Once the fungicide binds to the target site, it blocks fungal respiration so the cell eventually is starved of energy and dies (Avenot and Michailides 2010; Sierotzki and Scalliet 2013). These fungicides can range from locally systemic to systemic and their movement is usually translaminar and upwards (Mueller and Bradley 2008). Some SDHI fungicides in Alberta include Trivapro, Lance Ag, Aprovia Top and Priaxor (Alberta Agriculture and Forestry 2020a).

2.2. Fungicide resistance

The routine use of fungicides has allowed for an intensification of modern agriculture, boosting crop yields, improving quality and ensuring stable production (Hollomon 2015; Lucas et al. 2015). However, in any population there are rare distinct genetic individuals that will survive fungicide applications. Since fungicides are tools that disrupt fungal metabolism, it is inevitable that some pathogen populations will contain resistant or insensitive individuals (Hollomon 2015). Not until the 1970s did these resistant individuals become more common and result in the rapid loss of fungicide efficacy.

Some chemical classes of fungicides are more prone to development of resistance. FRAC has developed a list of fungicide groups that indicates the risk of resistance. For example, fungicide group 1, the methyl benzimidazole carbamates (MBCs), is at a high risk of resistance, DMIs are at a medium risk while multisite fungicides (chlorothalonil, coppers etc.) are at a low risk (Brent and Holloman 2007; FRAC 2019). Additionally, there are a few instances where resistance risks are shared. For instance, if a new fungicide product is from a high resistance risk class, then the high risk of resistance extends to the new fungicide product as well. This is also possible if two fungicides have closely related target sites but are from different classes. This is known as cross-resistance (Brent and Holloman 2007a).

2.2.1. Fungicide resistance in Europe

Fungicide resistance is more apparent in Europe, compared to North America, as more intensive fungicide programs were adopted earlier to increase crop yields (Lucas et al. 2015). The yield increase created an incentive for growers to use fungicides regularly. Eventually, nearly 100% of cereal crops in the UK received two to three fungicide applications per season, including seed treatments, and even more if there was high disease pressure. The introduction of new single site inhibitors such as DMIs, QoIs and SDHIs further accelerated the usage of fungicides, as they were more effective in targeting plant pathogens and protecting crop yield and quality. Eventually, many regions of Europe began to report the occurrence of fungicide resistant pathogen isolates.

A classic example is the development of resistance to methyl benzimidazole carbamates (MBCs) in eyespot of cereals, caused by *Oculimacula yallundae* and *O. acuformis,* in northwestern Europe. Previously, these fungi were thought to be at low risk of developing resistance due to their monocyclic (one generation per season) nature and short-dispersal distances via rain splash. However, studies in Germany found that MBC fungicide treatments resulted in a small population of resistant individuals. The UK also began to report resistant *Oculimacula* spp. at much higher levels (Lucas et al. 2015). Bierman et al. (2002) looked at the proportions of the fungal population sensitive to MBC (carbendazim) when this fungicide was applied in isolation vs. in combination with other fungicides over five seasons. They found that treatment with MBC or MBC + DMI resulted in almost a 100% resistant population within one or two seasons. In contrast, treatments with a DMI or a fungicide tank mix that included MBC had resistance levels of > 80% after five seasons, suggesting that the use of diverse fungicides mixtures delays fungicide resistance.

Septoria tritici blotch (STB) is a common wheat disease worldwide that can also be controlled with MBC fungicides. However, its causal agent, *Septoria tritici*, has also developed resistance to MBCs in Europe and had to be controlled with QoI fungicides. Unfortunately, the pathogen soon developed resistance to QoIs, and by 2004, neither fungicide was effective. QoI resistant isolates of *S. tritici* have also been found in New Zealand and in Oregon, USA (Lucas et al. 2015).

Fusarium head blight, caused by a complex of *Fusarium* and *Microdochium* species, is a threat to food safety and global cereal production due to production of toxic substances or mycotoxins in infected grain (Lucas et al. 2015). Some *F. graminearum* strains are insensitive to QoI fungicides and some are resistant to SDHIs in Europe and the USA. Hence, MBCs and some triazoles were used to control FHB, soon leading to widespread resistance of this fungus to MBCs. This is problematic as there are limited fungicide options to manage FHB.

2.2.2. Resistance mechanisms

Fungicide resistance is usually due an alteration at the site of action in the target pathogen. Modern fungicides are usually single site inhibitors or target a specific protein. Since single-site fungicides target a single protein, they are at a high risk of causing resistance, since only one change is needed for the pathogen to become resistant. Additionally, single site inhibitors kill or inhibit most sensitive pathogens in a population from completing their lifecycle, creating strong selection pressure for resistant individuals. This is a usually a qualitative change or resistance, where two distinct populations are present with a bimodal sensitivity (discrete pattern of resistance).

Several mechanisms are observed in the development of resistance to single-site fungicides. First is the alteration of the target protein due to mutations. This mechanism has been found in response to several single-site fungicides such as MBCs, DMIs, QoIs and SDHIs. Second, efflux of the fungicide by transporter pumps can occur in many plant pathogens. Third, overexpression of the target site/protein increases the concentration of fungicide needed for an effect. Fourth, the synthesis of an alternative enzyme that can substitute for the target protein may also result in fungicide resistance. Lastly, fungicides may, in some cases be degraded by metabolic enzymes (Lucas et al. 2015; Ma and Michailides 2005).

Multi-site fungicides, which are usually older contact fungicides, act on a range of cellular processes (Brent and Holloman 2007; Lucas et al. 2015). Multiple changes in the fungus are required to confer resistance to multi-site fungicides, and a unimodal distribution (multistep pattern of resistance) often is observed in the pathogen population. Resistance can be intrinsic or acquired. Intrinsic resistance is a natural (inert) ability of the pathogen to resist microbial or bacterial action through existing characteristics (e.g., life cycle, abundance of sporulation, ability to distribute spores, ability to mature, etc.). Acquired resistance is when the pathogen has to be exposed to the fungicide over time, such that it develops resistance (Brent and Hollomon 2007b; Lucas et al. 2015).

2.2.3. Resistance risk in DMIs, SDHIs and QoIs

Because of their widespread use, there has been a slow, quantitative, and progressive development of resistance to the DMIs (triazoles and imidazoles). The pathogen sensitivity and decline in disease control has been gradual (Brent and Holloman 2007a).

The FRAC code list describes SDHI as being at medium to high risk of fungicide resistance since single site mutations are required in the target enzyme for insensitivity. Resistance was first reported in the early SDHIs (carboxamides) in the 1970s. There are a variety of mutations in the target protein that result in SDHI insensitivity (Stammler et al. 2015), complicating effort to understand this insensitivity in particular cases. Some mutations result in complete loss of control to all SDHIs, whereas others result in reduced sensitivities to different SDHIs. Sometimes, mutations can have different effects in different pathogens. This makes it difficult to detect which mutations are occurring in the field. For example, there are several mutations in laboratory mutants of *S. tritici* that confer resistance. The introduction of new SDHI-based fungicide products to the market can further complicate the detection of mutations.

Since their introduction, QoI fungicides have been popular among farmers because of their broad-spectrum activity (Fernández-Ortuño et al. 2008). Hence, they are registered in many countries for use in different crops ranging from turf grasses and ornamentals to field crops. Resistance was detected within two years of commercial use for downy mildew control of grapes, underscoring the high risk of resistance associated with these products (Fernández-Ortuño et al. 2008; Sierotzki 2015).

2.2.4. Resistance management

Without successful fungicide resistance management, the efficacy and number of modern fungicides available to producers will decrease along with crop yield and quality. FRAC was established in 1981 to provide advice to producers, manufacturers and suppliers on best management practices to avoid or delay fungicide resistance (Leadbeater 2012). Brent and Holloman (2007) identified a number of strategies to manage fungicide resistance. First was not to use one product exclusively. When using a fungicide, it should be applied as a mixture with one or more other fungicides that have different MOA. Mixing of the fungicides will dilute the selection pressure imposed by the at-risk fungicides. The companion fungicide can be a low resistance risk, multi-site fungicide or any other single-site fungicide with a MOA that is not related to its partner and thus is not prone to cross-resistance. Second was the recommendation to restrict the number of treatments per season or to apply fungicides only when strictly necessary. This reduces the exposure of an at-risk fungicide and can favor the decline of resistant strains (Brent and Holloman 2007a). These two recommendations are used for the high resistant risk QoI fungicides. Specifically, for cereals, FRAC recommends that QoIs be mixed with a non-cross-resistant partner such as an SBI or multi-site fungicide and suggest a maximum of two applications per season. The third recommendation was to maintain the manufacturer's recommended dose. Historically, many growers have used lower rates of fungicide to reduce costs in areas where disease pressure is low. FRAC promotes the use of recommended doses because lowering the dose of an at-risk fungicide can enhance multi-step resistance, favoring survival of individuals with low levels of resistance, which could be eliminated with a full dose. These individuals then can mutate further and result in higher levels of resistance (Brent and Holloman 2007a). The fourth recommendation was to avoid use of systemic fungicides to eradicate or 'cure' existing infections, and to apply fungicides when there is a certain amount of disease present that will potentially cause economic losses to the crop (Brent and Holloman 2007a) .

Chemical diversity is also important in managing fungicide resistance. Using the same products over several years can contribute to increased risk of resistance, and the development of new products is important (Brent and Holloman 2007a). However, the number of registered products for one crop may be limited, which can increase the chances of resistance with the available fungicides. Additionally, legislation changes affecting registration and competition among manufacturers can also affect the speed at which new chemical compounds are found, registered and used (Hollomon 2015).

The implementation of an "integrated disease management" approach is also important in preventing or delaying fungicide resistance. This involves using all means of disease control (prevention, physical, biological, resistant varieties and chemical) available to a producer. Nevertheless, at present fungicides remain the most powerful tools for controlling plant diseases, and is many cases other control methods are not available or economically feasible (Brent and Holloman 2007a).

2.3. Fungicide timing and rates in wheat

2.3.1. Fungicide timing to control leaf disease

Traditionally, in the US and Australia, to control the septoria leaf complex and stem rust it is recommended to spray at flag leaf emergence (Milus 1994, Beard 2018). Beard (2018) found that delaying fungicide application by two weeks after leaf rust was detected did not provide effective disease control, as the leaves were left unprotected and lesions were already present. Leaf rust was best controlled two weeks after flag leaf (BBCH 55) or when symptoms were detected, leading Beard (2018) to recommend spraying for foliar leaf diseases between flag leaf (BBCH 39) and head emergence (BBCH 50-59). The same recommendation is made for tan spot, since the pathogen has a short latent period and the plant needs to be protected as soon as it emerges to avoid yield loss due lesion formation on the upper canopy (Jørgensen and Olsen 2007).

2.3.2. Fungicide timing to protect wheat yield

The optimum growth stages for protecting yield and preventing disease can sometimes differ. It has been found that spraying fungicides based on the plant structure may be more effective than spraying when a disease threshold has been reached. The disease threshold is the level of disease or damage to the crop where the financial benefit of control exceeds the cost of fungicide application (Jørgensen et al. 2017). However, in most cases, when the crop reaches this threshold, it is far too late for the fungicides to be effective. Therefore, fungicide thresholds are often defined according to the growth stages that are critical for yield and quality protection (Jørgensen et al. 2017).

It is crucial to know which leaves are responsible for maximizing yield. Generally, the top three leaves contribute about 70% of the grain filling (Poole and Arnaudin 2014). Specifically, in winter wheat, four leaves, the flag leaf (43% of grain filling), flag leaf-1 (23%), flag leaf-2 (7%) and flag leaf-3 (3%) contribute to the yield and quality. The green areas on the head then contribute another 22% (Poole 2009). The top four leaves begin to emerge during early stem elongation. However, it can be difficult to determine the emergence of these top four leaves. Nodal growth on the main stem can help to determine when the crucial leaves have emerged. In Europe, the following fungicide application times are used: Timing 1 (T1) is spraying between the first and second node emergence (BBCH 31-32), timing 2 (T2) is at flag leaf (BBCH 39) and timing 3 (T3) is during head emergence (BBCH 59) (Poole and Arnaudin 2014; Poole 2009).

Previous research conducted on spring wheat in Saskatchewan found that the optimal time to apply fungicide on spring wheat is between the flag leaf and medium milk growth stages (BBCH 41-72) (Meier 2018; Duczek and Jones-Flory 1994). Sometimes, fungicide applications at BBCH 61-63 (start of flowering: first anthers visible to 30% of anthers mature) can reduce head and foliar disease levels, and reduce deoxynivalenol (DON) in the grains, thus also protecting yield and quality (MacLean et al. 2018; Wiersma and Motteberg 2005).

2.3.3. Fungicide timing to protect wheat quality from Fusarium Head Blight (FHB)

Conventionally, the time to spray for FHB has been at flowering (BBCH 59-69) since this is when the infection occurs. A study by Edwards and Godley (2010) looked at timing of prothioconazole applications to reduce the FHB and DON levels. They found that FHB and DON were greatly reduced with a single fungicide application at BBCH 65 and least at BBCH 31-32. Multiple fungicide applications did not reduce FHB or DON. Prothioconazole application at BBCH 65 resulted in an 83% reduction in FHB and a 57% reduction in DON levels.

Wiersma and Motteberg (2005) found that a fungicide application at BBCH 39 significantly reduced DON levels by 40% in one of three trial years. This was attributed to the fungicide applications at this stage reducing *Fusarium* on young plants, debris, or sheaths. The fungicide deposited on the upper canopy at BBCH 39 could also protect flag-2, flag-1, and flag leaf due to the systemic properties of prothioconazole, which may also translocate to the wheat heads if the fungicide application is within 7-14 days of head emergence.

Edwards and Godley (2010) reported that wheat grain quality was greatest at a head timing fungicide application and yield was greatest at a flag leaf timing fungicide application. The increase in yield at flag leaf timing may be due to the suppression of leaf spots such as STB on the flag leaf, which is a key leaf in protecting yield, while improved quality may reflect FHB control.

2.3.4. Fungicide timing to protect both yield and quality

There may be some overlap of when fungicides can provide both leaf spot and FHB control. MacLean et al. (2018) looked at whether it is possible to control leaf spot diseases (septoria leaf complex) by spraying fungicide at anthesis (BBCH 61-63), when optimal FHB control is achieved. They found that fungicide application at anthesis provided adequate control of leaf spots. However, the severity of leaf diseases was higher than at flag leaf timing (BBCH 39), but this was only true for areas with high disease pressure and ultimately the yields were similar. In fact, test weights and thousand kernel weights (TKW) were improved with fungicide application at anthesis. This is a similar response to what Wiersma and Motteberg (2005) reported, and they concluded that the optimum timing to control leaf spots is at BBCH 60 rather than at BBCH 39 across eight cultivars. The change in timing did not reduce grain yield or leaf spot control. Overall, both studies show that a single fungicide application at anthesis will provide sufficient control of leaf diseases, have equivalent yield and improved grain quality compared with fungicide applications at flag leaf. This application timing is also beneficial since FHB is still controlled (MacLean et al. 2018; Wiersma and Motteberg 2005).

2.3.5. High versus low rates of fungicide

A study by Poole and Wylie (2011), in New Zealand, looked at the economic benefits of various fungicide rates. In that report, high fungicide rates gave significantly better disease control (87% control over the intermediate) for three of four fungicide products evaluated. The intermediate rates (below label rate) were more effective (76% disease control) than the lower fungicide rates (46 – 61% disease control). At one site, there was no significant economic difference between the treatments. Other sites saw economic benefits and the less costly products had better economic profit margins. It is also important to consider the dynamic nature of the disease triangle (interaction of the host, pathogen and environment) when establishing a holistic spray program (Poole and Arnaudin 2014), as this could have an impact of the development and severity of disease.

2.4. The Cereal Leaf Spots and Fusarium Head Blight

2.4.1. *Zymoseptoria tritici* syn. *Septoria tritici* (teleomorph *Mycosphaerella graminicola*)

Septoria tritici blotch (STB), also known as speckled leaf blotch, is caused by the fungus *Zymoseptoria tritici* (formerly *Septoria tritici*). This disease is associated with the development of necrotic lesions and dark pycnidia on affected leaves. The disease is severe in wheat production areas of the world characterized by cool, wet growing seasons. Over the years, the Canadian Prairies have seen an increased incidence of *S. tritici* infections, especially in Manitoba, where daytime temperatures are 22-25 °C (Chungu et al. 2001).

The first symptoms of infection are irregularly shaped chlorotic lesions. If the conditions are conducive for the pathogen (warm temperatures and high relative humidity (RH)), necrosis develops at the chlorotic sites. The lesions now appear sunken and grey-greenish. As they age, the lesions become light tan and black asexual fruiting bodies (pycnidia) begin to form; these are visible as black dots or specks on the lesions on either side of the leaf. Speckled leaf blotch starts to develop on the lower canopy of the crop, and gradually progresses to the flag leaf. In severe infections, the disease can spread to the heads and cause lesions on the glumes and awns (glume blotch) (De Wolf, Erick 2008; Eyal et al. 1987).

The pycnidiospores (asexual spores) are released from the pycnidia when the plant is wet. Usually, they germinate after 12 h and penetrate the host tissue within 24 hours. The sexual spores (ascospores) are produced early in the season from infected stubble and are responsible for primary infections; ascospores spread via wind under high humidity (Brennan et al. 2019). Moisture is needed at all stages of the infection and suitable moisture for 72 to 96 h results in a considerable amount of disease. If moisture is present for only 24 h then the disease levels are much lower, but symptoms still develop (Eyal et al. 1987). The maximum temperature for pycnidiospores germination is 33-37 °C, the minimum 2-3 °C and the optimal temperature is 20-25 °C. If the pathogen experiences temperatures < 7 °C for two consecutive nights, then germination can be halted, preventing mycelial growth, and lesion and pycnidial development. In general, the optimal conditions for a *S. tritici* infection is when it is cloudy, rainy and temperatures are between 20-25 °C (Eyal et al. 1987).

Infection by *S. tritici* consists of two broad phases. First is a latent phase or symptomless phase, where the pathogen transitions from the leaf surface to the substomatal cavities (1-3 days after inoculation). The pathogen then penetrates the host tissue via the stomata and colonizes the apoplastic spaces of the mesophyll. In the second or necrotrophic phase, the pathogen begins to feed on the efflux of nutrients released by the host cells as they start to die. At this point, symptoms begin to appear. The environment, host resistance and the virulence of the pathogen are key factors affecting disease development. If there is a susceptible cultivar, the period of humidity is short, but temperatures are optimal (25 °C), then there can still be severe levels of disease. Similarly, if temperatures are low and there are long periods of moisture, then high disease levels may also be observed.

2.4.2. *Stagnospora nodorum* (teleomorph *Phaeosphaeria nodorum*)

Stagonospora nodorum is another important pathogen of wheat, and with *S. tritici* forms what is sometimes referred to as the 'septoria complex' (MacLean et al. 2018). On its own, it causes septoria nodorum blotch (SNB). While *S. nodorum* is most closely associated with wheat, it also occurs on barley. Wild grasses can serve as alternative hosts (Solomon et al. 2006). The pycnidiospores germinate readily in water (after 4-36 h) and multiple germ tubes penetrate the cuticle or enter through the stomatal opening (within 24 h). Initially, chlorotic lesions develop and expand to form oval shaped lesions on the leaf. Necrotic patches develop within the chlorotic lesions and after a week under optimal conditions, pycnidia will begin to form. The pycnidia are initially hyaline (clear), but as they age, they turn a darker pale brown hue. The pycnidiospores are released in a pink cirrhus (mucous-bound, ribbon like mass of spores) that ruptures the cuticle. Eventually, severely affected leaves may die and the fungus will continue to sporulate asexually on the necrotic tissues (Shipton et al. 1971; Solomon et al. 2006).

S. nodorum is a particularly important pathogen in warm, moist conditions (Eyal et al. 1987). The fungal spores germinate within a temperature of 5-37 °C with an optimum temperature of 20-25 °C. Symptoms appear within 7-14 days after infection. In Wales, infection occurred when the RH was > 63%. Pycnidium maturation takes about 6 days from infection but can extend to 10 days if the crop experiences temperatures of 20 °C with RH between 85-90%. If there is an interruption to the latent period (i.e., dry period), then disease development can be delayed. Under field conditions, optimal or higher temperatures, long periods of leaf wetness and high spore densities can shorten the latent period and increase disease development.

Infected debris and seeds are the primary sources of SNB inoculum. Seed infection rates as high as 80% have been reported in Georgia, USA. In the southeastern USA, seed infection can range from 40-50% in susceptible cultivars. Only 10% seed infection is needed to provide sufficient inoculum to cause a severe epidemic (Eyal et al. 1987; Shipton et al. 1971; Solomon et al. 2006). The release of sexual ascospores from stubble and crop debris is initiated by low temperatures, rainfall, and high RH. The ascospores can travel long distances via wind dispersal. Secondary spread occurs by the dispersal of asexual pycnidiospores via rain splash and usually 2-4 cycles of asexual infection are needed for the pathogen to produce a significant amount of disease (Shipton et al. 1971; Solomon et al. 2006).

2.4.3. *Pyrenophora tritici-repentis* (anamorph *Dreschlera tritici-repentis*)

Tan spot of wheat is a destructive foliar disease caused by the fungus *Pyrenophora tritici-repentis* (anamorph *Dreschlera tritici-repentis*). The disease occurs frequently on the Prairies and has likely increased in recent decades due to the adoption of no-till practices and the cultivation of susceptible wheat varieties (Aboukhaddour et al. 2013). Tan spot develops in the spring and summer on both the lower and upper surfaces of the leaf. Like the septoria complex, tan spot can also infect the wheat spikes and eventually the kernels, causing the development of 'red smudge' (Wegulo 2011). The pathogen is known to have a wide range of hosts including bread wheat, durum wheat, barley, rye and several other grass species (De Wolf et al. 1998; Wegulo 2011).

On susceptible wheat cultivars, *P. tritici-repentis* causes oval to diamond shaped necrotic or chlorotic lesions. Initially, these appear as tan-colored spots that eventually turn into tan-colored, lens shaped lesions with yellow borders. The chlorotic borders are often described as a 'halo', but eventually lesions may coalesce together (De Wolf, E. D. et al. 1998; Wegulo 2011). When lesion development is severe, the leaf may die prematurely. If the lesions are exposed to wetness, then they can darken in the center due to the production of the asexual spores, conidia, and conidiophores of the fungus. The sexual fruiting structures of *P. tritici-repentis* are known as pseudothecia, which develop on mature wheat straw in the fall and winter (Wegulo 2011). In the spring, sexual ascospores are released from the pseudothecia, serving as the primary inoculum. Their discharge is favored by rainfall, high RH, and temperatures above 10 °C. Secondary infections are initiated by conidia produced in lesions. Conidia on infected residue, seed and volunteer crops can also serve as primary inoculum. Since these spores are lighter than ascospores, they can be dispersed over large distances and in large numbers by wind. Therefore, they are usually considered to be more important than ascospores (Wegulo 2011). Once a spore (conidia or ascospore) lands on the leaf surface, it germinates in the presence of moisture and produces a germ tube within 6 – 24 hours at 20 °C.

The pathogenicity of *P. tritici-repentis* (PTR) is mediated largely by the activity of three host-selective toxins (Ptr ToxA, Ptr ToxB and Ptr ToxC; Strelkov and Lamari 2003). Ptr ToxA and Ptr ToxB cause necrosis and chlorosis, respectively, on sensitive wheat. Ptr ToxC also causes chlorosis, but on different genotypes that Ptr ToxB. The eight known races of the tan spot pathogen are defined by the ability to produce the host-selective toxins, alone or in combination/: race 1 (ToxA+ToxC), race 2 (ToxA), race 3 (ToxC), race 4 (avirulent, no toxins), race 5 (ToxB), race 6 (ToxB+ToxC), race 7 (ToxA+ToxB) and race 8 (ToxA+ToxB+ToxC) (Strelkov and Lamari 2003; Lamari and Strelkov 2010). On resistant cultivars, the lesions can be smaller, and chlorosis and necrosis may be absent (De Wolf et al. 1998; Wegulo 2011), particularly if the cultivar is insensitive to the pathogen-produced toxins.

2.4.4 Fusarium head blight (*Fusarium spp.*)

Fusarium head blight (FHB), also known as scab, is a disease of wheat, barley, oats, and various other grass species. It is caused by several pathogens including *Fusarium avenaceum* (syns. *F. herbarum* var. *avenaceum* (Fr.:Fr.) Wollenw.; *Fusisporium avenaceum* Fr.:Fr.; *Gibberella avenacea* R. J. Cook), *F. culmorum* (syns. *Fusisporium culmorum* Wm.G. Sm.; *F. culmorum* var. *leteius*), *F. poae* (syns. *F. tricinctum* f. sp. *poae* (Peck) W. C. Snyder & H. N. Hansen; *Sporotrichum anthophilum* Peck; *S. poae* Peck) and *F. graminearum* (syn. *Gibberella zeae* (Schwein.) Petch). However, among these causal agents, *F. graminearum* is considered most important. Three of the fungi, *F. culmorum*, *F. avenaceum*, and *F. graminearum*, cause fusarium-damaged kernels (FDK), while only *F. culmorum* and *F. graminearum* produce the mycotoxin deoxynivalenol (DON), which is a threat to human and livestock health. The pathogen can produce up to 30 mg DON kg^{-1} in wheat and barley. On average, 1% of FDK will produce 1 mg DON kg^{-1} (can range from 0.5 – 4 mg DON kg^{-1}) (Alberta Agriculture 2003). Vomitoxin or DON in high concentrations results in reduced feed intake, weight gain and vomiting in animals. Nausea, vomiting, diarrhea, abdominal pains, headaches, dizziness and fevers have been reported in humans when they consume high concentrations of DON (Edwards and Godley 2010). Some beef cattle can tolerate up to 12 mg DON kg^{-1} and non-ruminants (i.e., pigs) can tolerate up to 1 mg DON kg^{-1}. Dairy cows can tolerate up to 8 mg DON kg^{-1} without an effect on milk production. Younger animals (calves, ewes, lambs) do not show any symptoms at 10 mg DON kg^{-1} or more, while adult cows, sheep and poultry can tolerate up to 15 mg DON kg^{-1} in feed grain. According to Agriculture and Agri-Food Canada guidelines, no more than 1 mg DON $kg^{-1}$is permitted in swine, dairy and horse feed, while up to 5 mg DON kg^{-1} is allowed for beef cattle, sheep, and poultry (Alberta Agriculture 2003).

Given it is importance in causing FHB, *F. graminearum* was a declared pest under the Alberta Agricultural Pests Act (Alberta Agriculture 2003) until June 2020, when it was removed following consultation with industry and government stakeholders. Over the past few years, *F. graminearum* has been isolated with increasing frequency in southern Alberta. In 2001, the number of counties in Alberta reporting the presence of *F. graminearum* was 9, increasing to 13, 22, and 26 in 2010, 2015 and 2016, respectively. By 2017, most of southern and some of central Alberta was considered to be at high risk of FHB (Alberta Agriculture and Forestry 2020b; Blois 2018).

In wheat, symptoms of FHB first appear immediately after flowering (BBCH 61-63). Infected spikelets present premature bleaching and over time, the entire heads can become bleached. If conditions are optimal (warm and humid) for the disease, then pink/salmon coloured spores (produced in asexual sporodochia) develop on the glumes and rachis of the spikelet. As the disease continues to develop, small blueish-black spherical bodies may appear, and these are the sexual structures (perithecia). In Canada, sexual reproduction by the fungus is relatively rare, and hence it is routine referred to by the anamorph name. Eventually, the pathogen attacks the grain, cause it to shrivel and wrinkle and turn pinkish-grey or light brown, resulting in the typical fusarium damaged kernels (sometimes called 'tombstone kernels') (Schmale and Bergstrom 2003). Infections are favored by long periods of high moisture or RH (>90%) and moderately warm temperatures (15-30 °C). If these conditions are present before, during and after flowering, then inoculum production, infections of the florets and grains are favored. The small window of infection associated with FHB (anthesis, BBCH 61-63) makes forecasting of this disease easier (Schmale and Bergstrom 2003).

F. graminearum overwinters on crop residue (wheat straw, alternate hosts) and produces asexual spores (macroconidia), which are dispersed to susceptible hosts via rain splash or wind. When conditions are warm and humid, the sexual stage, *Gibberella zeae*, develops on the debris and produces perithecia, which discharge ascospores into the air. If the wind picks up, the ascospores can travel long distances. Infections occur when the ascospores and asexual conidia land on wheat heads. The anthers present during flowering are thought to be the primary entry point for the fungus into the host. If the anthers become infected immediately after they emerge, then kernel development will not occur, as the fungus will be able to colonize the florets. Infected florets will produce tombstone kernels. Kernels that are infected late in their development may not show symptoms but may still be contaminated with DON (Schmale and Bergstrom 2003).

2.5. Genetic resistance of different wheat cultivars

2.5.1. Canadian wheat breeding

The deployment of resistant wheat cultivars is generally the most economical and simplest approach to mitigating the effects of foliar diseases and FHB. However, the availability and type of resistance to each pathogen varies, and in some cases no effective resistance sources are available. Resistance to STB (*S. tritici*) can be qualitative (often confers complete resistance) (Kushalappa et al. 2016) or quantitative (often confers partial resistance in the plant reducing pathogen multiplication or symptom severity) (Pilet-Nayel et al. 2017). Thirteen major qualitative genes for resistance to STB have been mapped and published to date, with several others noted but not yet published (Pilet-Nayel et al. 2017; Ponomarenko et al. 2011). Resistance to SNB (*S. nodorum*) is partial and associated with quantitative trait loci. Resistance to tan spot (*P. tritici-repentis*) is also available but only in durum and bread wheat cultivars (Mehra et al. 2019; Wegulo 2011). Considerable efforts have been made to introgress resistance against FHB into wheat, but at present, there are no lines with complete resistance. Nonetheless, some quantitative trait loci (QTL) have been used to confer partial resistance in wheat (Schmale and Bergstrom 2003; Brar et al. 2019)

Martens et al. (2014) examined 45 historical Canadian wheat cultivars registered between 1870 and 2001 and found that older cultivars (Red Fife (1885), Marquis (1909), Thatcher (1935)) were highly susceptible to foliar diseases, specifically leaf rust, and suffered significant yield losses compared with newer cultivars. The leaf rust susceptibility in the older cultivars was attributed to the absence of specific resistance genes, such as *Lr34*, *Lr16*, *Lr21*, *Lr22* effective against leaf rust. These resistance genes are present in newer cultivars such as AC Elsa (1996), AC Cadillac (1996), AC Minto (1991), Pasqua (1991), and AC Cora (1994). The results of

Martens et al. (2014) highlight the progress made in Canada over the past century in improving disease resistance in wheat. Nonetheless, resistance breeding takes years of effort and sometimes there can be field performance issues with resistant cultivars. For example, Kumar et al. (2019) looked at the yield responses of wheat and barley cultivars that varied in stripe rust resistance. They found one of the cultivars, Harvest, did poorly (40% yield loss) under severe disease pressure, despite its designation as resistant to moderately resistant to stripe rust.

The level of disease resistance in a crop can help to determine when fungicide application may be the most effective. If a crop is susceptible to moderately susceptible, then the application of fungicide based on the host developmental stage is effective, whereas for a resistant or moderately resistant cultivar, fungicide applications based on a disease threshold may be more successful (Poole and Arnaudin 2014).

2.5.2. AAC Viewfield

AAC Viewfield is a hard-red spring wheat cultivar developed by Agriculture and Agri-Food Canada at the Swift Current Research and Development Centre (SCRDC) and registered in 2017. It is a doubled haploid (DH) genotype from the cross of two cultivars, Stettler and Glen. This cultivar has intermediate resistance to FHB. It is classified as resistant to yellow rust and stem rust, and has moderate resistance to leaf rust and common bunt (Cuthbert et al. 2018). According to the Alberta seed guide (2020), AAC Viewfield is rated as having 'intermediate' resistance to leaf spots. In 2019, about 6% of CWRS acres were seeded to AAC Viewfield (Canadian Grain Commission 2019).

2.5.3. AAC Brandon

AAC Brandon is a slightly older cultivar than AAC Viewfield, having been registered in 2014. Like AAC Viewfield, however, AAC Brandon is a hard-red spring wheat cultivar developed at the SCRDC. It was derived from a cross between Superb/CDC Olser//ND744 in 2003. AAC Brandon is resistant to the prevalent races of leaf, stem, and yellow rust. It is moderately resistant to loose smut and susceptible to common bunt. This cultivar has moderate resistance to FHB (Cuthbert et al. 2017). Additionally, like AAC Viewfield, AAC Brandon is rated as having 'intermediate' resistance to leaf spots according to the Alberta seed guide (2020). In 2019, about 42% of CWRS acres were seeded to AAC Brandon (Canadian Grain Commission 2019).

While there have been great strides in breeding for disease resistance, yield and quality in Canadian wheat, there is a lack of recent information regarding the performance of newer CWRS cultivars, such as AAC Viewfield and AAC Brandon, in intensive management cropping systems.

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Chapter 3: Cultivar specific yield and economic advantages associated fungicide timing in Canadian Western Red Spring wheat

3.1 Introduction

Wheat (*Triticum* spp.) is the leading cereal grain produced, consumed, and traded globally. Canada was among the top 10 producers of wheat worldwide in 2018, growing about 32,201,100 MT, and was also one of the top three exporting countries in 2017 (FAOSTAT 2017; Statistics Canada 2019). Canadian Western Red Spring (CWRS) wheat (*T. aestivum* L.) has high protein levels and is valued for its superior milling and baking qualities (Canadian Wheat 2019). In 2019, the top five CWRS cultivars grown on the Canadian Prairies included AAC Brandon, AAC Elie, CDC Landmark, CDC Viewfield, and CDC Plentiful (Canadian Wheat 2019).

The most common fungal diseases of spring wheat across western Canada are leaf rust (*Puccinia triticina* Erikss) (Murray et. al 2015), stem rust (*Puccinia graminis* Pers.:Pers. f. sp. *tritici* Erikss. & E. Henning), stripe rust (*Puccinia striiformis* Westend), fusarium head blight (FHB) [*Fusarium graminearum* Schwabe *sensu lato* (syn. Gibberella zeae (Schwein.) Petch], tan spot [(*Pyrenophora tritici-repentis* (Died.) Drechsler], the septoria leaf complex [septoria tritici blotch (*Zymoseptoria tritici* syns. *Mycosphaerella graminicola* (Fuckel) J. Schröt.)] and stagnosporum nodorum blotch [*Parastagonospora nodorum* (Berk.) Quaedvlieg, Verkley, & Crous] (Manitoba Agriculture 2020; Pesticide Risk Reduction Program Canada 2019). In Alberta, Canada, yield loss in CWRS wheat is often primarily due to the leaf spot fungi. Under suitable conditions for disease development, trials in the US and Australia found that tan spot can cause yield losses of 50-70% (MacLean et al. 2018; Shabeer and Bockus 1988). Additionally, in severe epidemics, the septoria leaf complex can lead to yield losses of 31-35% (Eyal et al. 1987).

Previous research conducted on spring wheat in Saskatchewan found that the optimal time to apply fungicide on spring wheat is between the flag leaf and medium milk growth stages (BBCH 41-72) (Meier 2018; Duczek and Jones-Flory 1994). Sometimes, fungicide applications at BBCH 61-63 (start of flowering: first anthers visible to 30% of anthers mature) can reduce head and foliar disease levels, and reduce deoxynivalenol (DON) in the grains, thus also protecting yield and quality (MacLean et al. 2018; Wiersma and Motteberg 2005). However, there are many anecdotal reports of farmers applying fungicides much earlier (i.e., herbicide timing), presumably in an attempt to manage cereal diseases proactively, or for reasons of convenience or habit.

Traditional fungicide timing strategies may also need to be re-evaluated periodically as new cultivars are registered. A study by Iqbal et al. (2016) examined the effect of breeding on grain yield and agronomic traits (i.e., disease resistance, maturity, height and, lodging resistance) and found that newer cultivars had improved yield, quality and other agronomic traits. The research found that older CWRS cultivars were more susceptible to leaf rust than new cultivars, suggesting that new cultivars may show less response to fungicide applications. However, there has been no research to identify the optimum fungicide timing on current CWRS spring wheat cultivars with improved disease resistance packages.

In addition to genetics, the environment plays a major role in determining the incidence and severity of disease. A detailed disease triangle developed by McNew in the 1960s indicates that the interaction of the host, pathogen and environment can be used to determine how an epidemic might be predicted, limited or controlled (Scholthof 2007). McNew's disease triangle emphasized that the environment is one of the most important contributors to plant disease, but it is undervalued. Others agree that disease severity is highly influenced by changes in environmental conditions (Agrios 2004).

In light of the new wheat cultivars and recent incremental changes in climate and weather, repeated evaluations of fungicide effects at different wheat developmental stages on yield and quality were justified. Therefore, the objective of this study was to determine the yield and economic advantages of single fungicide applications at: BBCH 22-23 (2-3 tillers) [herbicide timing], BBCH 30-32 (stem elongation) [plant growth regulator (PGR) timing], BBCH 39-45 (flag leaf), and BBCH 61-63 (10-30% of anthers are mature) [FHB timing]. Two of the most commonly grown CWRS cultivars, AAC Brandon (Cuthbert et al. 2016) and AAC Viewfield (Cuthbert et al. 2018), were tested. The effects of fungicide application timing on wheat disease incidence and severity are provided in a separate report (Asif 2020).

3.2 Materials and methods

3.2.1 Field setup

Field experiments, designed as a split plot (Appendix 1), were conducted in Alberta, Canada, over two growing seasons in 2018 and 2019 at three rain-fed sites (Barrhead, Bon Accord, and Red Deer) and one irrigated site (Lethbridge). Precipitation, temperature, and relative humidity (RH) were obtained from the nearest Alberta Climate Information System (ACIS) station from seeding date to physiological maturity based on days to maturity (DTM). For the Barrhead 2018 (54.1 °N, -114.2 °W) and 2019 (54.1 °N, -114.3 °W) field sites, the Barrhead CS weather station was used, which was approximately 20 km and 11 km away from each site, respectively. The St. Albert weather station was about 48 km away from the Bon Accord 2018 site (53.8 °N, -113.3 °W) and about 11 km away from the Bon Accord 2019 field site (53.7 °N, -113.6 °W). The Lethbridge Farm IMCIN weather station was used for the

Lethbridge field site in 2018 and 2019 (49.7 ^{o}N, -112.7 ^{o}W), which was located 400 m from the site. For the Red Deer 2018 site (52.2 ^{o}N, -113.8 ^{o}W) and Red Deer 2019 site (52.2 ^{o}N, -113.9 ^{o}W), the Red Deer regional station was used as the source of RH and temperature data. Both sites were between 12 and 16 km away from the station, respectively. However, this weather station did not have FHB risk values, or observed precipitation data, so the Lacombe CDA station was used to collect these data. The Lacombe CDA station was approximately 47 km away from the Red Deer sites.

Trials were seeded into canola stubble at a depth of 2-5 cm on fields with a history of short wheat rotations (wheat-canola-wheat-canola), to increase chances of cereal leaf disease and to mimic the typical rotations used by many farmers in this region of western Canada. Certified seed was treated with tebuconazole (1-(4-chlorophenyl)-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentan-3-ol), prothioconazole (2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1*H*-1,2,4-triazole-3-thione) and metalaxyl (Methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl)alaninate) formulated as Raxil PRO at a rate of 325 mL 100 kg^{-1} of seed. Pre-seed or pre-emerge herbicide applications were 900 gai L ha^{-1} of glyphosate (N-(phosphonomethyl) glycine) tank mixed with Saflufenacil formulated as Heat LQ at a rate of 18 g ha^{-1} rate. Two herbicide products were applied in-crop for weed control. The first included florasulam (N-(2,6-difluorophenyl)-8-fluoro-5-methoxy (1,2,4)-triazolo- (1,5c) pyrimidine-2-sulfonamide), fluroxypyr (1-methylheptyl((4-amino-3,5-dichloro-6-fluoro-2- pyridinyl)oxy) acetic acid) and MCPA formulated as Stellar XL at rate of 988 mL ha^{-1}. The second product was pinoxaden formulated as Axial BIA at a rate of 1.2 L ha^{-1}. These herbicides were not tank-mixed but were applied to coincide with early fungicide applications at BBCH 22-23. The PGR trinexapac-ethyl was applied at a rate of 100 gai ha^{-1} to all plots at BBCH 30-32 (stem elongation) at 100 L ha^{-1}.

3.2.2 Fungicide treatments

The data reported herein is a sub-set of a larger data set resulting from an experiment with additional objectives. The study was set up as a split-plot design with four blocks (Appendix 1). In each block, there were two main plots for the two CWRS cultivars and 13 subplots per main plot for the 12 fungicide treatments and control. This paper will focus on the five single fungicide treatments and the non-treated control (Table 3.1) applied to two CWRS wheat cultivars. The fungicide products represent three fungicide groups: the triazoles (group 3), carboxamides (group 7) and the strobilurins (group 11).

3.2.3 Data collection

Ten main stem heads were collected from each plot at 30% (thumbnail does not dent kernel) to 40% (moisture comes out of kernel) grain moisture to determine the DTM according to Karamanos et al. (2008). Grain was harvested using Wintersteiger Delta small plot combines (Wintersteiger Inc., Saskatoon, Canada) equipped with a 2012 classic grain gauge automatic weigh system. Thousand kernel weight (TKW) was measured on an individual plot basis by weighing 500 kernels and multiplying by two. Test weight was determined either automatically by the combine weigh system or measured with a GAC 2100 Dickey-john grain moisture tester (Churchill Industries, Minneapolis, MN). Grain protein concentrations were determined with a DS2500 near infrared reflectance (NIR) spectrometer (FOSS, Eden Prairie, MN 55344, USA) and adjusted to standard grain moisture content and final protein levels were adjusted to 14.5% moisture. Plant heights were measured at BBCH 83 by taking the height of the main tiller from ground level to the top of the spike, not including the awns. Lodging was assessed at BBCH 89 on a 1-9 scale, where 1 = no lodging or 'erect' and 9 = severe lodging or 'flat'.

The economic analysis compared yields achieved with fungicide applications at various crop growth stages. Fungicide costs were based on the fee structure of a typical agri-chemical retail company in northcentral Alberta. Yield required to break even was calculated as: (total fungicide application cost + cost of fungicide) / (current wheat price) (Cornell 2020).

3.2.4 Statistical analysis

Data from the eight environments (site × year combinations) were analyzed using PROC GLIMMIX of SAS Studio v.3.8 (SAS Institute Inc. 2012-2018). Cultivar, fungicide treatments, site - year combinations (SiteYr) and their interactions were treated as fixed effects. Blocks and their interactions with all fixed effects were considered random effects.

Yang (2010) states that location effects should be treated as 'fixed' as they usually represent a physical property (e.g., soil type of a location) or a long-term average (precipitation, temperature, RH, etc.). Both O'Donovan et al. (2011) and Yang (2010) state that if a factor has greater than 10 levels and there is no structure to these levels, then it is best to declare the factor 'random', but if it has less than 10 levels, the variance of the factor may be unreliable and it would be better to consider the factor 'fixed'. Therefore, this study labels the site - year factor as 'fixed' since it has only eight levels. A mixed-effect model for combined split-plot experiments is as follows,

$$Y_{ijkl} = \mu + E_i + B_{j(i)} + A_k + AE_{ki} + (AB)_{kj(i)} + C_l + (AC)_{kl} + (CE)_{li} + (ACE)_{kli} + \varepsilon_{ijkl},$$

where μ is the overall mean, E_i is the effect of *i*th environment (site year), $B_{j(i)}$, is the effect of *j*th block within *i*th environment, A_k is the effect of the *k*th whole plot (cultivar), $(AB)_{kj(i)}$is the interactions between the effects of the *k*th cultivar and *j*th block within *i*th environment, or the whole plot error, C_l is the effect of the lth split plot (fungicide treatment), $(AC)_{kl}$ is the interaction between the effects of kth cultivar and lth fungicide treatments, $(CE)_{li}$ is the interaction between the effects of lth fungicide treatment and ith environment, and $(ACE)_{kli}$ is the interaction between the effects of kth cultivar, lth fungicide treatment and ith environment, and ε_{ijkl} is the split plot error.

DON data followed a Poisson distribution and were re-coded into a new discrete variable where the value was multiplied by 100,000 to create an integer value which can be analyzed via PROC GLIMMIX. The lsmeans were then back-transformed from log-scale using the 'ilink' option.

3.3 Results and discussion

3.3.1 Effect of environment

Sites were separated into those that showed a significant yield response to fungicide treatments, and hence are referred to as "responsive sites", and those that did not and are referred to as "non-responsive sites". The responsive sites included Barrhead 2019 (BR19), Bon Accord 2019 (BA19), Red Deer 2018 (RD18) and Red Deer 2019 (RD19). The non-responsive sites included Barrhead 2018 (BR18), Bon Accord (BA18), Lethbridge 2018 (LB18), and Lethbridge 2019 (LB19).

Average observed precipitation from seeding to physiological maturity at the responsive sites was 273 mm (194 – 364 mm), which was approximately 96% of the long-term average (LTA) (69 – 128% of the LTA) (Table 3.2). Of those sites with a significant yield response to fungicide treatments (responsive sites), the highest observed precipitation (364 mm) was recorded at BA19 and the lowest (194 mm) at RD18. The non-responsive sites received an average of 175 mm (121 – 214 mm) of observed precipitation, which was approximately 79% of the long-term average (LTA) (55 – 96% of the LTA). Of those non-responsive sites, Barrhead 2018 received the highest amount of precipitation (214 mm), with the lowest at LB18 (121 mm).

Relative humidity, on average, at the responsive sites was 70.2% (65.2 – 74.0%) and the non-responsive saw an average of 61.1%. (57.7 – 63.7%) (Table 3.2). The temperature difference between sites was minimal. The non-responsive sites had, on average, only 1.6°C higher temperature versus the responsive sites.

The wetter environment at the responsive sites affected plant height. At these sites, plants were about 17% taller than the plants at the non-responsive sites (data not shown). Lodging was also significantly greater (54%) at the responsive sites than at the non-responsive sites (data not shown). Previous studies have indicated that lodging is often associated with wetter summers (Berry et al. 2004).

3.3.2 Agronomic, yield and quality trends at responsive and non-responsive sites

Sites in Table 3.3 are referred to as "responsive sites" and the ones in Table 3.4 as "non-responsive sites".

For the non-responsive sites, yields varied between sites (Table 3.4). The highest was at LB18 (7.8 t ha^{-1}), followed by LB19 (6.9 t ha^{-1}), BA18 (6.6 t ha^{-1}) and finally BR19 (5.9 t ha^{-1}) (Table 3.4). These sites had an average of 2.7% lower yields (0.2 t ha^{-1}) than the responsive sites. Significant yield differences were also observed between the responsive sites. Red Deer 2019 had the highest yield (7.4 t ha^{-1}) followed by BA19 (7.1 t ha^{-1}), BR19 (7.0 t ha^{-1}) and RD18 (6.4 t ha^{-1}) (Table 3.3).

Both the non-responsive sites and responsive sites showed significant differences in DTM. At the responsive sites, DTM varied from 108.4 d at RD18 to 131.0 d at BA19 (average 120.3 d) (Table 3.3). At the non-responsive sites, DTM ranged from 103.4 d at BR18 to 120.0 d at LB19 (average 109.3) (Table 3.4). Responsive sites took 11 days longer to mature because of the generally better growing conditions (i.e., higher precipitation levels), which likely contributed to higher disease pressure and significant responses to the fungicide treatments.

The non-responsive sites showed significant test weight differences between sites (Table 3.4). LB18 had the highest test weights at 85.5 kg hL^{-1} with the lowest at BA18 with 76.0 kg hL^{-1}. For a grade No. 1 CWRS, the test weight needs to be at least 75 kg hL^{-1}, so test weights at the non-responsive sites met the minimum No. 1 grade requirement (Canadian Grain Commission 2019). The responsive sites also saw significant test weight differences (Table 3.3). BA19 had the highest test weight at 77.2 kg hL^{-1} and RD18 had the lowest with 69.1 kg hL^{-1}. Test weights at RD18 and RD19 would result in a No. 3 CWRS grade. On average, the responsive sites had about 9% lower test weights than the non-responsive sites. Lower test weights are usually due to stresses such as disease, insects, or unfavorable soil or environment conditions that can disrupt grain filling (Davidson 2018). The responsive sites had 25% higher foliar disease (Asif 2020) versus the non-responsive sites during grain filling, which could have resulted in the lower test weights at these sites.

For the non-responsive sites, significant differences in TKW were observed. LB18 had the lowest TKW at 37.7 g 1000 $seeds^{-1}$ and BA18 had the highest at 41.4 g 1000 $seeds^{-1}$ (Table 3.4). There were significant TKW effects attributed to the responsive sites, with the highest TKW (41.8 g 1000 $seeds^{-1}$) at RD18 and the lowest (36.5 g 1000 $seeds^{-1}$) at RD19 (Table 3.3). On average, the responsive sites had about 1.5% lower TKW than the non-responsive sites. This was unexpected, as lower precipitation levels or drought will reduce kernel weights (He et al. 2013). However, similar to the test weight results, the moderate to high level of foliar disease at the responsive sites could have led to lower TKW, while not significantly affecting yield.

For the non-responsive sites, protein levels were significantly different depending on the sites. BA18 had the highest protein levels at 167 mg g^{-1} and LB19 the lowest at 117 mg g^{-1} (Table 3.4). The responsive sites also had significant differences in protein levels between sites. RD19 had the highest protein level at 158 mg g^{-1}, while the lowest was 138 mg g^{-1} at RD18 (Table 3.3). On average, the responsive sites had about 3% lower protein than the non-responsive sites. This can be explained by the fact that initially, when there is a lot of available N, yield and protein increase simultaneously; however, when there is a lack of moisture (i.e., non-responsive sites), the yield increase halts but protein will continue to increase (Jones and Olson-Rutz 2012).

In summary, the responsive sites had 3.6% higher yields, 11 d longer maturity, 9% lower test weights, 1.5% lower TKWs and 3% lower protein content than the non-responsive sites.

3.3.3 Agronomic, yield and quality differences between cultivars

For the responsive sites, there was a significant cultivar × site interaction for yield (Table 3.3). This was attributed to yield differences between the two cultivars at two of four sites (BA19 and BR19), where AAC Viewfield averaged 13% higher yield than AAC Brandon. At BA19, AAC Viewfield yielded 0.6 t ha^{-1} more than AAC Brandon, and at BR19, AAC Viewfield yielded 1.4 t ha^{-1} more than AAC Brandon. There were no significant yield differences between cultivars at the non-responsive sites (Table 3.4).

At five of the eight site years, there was no significant difference in maturity between AAC Brandon and AAC Viewfield (Tables 3.3 and 3.4). This was expected and matches the maturity ratings of the cultivars (Table 3.5). The slight differences in maturities at BR18, RD18 and LB19 may be attributed to a genotype × environment interaction.

All responsive and non-responsive sites showed significant differences in TKW between cultivars. Overall, AAC Brandon had 8.1% heavier seeds compared with AAC Viewfield (Tables

3.3 and 3.4). This was also expected, since AAC Brandon is rated as having a heavier seed (41 g 1000 seeds^{-1}) relative to AAC Viewfield (40 g 1000 seeds^{-1}) (Alberta Seed Guide 2020) (Table 3.5). AAC Viewfield is reported to have a heavier test weight relative to AAC Brandon (Alberta Seed Guide 2020). Our data supported this trend at BA19, BR19, LB18 and LB19 (Tables 3.3 and 3.4).

The cultivars showed significant differences in protein content in four of eight site years, including at two responsive sites (BA19 and BR19) and two non-responsive sites (BR18 and LB19) (Tables 3.3 and 3.4). Specifically, AAC Brandon had a 5.2% and 8.7% higher protein than AAC Viewfield at the responsive and non-responsive sites, respectively. Again, this was expected, as AAC Viewfield is known to have lower protein levels compared with AAC Brandon (Alberta Seed Guide 2020) (Table 3.5).

3.3.4 Agronomic, yield and quality differences between fungicide treatments

The responsive sites had significant yield differences between fungicide treatments, but no significant fungicide × sites, or cultivar × fungicide × sites interactions (Table 3.3). As such, fungicide responses will be discussed based on fungicide treatment means averaged over the four responsive sites and both cultivars.

There were no significant yield differences between the non-treated control and fungicide applications at BBCH 22-23 or BBCH 30-32 (Table 3.3). This suggested there was no yield advantage associated with early fungicide applications, and this practice provides no economic benefit to growers. The non-treated control and earlier fungicide treatments, applied at BBCH 22-23 and BBCH 30-32, had lower yields (but not always significantly less yield) relative to the later fungicide applications at BBCH 39-45 and BBCH 61-63. Fungicide applications at BBCH 39-45 and BBCH 61-63 yielded significantly more than the non-treated control. This suggested that under responsive conditions, there may be an economic benefit associated with fungicide applications at these crop stages.

On average, there was a 0.62 t ha^{-1} yield decrease in earlier fungicide treatments versus later ones (Table 3.3). This trend was consistent with previous studies that have found that applying fungicides as a protectant according to plant growth stage can be a more effective approach in limiting yield loss (Poole and Arnaudin 2014). This is based on the premise that protecting the upper canopy (flag leaf, flag leaf-1, flag leaf-2, flag leaf-3), which contributes the most to grain yield, from foliar diseases is key to protecting yield, whereas lower canopy leaves that contribute very little to grain yield do not require disease protection. Our results showed that flag leaf timing (BBCH 39-45) and head timing (BBCH 61-63) for the application of fungicide treatments resulted in higher yields (on average an 8.4% increase) than any of the earlier applications. This was likely because the flag leaf had not yet emerged during the earlier application, whereas the later applications protected all the upper leaves, thus preventing major yield loss from fungal leaf spots.

The later fungicide applications at BBCH 39-45 and BBCH 61-63 resulted in significantly greater TKWs relative to the earlier fungicide treatments and the non-treated control (Table 3.3). The significant fungicide × sites interaction resulted from three sites (BA19, RD18 and RD19) showing significant responses to fungicide treatments, with later fungicide treatments (BBCH 39-45 and 61-63) having, on average, a 5% higher TKW relative to the earlier fungicide applications (BBCH 22-23 and 30-32) (data not shown). Fungicide applications did not impact DTM, test weight or protein. However, it should be noted that higher foliar disease is not always correlated with lower yields and vice versa.

3.3.5 Economic benefits of early versus later fungicide applications

The highest yield was achieved with the Prosaro XTR application at early anthesis (BBCH 61-63). It represented a 9.73% yield increase (or 0.72 t ha^{-1}) over the non-treated control (Table 3.3). The next highest yield came from spraying at the flag leaf stage with Trivapro A+B, representing a 9.4% yield increase (equivalent to 0.69 t ha^{-1}) relative to the non-treated control. The lowest yielding treatment was fungicide application with Trivapro A+B at BBCH 30-32, which was not significantly different from the non-treated control. Fungicide applications at BBCH 22-23 were 0.2 t ha^{-1} higher yielding than the non-treated control, representing a non-significant yield difference of 3% relative to the non-treated control. Overall, later fungicide applications at BBCH 39-45 and BBCH 61-63 were the most effective at increasing yield.

The cost of fungicides and their application costs need to be offset by increased yields. A grower applying Tilt 250 E must achieve an additional \$32.46 ha^{-1} in revenue to breakeven, which is equivalent to an additional 0.12 to 0.14 t ha^{-1} of grain yield (Table 3.6). A grower applying Trivapro A+B must achieve an additional \$58.62 ha^{-1} in revenue to breakeven, which is equivalent to an additional 0.23 to 0.25 t ha^{-1} of grain yield. The flag leaf (BBCH 39-45) application of Trivapro A+B would be profitable for a grower since it resulted in a yield increase of 0.69 t ha^{-1} at the responsive sites. In contrast, the application of Trivapro A+B at BBCH 22-23 or BBCH 30-32 would not be an economically sound decision, given that the yield increases relative to the non-treated control were +0.09 to -0.14 t ha^{-1} at these growth stages. A grower applying Prosaro XTR must achieve an additional \$71.46 ha^{-1} in revenue to breakeven, which is equivalent to an additional 0.28 to 0.31 t ha^{-1} of grain yield. At the responsive sites, Prosaro XTR applications resulted in an additional 0.72 t ha^{-1} in yield compared with the non-treated control. In this study, the Trivapro A+B treatment at flag leaf (BBCH 39-45) and the Prosaro XTR treatment at BBCH 61-63 would be two most economical choices for growers when environmental conditions were favorable for disease development.

3.3.6 Decisions for effective fungicide use

There are several factors that should be considered prior to spraying a fungicide. First, is the field expected to be responsive? Is there yield potential, and disease potential? If yield potential is compromised due to severe weather, such as drought, hail, or flooding, then there is no need for a fungicide. If there is no disease potential, then a fungicide is also not necessary.

Secondly, the relationship between the environment, pathogen and host/cultivar (disease triangle) is critical in determining whether or not to use fungicides (Scholthof 2007). For example, if the host is resistant or moderately resistant to the target pathogens, then the risk of yield loss decreases, and a fungicide may not be needed. In this study, the two CWRS cultivars did not have sufficient genetic resistance to foliar diseases. They both exhibited a response to fungicide application when disease pressure was high, as we observed at the responsive sites. As such, AAC Brandon and AAC Viewfield were responsive to fungicide applications given the disease pressure present in Alberta.

The environment also plays an important role in decision-making. Over the two growing seasons in this study, only half the sites were responsive to fungicide application. The responsive site years tended to have more precipitation and higher RH. Based on the present results, the best time to apply fungicides would be at growth stages BBCH 39-45 (flag leaf) and BBCH 61-63 (anthesis) to minimize yield and quality loss when observed season precipitation (194 – 364 mm) and RH (65.4 – 74.0%) are near the long-term average (Table 3.2). However, if disease incidence and severity are low throughout the growing season, such as we observed at the non-responsive sites (BA18, BR18, LB18 and LB19) with lower growing season precipitation (121 – 214 mm) and RH (57.7 – 63.7%), there may not be a need to spray, since the financial benefits are non-existent given that yield loss due to disease is non-significant.

3.4 Conclusion

When fungicide applications were made at BBCH 39-45 and 61-63, TKW was 5% greater and yields were 8.4% greater than when fungicide applications were made at BBCH 22-23 and 30-32. These data support earlier research showing that earlier fungicide applications provided no yield or quality benefits, even on new CWRS wheat varieties with improved genetic resistance to cereal diseases. Days to maturity, protein and test weight were not impacted by the fungicide treatments.

Our economic analysis indicated that applying propiconazole (Tilt 250 E) at BBCH 22-23 may be less costly, but also may not provide the best return on investment since it does not protect yield and quality compared to later fungicide applications. Propiconazole, benzovindiflupyr and azoxystrobin (Trivapro A+B) applications at BBCH 39-45 resulted in an additional 0.69 t ha^{-1} in yield compared with the non-treated control, when environmental conditions were conducive to disease development, and Trivapro A+B applications at BBCH 39-45 was the most profitable choice for growers. Applications of Prosaro XTR application at BBCH 61-63 also were profitable. Although this study found that later fungicide applications resulted in the highest yield, it is important to note that these yield responses occurred only at 50% of the site years. Site years where environmental conditions were not favorable for disease development, and fungicide applications were unnecessary, were characterized by low RH (57.7 - 63.7%) and an average observed precipitation of 175 mm.

Table 3.1: Six fungicide treatments applied at different growth stages at four locations (Barrhead, Bon Accord, Red Deer and Lethbridge) throughout Alberta, Canada in 2018 and 2019.

Fungicide product	FRAC[a] Fungicide Group	Active Ingredient	Fungicide rate (gai L ha^{-1})	Growth stage of fungicide treatment application (BBCH)
Tilt 250 E	3	Propiconazole	62.5	22-23
Trivapro A+B	3+7+11	Propiconazole + benzovindiflupyr + azoxystrobin	200 (TriA) + 30 (TriB)	22-23
Trivapro A+B	3+7+11	Propiconazole + benzovindiflupyr + azoxystrobin	200 (TriA) + 30 (TriB)	30-32
Trivapro A+B	3+7+11	Propiconazole + benzovindiflupyr + azoxystrobin	200 (TriA) + 30 (TriB)	39-45
Prosaro XTR	3	Prothioconazole + tebuconazole	200	61- 63
Non-treated control	-	-	-	-

[a]Fungicide Resistance Action Committee Code

Table 3.2: Observed precipitation, relative humidity, temperature, seeding dates, harvest dates and soil types for 8 site years over two growing seasons in 2018 and 2019. Barrhead 2018, Bon Accord 2018, Lethbridge 2018 and Lethbridge 2019 were considered non-responsive to fungicide treatments, while Barrhead 2019, Bon Accord 2019, Red Deer 2018 and Red Deer 2019 were considered responsive to fungicide treatments.

Location	Year	Seeding date	Harvest date	Physiological Maturity[a]	DTM	Relative humidity[b]	Air temperature[b]	Observed precipitation[b]	Long-term precipitation[bc]	Soil type[e]
					(days)	(%)	(°C)	(mm)		
Unresponsive Sites										
Barrhead	2018	May 2	Oct. 1	Aug 12	103	62.5	16.0	214	247	Solonetzic Dark Gray Chernozem
Bon Accord	2018	May 3	Oct. 4	Aug 20	110	63.7	16.0	207	250	Eluviated Black Chernozem
Lethbridge	2018	May 7	Sep. 6	Aug 15	104	57.7	17.2	121 [c]	182	Dark Brown Chernozem
Lethbridge	2019	Apr. 15	Sep. 4	Aug 12	120	60.3	14.2	159 [c]	207	
Average of non-responsive Sites					109	61.1	15.8	175	222	
Responsive Sites										
Barrhead	2019	May 6	Sep. 30	Aug 27	114	69.1	14.2	262	269	Solonetzic Dark Gray Chernozem
Bon Accord	2019	May 6	Sep. 23	Sept 13	131	72.3	14.0	364	284	Eluviated Black Chernozem
Red Deer	2018	May 21	Oct. 16	Sept 5	108	65.4	15.3	194[f]	275	Orthic Black Chernozem
Red Deer	2019	May 24	Oct. 19	Sept 28	128	74.0	13.4	272[f]	305	
Average of responsive sites					120	70.2	14.2	273	283	

[a]Physiological maturity was calculated by adding the recorded DTM from seeding date (eg: May 6th + 114 days = August 27th).
[b]Data were collected from Alberta Climate Iinformation System (ACIS) from each site's respective seeding date to physiological maturity based on days to maturity (DTM).
[c]Long term precipitation obtained from interpolated data.
[d]Growing season precipitation plus the average irrigation volume (29.5 mm) over 8 irrigation events in the growing season (May to Aug.) at the Lethbridge site.
[e]Soil description found via Alberta soil information viewer provided by (Alberta Agriculture and Forestry 2015).
[f]Observed rainfall data for Red Deer location obtained from Lacombe CDA station.

Table 3.3: *P* – values and least square means for the agronomic, yield and quality responses of the Canadian Western Red Spring (CWRS) wheat cultivars AAC Brandon and AAC Viewfield following foliar fungicide treatments at the responsive sites in Alberta, Canada, in 2018 and 2019.

Effects	DTM	Yield	Test weight	TKW	Protein
			P value		
SiteYr[a]	**<.0001*** **	**0.0013*** **	**<.0001*** **	**<.0001*** **	**<.0001*** **
	(days)	($t\ ha^{-1}$)	($kg\ hL^{-1}$)	($g\ 1000\ seeds^{-1}$)	($mg\ g^{-1}$)
Bon Accord 2019 (BA19)	131.0a	7.1a	77.2a	37.8b	140b
Barrhead 2019 (BR19)	113.8c	7.0a	76.3a	40.8a	139b
Red Deer 2018 (RD18)	108.4d	6.4b	69.1c	41.7a	138b
Red Deer 2019 (RD19)	128.0b	7.4a	73.2b	36.5b	158a
			P value		
Cultivar	0.1310	0.1274	0.2459	**0.0004*** **	**0.0414***
Cultivar X SiteYr[a]	**0.0004*** **	**<.0001*** **	**<.0001*** **	**0.0166***	0.6584
	(days)	($t\ ha^{-1}$)	($kg\ hL^{-1}$)	($g\ 1000\ seeds^{-1}$)	($mg\ g^{-1}$)
BA19 AAC Brandon	130.8A	**6.8A**	**76.4A**	**39.5A**	**145A**
BA19 AAC Viewfield	131.2A	**7.4B**	**78.1B**	**36.2B**	**136B**
BR19 AAC Brandon	113.6A	**6.3A**	**75.7A**	**43.3A**	**142A**
BR19 AAC Viewfield	114.1A	**7.7B**	**77.0B**	**38.4B**	**136B**
RD18 AAC Brandon	**107.2A**	6.2A	**69.9A**	**43.3A**	141A
RD18 AAC Viewfield	**109.6B**	6.4A	**68.3B**	**40.1B**	136A
RD19 AAC Brandon	128.0A	7.5A	72.9A	**38.3A**	161A
RD19 AAC Viewfield	127.9A	7.3A	73.5A	**34.8B**	156A
			P value		
Fungicide	0.0873	**0.0013***	0.9751	**<.0001*** **	0.3420
Fungicide X SiteYr[a]	0.2610	0.0896	0.2808	**0.0180***	0.2520
Fungicide X Cultivar X SiteYr[a]	0.4641	0.619	0.9758	0.0858	0.5470
	(days)	($t\ ha^{-1}$)	($kg\ hL^{-1}$)	($g\ 1000\ seeds^{-1}$)	($mg\ g^{-1}$)
Non-treated control	119.84	6.68c	73.78	38.5b	146
Tilt at BBCH 22-23	120.02	6.98abc	73.99	38.4b	143
Trivapro at BBCH 22-23	120.51	6.77bc	73.99	38.6b	144
Trivapro at BBCH 30-32	120.12	6.54c	74.04	38.7b	146
Trivapro at BBCH 39-45	120.30	7.37ab	74.03	40.7a	144
Prosaro XTR at BBCH 61-63	121.03	7.40a	74.03	40.5a	141
Adjusted CV%	1.3	9.0	1.9	1.3	6.7

[a]Site × Year

Significance indicated as *p<0.05, **p<0.01, ***p<0.001

Bolded letters = significance

Table 3.4: *P* – values and least square means for the agronomic, yield and quality responses of the Canadian Western Red Spring wheat cultivars AAC Brandon and AAC Viewfield following foliar fungicide treatments at non-responsive field sites in Alberta, Canada, in 2018 and 2019.

Effects	DTM	Yield	Test weight	TKW	Protein
			P value		
SiteYr[a]	**<.0001*****	**<.0001*****	**<.0001*****	**<.0001*****	**<.0001*****
	(days)	(t ha^{-1})	(kg hL^{-1})	(g 1000 seeds^{-1})	(mg g^{-1})
Bon Accord 2018 (BA18)	109.7b	6.6b	76.1d	41.4a	168a
Barrhead 2018 (BR18)	103.3c	5.9c	81.4b	40.6a	166a
Lethbridge 2018 (LB18)	104.1c	7.8a	85.5a	37.7c	144b
Lethbridge 2019 (LB19)	120.0a	6.9b	79.6c	39.6b	117c
			P value		
Cultivar	0.928	0.150	0.199	**0.002****	**0.027***
Cultivar X SiteYr[a]	**<.0001*****	0.133	**0.0001*****	0.345	**0.017***
	(days)	(t ha^{-1})	(kg hL^{-1})	(g 1000 seeds^{-1})	(mg g^{-1})
BA18 AAC Brandon	109.7A	6.5	**76.4A**	42.9	171A
BA18 AAC Viewfield	109.8A	6.7	**75.7B**	40.0	164A
BR18 AAC Brandon	**102.4A**	5.7	81.2A	41.6	**171A**
BR18 AAC Viewfield	**104.3B**	6.0	81.7A	39.7	**160B**
LB18 AAC Brandon	104.2A	7.6	**85.1A**	38.9	143A
LB18 AAC Viewfield	104.0A	8.0	**85.9B**	36.4	144A
LB19 AAC Brandon	**120.9A**	6.9	**79.2A**	40.9	**124A**
LB19 AAC Viewfield	**119.2B**	6.8	**80.0B**	38.4	**109B**
			P value		
Fungicide	0.862	0.232	0.241	0.078	0.582
Fungicide X SiteYr[a]	0.466	0.752	0.951	0.943	0.611
Fungicide X Cultivar X SiteYr[a]	0.134	0.530	0.459	0.977	0.715
	(days)	(t ha^{-1})	(kg hL^{-1})	(g 1000 seeds^{-1})	(mg g^{-1})
Non-treated control	109.0	6.85	80.8	39.8	152
Tilt at BBCH 22-23	109.1	6.57	80.3	39.6	148
Trivapro at BBCH 22-23	109.4	6.73	80.6	39.5	150
Trivapro at BBCH 30-32	109.6	6.88	80.6	39.5	147
Trivapro at BBCH 39-45	109.4	6.78	80.8	40.2	148
Prosaro XTR at BBCH 61-63	109.4	6.89	80.7	40.3	147
Adjusted CV%	1.3	7.9	1.2	3.4	8.6

[a]Site × Year

Significance indicated as *p<0.05, **p<0.01, ***p<0.001

Bolded letters = significance

Table 3.5: Ratings of two Canadian Western Red Spring (CWRS) cultivars compared with AC Carberry as reported in the spring 2020 Alberta Seed Guide

Cultivar	Yield	Maturity rating[a]	Protein	Test weight	TKW	Height	Lodging[b]	Leaf spots[c]	FHB[c]
	(t ha^{-1})		(%)	(kg hL^{-1})	(g)	(cm)			
AC Carberry	6.7	104	13.9	79	40	84	VG	MS	MR
AAC Brandon	7.1	0	-0.3	79	41	85	G	I	MR
AAC Viewfield	7.4	0	-0.4	80	40	80	VG	I	I

[a]Maturity rating +/- of days of Carberry
[b]Lodging: VG = Very good, G = Good
[c]Leaf spots: MS = Moderately susceptible, I = Intermediate, MR = Moderately resistant

Table 3.6: Estimated economic returns for a single application of Tilt 250 E, Trivapro A+B and Prosaro XTR to Canadian Western Red Spring (CWRS) wheat in Alberta, Canada based on grain prices from 2018 and 2019.

Year	Wheat price [a]	Application cost	Fungicide cost[bc]	Total application and fungicide cost	Yield needed
	($ t^{-1})[a]	($ ha^{-1})	($ ha^{-1})	($ ha^{-1})	($t\ ha^{-1}$)
			Single Application of Tilt 250 E		
2018	259.78	17.50	14.96	32.46	0.12
2019	230.04	17.50	14.96	32.46	0.14
			Single Application of Trivapro A + B		
2018	259.78	17.50	41.12	58.62	0.23
2019	230.04	17.50	41.12	58.62	0.25
			Single Application of Prosaro XTR		
2018	259.78	17.50	53.96	71.46	0.28
2019	230.04	17.50	53.96	71.46	0.31

[a]Average cost of wheat ($ t^{-1}) was collected from price & data quotes (PDG) in 2018 and 2019 from September 3rd to October 31st. The data was averaged each year for southern and northern regions in Alberta.
[b]Calculated costs ($ ha^{-1}) from retail values of fungicide and recommended label rates
[c]Tilt 250 E retail price was $401.87/8L jug with recommended herbicide timing rate to be 100 mL ac^{-1} (250 mL ha^{-1} or 0.25 L ha^{-1})
[c]Trivapro A+ B retail price was at $666.12/case in a co-pack including two 8.1L jugs of Trivapro A and two 2.43L jugs of Trivapro B. Recommended rates were 400 mL ac^{-1} (1 L ha^{-1}) of A and 120 mL ac^{-1} (0.3 L ha^{-1}) of B.
[c]Prosaro XTR retail price was $433/6.5L jug with recommended rate of 324 mL ac^{-1} (80 mL ha^{-1} or 0.81 L ha^{-1})

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Chapter 4: Evaluation of fungicide efficacies on common leaf spot diseases and fusarium head blight of wheat at different growth stages

4.1 Introduction

In Canada, there are about 20 different fungal pathogens that can infect wheat (Aboukhaddour et al. 2020). Since the early 2000s, tan spot [*Pyrenophora tritici-repentis* (Died.) Drechsler] (Murray et al. 2015), fusarium head blight (FHB) [*Fusarium spp,*] and stripe rust [*Puccinia striiformis* Westend] have been a major concern throughout Canada, with different geographic areas being at risk of different diseases. Historically, in Manitoba, FHB has been widespread and has yearly incidences with high severities (Manitoba Agriculture 2019). Over the years, Saskatchewan has also seen an increase in FHB (Canadian Grain Commission 2019a; Canadian Grain Commission 2019b). Comparatively, Alberta has seen an increase in FHB, but the disease is still limited to mainly southern Alberta with only a few cases in central and northern Alberta (Canadian Grain Commission 2019b; Harding et al. 2018).

Each pathogen can cause high levels of disease depending on the year and geographic area and are strongly influenced by weather conditions. Specifically, in Alberta, tan spot, stripe rust, septoria nodorum blotch (SNB) (*Parastagonospora nodorum* (Berk.) Quaedvlieg, Verkley, & Crous) and septoria tritici blotch (STB) (*Zymoseptoria tritici* (syns. *Mycosphaerella graminicola* (Fuckel) J. Schröt.) are diseases of concern. Surveillance for FHB is also a top priority as the risk of infection increases in the province (Pesticide Risk Reduction Program Canada 2019).

The disease triangle contains three main elements (host, pathogen, and the environment) that are crucial for disease development. If one factor is missing, then disease will not become established (Islam 2018). A more detailed disease triangle developed by McNew in the 1960s emphasizes that the environment is one of the most important causes of plant disease. Usually, the other factors can be manipulated; a cultivar can have genetic resistance to a disease and chemicals can be used to mitigate the disease, but the environment cannot be manipulated/managed for crops grown in large fields, such as wheat (Scholthof 2007). Therefore, the severity of plant disease is highly influenced by changes in environmental conditions (Agrios 2004).

The host's susceptibility to a pathogen is genetically controlled. In Canada, five fungal pathogens (the three rusts (stem [*Puccinia graminis* subsp. *graminis* Pers.:Pers] (Murray et. al 2015), stripe [*Puccinia striiformis* Westend] and leaf [*Puccinia triticina* Erikss]), common bunt [*Tilletia caries* (DC.) Tul. & C. Tul] and FHB [*Fusarium spp,*]) are 'Priority 1' diseases and are addressed in Canadian breeding programs (Aboukhaddour et al. 2020). Wheat breeding in western Canada from 1885 onwards has led to improvements in several agronomic traits and disease resistance (Iqbal et al. 2016)**.** The breeding for resistance to various diseases (stem, leaf and stripe rust and common bunt) has been highly successful in western Canada, selecting genes that improve disease resistance, early maturity and yield for many Canadian cultivars (Iqbal et al. 2016)**.** Specifically, the two cultivars, AAC Brandon and AAC Viewfield, are known to have 'intermediate' resistance to leaf spots and 'moderately resistant and 'intermediate' resistance, respectively to FHB (Alberta Seed Guide 2020).

Currently, there are no studies evaluating the impact of STB, SNB, tan spot and FHB on two of the more modern CWRS wheat cultivars in western Canada, with different genetic resistance to plant pathogens. Therefore, the objective of this study was to determine the effects of single fungicide applications timings at BBCH 22-23 [2-3 tillers (herbicide timing)] (Meier 2018), BBCH 30-32 [stem elongation (plant growth regulator (PGR) timing)], BBCH 39-45 (flag leaf), and BBCH 61-63 [10-30% of anthers are mature (head timing)] on plant disease severity and incidence. Two genetically different CWRS cultivars, AAC Brandon (Cuthbert et al. 2016) and AAC Viewfield (Cuthbert et al. 2018), were tested. The effects of fungicide application timing on yield, agronomic traits and grain quality were also measured and these results are presented in a separate report (Asif 2020).

4.2 Materials and methods

4.2.1 Field setup

Field experiments were conducted in Alberta, Canada, over two growing seasons in 2018 and 2019 at three rain-fed sites (Barrhead, Bon Accord and Red Deer) and one irrigated site (Lethbridge). Precipitation, RH and FHB risk were obtained from the nearest Alberta Climate Information System (ACIS) station from seeding date to physiological maturity (based on days to maturity (DTM) as mentioned in Chapter three under '3.2.1. Field Setup'.

4.2.2 Fungicide treatments

Like Chapter three, data reported herein are sub-set of data collected as part of a larger study. Five fungicide treatments and a non-treated control were compared (Table 3.1). The larger study was designed as a split-plot with four blocks (Appendix 1). The main plot was cultivar and the sub-plot was fungicide treatment. The fungicide treatments represented fungicide products from three groups: the triazoles (group 3), carboxamides (group 7) and the strobilurins (group 11), applied at four different growth stages: BBCH 22-23, BBCH 30-32, BBCH 39-45 and BBCH 61-63.

4.2.3 Data collection

Foliar disease symptoms were rated using the assessment scale of McFadden (1991) (Appendix 2). Disease ratings were conducted eight times over the growing season, immediately prior to each fungicide application and two weeks after the final fungicide application. Only plots receiving a fungicide application were rated prior to fungicide application.

To further quantify foliar disease levels, 10 representative flag leaves were collected two weeks after the fungicide applications at BBCH 61-63 which occurred in late July or early August. These leaves were taped to plastic sheets, scanned and assessed with Assess 2.0 Image Analysis Software for Disease Quantification (Lamari 2008). The calibration values were adjusted either manually or automatically according to picture quality and level of leaf disease.

At each time that a McFadden visual disease rating assessment was conducted, 10 representative, symptomatic young leaves were collected. For each symptomatic leaf, a small necrotic section or lesion was cut from the leaf and run under tap water for 1 h. Then, the leaf sections were surface-sterilized with 1% NaOCL for 1 min and plated on 1% water agar with 50 mg L^{-1} Streptomycin. Plates were incubated at room temperature under 12/12h light/dark for 2 days. Samples were then examined with a microscope to identify the foliar pathogens of concern: *Zymoseptoria tritici*, causal agent of STB; *Stagonospora nodorum* causal agent of SNB; and *Pyrenphora tritici-repenti* causal agent of tan spot, according to physical characteristics. To differentiate between *Z. tritici* and *S. nodorum* the fruiting bodies (pycnidia) were examined under a compound microscope. If the pycnidia ran parallel to leaf veins, they were considered *Z. tritici*; if the pycnidia did not run parallel to leaf veins, they were considered *S. nodorum*. To determine if a sample was *P. tritici-repenti*, the conidiophores were examined at under a dissecting microscope. If conidiophores were erect and not branched, then the pathogen was considered *P. tritici-repenti*. If conidiophores were hard to identify, then a slide was made to examine spores under a compound microscope to confirm whether or not it was *P. tritici-repenti*.

If spores were hyaline and cylindrical then it was *P. tritici-repenti.* If not, the samples were considered to be infected with an unknown pathogen.

Normalized Difference Vegetation Index (NDVI) was measured at BBCH 83-85 (early dough to soft dough: grain content soft but dry, fingernail impression not held) for each plot. Healthy vegetation reports higher NDVI values, while infected vegetation reports lower NDVI values. NDVI can be used as an objective method to quantify canopy health, as leaves with lesions, chlorosis and disease can present lower NDVI ratings (Kumar et al. 2016).

At BBCH 85, 10 heads in the non-treated control plots, for each cultivar, were assessed using the Stack and McMullen (1998) visual scale to estimate the severity of FHB. If the non-treated control plots showed symptoms, then all plots were rated. If the non-treated control showed no FHB, visual ratings ceased. The Barrhead 2019 site was the only site where visual FHB ratings were conducted on all plots.

An uncleaned 1 kg sample of harvested grain directly from the combine was saved for fusarium damaged kernels (FDK) and deoxynivalenol (DON)/mycotoxin analysis from each plot. Deoxynivalenol (DON) was analyzed via ELISA (enzyme-linked immunosorbent assay) using the Veratox® for DON5/5 kit according to the manufacturer's recommendations (Neogen, Lansing, MI, USA). The Veratox® kits are a valuable way of detecting DON for quantitative screening, but they cannot detect low DON concentrations and can sometimes have inadequate sensitivity to the antibodies used in the kits (Tangni et al. 2011). Therefore, liquid chromatography with tandem mass spectrometry (LC/MS/MS) was used as a reference method to detect lower DON concentrations (Tangni et al. 2011). The LC/MS/MS analysis was performed with an Agilent series 1260 Infinity Quaternary High Performance Liquid Chromatography (HPLC) system (Agilent Technologies, Mississauga, ON, Canada) coupled to an AB Sciex API 4000 hybrid triple quadrupole linear ion trap (QTRAP®) mass spectrometer (AB Sciex, Concord, ON, Canada) equipped with a Turboionspray™ interface on 1 kg of uncleaned harvested grain.

Fusarium damaged kernels (FDK) were hand-picked from a well-mixed, 500 g sub-sample of uncleaned harvested grain straight from the combine to ensure that FDK were properly represented in the sub-sample. Percent FDK was calculated by dividing the weight of FDK kernels by the total sample weight multiplied by 100. The FDK ratings were collected only on three fungicide treatments: the non-treated control, Trivapro A+B at BBCH 22-23 and Prosaro XTR at BBCH 61-63. These treatments were chosen to compare the FHB timing at BBCH 61-63 with the non-treated control and an early fungicide application at BBCH 22-23, where no FHB protection was expected.

FDK seed samples were then plated to identify the *Fusarium* species. Acidic potato dextrose agar (PDA) plates are used as they are ideal in growing fusarium colonies (32 g of PDA powder in 1 L bottle with 800 ml of water and 2.4 ml of lactic acid). At most 20 seeds per sample were collected with 5-10 seeds per plate. After 4-5 days, the PDA plates were examined under a compound microscope to identify fusarium colonies. The number of seeds that showed fusarium growth (out of at most 20) were recorded to confirm FDK count data.

4.2.4 Statistical analysis

The study was conducted over eight site years (site - year combinations) and was analyzed using PROC GLIMMIX of SAS Studio v. 3.8 (SAS Institute Inc. 2012-2018). The site year, cultivar, fungicide treatments, and their interactions were treated as fixed effects. Blocks and their interactions with all fixed effects were considered as random effects.

DON data followed a Poisson distribution and were re-coded into a new discrete variable where the value was multiplied by 100,000 to make an integer value which was analyzed via PROC GLIMMIX. The lsmeans were then back-transformed from log-scale using the 'ilink' option.

4.3 Results and discussion

4.3.1 Effect of environment

In 2018, the average observed precipitation was 184 mm (121 – 214 mm), which was about 77% of the LTA (50 – 89% of the LTA) (Table 4.1). Barrhead 2018 (BR18) had the highest observed precipitation (214 mm) and the lowest was at Lethbridge 2018 (LB18) (121 mm). Observed average precipitation at the 2019 sites was 264 mm (159 – 364 mm), which was 99% of the long-term average (LTA) (60 – 98% of the LTA). In 2019, Bon Accord 2019 (BA19) had the highest observed precipitation (364 mm) and the lowest was Lethbridge 2019 (LB19) (159 mm).

Relative humidity, on average, at the 2018 sites was 62.3% (57.7 – 65.4%) and the 2019 sites was 68.9% (60.3 – 74.0%) (Table 4.1). Temperature difference between sites was minimal. The 2018 sites had, on average, a 2.2°C higher temperature versus the 2019 sites.

To determine the risk of FHB, the ACIS fusarium disease severity risk prediction tool was used. The tool generated a risk value for FHB based on temperature and rainfall data and provided a risk score between 1–50 (1-9 =low risk; 10-20 = moderate risk; 21-30 = high risk; 31-50 = severe risk). It reported that the average risk score for 2019 was 23.3 (high FHB risk) and was 56% higher than the 2018 risk score of 12.3 (moderate FHB risk) (Alberta Agriculture and Forestry 2019). The wetter environment in Bon Accord 2019 (BA19) and Barrhead 2019 (BR19) led to severe FHB risk values two weeks after fungicide application at BBCH 61-63. The 'drier' sites, LB18, LB19 and Red Deer 2018 (RD18) sites saw very low FHB risk. FHB infections tend to thrive in conditions that have high RH (>90%) and moderately warm temperatures (15-30°C), thus explaining, in part, why the 'dry' site years (LB18 and LB19 and RD18) had lower FHB risk and infection compared with the more 'wet' site years (BA19 and BR19) (Schmale III and Bergstrom 2003).

4.3.2 Disease development and differences between fungicide treatments for the incidence, severity, and causal agent of leaf diseases

The average McFadden visual disease severity ratings, prior to fungicide application, increased throughout the season (Table 4.2). Averages for foliar disease severity at BBCH 22-23 and BBCH 30-32 were very low prior to any fungicide application compared with the later growth stages (BBCH 39-45 and BBCH 61-63).

The 2018 growing season saw low levels of foliar disease severity at early growth stages because of the low amount of rainfall (69 – 83 mm) in May and June (Table 4.1). The observed precipitation in 2018 was 16% lower than the long-term average in May and June. Foliar disease severity ratings at BBCH 39-45 were 73% and 29% higher, respectively, than at BBCH 22-23 and BBCH 30-32, prior to any fungicide application (Table 4.2). Foliar disease severities at BBCH 61-63 were 78% and 41% higher than at BBCH 22-23 and BBCH 30-32, respectively.

In 2019, the disease severity was low at the early growth stages, despite 19% higher observed precipitation levels relative to the long-term averages for May and June (Table 4.1). Later in the growing season, disease severity levels increased, with foliar disease severity at BBCH 39-45 being 89% and 87% higher than at BBCH 22-23 and BBCH 30-32, respectively (Table 4.2). Foliar disease severity at BBCH 61-63 was 97% higher compared to both of the earlier growth stages (BBCH 22-23 and BBCH 30-32).

The McFadden visual disease severity ratings two weeks after final fungicide application indicated significant differences between site-year, cultivar × site, fungicides, and fungicide × site interactions (Table 4.3). BA19, BR19 and LB19 had the highest foliar disease severity (4.64, 4.74, and 4.79 respectively), with the lowest foliar disease severity found at BA18, BR18 and RD18 (1.23, 1.87 and 1.96, respectively). On average, the 2019 growing season had about 53% higher visual leaf disease severity compared to 2018 by early to mid-August. This may be reflected in the higher levels of precipitation and RH in 2019 (Table 4.1). Most infections by plant pathogens appear and develop during wet, warm days and nights (Agrios 2004).

The Tilt 250 E (propiconazole) and Trivapro A+B (azoxystrobin + propiconazole + benzovindiflupyr) treatments at BBCH 22-23 along with the non-treated control had the highest foliar disease severities of any fungicide treatments in early to mid-August (Table 4.3). Fungicide treatment at BBCH 22-23 and BBCH 30-32 had 34% and 33%, respectively, higher foliar disease severity compared with the fungicide treatment at BBCH 39-45 (flag leaf). These results are consistent with Milus (1994), who found that a fungicide at BBCH 39-45 is far better at controlling leaf spots than earlier fungicide applications. The result also suggested that producers should avoid early fungicide applications at BBCH 22-23 and BBCH 30-32, as they do not result in significantly lower disease levels compared with the non-treated control. Unnecessary fungicide applications are economically unwise and, may contribute to the development of fungicide resistance.

The lowest leaf disease severities were observed for fungicide applications at BBCH 39-45 (Table 4.3). The fungicide application at BBCH 61-63 resulted in 5% more leaf disease than the application at BBCH 39-45, however, these levels of leaf disease were not significantly different. This suggests that a fungicide application at anthesis (BBCH 61-63) may also be helpful in controlling foliar diseases, giving producers a wider window for effective disease control from BBCH 39-45 to BBCH 61-63. Previous studies found that fungicide application at BBCH 61-63 can provide sufficient control against foliar disease without any reduction in grain yield while maintaining grain quality (Kutcher et al. 2018; MacLean et al. 2018; Wiersma and Motteberg 2005; Asif 2020).

Image analysis with the Assess 2.0 disease quantification analysis software was an alternate method to quantify foliar diseases. The software provided an objective, quantitative measure of the percent flag leaf disease, whereas the McFadden ratings provided a subjective, qualitative measure of disease severity for the whole plant. These two methods of foliar disease ratings resulted in slightly different trends. There was a significant difference in Assess 2.0 disease levels between sites, cultivars, and the cultivar × site and fungicide × site interactions (Table 4.3). The Assess 2.0 data indicated that BA19 had the highest percent flag leaf disease (5.33%), while RD19 and LB19 had the lowest at 0.77% and 0.98%, respectively.

Analysis of the Assess 2.0 data did not indicate significant differences between fungicide treatments; however, these data followed the same trend as the McFadden foliar disease severity ratings. Flag leaf disease in the fungicide treatment at BBCH 39-45 was 34% lower than in the non-treated control, and 28%, 23% and 27% lower than in the Tilt 250 E at BBCH 22-23, Trivapro A+B at BBCH 22-23, and Trivapro A+B at BBCH 30-32 treatments, respectively (Table 4.3). Again, this can be because the fungicide treatments at BBCH 39-45 are known to be the optimal time to control foliar diseases (Poole and Arnaudin 2014; Poole 2009). In our experience with Assess 2.0 and the McFadden disease severity rating systems, the latter are a more effective tool for evaluating disease levels in plots, because they evaluated a larger portion of the canopy. The Assess 2.0 method is better suited to detect small differences on single leaves.

For the fungicide × site interaction, only BA19 showed significant differences in the Assess 2.0 data (data not shown). The non-treated control had the highest disease (8.2%) and Trivapro A+B at BBCH 39-45 had the lowest (3.0%). The fungicide treatments at BBCH 22-23 and BBCH 30-32, on average, had 47% higher flag leaf disease than the fungicide application at BBCH 39-45.

For NDVI, there were significant differences between sites, cultivar × site interaction, fungicide treatments and fungicide treatment × cultivar × site interaction (Table 4.3). The highest NDVI reading at 0.46 was at LB18 and the lowest was at LB19, perhaps due at least in part to the severe hail seen at this site, and at BR18 at 0.30 and 0.32, respectively. Differences in NDVI between sites may also reflect slight differences in the growth stage when the NDVI data were collected. NDVI readings for the fungicide treatments, on average, were 5% higher than in the non-treated control. Tilt 250 E at BBCH 22-23 had an NDVI 10% lower than the fungicide treatments at BBCH 39-45 and BBCH 61-63. This was supported by both the McFadden foliar disease severity ratings and Assess 2.0 measurements of percent infection. While it was encouraging to see the NDVI values confirm the disease rating results, this is not always the case, as NDVI does not specifically measure disease. Any influence on the crop that affects absorbance/reflectance will affect NDVI, which is why NDVI values do not show as much distinction between fungicide treatments as the McFadden disease severity ratings.

The three foliar diseases, SNB caused by *Parastagonospora nodorum*, STB caused by *Zymoseptoria tritici* and tan spot caused by *Pyrenophora tritici-repenti* occurred in both 2018 and 2019 (Table 4.4). In 2018, tan spot was the most common disease and in 2019, STB was the most common disease.

4.3.3 Differences between fungicide treatments for the incidence, severity, and species of FHB

There was a significant difference between the sites for FDK (Table 4.3). BA19 and BR19 had the highest percent FDK (1.07 % and 1.03% respectively), while BR18 and LB19 had the lowest (0.12% and 0.16% respectively). This was expected as BA19 and BR19 received the highest observed precipitation (364 mm and 262 mm, respectively) (Table 4.1) and had the highest ACIS risk of FHB, while BR18 received lower levels of precipitation (214 mm) and had 38% lower FHB risk (Table 4.1). On average, the percent FDK was 49% higher in 2019 vs. 2018 (Table 4.3). However, these levels were still very low relative to other western Canadian provinces (Canadian Grain Commission 2019b).

There was also a significant fungicide × site year interaction for FDK (Table 4.3). Averaged over all sites, the non-treated control had 32% higher percent FDK compared with the two fungicide treatments. However, only three sites, LB18, BA19 and BR19, showed significant responses to fungicide treatments (data not shown). At the LB18 site, the non-treated control and the fungicide treatment at BBCH 30-32 had 21% higher FDK relative to the Prosaro XTR treatment at BBCH 61-63, which was expected. However, this trend was not observed for the other two sites. At BA19 and BR19, the percentage FDK for the Prosaro XTR (prothioconazole + tebuconazole) treatment at BBCH 61-63 fungicide was 31% and 42% higher FDK, respectively, than for the Trivapro (azoxystrobin + propiconazole + benzovindiflupyr) treatment at BBCH 22-23. We would have expected the treatments at BBCH 61-63 to have lower FDK than fungicide treatments at BBCH 22-23. Similar results were obtained in another study, where some fungicide treatments had higher levels of FHB and FDK than the unsprayed control in some site years (Fernandez et al. 2014). This is unusual, but there have been cases where earlier fungicide treatments can reduce FDK and the risk of FHB, due to a reduction of fusarium debris on younger plants and leaf sheaths (Edwards and Godley 2010).

The ELISA DON data differed between sites due to the elevated DON levels at RD19 (0.00126 mg g^{-1}) (Table 4.3). There was also a significant response attributed to fungicide treatment, fungicide × site and fungicide × cultivar × site. Averaged across all sites, the ELISA DON responses were not meaningful. To understand these data fully, it is necessary to investigate the fungicide × site interaction. RD19 was the only site that showed a response to fungicide treatments. At RD19, the Prosaro XTR (prothioconazole + tebuconazole) treatment at BBCH 61-63 had 48% higher DON than the non-treated control, and 67% higher DON than the average of the other treatments (data not shown). These results are highly unusual, but the RD19 samples were not adequately dried after harvest, possibly resulting in post-harvest disease development and mycotoxin production, which may explain the unexpected data trends. Harvested grain with high moisture content can lead to fungal growth and mycotoxin development in storage (Bolanos-Carriel et al. 2019). The maximum safe moisture level for wheat grain storage is 13.5% at 10°C (Bolanos-Carriel et al. 2019).

The LC/MS/MS method was used for the detection of DON at lower concentrations than possible by the ELISA. In 2018, there were no significant differences between fungicide treatments or their interaction with sites (Table 4.3). There was a site year difference, where the highest DON levels (3.2×10^{-4} mg g^{-1}) were found at BR18 and the lowest (8.0×10^{-5} mg g^{-1}) were at LB18. This was expected, since BR18 received the highest precipitation (214 mm) and LB18 the lowest (121 mm). This agrees with the ACIS prediction for BR18 as having a high risk of FHB versus LB18, which was at low risk. However, it is important to put these finding into context. The DON values at both sites were 403% below the 0.001 mg g^{-1} DON limit for cereal grains, reflecting very low FHB pressure at all sites. Analysis of the 2019 LC/MS/MS samples has been delayed due to the COVID-19 pandemic.

In 2018, *F. poae* was the most common *Fusarium* species identified, and represented 64% of the total number of infections (Table 4.5). In 2019, *F. avenaceum* was the most common *Fusarium* species (80% of the total infections). *Fusarium graminearum* was associated with the lowest number of infections in both years, representing about 0.9% and 9.4% of the total infections in 2018 and 2019, respectively. This species produces the mycotoxin, DON, which is detrimental to animal and human health (Tittlemier et al. 2020), so while many *Fusarium* species were found, the low proportion of *F. graminearum* indicates less human and animal health risk and explains why the DON levels in the samples were so low (Edwards et al. 2001).

4.3.4 Interaction of cultivar and leaf diseases

Leaf plating of foliar pathogens in 2018 indicated no STB or SNB infections on AAC Brandon, on which only PTR infections were found (Table 4.6). In contrast, AAC Viewfield was infected with STB, SNB, and PTR in 2018. Different trends were observed in 2019, with AAC Brandon being infected by all three foliar leaf pathogens, but AAC Viewfield only infected by STB and SNB. When compiling results over both years, both cultivars were infected at least once by all foliar pathogens. This confirmed that at least some infection can occur and is consistent with the leaf spot resistance ratings of these cultivars as ‘intermediate’. The irregularity of pathogen presence is likely a reflection of different field histories (e.g., crop rotation, crop sequence, previous cultivars).

There was a significant cultivar × site year interaction for the McFadden visual disease ratings (Table 4.3). At most sites, AAC Viewfield and AAC Brandon had similar foliar disease levels which was expected. In seed guides, AAC Brandon and AAC Viewfield were both rated as ‘intermediate’ for resistance to the leaf spot complex (STB, SNB and tan spot) (Table 3.5) (Alberta Seed Guide 2020; Government of Saskatchewan 2020). However, differences between cultivars were observed at three of eight sites. At BR19, LB19 and RD19, AAC Brandon had an average foliar disease 21% higher than AAC Viewfield (Table 4.3). This trend of AAC Brandon having more disease than AAC Viewfield was also supported by the image analysis data obtained with Assess 2.0, where AAC Brandon had 40% higher percent flag leaf disease than AAC Viewfield at the BA19, BR18, RD18 and LB18 sites.

The discrepancy between the seed guide disease ratings for each cultivar and the findings in our study may reflect variability in isolates or populations of fungi, disease pressure, and moisture and temperature conditions at each site and in different years. An ‘intermediate’ resistance rating covers a range of responses and it may be that both cultivars are considered ‘intermediate’, but AAC Viewfield is nonetheless slightly more resistant than AAC Brandon. It should be noted that since these are not Priority 1 pathogens, there is no mandatory requirement to breed for genetic resistance to the STB, SNB or tan spot pathogens in Canadian wheat breeding programs.

4.3.5 Interaction of cultivar and FHB

The percent FDK, ELISA and LC/MS/MS data indicated no significant difference between cultivars (Table 4.3). AAC Brandon is rated as ‘moderately resistant’ to FHB versus an ‘intermediate’ resistance rating for AAC Viewfield (Table 3.5). AAC Brandon is one of the five wheat cultivars in Canada related to Sumai 3, which is a highly resistant FHB cultivar due to the *Fhb1* gene (Zhu et al. 2019). The lack of difference in FDK and DON levels between the cultivars in this study is likely attributable to the very low FHB pressure, and even lower presence of mycotoxin-producing species, in the study environments.

Fusarium plating in 2018 indicated that AAC Brandon was infected with *F. poae* 74% of the time and with *F. graminearum* only 1.4% of the time (Table 4.7). Similar trends were observed with AAC Viewfield in 2018, with 65% of the infections attributed to *F. poae* and none to *F. graminearum.* In 2019, 78% of the infections on AAC Brandon appeared to be caused by *F. avenaceum*, while only 11% of the infections were caused by *F. graminearum*. As with AAC Brandon, *F. avenaceum* was the dominant species on AAC Viewfield, causing 82% of the infections; in contrast, only 8.2% of the infections on this host are were attributed to *F. graminearum*. These results confirm that levels of FHB in many parts of Alberta remain much lower than levels in the eastern Prairies, and that *F. graminearum* is not common across much of this province.

4.4 Conclusion

Precipitation across the experimental field sites was 43% higher in 2019 than in 2018, creating conditions that were more favorable for disease development. Overall disease levels were 53% higher in 2019 versus 2018. Fungicide applications at BBCH 22-23 and BBCH 30-32 resulted in foliar disease levels that were 33% and 19% higher, respectively, than at BBCH 39-45. Digital assessment of disease levels with Assess 2.0 software gave similar results, with disease levels on the flag leaf being 31-39% and 36% lower when fungicide treatment was at BBCH 39-45 versus at BBCH 22-23 and BBCH at 30-32, respectively. This confirms that a fungicide application at the flag leaf stage (BBCH 39-45) represents the optimal timing for control of foliar diseases. Additionally, percent FDK was 49% higher in 2019 than in 2018. Collectively, the results suggest that producers should avoid early fungicide applications at BBCH 22-23 and BBCH 30-32, since they do not result in significantly lower disease levels compared with the non-treated control.

Under environmental conditions favorable for disease development, both AAC Brandon and AAC Viewfield had reduced foliar disease symptoms when sprayed with a fungicide application at BBCH 39-45 and BBCH 61-63, meaning that a fungicide application will be beneficial for both cultivars in environments with high disease pressure.

Table 4.1: Seeding dates, harvest dates, environmental conditions and soil types at the 8 sites years where trials were conducted over two growing seasons in 2018 and 2019 at four different locations throughout Alberta (Barrhead, Bon Accord, Red Deer and Lethbridge).

Location	Year	Seeding date	Harvest date	Physiological Maturity	DTM	Relative humidity[a]	Air temperature[a]	Precipitation Observed[a]	Precipitation Long-term[ab]	May-June precipitation Observed	May-June precipitation Long-term	Soil type[d]
					(days)	(%)	(°C)	(mm)				
Barrhead	2018	May 2	Oct. 1	Aug 12	103	62.5	16.0	214	247	83	89	Solonetzic Dark Gray Chernozem
Bon Accord	2018	May 3	Oct. 4	Aug 20	110	63.7	16.0	207	250	75	81	Eluviated Black Chernozem
Red Deer	2018	May 21	Oct. 16	Sept 5	108	65.4	15.3	194[e]	275	82[e]	98	Orthic Black Chernozem
Lethbridge	2018	May 7	Sep. 6	15-Aug	104	57.7	17.2	121[e]	182	69	91	Orthic Dark Brown Chernozem
Average of 2018 sites					106	62.3	16.1	184	239	77	90	
Barrhead	2019	May 6	Sep. 30	Aug 27	114	69.1	14.2	262	269	132	89	Solonetzic Dark Gray Chernozem
Bon Accord	2019	May 6	Sep. 23	Sept 13	131	72.3	14.0	364	284	161	81	Eluviated Black Chernozem
Red Deer	2019	May 24	Oct. 19	28-Sep	128	74.0	13.4	272	305	71[e]	91	Orthic Black Chernozem
Lethbridge	2019	Apr. 15	Sep. 4	Aug 12	120	60.3	14.2	159[c]	207	71	91	Orthic Dark Brown Chernozem
Average of 2019 sites					123	68.9	14.0	264	266	109	88	

[a]Data is collected from ACIS from each site's respective seeding date to physiological maturity based on DTM.
[b]Long term precipitation obtained from interpolated data.
[c]Growing season precipitation plus the average irrigation volume (29.5 mm) over 8 irrigation events in the growing season (May to August) at the Lethbridge site.
[d]Soil description found via Alberta soil information viewer provided by (Alberta Agriculture and Forestry 2015).
[e]Observed rainfall data for Red Deer location obtained from Lacombe CDA station.

Table 4.2: Average observed Mc Fadden foliar disease severity ratings (0-11) from eight site years (Bon Accord 2018, Barrhead 2018, Lethbridge 2018, Red Deer 2018, Bon Accord 2019, Barrhead 2019, Lethbridge 2019, and Red Deer) at four growth stages, prior to fungicide treatments.

	Leaf assessment timing			
Site	**BBCH 22-23**	**BBCH 30-32**	**BBCH 39-45**	**BBCH 61-63**
		2018		
Bon Accord	0.60	0.00	0.33	0.93
Barrhead	0.14	1.97	2.16	2.64
Lethbridge	.	.	.	1.13
Red Deer	.	.	1.49	1.96
2018 average	**0.37**	**0.98**	**1.32**	**1.66**
		2019		
Bon Accord	0.09	0.01	0.28	1.41
Barrhead	0.01	0.00	0.19	1.58
Lethbridge	0.00	0.00	0.28	0.51
Red Deer	0.00	0.13	0.31	1.30
2019 average	**0.03**	**0.03**	**0.27**	**1.20**

Table 4.3: *P* – values and least square means of AAC Brandon and AAC Viewfield Canadian western red spring (CWRS) wheat, McFadden foliar disease severity, normalized difference vegetation index (NDVI), fusarium head blight (FHB) and deoxynivalenol (DON) levels response to foliar.

	Effects	McFadden	Assess 2.0	NDVI	FDK	DON	
	SiteYr[a]	**<.0001*** **	**0.006** **	**0.0005** **	**<.0001*** **	**<.0001*** **	**0.0003** **
		(0-11)	(%)	(0-1)	(%)	ELISA (mg g^{-1})	LCMSMS (mg g^{-1}) [b]
	Bon Accord 2018	1.23 c	2.24 ab	0.35 bc	0.55 b	0.00009 b	0.00021 b
	Bon Accord 2019	4.64 a	5.33 a	0.38 abc	1.07 a	0.00009 b	-
	Barrhead 2018	1.87 c	2.44 ab	0.32 c	0.12 c	0.00009 b	0.00032 a
	Barrhead 2019	4.74 a	2.63 ab	0.42 ab	1.03 a	0.00010 b	-
	Lethbridge 2018	3.23 b	2.54 ab	0.46 a	0.28 bc	0.00009 b	0.00008 c
	Lethbridge 2019	4.79 a	0.98 b	0.30 c	0.16 c	0.00009 b	-
	Red Deer 2018	1.96 c	2.97 ab	0.35 bc	0.39 bc	0.00009 b	0.00014 bc
	Red Deer 2019	3.38 b	0.77 b	-	0.35 bc	0.00126 a	-
	Cultivar	0.0843	**0.0211***	0.7745	0.204	0.130	0.224
	Cultivar X SiteYr[a]	**<.0001*** **	**0.0006** **	**<.0001*** **	0.685	0.745	0.140
		(0-11)	(%)	(0-1)	(%)	ELISA (mg g^{-1})	LCMSMS (mg g^{-1})
BA18	AAC Brandon	1.00 A	1.95 A	**0.32 A**	0.60	0.00009	0.000229
	AAC Viewfield	1.46 A	2.52 A	**0.39 B**	0.50	0.00009	0.000190
BA19	AAC Brandon	4.84 A	**5.88 A**	0.37 A	1.16	0.00009	-
	AAC Viewfield	4.43 A	**4.78 B**	0.39 A	0.98	0.00010	-
BR18	AAC Brandon	1.86 A	**3.40 A**	0.31 A	0.13	0.00009	0.000339
	AAC Viewfield	1.88 A	**1.48 B**	0.33 A	0.10	0.00009	0.000300
BR19	AAC Brandon	**5.09 A**	2.69 A	0.41 A	1.11	0.00009	-
	AAC Viewfield	**4.38 B**	2.57 A	0.43 A	0.94	0.00011	-
LB18	AAC Brandon	3.45 A	**3.40 A**	0.45 A	0.33	0.00009	0.000071
	AAC Viewfield	3.01 A	**1.68 AB**	0.48 A	0.23	0.00009	0.000085
LB19	AAC Brandon	**5.64 A**	1.46 A	**0.37 A**	0.16	0.00009	-
	AAC Viewfield	**3.94 B**	0.50 A	**0.24 B**	0.16	0.00009	-
RD18	AAC Brandon	1.78 A	**3.87 A**	0.34 A	0.33	0.00009	0.000175

	AAC Viewfield	2.13 A	**2.07 B**	0.35 A	0.46	0.00010	0.000118
RD19	AAC Brandon	**3.70 A**	0.51 A	-	0.39	0.00130	-
	AAC Viewfield	**3.06 B**	1.03 B	-	0.31	0.00122	-
Fungicide		**<.0001*** **	0.0637	**0.0028** **	**0.0092** **	**0.0055** **	0.4488
Fungicide × SiteYr[a]		**<.0001*** **	**0.0289***	0.0847	**<.0001*** **	**<.0001*** **	0.3299
Fungicide × Cultivar × SiteYr[a]		0.639	0.6224	**0.0387***	0.9423	**<.0001*** **	0.3657
		(0-11)	(%)	(0-1)	(%)	ELISA (mg g^{-1})	LCMSMS (mg g^{-1})
Non-treated control		4.01 a	2.99	0.35 b	0.63 a	0.00026 ab	0.00019
Tilt at BBCH 22-23		3.68 a	2.72	0.35 b	-	0.00017 ab	0.00018
Trivapro at BBCH 22-23		3.61 a	2.56	0.37 ab	0.39 b	0.00022 ab	0.00020
Trivapro at BBCH 30-32		3.00 b	2.67	0.36 ab	-	0.00021 ab	0.00020
Trivapro at BBCH 39-45		2.44 c	1.96	0.39 a	-	0.00016 b	0.00019
Prosaro XTR at BBCH 61-63		2.64 bc	2.03	0.39 a	0.46 b	0.00042 a	0.00016
Adjusted CV%		27.0	72.0	14.0	59.0		32.0

[a]Site × Year
[b]2019 sample analysis has been postponed due to COVID-19
Significance indicated as *p<0.05, **p<0.01, ***p<0.001
Bolded letters = significance

Table 4.4: Results of the most common foliar diseases found in 2018 and 2019 at different growth stages from Barrhead, Bon Accord, Red Deer and Lethbridge, AB.

Growth Stage of Disease Rating	Number of STB[a]	Number of SNB[b]	Number of Tan spot	**Total**
		2018		
BBCH 22-23	0	0	0	**0**
BBCH 30-32	0	0	0	**0**
BBCH 39-45	0	2	1	**3**
BBCH 61-63	0	0	6	**6**
2 weeks after BBCH 61-63	1	0	10	**11**
Total	**1**	**2**	**17**	**20**
		2019		
BBCH 22-23	0	0	0	**0**
BBCH 30-32	0	0	0	**0**
BBCH 39-45	0	0	0	**0**
BBCH 61-63	0	0	0	**0**
2 weeks after BBCH 61-63	12	7	2	**21**
Total	**12**	**7**	**2**	**21**

[a]STB = Septoria tritici blotch, caused by *Zymoseptoria tritici*
[b]SNB = Septoria nodorum blotch, caused by *Stagonospora nodorum*
[c]Tan spot, caused by *Pyrenphora tritici-repenti*

Table 4.5: Most common *Fusarium spp.* found in the 2018 and 2019 growing seasons when subjected to fungicide treatments at different growth stages.

Fungicide treatment	Number of *F.gr*[a]	Number of *F. cul*[b]	Number of *F. poae*[c]	Number of *F. ave*[d]	**Total**
			2018		
Non-treated control	1	5	24	15	**45**
Trivapro A + B at BBCH 22-23	0	1	24	9	**34**
Prosaro XTR at BBCH 61-63	0	1	24	8	**33**
Total	**1**	**7**	**72**	**32**	**112**
			2019		
Non-treated control	6	0	1	31	**38**
Trivapro A + B at BBCH 22-23	4	5	2	30	**41**
Prosaro XTR at BBCH 61-63	0	1	2	24	**27**
Total	**10**	**6**	**5**	**85**	**106**

[a]*F. graminearum*
[b]*F. culmorum*
[c]*F. poae*
[d]*F. avenaceum*

Table 4.6: Results of the most common foliar diseases in two Canadian western red spring (CWRS) cultivars across four different sites (Bon Accord, Barrhead, Lethbridge, Red Deer), AAC Brandon and AAC Viewfield, in 2018 and 2019.

Cultivar	Number of STB[a]	Number of SNB[b]	Number of Tan spot[c]
	2018		
AAC Brandon	**0**	**0**	**9**
Bon Accord	0	0	1
Barrhead	0	0	2
Lethbridge	0	0	1
Red Deer	0	0	5
AAC Viewfield	**1**	**2**	**6**
Bon Accord	0	0	1
Barrhead	0	0	1
Lethbridge	1	0	2
Red Deer	0	2	2
Total	**1**	**2**	**15**
	2019		
AAC Brandon	**1**	**6**	**2**
Bon Accord	0	1	2
Barrhead	1	2	0
Lethbridge	0	0	0
Red Deer	0	3	0
AAC Viewfield	**6**	**6**	**0**
Bon Accord	4	2	0
Barrhead	2	4	0
Lethbridge	0	0	0
Red Deer	0	0	0
Total	**7**	**12**	**2**
Grand Total	**8**	**14**	**17**

[a]STB = Septoria tritici blotch, caused by *Zymoseptoria tritici*

[b]SNB = Septoria nodorum blotch, caused by *Stagonospora nodorum*

[c]Tan spot, caused by *Pyrenphora tritici-repenti*

Table 4.7: Most common *Fusarium spp.*in two Canadian western red spring (CWRS) cultivars, AAC Brandon and AAC Viewfield, found in the 2018 and 2019 growing seasons.

Cultivar	Number of *F.gr*[a]	Number of *F. cul*[b]	Number of *F. poae*[c]	Number of *F. ave*[d]	**Total**
			2018		
AAC Brandon	1	3	52	14	**70**
AAC Viewfield	0	5	47.5	21	**73.5**
Total	**1**	**8**	**99.5**	**35**	
			2019		
AAC Brandon	8	4	4	57	**73**
AAC Viewfield	5	3	3	50	**61**
Total	**13**	**7**	**7**	**107**	
Grand Total	**18**	**34**	**350**	**216**	

[a]*F. graminearum*
[b]*F. culmorum*
[c]*F. poae*
[d]*F. avenaceum*

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Chapter 5: Intensified fungicide management: comparison of fungicides with multiple modes of action, higher rates, and multiple applications on Canada Western Red Spring wheat

5.1 Introduction

Fungicides are commonly used in cereal leaf spot management programs. Fungicides help protect against yield loss due to infection by fungal pathogens, and often provide a significant return on investment for cereal producers. However, after years of fungicide use, there are now global examples of fungicide insensitivity in a number of cereal pathogens. For example, septoria tritici blotch (STB) [*Zymoseptoria tritici* (*syns. Mycosphaerella graminicola* (Fuckel) J. Schröt.)] (Murray et. al 2015) is an important disease of wheat crops for which the quinone outside inhibitors (QoI) class of fungicides provided excellent control (Lucas et al. 2015). Repeated use of QoI fungicides resulted in strong selection pressure on fungal populations, which quickly shifted to mainly QoI insensitive individuals, resulting in a loss of the efficacy of this fungicide class in a number of cereal production regions (Lucas et al. 2015). Similarly, QoI-insensitive isolates have emerged in the UK, northwestern Europe, the USA, and New Zealand, with some jurisdictions imposing strict limitations on the use of QoI fungicides (i.e., one or two applications per season). In addition to STB, powdery mildew of cereals [*Blumeria graminis* (DC) Speer f. sp. *tritici* emend. É. J. Marchal] and tan spot [*Pyrenophora tritici-repentis* (Died.) Drechsler] also quickly became insensitive to QoI fungicides (Jørgensen and Thygesen 2006; Lucas et al. 2015). There were also reports of tan spot population insensitivity to group 3 and group 11 fungicides, because of frequent fungicide applications (Reimann and Deising 2005). Furthermore, stripe rust [*Puccinia striiformis* Westend] was found to be less sensitive to group 3 fungicides in the UK (Bayles et al. 2000) and an *in vitro* study showed that one phenotype of *Fusarium graminearum* exhibited insensitivity to this group (Becher et al. 2010).

The adoption of best management practices (BMPs) for fungicide use can delay the risk of fungicide insensitivity. A mixture of two or more fungicide modes of action (MOA) tends to dilute the risk of insensitivity for each individual at-risk fungicide by adding additional, unique requirements for insensitive individuals in the pathogen population (Brent and Holloman 2007). In addition to reducing the risk of insensitivity, commercial fungicides with more than one MOA may also result in improved disease control (Brent and Holloman 2007).

According to Fungicide Resistance Action Committee (FRAC), a BMP for avoiding fungicide insensitivity is restricting the number of applications per season, or spraying only when strictly necessary (Brent and Holloman 2007). This will minimize selection, reduce buildup of insensitive strains, and avoid the emergence of insensitive populations. However, in many cereal production areas, growers rely on multiple preventive fungicide applications to protect their crops from infection by fungal pathogens. In Luxembourg, for example, farmers must often apply two to three foliar fungicide applications per season to avoid significant yield loss (El Jarroudi et al. 2015). In situations where multiple applications are required to protect crop yield and/or quality, the rotation of fungicide MOA classes may reduce the risk of fungicide insensitivity (Brent and Holloman 2007). It is essential to avoid repeat applications of the same fungicide group, with the same MOA, in a single or subsequent season.

For many years, some growers used reduced rates of fungicides, especially under low disease pressure, to reduce input costs. However, FRAC suggests that reduced rates can enhance the risk of fungicide resistance (Brent and Holloman 2007). Lowering the dose may select for fungicide insensitivity by decreasing the overall effectiveness of the fungicide and exposing many individuals to a sub-lethal dose. Hence, the application of fungicides rates below that indicated on the product label is not recommended.

The BMPs for minimizing fungicide insensitivity raises the questions: ‘do multiple fungicide applications provide a return on investment?’, and ‘if sub-label rates are detrimental, are higher fungicide rates above the label rate beneficial?’. To our knowledge, there are no studies testing higher rates of triazole fungicides in wheat, but a study on canola found that higher fungicide rates increased yield while further reducing disease (Rempel and Hall 1995).

Therefore, the objective of the present report was to perform replicated, small-plot trials across Alberta and observe the yield, quality, and economic benefits associated with the application of multiple fungicide MOAs, higher than label rates, and multiple fungicide applications in a single season. Experiments were conducted on two of the most commonly grown Canadian Western Hard Red Spring (CWRS) wheat cultivars, AAC Brandon (Cuthbert et al. 2016) and AAC Viewfield (Cuthbert et al. 2018).

5.2 Materials and methods

5.2.1 Field setup

Field experiments were conducted at three rain-fed (Barrhead, Bon Accord and Red Deer) and one irrigated site (Lethbridge) in Alberta, Canada, over two growing seasons in 2018 and 2019. Data on precipitation and relative humidity (RH) were collected from the nearest Alberta Climate Information System (ACIS) station from seeding date to physiological maturity based on days to maturity (DTM) as mentioned in Chapter three under ‘3.2.1. Field Setup’.

5.2.2 Fungicide treatments

This study included 12 fungicide treatments and one non-treated control (Table 5.1) designed as a split plot (Appendix 1). The main plot was cultivar and the sub-plot was fungicide treatment. The treatments compared were: single, dual, and triple fungicide applications; recommended and 1.5x rates of fungicide; and a single versus multiple MOA on AAC Brandon and AAC Viewfield CWRS cultivars. The three fungicide products represented three fungicide MOAs: the triazoles (group 3), strobilurins (group 11) and carboxamides or SDHIs (group 7).

5.2.3 Data collection

Foliar disease was evaluated visually and rated on the assessment scale of McFadden (1991) (Appendix 2) on the same treatments and visual fusarium head blight ratings, percent FDK were collected and DON analysis as mentioned in Chapter four under section '4.2.3. Data Collection'.

Days to Maturity, TKW (g 1000 kernels ^{1}), test weights and grain protein were collected as well as mentioned in Chapter three under section '3.2.3. Data Collection'.

The economic analysis compared yield increases obtained with dual and triple fungicide applications relative to a single fungicide application. Fungicide costs were based on the fee structure of a typical agri-chemical retail company in northcentral Alberta. The yield required to breakeven was calculated as: (total fungicide application cost + cost of fungicide) / (current wheat price) (Cornell 2020).

5.2.4 Statistical analysis

The study was conducted over eight site years. However, this paper only reports data from sites where a statistically significant yield response to the fungicide treatment was observed (Bon Accord [BA19], Barrhead 2019 [BR19], Red Deer 2018 [RD18] and Red Deer [RD19]). Results from sites that were non-responsive to single fungicide applications are presented in Asif et al. (2020a and 2020b). Data from the four responsive site - year combinations were analyzed using PROC GLIMMIX of SAS Studio v. 3.8 (SAS Institute Inc. 2012-2018).

The cultivar, fungicide treatments, four environments (site - year combinations) and their interactions were treated as fixed effects. Blocks and their interactions with all fixed effects were considered as random effects.

The DON data followed a Poisson distribution and were re-coded into a new discrete variable, where the value was multiplied by 100,000 and analyzed via PROC GLIMMIX. The lsmeans were then back-transformed from log-scale using the 'ilink' option.

For both normal and non-normal data, single degree freedom orthogonal contrasts were constructed to partition the total variation of fungicide treatments and determine responses to specific fungicide treatment combinations at a significance level of $\alpha = 0.05$.

5.3 Results and discussion

5.3.1 Effect of environment

The average observed precipitation from seeding to physiological maturity was 273 mm (194 – 364 mm), or approximately 96% of the long-term average (LTA) (69-128% of the LTA) (Table 3.2 – Responsive Sites). Bon Accord 2019 had the highest observed precipitation (364 mm) and the lowest was at RD18 (194 mm).

Relative humidity, on average, was 70.2% (65.4 – 74.0%). Red Deer 2019 had the highest RH (74.0%) and RD18 had the lowest (65.4%) (Table 3.2 – Responsive Sites). Temperature was similar across the sites (average of 14.2 °C). In order to evaluate the effect of the environment on the risk of FHB infection, the ACIS fusarium disease severity risk prediction tool (Alberta Agriculture and Forestry 2019) was used. An extreme example of the effect of the environment on disease risk was observed at the Red Deer site, where the average risk score in 2019 was 30.3 which was 97% greater than the FHB risk in 2018 (1.0).

5.3.2 Single vs multiple MOA: Tilt 250 E (Group 3) vs Trivapro A + B (Group 3 + 7 + 11) effects on yield and foliar disease

There were no statistical differences between yields, DTM, test weights, TKW or protein for the fungicide applications with a single MOA [Tilt 250 E (group 3)] versus multiple MOA [Trivapro A+B (group 3, 7, and 11)] at BBCH 22-23 (2-3 tillers detectable) (Table 5.2).

The McFadden foliar disease severity ratings were significantly different when comparing the non-treated control versus the single MOA [group 3 (Tilt 250 E)] and multiple MOA [group 3 + 7 + 11 (Trivapro A+B)] fungicide treatments (Table 5.2). Foliar disease in the non-treated control was 13% higher than in the treatments receiving fungicide applications. However, there was no difference between the single MOA (group 3) and multiple MOA (group 3 + 7 + 11) fungicide treatments. Synergistic interactions for fungicides with different MOA tend to affect fungi at different biochemical sites and at different developmental stages (Gisi 1996). The similar results obtained with the Tilt 250 E (propiconazole) and Trivapro A+B (propiconazole, azoxystrobin, and benzovindiflupyr) treatments likely reflect that both of these fungicides are known to effectively reduce most foliar diseases of wheat (Nagelkirk and Chilvers 2018), and that disease pressure at BBCH 22-23 was very low. Foliar disease severity ratings were 0.09 at BA19, 0.01 at BR19, 0.00 at LB19 (Table 4.2), and there was no data recorded at RD18 due to lack of technical help. Previous reports indicate that a fungicide application at BBCH 22-23 does not improve yields (Asif 2020a; Kutcher et al. 2018). Thus, unless high disease pressure developed later in the growing season, it was unlikely that differences would be observed between the single and multiple MOA fungicide treatments. Despite no yield or agronomic benefits, however, multiple MOA fungicides are beneficial in delaying fungicide resistance (Brent and Holloman 2007).

5.3.3 Recommended vs. high fungicide rates: Trivapro B at 30 gai ha^{-1} (recommended rate) vs. 75 gai ha^{-1} (high rate)

There were no significant yield differences between the recommended and high fungicide rates when applied as either a single or a dual application (Table 5.3). This suggested that the recommended rate was sufficient and there was no additional yield benefit to using a higher rate of Trivapro B (active ingredient: benzovindiflupyr).

There was a significant DTM response (Table 5.3) attributed to trends at BA19 and RD18 (data not shown). At BA19, the dual fungicide treatment with the high fungicide rate took 0.6 days longer to mature. This was expected, as it known that fungicide applications, especially at a higher rate or a greater number of applications, tend to delay ripening of the crop (Jørgensen and Olesen 2002). However, at RD18, the recommended fungicide rate took 0.57 - 0.64 days longer to mature compared with the high fungicide rate. This was unexpected and perhaps resulted from an unusual fungicide x environment interaction.

Significant test weight differences between fungicide treatments occurred at two of the four sites, BA19 and BR19. At these sites, the non-treated control had 1.0% to 1.2% lower test weights versus the fungicide treatments (data not shown). However, there were no significant differences between high and recommended fungicide rates.

Significant thousand kernel weight differences occurred at two of the four sites, BA19 and BR 19. However, there were no differences between fungicide rates (data not shown). Significant differences were observed only for the non-treated control, which had a 4-5% lower TKW versus the fungicide treatments.

The McFadden foliar disease severity ratings indicated that the non-treated control had 33% higher foliar disease compared with the fungicide treatments (Table 5.3). However, there were no significant differences between the recommended and high fungicide rates.

DON concentrations in grain samples, as measured by ELISA, were significantly different (Table 5.3), which could be attributed to RD19 and BR19 trends (data not shown). At BR19, the non-treated control had 22% higher DON levels versus the fungicide 'rate' treatments, but there were no significant differences between the recommended and high fungicide rates. At RD19, the ELISA DON results did not follow expected trends; however, the RD19 samples were not dried adequately after harvest, possibly resulting in post-harvest fungal growth and mycotoxin production, which may explain the unexpected data trends (Bolanos-Carriel et al. 2019).

The findings from this study were not consistent with those of a canola study that found higher fungicide rates resulted in higher yields (Rempel and Hall 1995). However, a study testing the effects of fungicides on winter wheat yield indicated that half rates were not significantly different than full rates (Milus 1994). Most of the variability in the Milus (1994) study was attributed to the environment and its interaction with the fungicide. This interaction may explain the lack of response to higher fungicide rates in the current study. Furthermore, disease severity is highly variable in time and space, and greatly influenced by the environment (Gaunt, 1995). The current study had low foliar disease pressure at BBCH 30-32 (average disease severity ratings at BA19 = 0.01, BR19 = 0.00, RD19 = 0.13 and RD18 = not available) (Table 4.3). If disease pressure had been higher in the current study at BBCH 30-32, then perhaps there would have been a greater opportunity to observe any significant responses to the fungicide rate treatments.

5.3.4 Single vs. dual vs. triple fungicide applications

Yield differed significantly in response to the single, dual, and triple fungicide treatments (Table 5.4). Averaged over all sites, there were significant differences between the single versus dual and triple fungicide treatments. The single fungicide treatments had 3% lower yields (0.2 t ha^{-1}) versus dual and triple fungicide treatments. There also was a significant yield difference between dual and triple fungicide treatments. The triple fungicide treatments had 4% (0.3 t ha^{-1}) lower yields than the dual fungicide treatments. This was unexpected but may be explained by the trends seen at each individual site.

Each individual site had slightly different yield responses to single, dual and triple fungicide treatments, accounting for the significant fungicide × site year interaction (Table 5.4). At BR19 and BA19, the single fungicide applications resulted in 11% lower yield vs. the dual and triple treatments, and the dual treatments resulted in 5% lower yields than the triple treatments (data not shown). It is known that multiple fungicide applications increase yield by providing additional protection from foliar disease (Wiersma and Motteberg 2005). In the current study, a strong response to multiple fungicide applications was observed at BR19 and BA19, which had the highest levels of foliar disease. Yields at BR19 and BA19 were lowest for single application < dual applications < triple applications. Red Deer 2019 showed significant yield differences between the two triple fungicide applications with applications at BBCH 22-23 + 39-45 + 61-63 having 18% lower yields vs. the triple fungicide applications at BBCH 30-32 + 39-45 + 61-63. Unexpectedly, a triple application of fungicide at RD18 found resulted in 21% significantly lower yields than a dual application. However, the lower yields with triple fungicide application at this site may have reflected trampling damage associated with the additional fungicide pass.

Trends in yields were generally supported by the foliar disease severity ratings at three of the four sites. Averaged over all sites, there were significant differences in the McFadden disease severity ratings (Table 5.4). The single fungicide treatments had 45% higher foliar disease compared with the average of the dual and triple fungicide treatments. The dual fungicide treatments had 35% higher foliar disease compared with the triple fungicide treatments. More fungicide applications allowed for the 'green' of the leaves to be protected and losses from foliar diseases to be minimized (Kutcher et al. 2018). One example where yield and disease severity were not well correlated occurred at RD18. Disease severity ratings are a measure of the presence and virulence of the pathogen but are not a direct measurement of the plants' departure from normal function. Therefore, in some instances the measurement of disease severity does not predict yield (Gaunt, 1995). This is especially true in situations of low disease incidence or severity, where the pathogen presence remains below a threshold that is tolerated by the host plant with little or no effect on yield.

When comparing the timing of the individual applications in a dual or triple fungicide treatment, there was no statistical difference based on the timing of the first application (Table 5.4). This suggests that the timing of the first fungicide application (i.e., at BBCH 22-23 vs. 30-32 vs. 39-45) in the dual and triple fungicide applications did not matter. However, previous reports have concluded that the timing of fungicide applications does matter. Asif et al. (2020a, 2020b), for example, found that yields were 9.3% greater when single fungicide applications were made at BBCH 39-45 and 61-63 vs. BBCH 22-23 and 30-32. We suggest that the fungicide applications at BBCH 61-63 were exclusively responsible for protection against foliar disease, and therefore early fungicide application at BBCH 22-23 or 30-32 did not affect yield. If this was true, the timing of early season fungicide applications was irrelevant. A number of other reports have confirmed that early season fungicide applications do not affect yield. MacLean et al. (2018) and Wiersma and Motteberg (2005) both found that by spraying fungicide at BBCH 61-63 provided adequate control for leaf spots, especially under conditions similar to those reported herein. However, it should be noted that leaf diseases and yield are not correlated. As seen in chapter three and four, a high yielding fungicide treatment (Prosaro XTR at BBCH 61-63) was not significantly different in foliar disease levels when compared to the Trivapro A+B treatment at BBCH 30-32 (Table 3.3 and 4.3).

Averaged over all sites, the single fungicide treatments had 3% significantly lower TKW than the dual and triple treatments (Table 5.4). Individual sites followed the same trend with single fungicide treatments having significantly less TKW (BR19 = 4% lower, BA19 = 5%, RD19 = 2% and RD18 = 1%) than the dual and triple fungicide treatments (data not shown). This was similar to the results of El Jarroudi et al. (2015) on winter wheat, where they reported relatively higher TKW in dual and triple treatments. This is another example of higher foliar disease reducing yield and quality with single fungicide treatments compared with the dual and triple fungicide treatments. In comparing the timing of the individual fungicide applications that made up the dual fungicide treatments, the highest TKW (41.4 g 1000 seeds^{-1}) was achieved with the dual fungicide applications at BBCH 39-45 followed by a second fungicide application at BBCH 61-63. This was expected, as previous studies have shown that the best time for fungicide applications is at BBCH 39-45 or BBCH 61-63 (Asif 2020a; Milus 1994; Beard 2018). There were no differences between timings for triple fungicide applications.

Averaged over all sites, there was no difference in FDK among the single vs. dual and triple fungicide treatments (Table 5.4). Looking at each site individually, BA19 was the only location with a significant response to the single vs. dual and triple fungicide treatments (data not shown). The single treatments at BA19 had 34% higher FDK vs. the dual and triple fungicide treatments. This trend at BA19 seems reasonable since many of the single fungicide treatments were not applied after heading when we would anticipate FHB control. Additionally, the highest FHB risk (40 = severe FHB risk) was at BA19.

DON accumulation in grain samples differed depending on single, dual, and triple fungicide applications (Table 5.4). However, RD19 was the only site that showed a significant difference between the fungicide treatments (data not shown). At RD19, DON measurements from this site did not follow expected trends but harvested grain samples at this site were not adequately dried, possibly resulting in post-harvest fungal growth and mycotoxin production, which may explain the unexpected data trends (Bolanos-Carriel et al. 2019).

The DTM, test weight, protein and LC/MS/MS DON did not differ significantly between single, dual, or triple fungicide treatments (Table 5.4).

5.3.5 Economic benefits of single versus dual versus triple fungicide applications

The highest yield benefit was achieved with the dual fungicide treatments. These dual treatments resulted in a 5% yield increase (0.4 t ha^{-1}) compared with the single fungicide applications (Table 5.4). The revenue needed for the dual fungicide application of Trivapro A + B and Prosaro XTR to breakeven is \$130.08 ha^{-1} (Table 5.5). Compared with a single fungicide application, the dual fungicide application required, on average, an additional yield increase of 0.27 – 0.31 t ha^{-1} to be profitable. Therefore, based on the average single vs. dual yields obtained in this study, a dual fungicide application was profitable as the average yield increase for dual applications over single was 0.4 t ha^{-1}.

The triple fungicide applications yielded 0.10 t ha^{-1} more than the single fungicide applications (Table 5.4). However, the triple fungicide treatments required \$188.70 ha^{-1} in extra revenue or an average yield increase of 0.50 – 0.57 t ha^{-1} (over single fungicide yields) to breakeven (Table 5.5). Although the triple fungicide application reduced foliar disease by 35% compared with the dual fungicide treatments, it did not adequately increase yields to cover costs, another example of plant disease severity not strictly linked to yield (Gaunt, 1995). While triple fungicide applications may often provide a superior reduction in disease severity, they are not recommended due to the lack of positive financial return on the required investment.

Based on the yields, costs, and wheat prices in this study, theoretically, dual fungicide treatments seem reasonable. However, there are several factors that must be considered prior to making fungicide application decisions, namely the environment, disease incidence and severity, and the host/cultivar. In related papers (Asif 2020a; 2020b), significant responses to fungicides occurred at only four of eight site years, meaning that fungicide application was only beneficial half the time, mainly due to environmental effects on disease pressure. Under drier conditions, there was not significant disease pressure to offset the cost of a fungicide application. However, the two CWRS cultivars used in these studies did not have sufficient foliar disease resistance to prevent yield loss under all conditions and exhibited a significant yield response to the fungicide applications in the disease-conducive environments. Therefore, under conditions favorable for disease development, a dual fungicide application may be justified, as it resulted in an additional 0.40 t ha^{-1} of yield compared with a single fungicide application, and an additional 0.9 t ha^{-1} compared with the non-treated plots under the conditions in this study.

5.4 Conclusion

There were no statistical differences for leaf disease, DTM, yield or grain quality between application of one fungicide MOA (Tilt 250 E, group 3) and three MOA (Trivapro A+B, group 3 + 7 + 11) at BBCH 22-23. This contrasts with older studies, which found a diverse mix of fungicide groups provided more control over foliar disease and maintained yield and quality (Jørgensen and Olsen 2007). However, both Trivapro A+B and Tilt 250 E are both effective protectant fungicides, and disease pressure at BBCH 22-23 was very low, so it may have been difficult to detect any potential advantages of this mixture under these conditions. If single versus multiple MOA had been compared at a later growth stage, when more disease was present, there may have been a difference between the single and multiple MOA treatments. Furthermore, in cases were fungicide insensitivity to one of the fungicide groups is developing in a field, multiple MOA treatments may show increased efficacy since they would dilute the risk of fungicide insensitivity and delay fungicide resistance.

In the current study, no agronomic, yield or quality differences were detected between the recommended (30 gai ha^{-1}) versus the high rates (75 gai ha^{-1}) of Trivapro B (benzovindiflupyr) applied at BBCH 30-32. Again, if the recommended versus high rates of this fungicide had been compared at a later growth stage (such as at BBCH 39-45), when more disease was present, differences may have been observed.

There were agronomically relevant, yield and quality differences when comparing single, dual and triple fungicide applications. Single fungicide treatments saw 3% lower yields vs. the dual and triple fungicide applications. At BR19 and BA19, the single treatments had 11% significantly lower yield versus the dual and triple treatments and the dual treatments had 5% significantly lower yields than the triple fungicide treatments. In addition, the single fungicide treatments had 4% lower TKW versus the dual and triple treatments. These yield and quality trends highlight the benefit of multiple fungicide applications, at least under the conditions of this study.

The protection of yield and quality by the fungicides in this study generally reflected enhanced disease control. The most economically beneficial tactic at sites with significant disease pressure was the dual fungicide treatment. Under these conditions, dual applications were best able to reduce disease severity and minimize yield loss compared with a non-treated and single fungicide application. While a triple application of fungicide resulted in the lowest disease severity ratings at some sites, the cost of three applications was not recovered by the increases in yield under these conditions.

Table 5.1: Description of 12 fungicide treatments and a non-treated control applied on wheat at different growth stages and rates in field plot experiments at four locations in Alberta, Canada, in 2018 and 2019.

		First fungicide application			Second fungicide application			Third fungicide application		
Fungicide treatment		Fungicide Product	Rate	GS[a]	Fungicide Product	GS[a]	Rate	Fungicide Product	GS[a]	Rate
			gai ha^{-1}	BBCH		BBCH	gai ha^{-1}		BBCH	gai ha^{-1}
Single	1	Tilt 250 E[b]	62.5	22-23	-	-	-	-	-	-
	2	Trivapro A[c] + B[d]	200 + 30	22-23	-	-	-	-	-	-
	3	Trivapro A + B	200 + 30	30-32	-	-	-	-	-	-
	4	Trivapro A + B	200 + **75**	30-32	-	-	-	-	-	-
	5	Trivapro A + B	200 + 30	39-45	-	-	-	-	-	-
	6	Prosaro XTR[e]	200	61- 63	-	-	-	-	-	-
Dual	7	Trivapro A + B	200 + 30	22-23	Prosaro XTR	61- 63	200	-	-	-
	8	Trivapro A + B	200 + 30	30-32	Prosaro XTR	61- 63	200	-	-	-
	9	Trivapro A + B	200 + **75**	30-32	Prosaro XTR	61- 63	200	-	-	-
	10	Trivapro A + B	200 + 30	39-45	Prosaro XTR	61- 63	200	-	-	-
Triple	11	Trivapro A + B	200 + 30	22-23	Trivapro A + B	39-45	200 + 30	Prosaro XTR	61- 63	200
	12	Trivapro A + B	200 + 30	30-32	Trivapro A + B	39-45	200 + 30	Prosaro XTR	61- 63	200
	13	Non-treated control	-	-	-	-	-	-	-	-

[a]Growth Stage
[b]Fungicide group 3 = propiconazole
[c]Fungicide group 3 = propiconazole and Fungicide group 11 = azoxystrobin
[d]Fungicide group 7 = benzovindiflupyr
[e]Fungicide group 3 = prothioconazole and tebuconazole

Table 5.2: Analysis of variance and orthogonal contrasts for different fungicide modes of action (MOA) effects on disease severity, days to maturity (DTM), yield, test weight, thousand kernel weight (TKW), and protein at four sites that were responsive to fungicide application over two growing seasons in Alberta, Canada.

Effects	McFadden leaf disease	DTM	Yield	Test weight	TKW	Protein
	Analysis of variance P-value					
Fungicide	0.0928	0.3406	0.4747	0.7675	0.7918	0.5368
Fungicide × SiteYr[a]	0.3577	0.8485	0.4245	0.3795	0.1796	0.0775
Fungicide × Cultivar × SiteYr[a]	0.6728	0.3248	0.7372	0.7582	0.3097	0.3611
	Contrast P-value					
Non-treated control vs Tilt 250E (Gr 3) and Trivapro (Gr 3+7+11)	**0.0364***	0.2866	0.2618	0.4852	0.8966	0.3077
Tilt 250E (Gr 3) vs Trivapro (Gr 3+7+11)	0.8557	0.2858	0.7381	0.9711	0.5188	0.7404
	(0-11)	(days)	(t ha^{-1})	(kg hL^{-1})	(g 1000 $seeds^{-1}$)	(mg g^{-1})
Non-treated control	4.5	119.9	6.7	74.0	38.5	146
Tilt 250E (Gr 3)	4.0	120.0	6.9	74.0	38.4	143
Trivapro (Gr 3 + 7 + 11)	4.0	120.5	6.9	74.0	38.6	144

[a]Site x Year

Significance indicated as *p<0.05, **p<0.01, ***p<0.001

Bolded letters = significance

Table 5.3: Analysis of variance and orthogonal contrasts comparing different fungicide rates applied on wheat at BBCH 30-32 for their effects on disease severity, days to maturity (DTM), yield, test weight, thousand kernel weight (TKW), protein, and deoxynivalenol (DON) at four responsive sites over two growing seasons in Alberta, Canada.

							DON	
Effects	McFadden	DTM	Yield	Test weight	TKW	Protein	ELISA	LC/MS/MS[d]
				Analysis of variance *P*-value				
Fungicide	**0.0004****	0.0666	**0.0005****	0.7256	**<.0001*****	0.0819	**0.0307***	0.8646
Fungicide × SiteYr[a]	0.3143	**0.0151***	0.1206	**0.0183***	**0.0001*****	0.6297	**0.0104****	-
Fungicide × Cultivar × SiteYr[a]	0.4550	0.6256	0.6463	0.6410	**0.0005****	0.3113	**0.0049**	0.2945
				Contrast *P*-value				
Non-treated control vs Fungicide treatments (high and recommended rates)	**0.0001*****	0.2398	**0.0167****	0.2569	**0.0003****	0.1453	0.3790	0.5177
Single (high vs recommended rates)	0.8716	0.0922	0.1207	0.5303	0.9465	0.7524	**0.0173***	0.4414
Dual (high vs recommended rates)	0.8998	0.0613	0.7041	0.9371	0.4125	0.1851	**0.0410***	0.9159
	(0-11)	(days)	(t ha^{-1})	(kg hL^{-1})	(g 1000 $seeds^{-1}$)	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})
Non-treated control	4.5	119.9	6.7	73.8	38.4	146	0.00030	0.000178
Trivapro (RR)[b]	3.7	120.1	6.5	74.0	38.7	146	0.00028	0.000125
Trivapro **(HR)**[c]	3.8	119.7	6.8	74.3	38.7	145	0.00045	0.000163
Trivapro (RR)[b] at BBCH 30-32 + Prosaro at BBCH 61-63	3.0	120.9	7.5	74.0	40.3	142	0.00042	0.000154
Trivapro **(HR)**[c] at BBCH 30-32 + Prosaro at BBCH 61-63	3.0	120.0	7.4	74.1	40.5	139	0.00023	0.000160

[a]Site x Year
[b]Recommeded fungicide rate (30 gai ha^{-1})
[c]High fungicide rate (75 gai ha^{-1})

[d]Only the RD18 data has been analyzed. Analysis of the 2019 samples has been delayed due to COVID-19
Significance indicated as *p<0.05, **p<0.01, ***p<0.001
Bolded letters = significance

Table 5.4: Analysis of variance and orthogonal contrasts for single, dual and triple fungicide treatments for their effects on wheat disease severity, days to maturity (DTM), yield, test weight, thousand kernel weight (TKW), protein, fusarium damaged kernels (FDK), and deoxynivalenol (DON) at four sites responsive to fungicide treatment over two growing seasons in Alberta, Canada.

Effects	Disease severity	DTM	Yield	Test weight	TKW	Protein	FDK	DON (ELISA)	DON[b] (LC/MS/MS)
					Analysis of variance *P*-value				
Fungicide	**<.0001*** **	0.2766	**<.0001*** **	0.9803	**<.0001*** **	0.3636	**0.0267***	**<.0001*** **	0.1677
Fungicide × SiteYr[a]	0.0954	0.4127	**<.0001*** **	0.4704	**0.0071** **	0.8529	**0.0036** **	**<.0001*** **	-
Fungicide × Cultivar × SiteYr[a]	0.3298	0.3306	0.053	0.9599	0.1755	0.2063	0.7764	**<.0001*** **	0.1805
					Contrast *P*-value				
Single vs. (Dual + Triple)	**<.0001*** **	0.4669	**<.0001*** **	0.9305	**<.0001*** **	0.2599	0.0991	0.404	**0.0156***
Dual vs. Triple	**<.0001*** **	0.4731	**0.0079** **	0.833	0.1978	0.1315	-	**0.0249***	0.9574
	(0-11)	(days)	($t\ ha^{-1}$)	($kg\ hL^{-1}$)	(g 1000 $seeds^{-1}$)	($mg\ g^{-1}$)	(%)	($mg\ g^{-1}$)	($mg\ g^{-1}$)
Single Fungicide	3.4	120.5	7.2	74.0	39.6	144	0.58	0.00041	0.00014
Dual Fungicide	2.7	120.7	7.6	74.1	40.8	143	0.50	0.00033	0.00020
Triple Fungicide	2.0	120.5	7.3	74.0	41.1	141	-	0.00055	0.00020
					Contrast *P*-value				
Dual at BBCH 30-32 + BBCH 61-63 vs BBCH 22-23 + BBCH 61-63 and BBCH 39-45+ BBCH 61-63)	**0.0162** **	0.4461	0.5793	0.8855	**0.0187***	0.4802	-	**0.0052** **	0.1299
Dual at BBCH 22-23 + BBCH 61-63 and BBCH 39-45+ BBCH 61-63	**0.0016** **	0.1042	0.4339	0.2822	**0.0447***	0.8691	-	**<.0001*** **	0.6552
	(0-11)	(days)	($t\ ha^{-1}$)	($kg\ hL^{-1}$)	(g 1000 $seeds^{-1}$)	($mg\ g^{-1}$)	(%)	($mg\ g^{-1}$)	($mg\ g^{-1}$)
Dual Fungicide at BBCH 22-23 + BBCH 61-63	2.9	120.0	7.65	73.8	40.7	144	0.50	0.00027	0.00021

Dual Fungicide at BBCH 30-32 + BBCH 61-63	3.0	120.9	7.52	74.0	40.3	142	-	0.00041	0.00015
Dual Fungicide at BBCH 39-45 + BBCH 61-63	2.2	120.3	7.54	74.3	41.4	144	-	0.00030	0.00024
					Contrast *P*-value				
Triple at BBCH 22-23 + BBCH 39-45 + BBCH 61-63 vs Triple at BBCH 30-32 + BBCH 39-45 + BBCH 61-63	0.1967	0.5456	0.0691	0.4621	0.7031	0.8932	-	**<.0001*****	0.4191
	(0-11)	(days)	(t ha^{-1})	(kg hL^{-1})	(g 1000 $seeds^{-1}$)	(mg g^{-1})	(%)	(mg g^{-1})	(mg g^{-1})
Triple Fungicide at BBCH 22-23 + BBCH 30-32 + BBCH 61-63	2.2	120.4	7.15	74.2	41.2	141	-	0.00023	0.00018
Triple Fungicide at BBCH 30-32 + BBCH 39-45 + BBCH 61-63	1.9	120.7	7.44	73.9	41.0	141	-	0.00090	0.00022

[a]Site x Year
[b]Only the RD18 data has been analyzed. Analysis of the 2019 samples has been delayed due to COVID-19
Significance indicated as *p<0.05, **p<0.01, ***p<0.001
Bolded letters = significance

Table 5.5: Estimated economic returns required for dual and triple fungicide applications of Trivapro A+B and Prosaro XTR compared with a single fungicide application of Trivapro A + B on wheat at BBCH 39-45 at four sites responsive to fungicide treatment over two years in Alberta, Canada.

Year	Wheat price [a]	Application cost	Fungicide cost[bc]	Total application and fungicide cost	Yield[d] increase needed to breakeven with dual or triple fungicide applications	Yield[d] increase needed relative to a single fungicide application of Trivapro A+B at BBCH 39-45[e]	Additional yield[d] needed to breakeven over a single fungicide application[f]
	($ t^{-1})[a]	($ ha^{-1})	($ ha^{-1})	($ ha^{-1})	(t ha^{-1})	(t ha^{-1})	(t ha^{-1})
	Dual Application of Trivapro A+B at BBCH 39-45 and Prosaro XTR at BBCH 61 – 63						
2018	259.78	35.00	95.08	130.08	0.50	0.23	0.27
2019	230.04	35.00	95.08	130.08	0.56	0.25	0.31
	Triple Application of Trivapro A+B at BBCH 22-23 and BBCH 39-45 and Prosaro XTR at BBCH 61-63						
2018	259.78	52.50	136.20	188.70	0.73	0.23	0.50
2019	230.04	52.50	136.20	188.70	0.82	0.25	0.57

[a]Average cost of wheat ($ t^{-1}) was collected from price & data quotes (PDG) in 2018 and 2019 from September 3rd to October 31st. The data was averaged each year for southern and northern regions in Alberta.
[b]Calculated costs ($ ha^{-1}) from retail values of fungicide and recommended label rates.
[c]Trivapro A+ B retail price was $666.12/case in a co-pack including two 8.1L jugs of Trivapro A and two 2.43L jugs of Trivapro B. Recommended rates were 400 mL ac^{-1} (1 L ha^{-1}) of A and 120 mL ac^{-1} (0.3 L ha^{-1}) of B.
[c]Prosaro XTR retail price was $433/6.5L jug with recommended rate of 324 mL ac^{-1} (80 mL ha^{-1} or 0.81 L ha^{-1}).
[d]Yield is based on 'AAC Brandon' and 'AAC Viewfield' CWRS wheat under the conditions reported in Table 3.2 – Responsive Sites.
[e]A single application of Trivapro A +B costs $99.74 ha^{-1}.
[f]Additional yield required to breakeven was calculated by subtracting the yield needed to breakeven in a single fungicide application from the yield needed to break even in the dual or triple fungicide applications.

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Chapter 6: Conclusion

6.1. Summary of results

There was a distinct difference between the eight site years in response to fungicide applications. Four of the eight site years (or 'responsive site years') showed yield differences among fungicide treatments, likely because the environment was more conducive for disease development. Overall, at these sites, the earlier fungicide applications at BBCH 22-23 and 30-32 resulted in lower yield and quality than the later fungicide applications at BBCH 39-45 and 61-63. As expected, foliar disease and flag leaf disease was also higher at the earlier versus later applications. The highest yields were achieved with fungicide applications at BBCH 39-45 and 61-63, as earlier fungicide applications did not protect the flag leaf (which contributes 43% to grain filling) since it had not yet emerged. Generally, the single fungicide applications had lower yields and higher foliar disease compared with the dual and triple fungicide applications. However, it should be noted that leaf disease levels and yield are not correlated. As seen in Chapters three and four, the high yielding (7.4 t ha^{-1}) fungicide treatment (Prosaro XTR at BBCH 61-63) had statistically similar levels of leaf disease compared to the low yielding (6.54 t ha^{-1}) Trivapro A+B treatment at BBCH 30-32 (Tables 3.3 and 4.3). This suggests that levels of leaf disease cannot be used to predict yield.

At most sites, no difference was observed between the cultivar responses to fungicide treatments with respect to leaf spot severity, FDK, ELISA and LC/MS/MS data. However, AAC Brandon did have an overall higher level of flag leaf disease at four of eight site years, averaged over all fungicide treatments. These findings, obtained with modern cultivars (AAC Brandon and AAC Viewfield), are generally consistent with previous fungicide studies using older CWRS cultivars.

Based on the results from our study, later fungicide applications at BBCH 39-45 and BBCH 61-63 are recommended as the best time to spray to protect yield and quality of wheat. However, the question 'to spray or not to spray?' is highly dependent on whether or not environmental conditions are conducive for disease development. Four site years in this study experienced conditions conducive to disease development (average relative humidity = 70.2%, average observed precipitation = 273 mm), so the application of fungicides to protect yield and quality at these sites was justified. In addition, at these sites, dual fungicide applications were economically justified. At the remaining site years, however, conditions were not conducive to disease (average relative humidity = 61.1%, average observed precipitation = 175 mm) and fungicide applications were not necessary.

6.2. Summary of results according to each objective

6.2.1. To determine the yield and economic advantages of single fungicide applications at BBCH 22-23 (herbicide timing), BBCH 30-32 (plant growth regulator timing), BBCH 39-45 (flag leaf), and BBCH 61-63 (10-30% of anthers are mature) on two wheat cultivars

The non-treated control and the earlier single fungicide applications at BBCH 22-23 (2 – 3 tillers detectable) and 30-32 (early stem elongation) had significantly lower yields than the later single fungicide applications at BBCH 39-45 (flag leaf) and BBCH 61-63 (head timing). On average, there was a 0.62 t ha^{-1} (or a 9.2%) yield decrease for the earlier fungicide applications compared with the later ones. The later fungicide applications also resulted in significantly greater thousand kernel weights (TKW) compared with the non-treated control and the earlier fungicide applications. On average, TKW was 5% higher in the fungicide applications at BBCH 39-45 and at BBCH 61-63. The single fungicide applications did not have an impact on the days to maturity (DTM), test weight or protein.

The costs of fungicide applications need to be offset by increased yield. From our results, the highest yield was achieved by a single application of Prosaro XTR at BBCH 61-63 (9.73% yield increase), and the second highest was a single application of Trivapro A+B at BBCH 39-45 (9.4% yield increase). A grower applying Prosaro XTR must earn an additional \$71.46 ha^{-1} in revenue to breakeven or produce an additional 0.28 to 0.31 t ha^{-1} of yield compared with the non-treated control. Comparatively, a single application of Trivapro A+B needs to earn an additional \$58.62 ha^{-1} or produce an additional 0.23 to 0.25 t ha^{-1} to breakeven.

There are several factors that need to be accounted for prior to fungicide application. The most important is to consider the relationship between the environment, plant pathogen and host/cultivar (disease triangle). This study found that later fungicide applications resulted in higher yields, but it is important to note that this was only true for half the site years. In site years where environmental conditions were not conducive for disease, fungicide applications were unnecessary.

6.2.2. To determine the effects of single fungicide applications timings at BBCH 22-23 (herbicide timing), BBCH 30-32 (plant growth regulator timing), BBCH 39-45 (flag leaf), and BBCH 61-63 (10-30% of anthers are mature) on disease severity on two wheat cultivars

Precipitation in 2019 was 43% greater than in 2018. These environmental conditions were more favorable for disease development, with 53% higher disease levels observed in 2019 versus 2018.

Fungicide applications at BBCH 22-23 and BBCH 30-32 had 33% and 19%, respectively, higher foliar disease ratings than fungicide treatments at BBCH 39-45. These trends were supported by quantification of foliar lesion area by image analysis and NDVI data. The results suggest that early fungicide applications at BBCH 22-23 and BBCH 30-32 should be avoided by producers, as they did not result in significantly lower disease compared with the untreated control; indeed, they could be considered an unnecessary economic input which could contribute to the development of fungicide resistance.

Consistent with Alberta Climate Information Services (ACIS) FHB risk predictions, in our field trials showed Fusarium damaged kernels (FDK) were 49% higher in 2019 than 2018. Despite the high FHB risk in 2019, however, there were low FDK and DON levels at all site years, and DON levels were considerably below the Canadian Grain Commission limit. The most common *Fusarium* species found were *F. poae* in 2018 and *F. avenaceum* in 2019. There were no significant differences between cultivars for FDK, ELISA and LC/MS/MS DON.

At most of the sites, AAC Viewfield and AAC Brandon had similar foliar disease levels, which was expected since both cultivars have an 'intermediate' leaf spot resistance rating. Nevertheless, at 38% of the sites, AAC Brandon had significantly higher foliar disease levels than AAC Viewfield. Although both have 'intermediate' leaf spot resistance, AAC Viewfield appears to be slightly more resistant than AAC Brandon. The re-isolation of fungi recovered from the plant tissues also confirmed that both cultivars are susceptible to STB, SNB and tan spot at least to some extent. Under environmental conditions favorable for disease development, both cultivars had reduced foliar disease symptoms when treated with fungicide at BBCH 39-45 and BBCH 61-63, meaning that a fungicide application would be beneficial in environments with high disease pressure.

6.2.3. To observe the yield, quality, and economic benefits associated with the application of multiple fungicide MOAs, higher than label rates, and multiple applications in a single season on two wheat cultivars

There was no statistical difference in yields, DTM, test weights, TKW and protein for the fungicide applications with a single mode of action (MOA) [Tilt 250 E (group 3)] versus multiple MOA [Trivapro A+B (group 3, 7, and 11)] at BBCH 22-23 (2-3 tillers detectable). The lack of a difference may reflect the low disease pressure observed at BBCH 22-23 and the fact that both Tilt 250 E and Trivapro A+B are known to reduce most foliar diseases effectively in Alberta.

There were no significant yield or quality differences between the recommended (30 gai ha^{-1}) and high (75 gai ha^{-1}) Trivapro B (list the active ingredient here) fungicide rates, when these were applied as a single or dual treatment at BBCH 30-32. This suggests that the recommended or label rate of fungicide was sufficient and there was no added benefit associated with a higher rate. If the comparison between recommended and higher rates had been made at a later growth stage (i.e., BBCH 39-45), when disease pressure was greater, then perhaps a difference could have been noted.

There were relevant agronomic, yield and quality differences observed between single, dual, and triple fungicide applications. Overall, single fungicide treatments had 3% (0.2 t ha^{-1}) lower yields versus the dual and triple fungicide treatments. Additionally, the triple fungicide treatments had 4% (0.3 t ha^{-1}) lower yields compared with the dual treatments. This was unexpected but may have been reflected a 21% lower yield at the Red Deer 2018 site in the triple vs. dual fungicide application treatment. The lower yields may have been due to trampling damage associated with the additional fungicide passes due to lack of experience in the technical staff at this site. At all other sites, yield trends were generally supported by the foliar disease severity ratings. The single fungicide treatments had 45% higher foliar disease compared with the dual and triple treatments, and the dual treatments had 35% higher foliar disease compared with the triple fungicide treatments. Additionally, the TKW was 4% lower in the single versus the dual and triple treatments. These results appear to highlight the benefits of multiple fungicide applications.

The highest yield and economic benefit were achieved with the dual fungicide treatments. The increased revenue needed for a dual fungicide (Trivapro A+B and Prosaro XTR) to breakeven is \$130.08 ha^{-1}. This is equivalent to an additional yield increase of 0.50 to 0.56 t ha^{-1}. As noted earlier, however, only four out eight site years saw significant responses to fungicide applications. Under drier conditions, disease pressure was low and fungicide applications were unnecessary.

6.3. Future research

The research in this thesis provided a good foundation for further analysis of fungicide timing in wheat. Several follow up studies could immediately stem from this work.

- The comparison of recommended rates (30 gai ha^{-1}) and higher rates (75 gai ha^{-1}) at BBCH 30-32 did not detect distinct differences among the treatments, due to the low disease pressure at these early growth stages. Therefore, a comparison of the higher and recommended rates of fungicide applications at later growth stages (BBCH 39-45) should be undertaken. Higher disease pressure at the later growth stages may allow for clearer detection of differences between treatments.
- Similarly, there were no differences between single MOA [Tilt 250 E (group 3)] versus multiple MOA [Trivapro A+B (group 3, 7, and 11)] at BBCH 22-23 (2-3 tillers detectable) due to the low disease pressure. Therefore, a comparison of multiple MOA at later growth stages (BBCH 39-45 or BBCH 61-63) should be explored.

This findings from this study will be valuable to Alberta farmers in several ways. The yield and quality data on the two newly registered CWRS cultivars under intensive management and different environmental conditions showed that, in some cases, fungicide applications are unnecessary and will not be profitable for the grower. Hopefully, by providing insights into fungicide timing, this study will help growers to develop an understanding of good fungicide stewardship practices and avoid early fungicide applications, which are important in delaying fungicide resistance while increasing wheat yield, quality, and profit and avoid low rate fungicide applications at early growth stages.

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Appendix

Appendix 1: Split-plot design for the Value of Early Fungicide project in 2018 and 2019 used at four sites (Barrhead, Bon Accord, Red Deer and Lethbridge) in Alberta, Canada. There are four blocks or replicates shown by the orange border. The main plot is the cultivar (AAC Brandon and AAC Viewfield) which is differentiated by the 'blue' and 'green' coloured plots. The sub-plot are the fungicide treatments which are listed from 1-13 on each of the plots.

Replicate/Block 4

Trt 6	Trt 9	Trt 4	Trt 7	Trt 8	Trt 5	Trt 1	Trt 13	Trt 10	Trt 3	Trt 12	Trt 2	Trt 11	Trt 15	Trt 26	Trt 22	Trt 24	Trt 21	Trt 25	Trt 18	Trt 16	Trt 23	Trt 17	Trt 19	Trt 14	Trt 20
Fung Trt 6	Fung Trt 9	Fung Trt 4	Fung Trt 7	Fung Trt 8	Fung Trt 5	Fung Trt 1	Fung Trt 13	Fung Trt 10	Fung Trt 3	Fung Trt 12	Fung Trt 2	Fung Trt 11	Fung Trt 2	Fung Trt 13	Fung Trt 9	Fung Trt 11	Fung Trt 8	Fung Trt 12	Fung Trt 5	Fung Trt 3	Fung Trt 10	Fung Trt 4	Fung Trt 6	Fung Trt 1	Fung Trt 7
AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield
401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426

Replicate/Block 3

Trt 21	Trt 25	Trt 19	Trt 18	Trt 20	Trt 24	Trt 23	Trt 17	Trt 14	Trt 22	Trt 15	Trt 26	Trt 16	Trt 12	Trt 6	Trt 4	Trt 1	Trt 8	Trt 9	Trt 2	Trt 7	Trt 5	Trt 13	Trt 3	Trt 11	Trt 10
Fung Trt 8	Fung Trt 12	Fung Trt 6	Fung Trt 5	Fung Trt 7	Fung Trt 11	Fung Trt 10	Fung Trt 4	Fung Trt 1	Fung Trt 9	Fung Trt 2	Fung Trt 13	Fung Trt 3	Fung Trt 12	Fung Trt 6	Fung Trt 4	Fung Trt 1	Fung Trt 8	Fung Trt 9	Fung Trt 2	Fung Trt 7	Fung Trt 5	Fung Trt 13	Fung Trt 3	Fung Trt 11	Fung Trt 10
AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon
301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326

Replicate/Block 2

Trt 23	Trt 25	Trt 21	Trt 22	Trt 14	Trt 15	Trt 19	Trt 20	Trt 18	Trt 26	Trt 24	Trt 17	Trt 16	Trt 8	Trt 2	Trt 1	Trt 9	Trt 12	Trt 6	Trt 7	Trt 10	Trt 11	Trt 4	Trt 13	Trt 3	Trt 5
Fung Trt 10	Fung Trt 12	Fung Trt 8	Fung Trt 9	Fung Trt 1	Fung Trt 2	Fung Trt 6	Fung Trt 7	Fung Trt 5	Fung Trt 13	Fung Trt 11	Fung Trt 4	Fung Trt 3	Fung Trt 8	Fung Trt 2	Fung Trt 1	Fung Trt 9	Fung Trt 12	Fung Trt 6	Fung Trt 7	Fung Trt 10	Fung Trt 11	Fung Trt 4	Fung Trt 13	Fung Trt 3	Fung Trt 5
AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon
201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	216	217	218	219	220	221	222	223	224	225	226

Replicate/Block 1

Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Trt 7	Trt 8	Trt 9	Trt 10	Trt 11	Trt 12	Trt 13	Trt 14	Trt 15	Trt 16	Trt 17	Trt 18	Trt 19	Trt 20	Trt 21	Trt 22	Trt 23	Trt 24	Trt 25	Trt 26
Fung Trt 1	Fung Trt 2	Fung Trt 3	Fung Trt 4	Fung Trt 5	Fung Trt 6	Fung Trt 7	Fung Trt 8	Fung Trt 9	Fung Trt 10	Fung Trt 11	Fung Trt 12	Fung Trt 13	Fung Trt 1	Fung Trt 2	Fung Trt 3	Fung Trt 4	Fung Trt 5	Fung Trt 6	Fung Trt 7	Fung Trt 8	Fung Trt 9	Fung Trt 10	Fung Trt 11	Fung Trt 12	Fung Trt 13
AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Brandon	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield	AAC Viewfield
101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121	122	123	124	125	126

Appendix 2: McFadden Scale: McFadden, W. 1991. Etiology and epidemiology of leaf spotting diseases in winter wheat in Saskatchewan. Ph.D. thesis, University of Saskatchewan, Saskatoon, 151 pp.

	Intensity of foliar symptoms on leaves											
Leaf level	0	1	2	3	4	5	6	7	8	9	10	11
Upper	0[a]	0	0	0	0	0		0	0-1	2-5	6-10	11-25
Middle	0	0	0	0	0-1	2-5	6-10	6-10	11-25	26-50	>50	>50
Lower	0	0-1	2-5	6-10	11-25	26-50	>50	>50	>50	>50	>50	>50

[a]percentage of leaf area with lesions in the upper, middle, and lower leaf canopies