Stripe rust management in spring and winter wheat by varietal resistance and fungicide application

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By

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ABSTRACT

Stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* Eriks., is prevalent throughout western Canada in some years. As a result, there is a need to study stripe rust control for winter wheat grown in western Canada. The objective of the first thesis project was to evaluate the effects of fall fungicide application (metconazole and pyraclostrobin) on stripe rust and leaf spot severity, as well as yield and quality of winter wheat. The effects of fungicide application in the fall, spring, or both fall and spring (dual) on four winter wheat cultivars varying in disease resistance: 'AC Bellatrix,' 'Moats,' 'Radiant,' and 'CDC Osprey' were evaluated at four sites: Saskatoon and Indian Head in SK and Lethbridge and Lacombe in AB. Stripe rust severity on susceptible cultivars, 'AC Bellatrix' and 'CDC Osprey,' and leaf spot severity on these cultivars and 'Radiant,' were reduced by a single spring or dual fungicide applications (fall and spring) but not by fall application when over-all disease severity was high. Single spring and dual fungicide applications on stripe rust susceptible cultivars maintained yield potential compared to the unsprayed treatment, which was reduced by nearly 30%; grain quality was also maintained by the same treatments. The stripe rust resistant cultivar 'Moats' was not affected by fungicide application at any timing. Fall fungicide application had no effect on disease severity, yield or grain quality during the study. The dual fungicide application did not offer additional benefits.

Race-specific stripe rust resistance genes (*Yr* genes) are widely deployed in wheat cultivars to control stripe rust. However, race-specific resistance break-down by the pathogen occurs frequently. The residual effects, the expression of partial, non race-specific resistance to virulent pathogen races by defeated race-specific disease resistance genes, is a potential source of durable stripe rust resistance. In the second project, the residual effects of race-specific *Yr* genes

in wheat were evaluated. The parental near isogenic lines (NILs) with defeated single *Yr* genes (*Yr10, Yr26,* and *Yr32*) in the 'Avocet' background, F_5 NILs with two *Yr* genes in the combinations of *Yr26/Yr10, Yr32*/*Yr10* and *Yr32*/*Yr26* and the susceptible cultivar 'Avocet-S' were inoculated with each *Pst* isolate or isolate mixtures (W020, W049, T034/W052) virulent to all three *Yr* genes in growth chambers. Infection type (IT), infection area (IA) and latent period (LP) were assessed and recorded. An increased number of *Yr* genes was correlated with reduced IT and IA and longer LP. The same NILs were evaluated in stripe rust field nurseries at Saskatoon, SK and Lethbridge, AB in 2018. There was an interaction between genotypes and isolates, and correlations between increasing numbers of *Yr* genes and all parameters. When inoculated with T034/W052, *Yr32*/*Yr10* and *Yr32*/*Yr26* wheat genotypes had lower IT and IA and longer LP than 'Avocet-S'. With two other isolates, *Yr32*/*Yr10* and *Yr32*/*Yr26* wheat genotypes tended to have lower IA and longer LP than 'Avocet-S' but not always. The *Yr26/Yr10* wheat genotype often showed no residual effect. The results from field disease nurseries were inconclusive due to the avirulent nature of the *Pst* population to *Yr10* and *Yr26* wheat genotypes. The results indicated that some defeated *Yr* genes have residual effects in certain combinations and further study is required for a better understanding of the mechanism of residual effects.

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DEDICATION

I would like to dedicate my thesis to wheat breeders in western Canada.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AAFC	Agriculture and Agri-Food Canada
AB	Alberta
ANOVA	Analysis of variance
APR	Adult-plant resistance
ASR	All-stage resistance
Avr	Avirulence
CDC	Crop Development Centre
cv.	Cultivar
dpi	Day post inoculation
FRAC	Fungicide Resistant Action Committee
FRET	Fluorescent resonant energy transfer
HMC	Haustorial mother cell
hpi	Hour post inoculation
IA	Infection area
IH	Infection hyphae
IT	Infection type
KASP	Kompetitive allele specific PCR
LP	Latent period
MAS	Marker assisted selection
NIL	Near isogenic line
NTC	No template cell
PCR	Polymerase chain reaction
Pst	*Puccinia striiformis* f. sp. *tritici*
QTL	Quantitative trait loci
R	Resistance
RFU	Relative fluorescence unit
RIL	Recombinant inbred line

SK	Saskatchewan
SNP	Single nucleotide polymorphism
SV	Substomatal vesicle
TKW	Thousand kernel weight
TW	Test weight
Yr	Yellow rust/stripe rust resistance

CHAPTER 1.

1 INTRODUCTION

Stripe rust of wheat caused by *Puccinia striiformis* f. sp. *tritici* Eriks. (*Pst*) is a globally important and devastating disease of cereal crops (Chen 2005). In western Canada, stripe rust was a minor problem restricted to southern Alberta and British Columbia prior to 2000 (Su et al. 2003). Since the appearance of more aggressive and warm-temperature tolerant strains in North America in 2000 (Hovmøller et al. 2008; Milus et al. 2009), possibly associated with climate change (Lyon & Broders 2017), stripe rust of wheat has become prevalent throughout Canada (Holtz et al. 2013; Rioux et al. 2015). There was a major stripe rust epidemic in western Canada in the 2010/2011 winter wheat growing season and stripe rust incidence has been observed in many areas including the three Prairie Provinces: Alberta, Saskatchewan and Manitoba, and in Quebec. Although no comparable disease pressure and wide spread economic loss by this disease has been reported in Saskatchewan since 2011, the high prevalence of the disease is concerning and a year with stripe rust favorable weather conditions could result in another damaging epidemic. The ever-evolving population of *Pst* and unpredictable weather conditions due to climate change increases the risk of stripe rust epidemics in this region.

Winter wheat in western Canada is a relatively minor crop comprised of 3.6% of the total area seeded to wheat on average over the last 10 years (Stats Canada 2018). Despite limited production, winter wheat has multiple benefits for the grower such as higher yield than spring wheat, more efficient water utilization in early spring, highly competitive growth against weeds, diversification of crop rotations and undisturbed nesting in spring for ducks (Entz et al. 1992;

Devries et al. 2008; Beres et al. 2010a; Stats Canada 2018). While winter wheat production may provide many advantages for growers, there are several major issues with production. One of the issues is disease management as winter wheat may facilitate the overwintering of *Pst* and provide a green bridge for early season infection of spring wheat grown nearby, as has been witnessed in southern Alberta (Kumar et al. 2013). The infection of winter wheat seedlings by *Pst* in the fall is a concern due to the potential for increased winterkill, which is also an adverse factor for weed-competitive ability and high yield potential (Thurston 1962; Beres et al. 2010a). A study of fall fungicide application on winter wheat infected with stripe rust demonstrated a yield benefit (Turkington et al. 2016); however, the consistency of benefits from fall fungicide application has not been studied in detail. Besides stripe rust, the leaf spot disease complex of wheat is common and can cause yield loss by reducing photosynthesis. Leaf spotting diseases are detrimental to seedling health and winter survival, affect the same plant parts of the host crop as stripe rust, have similar infection timings as stripe rust, and can compound the damage to the host (Menzies & Gilbert 2003). Therefore, the effects of a control method on one disease would likely influence the other and the result needs to carefully account for all diseases.

The management strategies for stripe rust of wheat focus mainly on chemical control and genetic varietal resistance in the host crop. Seedling resistance or all-stage resistance (ASR) has been widely deployed as a disease control method that is effective, economical and environmentally sustainable in modern agroecosystems. However, the widespread use of a few race-specific qualitative seedling resistance genes makes ASR vulnerable to break down by new strains of *Pst* with virulence to these resistance genes. While the research for new resistance genes is on-going, some studies suggest that defeated disease resistance genes express residual resistance to the virulent strains of pathogen species that may be significant when multiple defeated genes are pyramided into a line (Nass et al. 1981; Brodny et al. 1986; Dowkiw &

Bastien 2007). There have been no studies of the residual resistance of defeated stripe rust resistance genes in wheat to this day.

This thesis focuses on stripe rust control in winter wheat through fungicide application in fall and spring on cultivars varying in resistance to stripe rust and leaf spot diseases, and an evaluation of residual resistance from defeated stripe rust resistance genes in wheat.

1.2 Hypotheses

1. Fall fungicide application will improve yield and grain quality of winter wheat by managing stripe rust at the seedling stage in western Canada.
2. Defeated stripe rust resistance genes, *Yr10*, *Yr26* and *Yr32* in wheat have residual disease resistance to *Pst* virulent to these resistance genes.

1.3 Objectives

1. To determine the effectiveness of cultivar resistance, timing of fungicide application and their interaction to improve yield and grain quality of stripe rust infected winter wheat.
2. To detect and evaluate residual resistance in spring wheat lines with defeated stripe rust resistance genes.

CHAPTER 2.

2. LITERATURE REVIEW

2.1 Wheat

Common wheat, *Triticum aestivum* L., is a cereal crop in the family Poaceae, subfamily Pooideae, and tribe Triticeae. Common wheat is also called bread wheat and mainly milled into flour to produce baked goods or used for animal feed and sometimes ethanol. Common wheat is hexaploid (2n = 42), meaning it has three homologous groups of seven pairs of chromosomes (AABBDD). Hexaploid wheat resulted from two hybridizations, first between progenitors with the A and B genomes (2n = 28, AABB), and second between the tetraploid hybrid (AABB) and the progenitor of the D genome (Kimber & Sears 1987). Beside hexaploid wheat, the cultivated tetraploid wheat species is widely grown as durum wheat, *T. turgidum* spp. *durum.* Durum wheat flour is used mainly for pasta and couscous production. Global wheat production (772 million tonnes) ranks third after sugarcane and maize and it is the third most valuable crop in the world (FAO 2019). In western Canada, bread wheat is grown as spring wheat or winter wheat with different wheat classes and varying numbers of cultivars within a class. In 2016, wheat production in Canada, spring, winter, and durum wheat combined, was 31,769,100 metric tonnes, which was the highest production of all field crops in Canada (Stats Canada 2018).

2.2 Production and importance of winter wheat in western Canada

Winter wheat is sown in the fall to overwinter as seedlings and mature the following summer (Fowler). Unlike spring wheat, vernalisation with a period of cold conditions is required for winter wheat to head. The long growing season results in a higher number of kernels per

spikelet, and equal or higher harvest index than spring wheat (Beres et al. 2010a), which usually results in higher yield than spring wheat in western Canada (Fig. 2.1). Winter wheat also has better weed-competitive ability than spring wheat due to the early development of the crop in spring, and as a result, it requires less herbicide compared to spring wheat production (Thurston 1962; Beres et al. 2010a; Harker et al. 2016). The ability to suppress weed growth in early spring, as well as the diverse seeding and herbicide application timings of winter wheat, disturbs weed lifecycles resulting in improved weed control (Liebman & Dyck 1993). It may also mitigate development of herbicide resistance in weed species due to reduced herbicides uses when winter wheat is included in the crop rotation. Another advantage of winter wheat over spring wheat is more efficient moisture utilization through extraction of soil water in early spring, which is advantageous in dryland regions of the prairies (Entz et al. 1992). Winter wheat also provides suitable nesting sites for particular waterfowl and helps to conserve wild bird populations in North America (Skone et al. 2016).

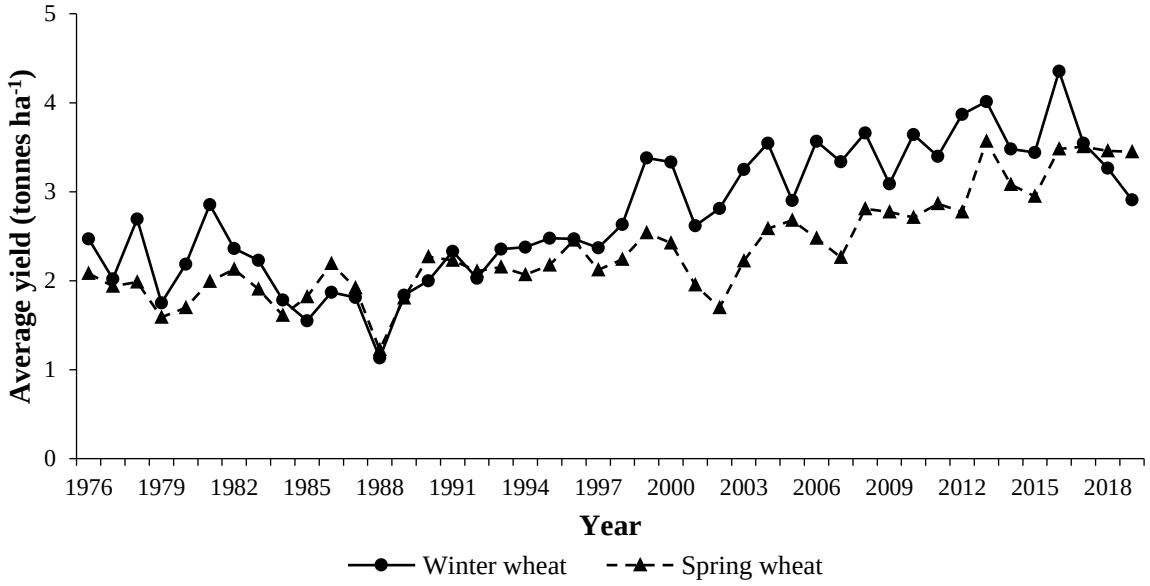

Figure 2.1 Average yield (metric tonnes per hectare) of spring and winter wheat in the Prairie Provinces of Canada, 1976 to 2018 (Stats Canada 2018).

Winter wheat was introduced to western Canada in the late 1800's and commercial production started in 1902 in southern Alberta (Hay et al. 1950). Production in western Canada has been consistent yet limited to a fraction of spring wheat production despite the economic and environmental advantages. In the growing season of 2016/2017, there were 165,100 hectares seeded to winter wheat in western Canada, while more than 6,192,500 hectares were seeded to spring wheat in 2017 (Stats Canada 2018). The area seeded to winter wheat has fluctuated (Fig. 2.2) with the challenges of growing the crop in the semiarid grassland, under harsh winter conditions, and due to market limitations.

Overcoming winterkill and managing plant density in the spring is critically important for winter wheat production in western Canada. Winterkill not only reduces yield by decreasing the plant population, but it also compromises the weed-competitive ability of winter wheat at less than optimal crop plant density (Thurston 1962; Beres et al. 2010a). Although the cold hardiness of the plant itself has been improved by breeding, it appears that maximum cold hardiness for winter wheat cultivars in western Canada was reached decades ago (McLeod 1980). Cold weather

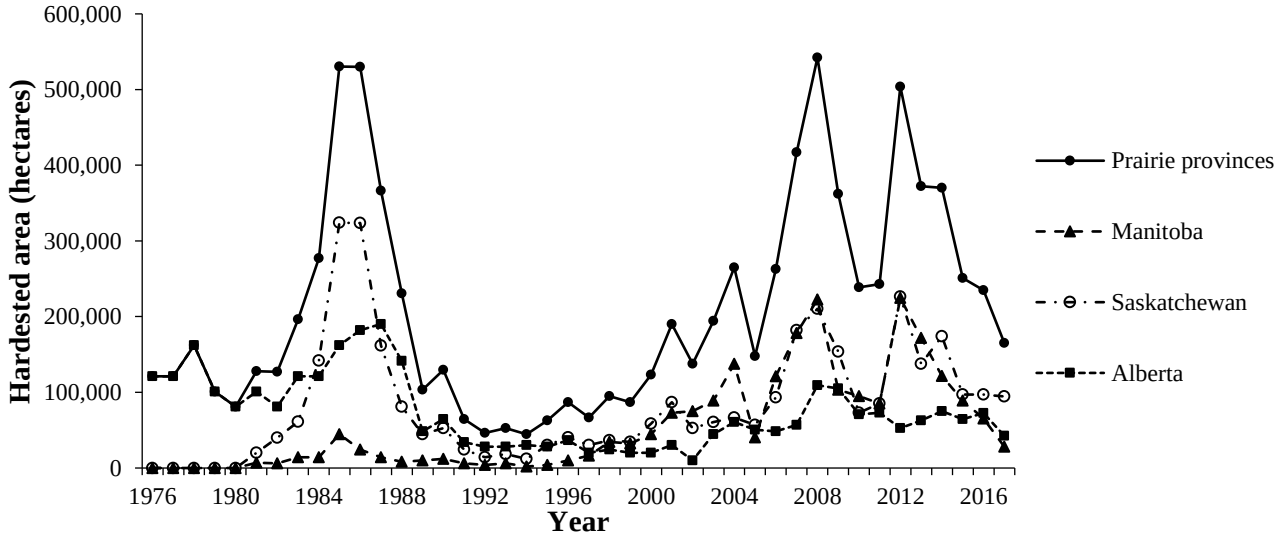

Figure 2.2 The harvested area of winter wheat in the Prairie Provinces of Canada from 1976 to 2017 (Stats Canada 2018).

in the prairies often devastated winter wheat sown into summer fallow in the 1980's (Fowler 2012). Winter survival in western Canada is improved with adequate snow cover in winter wheat fields (Savdie et al. 1991). With the adoption of reduced or no-till, more snow is trapped than in conventional tillage, and the potential area for winter wheat production in western Canada was greatly expanded.

Winter wheat survival is improved through optimal seeding dates, depth, and rate; better fertilizer management; and expanding stubble crop options (McLeod et al. 1996; Beres et al. 2010b; Irvine et al. 2010; Irvine et al. 2013). High seeding rates (300-450 seeds/m^2) improve winter survival and yield stability (Beres et al. 2010b). Canola stubble is the most common stubble option for winter wheat since it can trap more snow that other crop stubbles. However, pea and barley silage are reported to be potential stubbles with similar snow trapping ability, nutrient benefits and disease mitigation benefits (Irvine et al. 2013).

Disease control in winter wheat is another important issue that requires further research. Many common diseases of spring wheat such as common root rot, leaf spots, rusts, and Fusarium head blight also affect winter wheat (Menzies & Gilbert 2003). Disease surveys in western Canada indicate stripe rust incidence and severity could sometimes be higher in winter wheat fields than they are in spring wheat fields (Rioux 2017; McCallum et al. 2017). Seed treatment and fungicide application in fall improve winter survival and protect yield of winter wheat by reducing foliar disease severity (Turkington et al. 2016). *Puccinia striiformis* f. sp. *tritici* (*Pst*) overwinters on winter wheat in southern Alberta and may contribute to early infection of spring wheat sown nearby (Kumar et al. 2013). Fall infection of winter wheat seedlings with *Pst* also has the potential to cause a reduction of overall plant health and winter survival rate. In the late 1800's and early 1900's, in the case of leaf rust of wheat, infection of winter wheat seedlings in the fall was reported to reduce seedling vitality and increase winter kill (Chester 1946). Leaf rust

control in fall was considered beneficial to mitigate this negative effect on the seedlings and this could well be applied to the case with stripe rust of winter wheat. Prior to 2000, stripe rust was a minor disease outside of southern Alberta and only recently became common in Saskatchewan and Manitoba; therefore, research on the reaction of winter wheat cultivars to stripe rust has been limited. There is a need for more information on stripe rust management to provide improved knowledge and disease control measures to winter wheat producers.

2.3 Stripe rust of wheat

Stripe rust of wheat is caused by *Pst*, a rust fungus of the phylum Basidiomycota, class Urediniomycetes, order Uredinales, family Pucciniaceae, and genus *Puccinia*. *Puccinia striiformis* is separated into nine *formae speciales* and *Pst* is classified as a *tritici* that causes stripe (yellow) rust of bread (hexaploid) wheat, durum (tetraploid) wheat, cultivated emmer wheat (*T. dicoccum* Schrank), wild emmer wheat (*T. dicoccoides* Korn), and triticale (x *Triticosecale* Wittmack). The pathogen is a biotrophic, polycyclic and heteroecious fungus with wheat as the primary host and, for a long time, no known alternate host. Recent studies reported that barberry (*Berberis* sp.) (Jin et al. 2010) and Oregon grape (*Mahonia aquifolium*) function as alternate hosts of *Pst* (Wang & Chen 2013). A study with common barberry (*Berberis vulgaris*) demonstrated that the short dormancy period and unfavorable conditions prohibited *Pst* from infecting its alternate hosts in the Pacific Northwest region of the United States (Wang & Chen 2016). The discovery of alternate hosts explains the high genetic diversity found in Gansu province in China, the Himalayas (Nepal, Pakistan) and near Himalayan regions (the mountainous region in China) (Ali et al. 2014; Thach et al. 2016). The lack of functional alternate hosts for *Pst* populations in North America supports the reports of clonal *Pst* populations of this region (Ali et al. 2014; Chen et al. 2014; Thach et al. 2016). However, the clonal *Pst* populations in North America and Australia are still able to rapidly gain virulence through single-step

mutation and somatic recombination (Hovmøller et al. 2011; Wellings 2011; Chen 2014). The rise and spread of new virulence in the *Pst* populations in recent years are also attributed to the introduction of new races from different geographic areas by wind or human travel (Chen 2005; Hovmøller et al. 2008; Brar & Kutcher 2016). These factors make control of stripe rust difficult and make the disease one of the most devastating and widespread problems of wheat in the world. The disease can cause up to 100% yield loss if conditions favor severe infection at an early crop growth stage and no control measure is taken (Chen 2005; Wellings 2011).

2.3.1 Lifecycle

Puccinia striiformis f. sp. *tritici* is heteroecious and has five spore stages including an asexual urediniospore stage on its primary host, wheat, and a sexual stage on the alternate host. Overwintering of *Pst* occurs as asexual urediniospores or dormant mycelium on primary hosts, or in the sexual stage as aeciospores in aecia on the alternate host if the alternate host species is present (Chen & Kang 2017). In spring, urediniospores from uredia on primary hosts or aeciospores from alternate hosts are released and dispersed by wind. The infection process starts once the spore lands on the plant surface, usually the leaf of the primary host species (Chen & Kang 2017). In western Canada, urediniospores do not commonly overwinter due to the extremely cold weather outside of southern Alberta; however, the spores are carried by air currents from the southern United States and arrive in the spring (Chen 2005). Urediniospores germinate on the plant surface and infect leaf tissues after at least 3 hours of continuous moisture (Rapilly 1979) at optimal temperatures between 9-12°C (Menzies & Gilbert 2003). Initial infection leads to colonization of leaf tissue and development of pustules after approximately two weeks under optimal environmental conditions. Urediniospores are produced within pustules called uredia, extruded from the pustule as they mature and are dispersed by rain splash and wind to uninfected hosts nearby, with multiple subsequent cycles possible in spring and summer.

Towards maturity of the wheat crop, telia form on infected wheat plants and produce two-celled teliospores at the beginning of the sexual cycle. Teliospores germinate under 24 hours of moist conditions at 12°C and produce a four-celled promycelium without being dispersed from the telia. Basidiospores are produced from promycelia and readily dispersed to the alternate host (Chen et al. 2014). On the alternate host, basidiospores germinate and infect the plant tissue to undergo sexual reproduction and produce pycniospores from pycnia. Pycniospores are transferred between male and female pycnia, usually by insects to cross-fertilize and initiate aecia development, which then produces aeciospores and completes the sexual cycle. Urediniospores from overwintered mycelia and aeciospores start another disease cycle the next spring (Chen & Kang 2017).

Infection frequency and spore survival of *Pst* and disease severity of stripe rust are all influenced by environmental factors such as temperature, wind, and moisture availability (Chen 2005). Before the year 2000, most strains of *Pst* commonly favored low temperatures (9-12°C) as optimal conditions for infection and disease development and activity was inhibited by warmer temperatures (Line 2002). Since 2000, new *Pst* strains that are more aggressive and warm temperature tolerant have become dominant within the pathogen population in North America. This change has led to increased prevalence and severity of stripe rust of wheat in western Canada where the disease was historically of minor importance (Holtz et al. 2013). The pathogen caused epidemics in western Canada in 2005, 2006, and 2011 (McCallum et al. 2007; Holtz et al. 2013) and future epidemics are likely.

2.3.2 Infection process

In the optimal environment of 100% humidity and 9-13°C temperatures, a urediniospore of *Pst* that lands on the wheat leaf surface germinates and extends a narrow germ tube across the leaf surface perpendicular to leaf veins until it reaches a stoma (Mares & Cousen 1977). Upon

contacting the stoma, the germ tube enters the host tissue often without forming an appressorium or occasionally with a weak appressorium, and then forms a substomatal vesicle (SV) in the substomatal space (Marryat 1907; Mares & Cousen 1977; Niks 1986). From the SV, a few primary infection hyphae (IH) form and enter the host mesophyll cells (Moldenhauer et al. 2006; Zhang et al. 2012).

The SVs and primary IH may appear as early as 12 hours post inoculation (hpi) on susceptible wheat lines, which indicate the establishment of an infection site by the pathogen (Zhang et al. 2012). Once in contact with a host mesophyll cell, the tip of the primary IH is delimited by a septum and a haustorial mother cell (HMC) forms (Niks 1986; Moldenhauer et al. 2006; Zhang et al. 2012), while branching and extension of secondary IH are also initiated from primary IH. From an HMC, a penetration peg forms and perforates the cell wall to develop a haustorium that invaginates the cell membrane inside of a host mesophyll cell at 12 hpi (Mares 1979; Zhang et al. 2012). The haustoria then take up nutrients and water from the host cell while the IH continue growing and branching to create more HMC and haustoria to colonize a wider area of host tissue at 48 hpi (Zhang et al. 2012). At the same time, IH closer to the infection site start filling intercellular spaces as well (Mares 1979). Some secondary IH, called “runners”, grow longer with less branching parallel to the plane of the epidermis and along the length of the wheat leaf to reach uncolonized tissues (Evans 1907; Hanes 1936). Later in the infection process, sporulation occurs along the length of the “runners” and this gives the characteristic pattern of stripes of uredia of *Pst*.

By 120 hpi, microcolonies are formed in host leaf tissue and the first visible signs of infection, minute white flecks, appear on the infected leaf 6-7 days post inoculation (dpi) (Mares & Cousen 1977; Zhang et al. 2012). Under a controlled environment, pustule formation and sporulation are often observed at 10-12 dpi (Marryat 1907; Mares 1979). Sporulation is initiated

by the formation of sporogenous cells, from which spore-buds grow inside pustules. The spore-buds expand to form septa, and then successively develop into urediniospore initials, pedicel cells and primary urediniospore cells. Mature urediniospores will be released through the ruptured epidermis and dispersed to infect more wheat plants.

2.3.3 Disease symptoms

When wheat leaves are infected by *Pst*, long narrow rows or stripes of pustules form along leaf veins and often extend the full length of the leaf blade (Fig. 2.3). Chlorosis may occur on tissue surrounding the pustules. When mature, pustules release bright yellow to orange urediniospores. Dark black telia containing teliospores often replace the uredia later in the season. Heavy infection of the leaves causes defoliation and desiccation, compromises photosynthesis required for grain filling, and reduces yield and grain quality (Menzies & Gilbert 2003). When infection is severe, pustules sometimes appear on the lemma and palea of the florets.

Figure 2.3 Winter wheat leaves with stripe rust symptoms.

2.4 Leaf spot disease complex of wheat

As a foliar disease that can damage the wheat leaves around the same time as stripe rust disease does, the leaf spot disease complex of wheat may need to be controlled to protect yield potential and grain quality. Leaf spot diseases of spring and winter wheat are caused by multiple pathogens, which include Septoria tritici blotch caused by *Septoria tritici* Rob. and Desm.; Stagonospora nodorum blotch caused by *Stagonospora nodorum* Berk.; Stagonospora leaf blotch caused by *Septoria avenae* Frank f. sp. *triticea* Johnson; spot blotch caused by *Cochliobolus sativus* (S. Ito and Kurib.) Drechs ex Dastur; and tan spot caused by *Pyrenophora tritici-repentis* (Died.) Drechs (Menzies & Gilbert 2003). All diseases can cause yield loss and grain quality reduction by reducing photosynthesis of the leaves. The damage is greatest when the complex severely infects the flag and penultimate leaves. It has been reported that Stagonospora nodorum blotch may cause up to 40% yield loss, and losses caused by the entire disease complex range from 10 to 50% (King et al. 1983; Bhathal et al. 2003).

2.4.1 Lifecycle

Some pathogens, mainly *S. nodorum*, can be seed-borne; however, the main source of primary inoculum is infected crop residues. *Septoria* spp. and *P. tritici-repentis* spread as conidiospores or ascospores in spring by water splash or wind, while *C. sativus* produces only conidiospores as the primary inoculum. Infection of winter wheat by the pathogens can begin in the fall and resume in the spring. Wet conditions in spring and summer is favored by all species for inoculum dispersal and infection. Optimal temperature for disease development by *Septoria* spp. is 15-27°C, while *P. tritici-repentis* is favored at 20-28°C. Hot and dry conditions can inhibit disease development by *P. tritici-repentis* (Menzies & Gilbert 2003). When conditions are favorable, conidiospores are produced on infected leaf surfaces, dispersed by rain splash or wind and repeat infection and disease development as polycyclic pathogens.

2.4.2 Symptoms

Leaf blotch caused by *Septoria* spp. first appears as yellow flecks, which then develop into greyish, yellow, white or brown lesions on any above ground plant part. Typically, *S. nodorum* causes light coloured lesions with dark margins on leaves. Spot blotch appears as dark brown blotches on leaves that do not fuse. Tan spot begins as small dark brown spots on leaves and develops into irregular shaped blotches, similar to Septoria leaf blotch (Menzies & Gilbert 2003). In many cases, it is difficult to identify the pathogen by observing the symptoms since they are similar to each other and multiple pathogens could be present on the same leaf. Laboratory diagnostics will be required to confirm pathogen identification.

2.4.3 Disease management

The primary inoculum of leaf spotting pathogens occurs on previous crop residue; therefore, crop rotation with non-host crop species is recommended as a control measure. Some cultivars with limited leaf spot resistance are available (Fernandez et al. 2014a). Fungicide application is commonly used as an effective disease control measure. Fungicides registered for stripe rust control for wheat also control most of the leaf spot diseases.

2.5 Stripe rust management by fungicide application

When host resistance is not available or has been overcome by the *Pst* population, fungicide application is another option for stripe rust control. Chemical control of rust diseases of wheat has been a focus of research in North America since the 1950's and 1960's (Dickson 1959; Rowell 1968). Before the development of effective systemic fungicides, protectant fungicide application was not economically beneficial due to the necessity of multiple applications (Rowell 1968; Line 2002). Another reason chemical control was not feasible was the lack of understanding of the epidemiology and knowledge of optimal application timings. Also, many fungicides tested in the 1950's and 1960's contained phytotoxic heavy metals such as nickel,

which also raised public health and environmental concerns. Systemic fungicides were found to be more promising than protectant fungicides (Rowell 1968); triadimefon was the first systemic fungicide that was registered for cereal rust control in the USA in 1981 (Line 1993). Since then, newer and more effective fungicides have been created and tested in intensive trials in North America. Currently, most fungicides used against stripe rust of wheat are in the triazole (group 3) and strobilurin (group 11) chemical groups. The 2018 Plant Disease Management Report (https://www.plantmanagementnetwork.org/pub/trial/pdmr/) reported that the application of Quilt® (azoxystrobin and propiconazole), Trivapro® (azoxystrobin, propiconazole, and benzovindiflupyr), Topguard® (flutriafol), and Tilt® (propiconazole) on winter wheat at the early jointing stage, the boot stage or both, effectively controlled stripe rust (Chen et al. 2018). Fungicide use in other parts of the world differs from western Canada, depending on environmental and economic factors. Seed treatment with triadimefon and foliar fungicides are widely used for susceptible cultivars in China to protect yield under high disease pressure (Wan et al. 2007; Kang et al. 2010; Chen & Kang 2017). The overwintering of the *Pst* population and heavy winter wheat seedling infection in fall warrants the use of seed treatment in Gansu Province, China. In Europe, chemical fungicides were used in the intensive wheat production regions in the 1970's and 1980's and it became more popular in the 1980's (Chen & Kang 2017). To this day, triazole, strobilurin or a mix of both chemical groups are commonly used in Europe (Blake et al. 2011; Matzen et al. 2019).

In Canada, there are currently multiple fungicide products registered for stripe rust control although most of them are group 3 (metconazole, propiconazole, prothioconazole, and tebuconazole) and group 11 fungicides (azoxystrobin, fluoxastrobin, picoxystrobin, and pyraclostrobin), and a few of these are mixed with group 7 fungicides (carboxamides) based on recommendations by the FRAC (Fungicide Resistant Action Committee) (Guide to Crop

Protection 2019). Although fungicides are effective on susceptible host plants, they are not the most economical or the easiest method to control stripe rust due to variable economic costs and returns, and the difficulty of forecasting disease severity to warrant fungicide application (Poole & Arnaudin 2013). Even when disease pressure is high, the benefit of fungicide application may not be warranted if either the price of wheat is low, the price of the fungicide is high, or both (Wegulo et al. 2011; Poole & Arnaudin 2013; Lopez et al. 2015). Environmental concerns with fungicide application in large scale agricultural systems, the common wheat production systems in western Canada, is another important issue. Another issue with widespread use of fungicides for stripe rust control is fungicide resistance development of *Pst* populations. Although fungicide resistance has not been observed in the *Pst* population so far, Kang et al. (2019) reported differences in sensitivity to pyraclostrobin and propiconazole at reduced fungicide concentrations or delayed application timings among multiple *Pst* isolates. Both fungicide groups completely suppressed spore germination at full concentration, but varying sensitivity was observed within the pathogen populations and strong selection pressure from extensive use of the fungicides could result in pathogen resistance to these fungicides. Fungicides need to be used with cautions, and if possible, as the secondary method of management of wheat stripe rust to address the many issues that come with use.

2.6 Stripe rust management by host resistance

Host resistance is the most effective and economical control measure for stripe rust of wheat. The gene-for-gene model of host-pathogen interactions suggested by Flor (1942) is one of the principle theories utilized in stripe rust resistance in wheat. The model states that for each resistance (R) gene in the host plant, there is a corresponding avirulence (Avr) gene in the pathogen that triggers an incompatible (resistant) reaction. A resistant reaction can be called vertical or qualitative disease resistance. Qualitative resistance to stripe rust of wheat, which is

effective at all growth stages, is also called all-stage resistance (ASR) and detectable at the seedling stage. Resistance that is regulated by multiple genes without gene-for-gene interactions with a pathogen is called horizontal or quantitative resistance. Quantitative resistance does not cause an all-or-nothing reaction as qualitative resistance does, but mitigates disease severity in a non-race-specific manner. Adult-plant resistance (APR) in wheat is generally indicative of quantitative resistance that is regulated by major genes and/or multiple minor genes. Quantitative trait loci (QTL) are the areas on a chromosome that have a statistically significant correlation with a specific phenotype. The QTL are found by using genetic markers and populations segregating for variable phenotypes and further study of QTL can lead to the identification of minor genes that together regulate APR reactions (Bariana et al. 2010; Hao et al. 2011; Dong et al. 2017; Li et al. 2017). This kind of resistant reaction often reduces stripe rust severity at the tillering stage and becomes strongest at the booting stage (Zhang et al. 2012). However, APR can be race specific (Milus et al. 2015) or non-race specific such as HTAP (high temperature, adult plant) resistance (Chen 2007).

The numerous stripe rust resistance genes (*Yr*, yellow rust resistance gene) in wheat, both qualitative and quantitative, are widely used in wheat breeding programs and have been successful at reducing disease incidence and severity worldwide (Johnson 1992; Chen 2005). To date, there are 83 genes officially designated as *Yr* genes (McIntosh et al. 2013), and the number is growing as the technology to detect resistance genes and QTL has advanced. With the rapid evolution of pathogen races, the breakdown of race specific ASR often follows shortly after the introduction of *Yr* genes into commercial cultivars (Line & Qayoum 1992; Line & Chen 1995).

In North America and many other parts of the world, *Yr9*, an ASR gene, was often deployed in breeding programs and was present in numerous commercial wheat cultivars for decades (Graybosch 2001). Virulence to *Yr9* in *Pst* was first detected in 1997 and the frequency

of virulence rapidly increased in the next two decades in North America and other countries (Liu et al. 2017). Along with many other *Yr* genes that have broken down, *Yr9* is no longer useful in wheat breeding programs (Wan et al. 2015). The *Yr10* gene was another effective ASR gene until 2010 in western Canada, but was overcome by virulent *Pst* races and became ineffective over a short period of time (Puchalski & Gaudet 2011). This boom-and-bust pattern of host resistance led to the search for more durable resistance, along with deployment of APR *Yr* genes, in addition to all-stage, race-specific *Yr* genes in wheat breeding programs.

More than half of the stripe rust resistant spring wheat cultivars in western Canada carry *Yr18*, which is an APR gene, followed by a few cultivars with *Yr17*, *Yr36* and other unknown genes not detected with molecular markers (Randhawa et al. 2012). Although quantitative adult plant resistance is often more durable than race-specific resistance, it may not be sufficient when the environment is favorable for disease development (Line & Chen 1995) and more aggressive *Pst* races become prevalent in pathogen populations (Vanderplank 1968). With climate change that causes unpredictable weather conditions, and the appearance of warm-temperature tolerant, aggressive *Pst* races (Hovmøller et al. 2008; Milus et al. 2009), the need for more durable resistance genes to prevent future wheat stripe rust epidemics is greater than ever.

2.6.1 *Yr10*

The dominant resistance gene *Yr10* is located on chromosome 1BS; it likely originated from wheat line PI178383 and is linked to the gene that controls brown glume colour (Metzger & Silbaugh 1970; Smith et al. 2002). It is a single gene with ASR to avirulent *Pst* races. The same gene was also found in Iranian spelt accession 415, as well as cv. Moro (Kema & Lange 1992). Several molecular markers were developed for *Yr10* (Shao et al. 2001; Smith et al. 2002) and the gene was once cloned and characterized as a gene that encode a nucleotide-binding-site leucine-rich repeat protein by Liu et al. (2014). Since then, it has been remapped and found to be located

1.2-cM away from the location of the previously cloned gene was reported; however, the true identity of the protein encoded by *Yr10* remains to be discovered (Yuan et al. 2018).

2.6.2 *Yr26*

Another all-stage, highly effective dominant resistance gene is *Yr26*, which likely originated from Chinese landrace γ80-1 (*Triticum turgidum*) and is located close to the centromere on chromosome 1BL (Ma et al. 2001; Wang et al. 2008; Zhang et al. 2013). Another gene identified as *Yr24* was later discovered to be the same gene as *Yr26* (Li et al. 2006). This gene was deployed in many Chinese wheat cultivars, but a race of *Pst* virulent to *Yr26* was found in China in 2008 (McIntosh et al. 2018). This is another gene extensively studied for its chromosomal location and genetic identity. Several expressed sequence tag – sequence-tagged site markers have been identified that are closely linked to *Yr26* at a genetic interval of <1.16 cM (Zhang et al. 2013). A recent study with SNP-based pool genotyping identified a 0.003-cM interval where *Yr26* is located (Wu et al. 2018).

2.6.3 *Yr32*

Gene *Yr32* is one of three *Yr* genes discovered in cv. 'Carstens V' located on chromosome 2AL (Eriksen et al. 2004). It was characterized as a race-specific resistance gene with incomplete resistance against *Pst*, which required another additive resistance gene in 'Carstens V' to exert highly effective resistance (Chen & Line 1993; Calonnes et al. 2002). The resistant reaction of the additive resistance genes in 'Carstens V' were discovered to be sensitive to light intensity and resistance was reduced under low light intensity (Calonnec et al. 2002). There is no information on the identification or exact location of *Yr32* to date.

2.7 Residual effects of defeated resistance genes

Defeated resistance genes are race-specific resistance genes that have been overcome by emerging virulent races of a pathogen. Defeated genes may have residual resistant effects that

may be of consequence when pyramided into one genotype (Nelson et al. 1970; Li et al. 1999). The theory suggests that quantitative resistance is conditioned by defeated qualitative genes that have accumulated in the host plant over time and act additively (Nelson et al. 1970). Residual effects of defeated qualitative resistance genes have been reported for leaf rust of poplars (*Melampsora larici-populina*) (Dowkiw & Bastien 2007), powdery mildew of wheat (*Erysiphe graminis* DC. f. sp. *tritici* E. Marchal) (Nass et al. 1981), and stem rust of wheat (*Puccinia graminis* Pers. f. sp. *tritici*) (Brodny et al. 1986). As pointed out by Johnson (1984), the earlier studies of residual effects with isogenic lines could not completely reject the possibility that the putative residual effect could be the result of other unknown minor resistance genes linked to the defeated major resistance gene. Another criticism of the early study by Nass et al. (1981) was that only a single virulent isolate was used, and the study lacked strong evidence for "residual effect". Pedersen and Leath (1988) reviewed multiple studies on the residual effects and their possible value as durable resistance by pyramiding them. While they reported on some studies that found possible residual effects by defeated genes in a few pathosystems, they found a lack of research under field conditions and a lack of clear evidence for the existence of residual effects. In a more recent study with the rice-*Xanthomonas oryzae* pv. *oryzae* pathosystem (Li et al. 1999), a set of recombinant inbred lines (RILs) was inoculated with three strains of virulent *Xanthomona oryzae* pv. *oryzae*. The study found that $Xa4^T$, an allele of disease resistance locus *Xa4*, was located in the same region as a recessive QTL for resistance against a virulent strain and this indicated that a major resistance gene could function as a minor resistance gene. On the other hand, a study on the poplar-rust pathosystem found no evidence of residual effect when the F_2 progeny of poplar clones were tested in the field, while the same clones inoculated with an isolate of *Melampsora medusae* in the greenhouse showed a longer latent period associated with a defeated resistance gene (Woo & Newcombe 2003). In the wheat-stripe rust pathosystem, a positive correlation was

found between the number of pyramided *Yr* genes in the wheat genotypes and *Pst* resistance (Zheng et al. 2017). Furthermore, the same study showed some combinations of two *Yr* genes, such as *Yr9* + *Yr18* (APR) and *Yr30* (APR) + *Yr46* (APR), resulted in improved resistance compared to the lines in which these genes were deployed singly. However, it was also found that two *Yr* genes in different combinations resulted in no improvement or higher susceptibility than when the genes were deployed singly. Differences in residual effect among defeated genes was observed in the potato-*Phytophthora infestans* pathosystem as well (Stewart et al. 2003). The research on the residual effects of defeated genes is still in progress as its existence is not fully proven in any pathosystem and the mechanism of the effect is to be examined. There are no studies evaluating the residual effect of defeated genes in the wheat-stripe rust pathosystem under controlled conditions or in the field to date.

2.8 Single nucleotide polymorphism (SNP) and marker assisted selection (MAS) by KASP assay

Wheat breeding has been improved from tedious, labour intensive phenotypic screening to less time-consuming genotypic screening with molecular marker assisted selection (Babu et al. 2004). There are a number of different types of markers and selection processes used in wheat breeding programs (Paux et al. 2012). A single nucleotide polymorphism (SNP) is a single base difference in a DNA sequence at a given position (Vignal et al. 2002) that can be linked to a specific gene or a region where the gene is located. The SNP markers are used to distinguish allelic differences among individuals within a species. One PCR-based genotypic method that uses SNP markers is kompetitive allele specific PCR (KASP). The KASP protocol consists of two PCR phases: the first creates copies of target genes with unique, unlabeled tails and the second phase creates copies of target genes with one of two fluorescent labels that corresponds to the unique tail sequence created in the first phase. When SNP markers are homozygous for the

targeted gene, only one fluorescent signal is generated. Heterozygous genotypes create a mix of two fluorescent signals (He et al. 2014). The KASP method has been successfully used in marker assisted selection (MAS) of wheat for physiological traits and disease resistance (Liu et al. 2015; Babiker et al. 2016; Cai et al. 2016; Rasheed et al. 2016; Tan et al. 2017).

2.9 Summary

The increased prevalence and frequency of stripe rust epidemics caused by virulent and aggressive *Pst* races are of critical concern in North America as well as other wheat growing regions in the rest of the world. Farmers in western Canada must manage stripe rust, and winter wheat, in particular, needs to be a research focus due to the lack of attention to stripe rust resistance in past breeding programs (Brian Fowler, personal communication). The potentially damaging fall infection by *Pst* on winter wheat raises the question of whether fall fungicide application has a positive effect on winter wheat production. A field study of fungicide application on several winter wheat cultivars with different levels of resistance against stripe rust will provide useful information on stripe rust control in winter wheat.

While fungicides are highly effective and widely used to control stripe rust worldwide, host disease resistance is a more cost effective and environmentally sustainable method. The lack of highly effective and durable stripe rust resistance is a long-term issue for wheat breeding programs. The residual effects of defeated resistance genes in wheat could provide another tool to develop durable resistance with existing *Yr* genes and this study could provide much needed insight into the presence and effectiveness of this effect in the wheat-stripe rust pathosystem.

CHAPTER 3

3. FALL FUNGICIDE APPLICATION DOES NOT IMPROVE YIELD OR GRAIN QUALITY OF WINTER WHEAT IN THE ABSENCE OF STRIPE RUST

3.1 Introduction

Winter wheat has been produced in western Canada for decades, although it is planted on a fraction (~5%) of the hectares seeded to wheat (Stats Canada 2018). Winter wheat has many benefits for growers such as more efficient soil moisture utilization and higher yield potential than spring wheat, diversification of the crop rotation, improved weed control by diversified seeding date and herbicide application timings, and undisturbed nesting sites for some waterfowl populations (Thurston 1962; Entz et al. 1992; Liebman & Dyck 1993; Beres et al. 2010a; Harker et al. 2016). Despite some production issues and unpredictable and extreme weather conditions due to global climate change, it is important to develop a highly diversified and high yielding cropping system. With improved winter survival through breeding, better agronomic practices and the introduction of reduced- or no-till agriculture in western Canada, winter wheat production has become more practical and of greater benefit (McLeod et al. 1996; Beres et al. 2010b; Irvine et al. 2010; Irvine et al. 2013).

Within the last two decades, stripe rust epidemics have become a more prominent issue in western Canada due to wider virulence, higher aggressiveness and warm-temperature tolerance in recent *Pst* races in North America (Hovmøller et al. 2008; Milus et al. 2009). The occasional overwintering of *Pst* on winter wheat acts as a green bridge, as observed in southern Alberta (Kumar et al. 2013). As a result, early infection of nearby spring wheat from infected winter

wheat is a concern. Fall infection of winter wheat with *Pst* also raises concerns for reduced seedling survival, which is important for winter wheat to achieve high yield and strong competition against weeds in early spring (Thurston 1962; Beres et al. 2010a). Fall fungicide application to control stripe rust was reported to be beneficial to winter wheat in western Canada, resulting in improved yield (Turkington et al. 2016). This study focused on the effect of fall fungicide application on winter wheat for stripe rust and other foliar diseases and ultimately on yield and grain quality improvement.

3.2 Material and methods

3.2.1 Plant and pathogen material

Four winter wheat cultivars, 'AC Bellatrix' (Thomas et al. 2011a), 'Moats' (Fowler 2011), 'CDC Osprey' (Fowler 1997), and 'Radiant' (Thomas et al. 2011b) were selected according to disease resistance against stripe rust and leaf spot diseases (Table 3.1). Three *Pst* isolates (ID: W002, W003 and W004) collected from various locations in Saskatchewan in 2011 (Brar 2015) were mixed and used to inoculate the experimental field plots in fall and spring of each growing season at Saskatoon, SK. Urediniospores of each isolate were increased on seedling plants of the susceptible wheat cultivar 'Avocet-S' in growth chambers at the University of Saskatchewan and stored at -80 ºC until the plots were ready to be inoculated.

Table 3.1 Stripe rust and leaf spot disease reactions of winter wheat cultivars (Varieties of Grain Crops in Saskatchewan 2018).

Cultivar	Stripe rust	Leaf spot complex
AC Bellatrix	S	MR
CDC Osprey	S	S
Moats	MR	I
Radiant	MR/MS*	S

Reactions to stripe rust and leaf spots: "S" = susceptible, "MS" = moderately susceptible, "I" = intermediate, "MR" = moderately resistant, "R" = resistant.
* reaction varied from MR to MS depending on location

3.2.2 Field experiment

The cultivars of winter wheat were planted at four locations: Kernen Crop Research Farm in Saskatoon (52°17N, 106°52W; in 2013/2014 – 2016/2017); Agriculture and Agri-Food Canada (AAFC) at the Indian Head Research Farm in Indian Head, SK (50°54N, 103°65W; in 2015/2016); the AAFC Lacombe Research and Development Centre in Lacombe (52°45N, 113°75W; in 2014/2015 – 2016/2017); and the AAFC Lethbridge Research and Development Centre in Lethbridge, AB (49°70N, 112°76W; in 2014/2015 – 2016/2017). Saskatoon and Lethbridge have Dark Brown Chernozem soil type and Indian Head and Lacombe had Black Chernozemic soil type. At each site, winter wheat was direct seeded into an experimental plot with canola stubble in the fall at a seeding rate to achieve a target plant density of 275 plants m^{-2} in the spring of the following year. The location of the experimental plots was rotated within each research farm every year. Nitrogen and phosphorus fertilizers were side-banded at seeding and nitrogen fertilizer was broadcasted in spring at rates appropriate for each site based on soil tests to achieve the target yield. Each subplot was 2 X 8 m at Saskatoon, SK and was similar but varied slightly at each site. Plot borders were seeded with the stripe rust susceptible cultivar 'AC Bellatrix', which was used as a disease spreader. The foliar fungicide Twinline® (active ingredients: metconazole and pyraclostrobin, BASF Corporation, Research Triangle Park, NC) was applied for stripe rust and leaf spot control at the recommended rate of 500 mL ha^{-1} in 100 L ha^{-1} of water. Each cultivar was assigned one of four fungicide treatments: (i) an unsprayed control, (ii) a single application in fall at seedling stage, growth stage (GS) 15–19 (Zadoks et al. 1974), (iii) a single application in spring at the flag leaf stage (GS 39-47), and (iv) a dual application consisting of applications in both fall and spring. The experiment was designed as a two-way factorial randomized complete block design (RCBD) with four replications of 16

treatments (four cultivars and four fungicide treatments, Table 3.2) at all locations except Lethbridge, AB where a split-plot design arranged in blocks was used due to limited space availability. At Saskatoon, the spreader rows between plots were inoculated in fall at the seedling stage and in spring at the tillering stage with a mixture of urediniospores of the three *Pst* isolates to increase the risk of stripe rust infection and development. Urediniospores were mixed in Bayol® 35 mineral oil (Imperial Oil, Calgary, AB) and applied on foliage with a hand-held sprayer followed by an application of water to the plants to add moisture. Tarps were laid over the spreader rows overnight following inoculation to create humid conditions for 24 hours to induce infection. Herbicides were applied as necessary and appropriate for each location. Experiments relied on natural infection at Lethbridge, Lacombe or Indian Head. In late August, winter wheat was harvested using a straight cut combine harvester.

3.2.3 Disease rating and data collection

Plant emergence was recorded in fall and spring to quantify winter survival each year at Saskatoon. In fall, stripe rust and leaf spot incidence in the unsprayed check plots were rated at the fall fungicide application timing (seedling stage, GS 16-19) and before snow fall/ground freeze. Disease severity in unsprayed check plots was rated at the time of spring fungicide application (flag leaf stage) by assessing 10 flag and 10 penultimate leaves at random for stripe rust infection type (IT) and severity using the modified Cobb scale (Peterson et al. 1948). Leaf spot severity was rated by assessing the same leaves using the Horsfall-Barratt scale (Horsfall & Barratt 1945) and assessing the whole plant with the McFadden scale (McFadden 1991). The final disease rating was conducted at the soft-dough stage (GS 85) on flag and penultimate leaves from all treatments. Ten flag and penultimate leaves with leaf spot symptoms from unsprayed check plots were collected, dried, and stored at room temperature until processed in the lab for

leaf spot pathogen identification. The average rating of both flag and penultimate leaves of four replicates for each treatment were calculated and recorded. The harvested grain was dried, cleaned and processed to determine yield (kg ha^{-1}), test weight (TW; kg hL^{-1}), thousand kernel weight (TKW; g), and protein content (%) by LECO analysis (LECO Corporation, St. Joseph, MI, USA) for each treatment.

3.2.4 Leaf spot pathogen identification

Each flag and penultimate leaf collected at the soft dough stage was cut into 10 2-cm long pieces and plated onto water agar media in 9-cm diameter Petri dishes. The leaf pieces in petri dishes were incubated at room temperature under constant light and examined for the presence of leaf spot pathogens; *Pyrenophora tritici-repentis*, *Septoria tritici*, *Stagonospora nodorum*, *Septoria avenae* f. sp. *tritici*, and *Cochliobolus sativus*, at 7 to 14 days after plating. Pathogens were identified by conidiospore or ascospore morphology. The percentage of leaf samples with a specific pathogen, of the total leaf samples from which all pathogens were recovered was recorded as the frequency of a specific pathogen. The prevalence of pathogen recovery was measured as the percentage of plots from which a specific pathogen was recovered out of the total number of plots tested.

3.2.5 Statistical analysis

All data collected were analyzed using PROC MIXED with SAS 4.9 software (SAS Institute Inc., Cary, NC, USA) with the Kenward-Roger method for degrees of freedom; spray timing and cultivars were considered fixed factors and replication a random factor. The assumptions of normal distribution of residuals and variance homogeneity were tested for each set of dependent variables by using PROC UNIVARIATE and Levene's test. When the assumption of normal distribution of residuals was not satisfied, data were modified by

transformation or removing outliers with the Studentized residual value greater than ±3. Heterogeneous variance was accounted for during the analysis by using the REPEATED statement. When data transformation or removal of outliers did not satisfy the assumptions, PROC GLIMMIX was used with the same method for degrees of freedom and fixed and random factors as PROC MIXED. A data distribution model that best fit the data set and minimized Akaike information criteria was selected by changing DIST option under the MODEL statement.

RCBD model: Y = mean + block + timing + cultivar + timing*cultivar + error

$$y_{ijk} = \mu + r_k + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

The data from Lethbridge were analyzed with PROC MIXED with fungicide application timing as the main fixed effect of the main plots and cultivar as the second fixed effect of the subplots within a main plot. The main effect was assigned randomly to the plots within a block and block was assumed as random effects. The Satterthwaite method was used to calculate the degrees of freedom.

Split-plot model: Y = mean + block + timing + error1 (=block*timing) + cultivar + (timing*cultivar) + error2

$$y_{ijk} = \mu + r_k + \alpha_i + w_{ik} + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

The assumptions of normally distributed residuals and homogeneity of variance were tested and modifications were made when necessary as described above.

Stripe rust severity and leaf spot severity were analyzed for correlation with yield, TW, TKW and protein content separately for each site-year using PROC CORR of SAS 9.4.

Table 3.2 Treatment list of the winter wheat cultivars and fungicide treatments for the fungicide timing x cultivar trial. Fungicide applied was metconazole and pyraclostrobin (500 mL ha^{-1} in 100 L ha^{-1} water).

Treatment	Cultivar	Fungicide Timing
1	CDC Osprey	Unsprayed
2	CDC Osprey	Fall
3	CDC Osprey	Spring
4	CDC Osprey	Both
5	AC Bellatrix	Unsprayed
6	AC Bellatrix	Fall
7	AC Bellatrix	Spring
8	AC Bellatrix	Both
9	Moats	Unsprayed
10	Moats	Fall
11	Moats	Spring
12	Moats	Both
13	Radiant	Unsprayed
14	Radiant	Fall
15	Radiant	Spring
16	Radiant	Both

3.3 Results

3.3.1 Stripe rust severity

Stripe rust severity was affected by both cultivar and fungicide timing at four of 11 site-years and by their interaction at seven site-years (Table 3.3). 'AC Bellatrix' and 'CDC Osprey' were both highly susceptible to stripe rust and 'Moats' was resistant to stripe rust at all site-years (Fig. 3.1 and 3.2). The differences among site-years depended on the resistance or susceptibility of 'Radiant' at each site-year and the level of stripe rust severity. Stripe rust severity of unsprayed 'AC Bellatrix' was designated as the "check" treatment to compare stripe rust severity among site-years. Site-years were separated or combined based on severity of unsprayed 'AC Bellatrix': high (≥70%), intermediate (20-69%) or low (≤19%), and the stripe rust susceptibility of 'Radiant' (susceptible, intermediate or resistant) (Table 3.4). Combinations of stripe rust severity of unsprayed 'AC Bellatrix' at each site-year and 'Radiant' resistance or susceptibility resulted in five groups. There were interactions between cultivar and fungicide application timing on stripe rust severity in the high stripe rust severity/resistant 'Radiant' group, high stripe rust severity/susceptible 'Radiant' group and intermediate stripe rust severity /susceptible 'Radiant' group. Both cultivar and application timing affected disease severity in the intermediate stripe rust severity/intermediate 'Radiant' group and in the low stripe rust severity/intermediate 'Radiant' group.

In the high stripe rust severity/resistant 'Radiant' group (Saskatoon in 2013/2014, and Lacombe in 2014/2015 and 2015/2016), the check treatment had stripe rust severity of 79% and unsprayed 'Radiant' was resistant to moderately resistant (13% severity) (Fig. 3.1a). The interaction effect was due to differences in disease severity among cultivars in response to fungicide treatment. A single spring fungicide application reduced disease severity from 79 to

5% for ‘AC Bellatrix’ and from 57 to 5% on ‘CDC Osprey’. Dual fall and spring applications did not improve control of stripe rust compared to a single spring application. Although disease severity of ‘Radiant’ was also reduced by the same fungicide treatments (2% with spring fungicide application), the severity of unsprayed ‘Radiant’ was lower than other unsprayed susceptible cultivars and severity reduction by fungicide application was significant but unnecessary. None of the fungicide treatments lowered disease severity of the resistant cultivar ‘Moats’.

The high stripe rust severity/susceptible ‘Radiant’ group (Lethbridge in all years) had stripe rust severity higher than 80% for both the check treatment (88%) and unsprayed ‘Radiant’ (81%) (Fig. 3.1b). The interaction was explained by the benefit of spring applied fungicide to reduce stripe severity of ‘AC Bellatrix’, ‘CDC Osprey’ and ‘Radiant’, but not ‘Moats’. Stripe rust severity of ‘AC Bellatrix’, ‘CDC Osprey’ and ‘Radiant’ ranged from 81 to 88% and was reduced by spring and both fall and spring fungicide application to between 6 to 13%. ‘Moats’ was resistant (3.4% unsprayed) to stripe rust and none of the fungicide treatments affected disease severity of this cultivar.

The intermediate stripe rust severity/susceptible ‘Radiant’ group (Saskatoon in 2015/2016) had stripe rust severity of 21% on the check treatment and unsprayed ‘Radiant’ had comparable disease severity (12%) to the check (Fig. 3.1c). Again, a single spring and the dual fall and spring fungicide applications lowered disease severity of all cultivars except ‘Moats’. Contrary to the moderately resistant reaction on ‘Radiant’ at Saskatoon in 2013/2014, ‘Radiant’ in 2015/2016 was as susceptible to stripe rust as ‘AC Bellatrix’.

Stripe rust severity of the intermediate stripe rust severity/intermediate ‘Radiant’ group (Saskatoon and Lacombe in 2016/2017) was 49% on the check treatment and affected by both

cultivar and fungicide application timing; however, there was no interaction (Fig. 3.2a). The average stripe rust severity of all cultivars with no fungicide application (26%) was reduced by single spring and dual fall and spring fungicide applications to 8%. When the cultivars were compared, 'AC Bellatrix' and 'CDC Osprey' were similarly susceptible to stripe rust and 'Radiant' was intermediate as disease severity (10%) was lower than 'AC Bellatrix' (36%) but higher than 'Moats' (4%). The resistant reaction of 'Radiant' to stripe rust at Saskatoon and Lacombe in 2016/2017 was similar to the reaction observed at Saskatoon in 2015/2016.

The low stripe rust severity/intermediate 'Radiant' group (Saskatoon in 2014/2015 and Indian Head 2015/2016) had low stripe rust severity because of the dry spring and summer weather for those site-years (Fig. 3.2b). There were significant effects of both cultivar and fungicide application timing but no interaction. The disease severity reduction by fungicide application in spring and by the dual application for all cultivars was significant. Cultivars 'AC Bellatrix' and 'CDC Osprey' had similar stripe rust severity, which was higher than 'Radiant' and 'Moats'. There was no difference between 'Radiant' and 'Moats' in this group. The over-all severity was extremely low (<5% on the check) and any disease severity reduction as a result of fungicide or lower severity by cultivar difference was not biologically significant.

3.3.2 Leaf spot severity

During this study, Lacombe in 2015/2016 was the only site-year that had substantial leaf spot severity (72% for the unsprayed treatment, average of all cultivars). For other site-years, the leaf spot severity of the unsprayed treatment averaged over all cultivars or a susceptible cultivar ('CDC Osprey' and 'Radiant') was <26%. Leaf spot severity was not assessed at Lethbridge in 2015/2016 and 2016/2017 due to the severe stripe rust symptoms. Leaf spot severity was

influenced by the interaction of cultivar and fungicide application timing at four site-years and by cultivar and/or application timing, but with no interaction, at five site-years (Table 3.3).

When there was an interaction between cultivar and fungicide treatment, the effects were inconsistent among the site-years (Table 3.5). At Lacombe 2014/2015, the severity of the unsprayed treatment ranged from 6 to 8%, and the spring and dual fungicide treatments reduced leaf spot severity of all cultivars, except 'Moats', to between 2 and 4%. At Saskatoon in 2015/2016, the disease severity of 'AC Bellatrix' and 'CDC Osprey' was reduced from 11 and 8% to 5 and 4%, respectively by the dual fungicide treatment. For the same cultivars at the same site-year, disease severity tended to be lower with single spring fungicide application, but the difference was not significant compared to the severity of the unsprayed treatment nor to the dual fungicide treatment. Disease severity of 'Radiant' was reduced from 17 to 4% by the single spring application and the dual fungicide treatment. None of the fungicide treatments had any effect on leaf spot severity of 'Moats' at this site-year. Indian Head in 2015/2016 had comparable leaf spot severity to Saskatoon 2015/2016; however, 'Radiant' was the only cultivar on which disease severity was reduced from 27 to 8% by the single spring application and to 9% by the dual fungicide treatment. At Saskatoon in 2016/2017, leaf spot severity was the lowest among site-years (4% on unsprayed 'Radiant' was the highest); the single spring and dual treatments reduced disease severity of 'CDC Osprey' and 'Radiant' and the single spring application only on 'Moats'. No fungicide treatment had an effect on leaf spot severity of 'AC Bellatrix' at this site-year. 'Radiant' was the only cultivar on which leaf spot severity was reduced by single spring and the dual applications at all four site-years. Fall fungicide treatment had no consistent effect on leaf spot severity compared to unsprayed treatments on any cultivar at any site-year.

Two site years (Saskatoon 2013/2014, Lacombe 2016/2017) had leaf spot severity affected by both cultivar and fungicide application timing, and only fungicide treatment had a significant effect on disease severity at the other two site-years (Saskatoon 2014/2015 and Lacombe 2015/2016) (Table 3.6). Fungicide application reduced leaf spot severity of all cultivars with spring or both fall spring timings at all four site-years. Disease severity reduction by fungicide treatment was significant and effective under high disease severity, such as at Lacombe 2015/2016; however, leaf spot severity remained at 43% even with single spring and dual fungicide treatment and disease control was not as effective as stripe rust control by the same treatments. Fall fungicide application was not effective for leaf spot control on any cultivar at any site-year. The difference in leaf spot susceptibility by cultivar was inconsistent between two site-years that had a cultivar effect. 'Radiant' (24%) was the most susceptible cultivar at Saskatoon 2013/2014, while 'CDC Osprey' (10%) was the most susceptible at Lacombe 2016/2017.

The pathogens that caused the leaf spot disease complex were identified in all years at Saskatoon and in 2016/2017 at Lacombe (Table 3.7). Disease frequency of each pathogen was calculated as the percentage of leaf pieces with the pathogen of the total number of leaf pieces examined, and disease prevalence was calculated as the percentage of plots in which the pathogen was found of the total plots assessed. In 2013/2014 at Saskatoon, *P. tritici-repentis* and *S. tritici* were the most common leaf spot pathogens (frequency of isolation from leaves was 50 and 48.5%, respectively). The incidence of these pathogens was also high (percentage of plots from which the pathogen was recovered was 75 and 81.3%, respectively); however, *S. tritici* (53.3% frequency) and *C. sativus* (33.3% frequency) were the most common in 2014/2015 at Saskatoon. In 2015/2016, *P. tritici-repentis* was not detected at Saskatoon, nor at either

Saskatoon or Lacombe in 2016/2017. Similarly, *S. tritici* was not detected at Saskatoon in 2015/2016 or Lacombe in 2016/2017, and only detected at 11.5% frequency at Saskatoon in 2016/2017. In contrast, *St. nodorum* and *S. avenae* were not detected at Saskatoon in 2013/2014 or 2014/2015 but were common at Saskatoon in 2015/2016 and at both locations in 2016/2017. *Cochliobolus sativus* was detected at all five site-years with a frequency ranging from 1.5 to 33.3% and incidence ranging from 6.3 to 40%.

3.3.3 Yield

There were significant effects of cultivar and fungicide application timings on yield at five site-years and an interaction between them at three site-years (Table 3.3). At Lethbridge and Indian Head in 2015/2016, only cultivar differences had an effect on yield, while yield was affected only by fungicide treatment at Lacombe in 2015/2016. Yield at Lethbridge in 2014/2015 was not affected by any factor.

The interaction between cultivar and fungicide application timing indicated that the reaction to fungicide treatment varied among cultivars (Table 3.8). Saskatoon in 2013/2014 had low over all yield (ranging from 975 to 2895 kg ha^{-1}); dual fungicide application maintained yield potential of 'AC Bellatrix' at 2183 kg ha^{-1} (124% higher) compared to the unsprayed treatment (975 kg ha^{-1}). Yield potential of 'CDC Osprey' was maintained at 2621 kg ha^{-1} (113% higher) with a single spring application compared with 1231 kg ha^{-1} for the unsprayed treatment. None of the fungicide treatments had an effect on yield of 'Radiant' or 'Moats' at this site-year. The effect of fungicide application on yield at Saskatoon in 2015/2016 was similar to Saskatoon in 2013/2014, except that yield was higher (ranging from 4461 to 6523 kg ha^{-1}). The yield of 'CDC Osprey' was maintained at 5688 kg ha^{-1} (16% higher) with a single spring fungicide

application compared with 4797 kg ha^{-1} and by the same amount by the dual fungicide applications. 'Radiant' and 'Moats' had no yield improvement by fungicide treatment.

The yield at Lethbridge in 2016/2017 was the highest among three years at that site (ranging from 1734 to 8071 kg ha^{-1}) and unlike the two site-years at Saskatoon, yield of 'Radiant', along with 'AC Bellatrix' and 'CDC Osprey', was maintained higher by a single spring fungicide application and by the dual fungicide treatment. For 'AC Bellatrix', no fungicide application reduced yield from 5111 kg ha^{-1} with a single spring fungicide application or 5697 kg ha^{-1} with the dual fungicide treatment to 1734 kg ha^{-1}, which was a difference of 195%. Similarly, yield of 'CDC Osprey' was maintained at 6816 kg ha^{-1} (89% higher) with a single spring treatment and at 6515 kg ha^{-1} (78% higher) with a dual fungicide treatment compared to 3610 kg ha^{-1}. Yield of 'Radiant' was also maintained at 6247 kg ha^{-1} (127% higher) with a single spring fungicide application and at 6002 kg ha^{-1} (118% higher) with a dual fungicide treatment from 2753 kg ha^{-1} of the unsprayed treatment. 'Moats' was the highest yielding cultivar but there was no benefit from the fungicide treatments.

The yield protection corresponded to stripe rust severity reduction by the same fungicide application treatments. On the other hand, stripe rust severity of 'Radiant' at Saskatoon 2015/2016 was reduced by fungicide application; however, the effect was not reflected in terms of yield improvement at this site-year. At Lethbridge in 2016/2017, the yield protection of three cultivars corresponded to the high susceptibility of 'AC Bellatrix' and 'CDC Osprey' and the susceptibility of 'Radiant' to stripe rust at this site-year and the successful disease control by fungicide treatments. While single fall fungicide application did not maintain yield at any site-year, the yield of 'AC Bellatrix' at Saskatoon in 2013/2014 and 2015/2016 was protected by the dual fungicide application when the single spring application did not, compared to the unsprayed

treatment. However, the yield of the dual fungicide application treatment was not significantly higher than the single spring application.

At four site-years, the effects of both cultivar and fungicide application timing were detected, at two site-years only the effect of cultivar was detected and at one site-year only the effect of fungicide application timing was observed (Table 3.9). The yield differences due to fungicide treatment were similar among site-years but the yield differences due to cultivar were not consistent over the site-years. The magnitude of the yield among seven site-years was comparable and ranged from 3236 to 5832 kg ha^{-1}. Single spring application effectively maintained higher yield at Saskatoon 2014/2015 at 4563 kg ha^{-1} (7.4% higher), Lacombe in 2015/2016 at 5045 kg ha^{-1} (13% higher) and Lacombe 2016/2017 at 5832 kg ha^{-1} (23% higher) compared to the unsprayed treatment. The single spring application was as effective as the dual fungicide treatment at the same site-years. At Lacombe in 2014/2015, the dual fungicide application was the only treatment that maintained yield (at 4194 kg ha^{-1} or 11% higher) compared to the unsprayed treatment (3557 kg ha^{-1}), although the yield was not higher than that of the single spring or single fall application.

At Saskatoon 2016/2017, the yield of the unsprayed treatment did not differ from that of the single spring or the dual fungicide treatment; however, the yield of the single fall application (3354 kg ha^{-1}) was lower than the yield of the dual fungicide treatment (4126 kg ha^{-1}). Cultivar was the only factor that affected yield at Indian Head and Lethbridge in 2015/2016, although there was no effect of cultivar at Lacombe in 2015/2016. At Saskatoon in 2014/2015, the stripe rust susceptible cultivars, 'AC Bellatrix' (4534 kg ha^{-1}) and 'CDC Osprey' (4483 kg ha^{-1}), and 'Radiant' (4469 kg ha^{-1}), which had an intermediate reaction to stripe rust, had higher yield than the resistant cultivar 'Moats' (4150 kg ha^{-1}). At Indian Head in 2015/2016, 'Moats' had the

highest yield (4269 kg ha^{-1}), which was comparable to 'AC Bellatrix' and 'CDC Osprey', and 'Radiant' had the lowest yield (3536 kg ha^{-1}). The two stripe rust susceptible cultivars were the lowest yielding at the rest of the site-years (Lacombe 2014/2015, Lethbridge 2015/2016, Saskatoon and Lacombe 2016/2017). 'Radiant' was the highest yielding cultivar at Lacombe in 2014/2015 (4638 kg ha^{-1}) and at Lethbridge in 2015/2016 (4702 kg ha^{-1}). Yield of 'Radiant' was higher (4164 kg ha^{-1}) than the susceptible cultivars (3235 kg ha^{-1} for 'AC Bellatrix', 3406 kg ha^{-1} for 'CDC Osprey') at Saskatoon in 2016/2017, and both 'Radiant' (5620 kg ha^{-1}) and 'Moats' (5730 kg ha^{-1}) yielded higher than the susceptible cultivars (4806 kg ha^{-1} for 'AC Bellatrix, 4844 kg ha^{-1} for 'CDC Osprey') at Lacombe in 2016/2017. There was no significant effect on yield at Lethbridge in 2014/2015 (data not shown) by any factor despite the high stripe rust severity at this site-year.

The correlation analysis of yield as the dependent variable and stripe rust and leaf spot severities as independent variables for each site-year revealed that the correlation of each disease with yield was significant when disease severity was high (Table 3.10). Stripe rust severity was not correlated with yield at Saskatoon 2014/2015, Indian Head 2015/2016 or Lethbridge 2015/2016. There was an inverse correlation between stripe rust and yield ($p \leq 0.0006$) at the rest of the site-years and the correlation coefficients (r) ranged from -0.418 to -0.913. Lethbridge in 2016/2017 had severe stripe rust and had the highest coefficient of determination. Leaf spot severity was inversely correlated to yield at Saskatoon in 2014/2015 (r = -0.267, $p = 0.0326$), and at all three years at Lacombe. The highest inverse correlation coefficient (r = -0.644, $p < 0.0001$) was observed at Lacombe in 2015/2016, which had the highest leaf spot severity among site-years. Except Lethbridge in 2016/2017, the correlation between yield and the severity of both diseases was ≥ -0.700 for the rest of the site-years.

3.3.4 Test weight (TW)

Test weight was affected by the interaction of cultivar and fungicide application timing at three site-years, by both factors at four site-years and by cultivar only at two site-years (Table 3.3). There was no effect on TW from either factor at Indian Head in 2015/2016. Data on TW was not collected at Lacombe in 2014/2015.

The site-years with interaction effects for TW did not match the site-years with interaction effects on yield except Lethbridge in 2016/2017 (Table 3.11). Over all site-years, the TW varied from 65.5 to 81.8 kg hL^{-1}. Similar to yield, the interaction for TW arose from the inconsistent response of cultivar to fungicide treatment. A single spring and the dual fungicide applications improved TW of 'AC Bellatrix' by 5.6 kg hL^{-1} (7.8%) at Lacombe, by 2.7 kg hL^{-1} (3.5%) at Saskatoon in 2015/2016, and by 12.7 kg hL^{-1} (19.4%) at Lethbridge in 2016/2017. The same fungicide treatments were effective in increasing TW of 'CDC Osprey' at Saskatoon in 2015/2016 (increased by 2.0 kg hL^{-1}, or 2.6%) and Lethbridge in 2016/2017 (increase by 10.0 kg hL^{-1}, or 14.7%). The TW increase by fungicide treatments on 'Radiant' occurred only at Lethbridge in 2016/2017 (increased by 10.0 kg hL^{-1}, or 15.0%). There was no difference between the effects of a single spring fungicide application and the dual fungicide application on TW for any cultivar at any site-year. No fungicide treatment affected TW of 'Moats' at three site-years.

There was no interaction between cultivar and fungicide treatment at six site-years (Table 3.3). At all six site-years, TW was affected by cultivar differences, while four of six site-years were influenced by fungicide treatment (Table 3.12). Except for Lethbridge in 2015/2016, a single spring fungicide application or dual application improved TW compared to the unsprayed check for all cultivars. The increase ranged from 1.0 to 2.6 kg hL^{-1}. 'Moats' tended to have higher TW than other cultivars; although its TW was lowest at Saskatoon in 2014/2015 when

disease severity was low, and TW of all cultivar was high. ‘CDC Osprey’ tended to have lower or the lowest TW over the six site-years. There was no strong trend in TW difference among cultivars from site-year to site-year.

The correlation analysis between stripe rust severity and TW and leaf spot severity and TW indicated that stripe rust severity was inversely correlated with TW at most site-years, except at Saskatoon in 2014/2015, which had a positive correlation ($r = 0.326$), and Lethbridge in 2015/2016 (non-significant correlation) (Table 3.13). However, correlation coefficients between stripe rust severity and TW varied from weak to strong ($-0.8 < r < -0.3$) among most site-years. The highest coefficient ($r = -0.945$) was recorded at Lethbridge in 2016/2017, similar to the coefficient for yield. The correlation between TW and leaf spot severity was significant at four of eight site-years. The TW at Lethbridge in 2015/2016 had a positive correlation with leaf spot severity. For the rest of site-years, the correlation coefficient between TW and leaf spot severity was weak to moderate ($-0.6 < r < -0.3$).

3.3.5 Thousand kernel weight (TKW)

At seven out of 10 site-years there was an interaction between cultivar and fungicide application timing, and at three site-years there were effects of both cultivar and fungicide timing on TKW (Table 3.3). The interaction of factors on TKW was due to the reaction of cultivars to the fungicide treatment, but the effect of fungicide treatment was not as consistent with TKW as it was with yield and TW (Table 3.14). A single spring and the dual fungicide treatments improved TKW of ‘AC Bellatrix’ at Saskatoon in 2013/2014 and in 2015/2016, at Lacombe in 2015/2016 and Lethbridge in 2016/2017. The largest increase was observed at Lethbridge in 2016/2017, where it increased from 20.8 to 33.0 g with a single spring or the dual fungicide applications, which was an increase of 58.7%. The TKW of ‘CDC Osprey’ was increased over

the unsprayed treatment by the same fungicide treatments at Lacombe in 2015/2016 from 27.3 to 30.7 g (13%) and Lethbridge in 2016/2017 from 23.9 to 30.7 g (29%). 'Radiant' had an increase in TKW compared to the unsprayed treatment at Saskatoon 2015/2016 from 35.3 to 37.9 g (7%) with the dual fungicide applications and at Lethbridge in 2016/2017 from 23.2 to 29.6 g (28%) with a single spring application and to 30.3 g (31%) with the dual applications. The change in TKW between sprayed and unsprayed treatments was not significant for any cultivar at Saskatoon in 2014/2015, Lethbridge in 2014/2015 or Lacombe in 2016/2017. 'Moats' was the only cultivar whose TKW was not affected by fungicide treatment at any site-year. The single fall fungicide application did not improve TKW for any cultivar at any site-year.

The TKW was affected by cultivar at three site-years and by fungicide treatment at two of 10 site-years. The differences among cultivars did not follow a strong trend. 'CDC Osprey' tended to have the lowest TKW among the cultivars at these site-years (Table 3.15). The highest TKW was that of 'Radiant' at Saskatoon in 2016/2017, but the TKW of 'AC Bellatrix' and 'Moats' were the same as 'Radiant' at Indian Head in 2015/2016, as was 'Moats' at Lethbridge in 2015/2016. The increase in TKW due to the dual fungicide treatment at Indian Head in 2015/2016 was from 31.6 to 33.3 g (5%) and by a single spring and the dual fungicide treatments at Saskatoon in 2016/2017 from 32.5 to 35.8 g (10%). Although TKW was improved by the dual fungicide application compared to the unsprayed treatment, a single spring application did not improve TKW at Indian Head, and the single fall application was not effective in improving TKW.

The inverse correlation between TKW and stripe rust severity was significant at seven of 10 site-years, and the inverse correlation with leaf spot severity was significant at four of eight site-years (Table 3.16). Despite the highly significant correlation between the parameters, the

correlation coefficient was moderate ($r > -0.7$) at most site-years. As detected with the correlation coefficient for stripe rust severity and TKW at Lethbridge in 2016/2017 ($r = -0.905$), the site-years with high disease severity tended to have a higher coefficient similar to that observed with correlation analysis of yield and TW. The correlation between TKW and leaf spot severity was significant at four of eight site-years and the coefficient ranged from weak to moderate ($-0.6 < r < -0.3$).

3.3.6 Protein content

Of 10 site-years, only at Lethbridge in 2016/2017, was the interaction between cultivar and fungicide treatment significant for protein content. At the nine other site-years, there was an effect of cultivar and/or fungicide treatment but no interaction (Table 3.3).

Protein content of wheat at Lethbridge in 2016/2017 ranged from 10.2 to 12.6% and the effect of fungicide treatment varied among cultivars (Table 3.17). The reaction to fungicide treatment, however, was not consistent with the pattern observed with other parameters. 'AC Bellatrix' was the only cultivar that was affected by fungicide application; the dual fungicide application reduced protein content from 12.6 to 10.2%. Protein content of the rest of the cultivars was >11% regardless of fungicide treatment.

Among site-years with no interaction, protein content at two site-years was affected by both cultivar and fungicide application timing. At another site-year, only cultivar had an effect on protein content (Table 3.18). Contrary to the observation at Lethbridge in 2016/2017, protein content was improved slightly by the dual fungicide treatment from 11.7 to 12.0% at Saskatoon in 2014/2015 and by a single spring application, but not by the dual application from 10.3 to 10.6% at the same site in 2015/2016. The improvement was statistically significant, but the increase was small (0.3%). Protein content of cultivars varied by site-year, although 'Moats'

tended to have the highest especially when stripe rust severity was substantial, such as at Lethbridge in 2014/2015 and 2015/2016. ‘AC Bellatrix’ and ‘CDC Osprey’ had the lowest protein content at four site-years (Saskatoon, Indian Head and Lacombe in 2015/2016, Saskatoon in 2016/2017). Protein content differed more often among cultivars than fungicide treatment.

The inverse correlation was significant between protein content and stripe rust severity at four site-years and between protein content and leaf spot severity at one site-year (Table 3.19). The correlation coefficient values ranged from low (-0.2) to moderate (-0.6) for the correlation between protein and both stripe rust severity and leaf spot severity, which did not have a large influence on protein content at many site-years. Lethbridge in 2015/2016 was the only site-year that had a positive correlation for stripe rust severity and protein content.

Table 3.3 The *p* values from the ANOVA of winter wheat cultivar (C), fungicide application timing (T) and their interaction (C x T) on each variable at all site-years.

Site and Year	Factor	Stripe rust severity	Leaf spot severity	Yield	TW[a]	TKW[b]	Protein
Saskatoon							
2013/2014	C	<.0001***	0.0047**	0.0008***	<.0001***	<.0001***	0.028*
	T	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	ns
	C x T	0.0028**	ns	0.0325*	ns	0.0401*	ns
2014/2015	C	<.0001***	ns	0.0006***	<.0001***	ns	0.0084**
	T	<.0001***	<.0001***	0.0007***	ns	<.0001***	<.0001***
	C x T	ns	ns	ns	ns	0.0024**	ns
2015/2016	C	<.0001***	ns	<.0001***	<.0001***	<.0001***	0.0031**
	T	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	0.0004**
	C x T	0.0026**	<.0001***	<.0001***	<.0001***	0.0003***	ns
2016/2017	C	<.0001***	0.002**	<.0001***	<.0001***	<.0001***	<.0001***
	T	<.0001***	<.0001***	0.0075**	0.0075**	<.0001***	ns
	C x T	ns	0.0091**	ns	ns	ns	ns
Lacombe							
2014/2015	C	<.0001***	<.0001***				
	T	<.0001***	<.0001***				
	C x T	0.0008***	0.0153*				
2015/2016	C	<.0001***	ns	<.0001***	<.0001***	<.0001***	0.0016**
	T	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	ns
	C x T	0.0003***	ns	0.0082**	0.0082**	0.0318*	ns
2016/2017	C	0.0033**	<.0001***	<.0001***	<.0001***	<.0001***	0.0108*
	T	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	ns
	C x T	ns	ns	ns	ns	0.0362*	ns
Lethbridge							
2014/2015	C	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***
	T	<.0001***	0.0364*	ns	ns	0.0034**	ns
	C x T	0.0004***	ns	ns	ns	0.0444*	ns
2015/2016	C	<.0001***		<.0001***	<.0001***	<.0001***	<.0001***
	T	<.0001***		<.0001***	<.0001***	0.0191*	ns
	C x T	ns		ns	ns	ns	ns
2016/2017	C	<.0001***		<.0001	<.0001	<.0001***	ns
	T	<.0001***		<.0001	<.0001	<.0001***	<.0001***
	C x T	<.0001***		<.0001***	<.0001***	<.0001***	0.0127*
Indian Head							
2015/2016	C	0.0001**	0.0264*	ns	ns	<.0001***	0.0148*
	T	<.0001***	ns	ns	ns	<.0001***	ns
	C x T	0.0359*	0.0435*	ns	ns	ns	ns

[a] TW = test weight

[b] TKW = thousand kernel weight

Table 3.4 The *p* values from the ANOVA of winter wheat cultivar, fungicide application timing and their interaction on stripe rust severity for the grouped site-years according to the stripe rust severity on "check" and resistance reaction of 'Radiant'.

'Radiant'[a]	R	S	S	I	I
Stripe rust severity[b]	H	H	I	I	L
Site year Factor	SK[c]2014, LC2015, LC2016	LB all years	SK2016	SK2017, LC2017	SK2015, IH2016
C	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***
T	<.0001***	<.0001***	<.0001***	<.0001***	<.0001***
C x T	<.0001***	<.0001***	0.002**	ns	ns

[a] "Radiant" susceptibility, R = resistant, I = intermediate, S = susceptible

[b] Stripe rust severity on "check" (unsprayed 'Bellatrix'), H = high, I = intermediate, L = low

[c] IH=Indian Head, LB=Lethbridge, LC=Lacombe, SK=Saskatoon

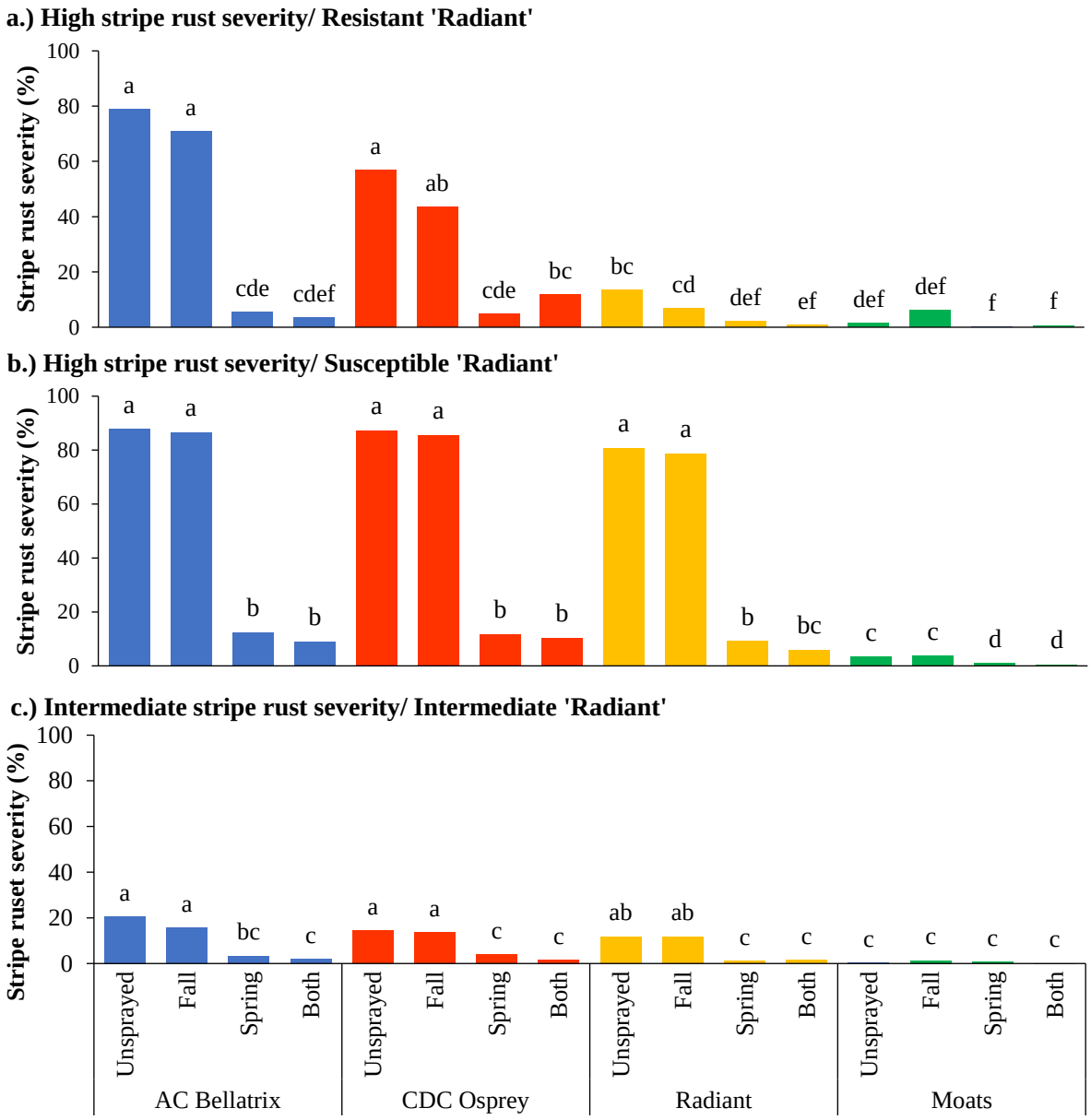

Figure 3.1 Stripe rust severity of grouped site-years affected by the interaction between winter wheat cultivar and fungicide application timing. Data groups: a.) High stripe rust severity with resistant 'Radiant' (Saskatoon in 2013/2014 and Lacombe in 2014/2015 and 2015/2016), b.) High stripe rust severity with susceptible 'Radiant' (Lethbridge in 2014/2015, 2015/2016 and 2016/2017), c.) Intermediate stripe rust severity with intermediate 'Radiant' (Saskatoon in 2015/2016). Treatment with different letters indicate significant differences among fungicide treatments for each cultivar according to the Tukey-Kramer test, $p < 0.05$.

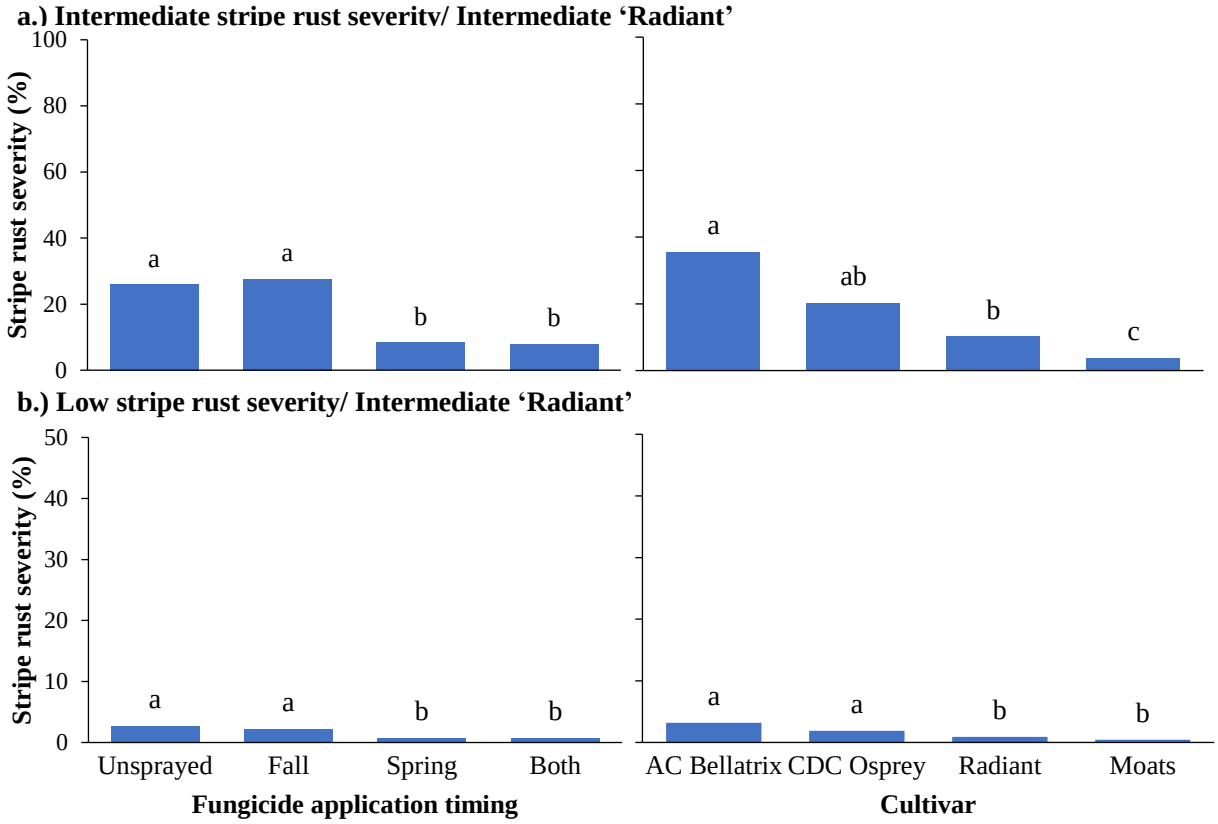

Figure 3.2 Stripe rust severity of grouped site-years affected by winter wheat cultivar and fungicide application timing. Data groups: a.) Intermediate stripe rust severity with intermediate 'Radiant' (Saskatoon in 2016/2017 and Lacombe in 2016/2017) and b.) Low stripe rust severity with intermediate 'Radiant' (Saskatoon in 2014/2015 and Indian Head in 2015/2016). Treatment with different letters indicate significant differences among fungicide treatments for each cultivar according to the Tukey-Kramer test, $p < 0.05$.

Table 3.5 Leaf spot severity (%) of winter wheat at four site-years affected by interaction between cultivars and fungicide application timings.

		2014/2015	2015/2016		2016/2017
Cultivar	Time	Lacombe	Saskatoon	Indian Head	Saskatoon
AC Bellatrix	Unsprayed	7.3 a[a]	11.1 ab	16.6 ab	2.4 cde
	Fall	6.9 a	8.3 abc	8.4 b	2.4 cde
	Spring	3.1 b	5.8 bcd	11.0 ab	1.9 e
	Both	3.4 b	5.1 cd	9.4 b	2.3 cde
CDC Osprey	Unsprayed	8.1 a	8.4 abc	11.6 ab	3.3 abc
	Fall	7.1 a	11.2 ab	11.4 ab	2.5 abcde
	Spring	2.1 b	4.3 cd	9.8 ab	1.9 e
	Both	2.9 b	4.0 d	14.9 ab	1.7 e
Radiant	Unsprayed	6.4 a	16.6 a	26.8 a	3.8 a
	Fall	7.2 a	15.3 a	20.6 ab	3.6 ab
	Spring	3.2 b	3.5 d	7.6 b	2.0 de
	Both	3.6 b	3.9 d	8.6 b	2.1 de
Moats	Unsprayed	7.5 a	6.5 bcd	9.4 b	3.0 abcd
	Fall	7.8 a	7.3 bcd	13.1 ab	2.4 bcde
	Spring	6.3 a	4.9 cd	8.9 b	1.9 e
	Both	5.8 a	6.1 bcd	8.7 b	2.1 de

[a] Treatments with different letters indicate significant differences among fungicide treatment for each winter wheat cultivar according to the Tukey-Kramer test, $p = 0.05$.

Table 3.6 Leaf spot severity (%) of winter wheat at four site-years affected by cultivars and/or fungicide application timings.

	2013/2014	2014/2015		2015/2016	2016/2017
Cultivar	Saskatoon	Saskatoon	Lethbridge	Lacombe	Lacombe
AC Bellatrix	13.1 b[a]	5.1 [ns]	4.3 b	60.0 [ns]	3.7 b
CDC Osprey	19.6 ab	3.8	4.3 b	56.6	10.3 a
Radiant	24.4 a	5.3	3.9 b	52.1	2.5 c
Moats	18.8 ab	4.7	6.0 a	59.0	1.8 c
Time					
Unsprayed	26.2 a	7.8 a	5.2 [ns]	72.0 a	7.6 a
Fall	28.0 a	6.0 a	5.4	69.2 a	8.7 a
Spring	12.6 b	2.5 b	4.3	43.4 b	1.0 b
Both	8.9 b	2.6 b	4.2	43.2 b	1.2 b

[a] Treatments with different letters indicate significant differences among fungicide treatment and/or cultivars according to the Tukey-Kramer test, $p = 0.05$.
"ns" = non-significant difference among means.

Table 3.7 The frequency and incidence rates of leaf spot disease pathogen species found on the leaf samples winter wheat from check plots at Saskatoon from 2013/2014 to 2016/2017 and at Lacombe in 2016/2017.

Site-year		N (plot)	*Pyrenophora tritici-repentis*	*Septoria tritici*	*Stagonospora nodorum*	*Septoria avenae*	*Cochliobolus sativus*	Total
Saskatoon	Frequency[a] (%)	16	50.0	48.5	0	0	1.5	100
2013/2014	Incidence[b] (%)	16	75.0	81.3	0	0	6.3	-
Saskatoon	Frequency (%)	15	13.3	53.3	0	0	33.3	100
2014/2015	Incidence (%)	15	26.7	73.3	0	0	40.0	-
Saskatoon	Frequency (%)	16	0	0	72.2	11.1	16.7	100
2015/2016	Incidence (%)	16	0	0	62.5	12.5	25.0	-
Saskatoon	Frequency (%)	16	0	11.5	73.1	3.8	11.5	100
2016/2017	Incidence (%)	16	0	12.5	56.3	6.3	25.0	-
Lacombe	Frequency (%)	16	0	0	89.2	5.4	5.4	100
2016/2017	Incidence (%)	16	0	0	93.8	18.8	12.5	-

[a] 'Frequency': Pathogen isolation frequency as % of leaf pieces that a pathogen was detected on out of all leaf pieces that all leaf spot pathogens were isolated from.
[b] 'Incidence': Disease incidence as % of plot that a pathogen was detected out of all plots.

Table 3.8 Yield (kg ha^{-1}) of winter wheat at site-years with an interaction between cultivar and fungicide application timing.

		2013/2014	2015/2016	2016/2017
Cultivar	Time	Saskatoon	Saskatoon	Lethbridge
AC Bellatrix	Unsprayed	975 d[a]	4461 d	1734 h
	Fall	982 d	5068 bcd	2641 gh
	Spring	1961 abcd	5652 abcd	5111 de
	Both	2183 abc	5955 abc	5697 cd
CDC Osprey	Unsprayed	1231 cd	4797 d	3610 efg
	Fall	1475 bcd	5163 cd	3714 ef
	Spring	2621 ab	5688 abc	6816 abc
	Both	2105 abcd	5681 abc	6415 bcd
Radiant	Unsprayed	1718 abcd	5872 abcd	2752 fgh
	Fall	1476 bcd	5687 abcd	2908 gh
	Spring	1908 abcd	6465 ab	6247 c
	Both	2674 a	6523 a	6002 cd
Moats	Unsprayed	1979 abcd	5649 abc	7280 abc
	Fall	2103 abcd	5698 abc	7163 abc
	Spring	2895 a	5875 ab	7950 ab
	Both	2062 abcd	5912 ab	8071 a

[a] Treatments with different letters indicate significant differences among fungicide treatment for each winter wheat cultivar according to the Tukey-Kramer test, $p = 0.05$.

Table 3.9 Yield (kg ha^{-1}) of winter wheat at site-years with an effect of cultivar and/or fungicide application timing.

	2014/2015		2015/2016			2016/2017	
Cultivar	Saskatoon	Lacombe	Indian Head	Lethbridge	Lacombe	Saskatoon	Lacombe
AC Bellatrix	4534 a[a]	3786 b	4192 ab	3462 b	4714 [ns]	3235 b	4806 b
CDC Osprey	4483 a	3702 b	4069 ab	3441 b	4782	3406 b	4844 b
Radiant	4469 a	4638 a	3563 b	4702 a	5140	4164 a	5620 a
Moats	4150 b	3456 b	4269 a	3346 b	4963	3805 ab	5730 a
Time							
Check	4248 b	3557 b	4078 [ns]	3439 [ns]	4475 c	3414 ab	4752 b
Fall	4267 b	3839 ab	3730	3756	4808 bc	3354 b	4722 b
Spring	4563 a	3993 ab	4135	3789	5045 ab	3716 ab	5832 a
Both	4559 a	4194 a	4191	4001	5271 a	4126 a	5694 a

[a] Treatments with different letters indicate significant differences among fungicide treatment and/or cultivars according to the Tukey-Kramer test, $p = 0.05$.
"ns" = non-significant difference among means.

Table 3.10 The *p* values and correlation coefficients (r) from correlation analysis between yield and stripe rust severity, and yield and leaf spot severity of winter wheat at each site-year.

	Variables	Stripe rust severity			Leaf spot severity		
Site	Year	n	*p* value	r	n	*p* value	r
Saskatoon	2013/2014	64	<.0001***	-0.700	64	ns	-
	2014/2015	64	ns	-	64	0.0326*	-0.267
	2015/2016	64	<.0001***	-0.495	64	ns	-
	2016/2017	64	<.0001***	-0.560	64	ns	-
Indian Head	2015/2016	62	ns	-	62	ns	-
Lethbridge	2014/2015	63	0.0006***	-0.418	53	ns	-
	2015/2016	63	ns	-			
	2016/2017	63	<.0001***	-0.913			
Lacombe	2014/2015	64	0.0008***	-0.409	57	0.007**	-0.353
	2015/2016	63	<.0001***	-0.685	64	<.0001***	-0.644
	2016/2017	64	<.0001***	-0.591	64	0.0009***	-0.409

* = $0.01 \leq p < 0.05$, ** = $0.001 \leq p < 0.01$, *** = $p < 0.001$
"ns" = non-significant ($P > 0.05$).

Table 3.11 Test weight (kg hL^{-1}) of winter wheat at site years with an interaction between cultivar and fungicide application timing.

		2015/2016		2016/2017
Cultivar	Time	Saskatoon	Lacombe	Lethbridge
AC Bellatrix	Check	76.2 e[a]	71.7 d	65.5 f
	Fall	76.5 e	74.9 abcd	65.8 f
	Spring	78.9 abc	77.3 ab	78.2 bcd
	Both	78.9 abc	77.2 ab	77.4 cd
CDC Osprey	Check	76.1 e	73.8 cd	67.9 ef
	Fall	76.0 e	74.5 bcd	69.7 e
	Spring	77.7 d	75.6 abc	77.8 bcd
	Both	78.1 bcd	76.0 abc	77.9 abcd
Radiant	Check	78.2 abcd	76.4 abc	66.5 ef
	Fall	78.0 cd	76.7 abc	66.0 f
	Spring	78.9 abcd	76.8 abc	76.5 d
	Both	78.9 abcd	77.5 ab	75.7 d
Moats	Check	79.0 abc	77.0 abc	80.8 abc
	Fall	79.0 abc	77.2 ab	81.2 abc
	Spring	79.2 a	77.6 ab	81.8 a
	Both	79.2 ab	77.8 a	81.3 ab

[a] Treatments with different letters indicate significant differences among fungicide treatment for each winter wheat cultivar according to the Tukey-Kramer test, $p = 0.05$.

Table 3.12 Test weight (kg hL^{-1}) of winter wheat at site years with an effect of cultivar and/or fungicide application timing.

	2013/2014	2014/2015		2015/2016	2016/2017	
Cultivar	Saskatoon	Saskatoon	Lethbridge	Lethbridge	Saskatoon	Lacombe
AC Bellatrix	77.4 b[a]	81.8 a	68.2 b	73.7 b	77.5 a	77.8 ab
CDC Osprey	76.6 c	79.2 b	67.3 b	74.0 b	75.4 b	76.8 b
Radiant	77.3 bc	80.1 b	67.9 b	73.8 b	78.4 a	76.8 b
Moats	78.7 a	79.8 c	72.8 a	76.5 a	78.0 a	80.2 a
Time						
Check	76.9 b	80.0 [ns]	68.8 [ns]	75.2 a	76.8 b	76.6 b
Fall	77.1 b	80.2	68.3	75.0 a	77.0 b	76.4 b
Spring	78.1 a	80.3	69.1	73.7 b	77.9 a	79.2 a
Both	77.9 a	80.4	70.0	74.1 b	77.6 ab	79.2 a

[a] Treatments with different letters indicate significant differences among fungicide treatment and/or cultivars according to the Tukey-Kramer test, $p = 0.05$.
"ns" = non-significant difference among means.

Table 3.13 The *p* values and correlation coefficients (r) from correlation analysis between test weight and stripe rust severity, and TW and leaf spot severity of winter wheat at each site-year.

	Variables	Stripe rust severity			Leaf spot severity		
Site	Year	n	*p* value	r	n	*p* value	r
Saskatoon	2013/2014	63	<.0001***	-0.643	63	ns	-
	2014/2015	64	0.0085**	0.326	64	ns	-
	2015/2016	64	<.0001***	-0.777	64	0.002**	-0.380
	2016/2017	64	<.0001***	-0.490	64	ns	-
Indian Head	2015/2016	64	0.0131*	-0.309	64	ns	-
Lethbridge	2014/2015	64	0.0001***	-0.462	54	0.0054**	0.374
	2015/2016	64	ns	-			
	2016/2017	64	<.0001***	-0.945			
Lacombe	2014/2015						
	2015/2016	63	<.0001***	-0.793	64	<.0001***	-0.553
	2016/2017	64	<.0001***	-0.688	63	0.0012**	-0.399

* = $0.01 \leq p < 0.05$, ** = $0.001 \leq p < 0.01$, *** = $p < 0.001$

"ns" = non-significant ($P > 0.05$).

Table 3.14 Thousand kernel weight (g) of winter wheat at site-years with an interaction between cultivar and fungicide application timing.

		2013/2014	2014/2015		2015/2016		2016/2017	
Cultivar	Time	Saskatoon	Saskatoon	Lethbridge	Saskatoon	Lacombe	Lacombe	Lethbridge
AC Bellatrix	Unsprayed	32.8 ef[a]	36.4 b	21.9 bcd	30.2 e	29.4 defg	29.3 abcd	20.8 d
	Fall	33.6 def	37.0 ab	21.8 bcd	31.4 de	32.4 abcdef	26.0 d	23.8 c
	Spring	36.6 abc	37.4 ab	26.3 ab	35.7 abc	35.9 a	34.8 ab	33.0 ab
	Both	37.4 a	38.0 ab	23.7 abcd	35.6 abc	36.1 a	36.2 a	33.0 ab
CDC Osprey	Unsprayed	31.8 f	34.4 c	20.7 cd	31.0 de	27.3 g	25.8 d	23.9 c
	Fall	32.0 f	33.6 c	19.0 d	30.9 de	28.4 fg	27.7 cd	24.4 c
	Spring	34.1 bcdef	33.9 c	23.0 abcd	33.4 cd	30.7 cdef	29.0 bcd	30.7 b
	Both	33.3 def	33.1 c	23.2 abcd	33.4 cd	30.8 cdef	31.0 abcd	31.5 ab
Radiant	Unsprayed	34.4 abcdef	38.2 a	22.6 abcd	35.3 bc	32.5 abcd	30.4 abcd	23.2 cd
	Fall	33.8 cdef	36.6 ab	20.2 cd	35.0 c	31.9 bcde	31.9 abcd	25.5 c
	Spring	35.1 abcde	37.8 ab	24.7 abc	37.7 ab	33.5 abc	35.4 ab	29.6 b
	Both	37.2 ab	37.6 ab	24.6 abc	37.9 a	33.9 ab	34.1 abc	30.3 b
Moats	Unsprayed	33.1 def	33.0 c	25.3 ab	31.8 de	29.1 fg	31.5 abcd	33.4 ab
	Fall	32.9 ef	32.8 c	25.9 ab	30.8 de	29.7 efg	32.4 abc	33.8 ab
	Spring	35.8 abcd	33.1 c	24.8 abc	32.0 de	31.2 cdef	33.9 abc	35.0 a
	Both	34.4 bcdef	34.3 c	27.0 a	31.2 de	30.6 def	33.8 abc	33.1 ab

[a] Treatments with different letters indicate significant differences among fungicide treatment for each winter wheat cultivar according to the Tukey-Kramer test, $p = 0.05$.

Table 3.15 Thousand kernel weight (g) of winter wheat at site-years with effects of cultivar and/or fungicide application timing.

	2015/2016		2016/2017
Cultivar	Indian Head	Lethbridge	Saskatoon
AC Bellatrix	33.5 a[a]	35.1 bc	33.5 bc
CDC Osprey	30.3 b	33.9 c	31.6 c
Radiant	32.9 a	37.5 a	37.6 a
Moats	31.7 ab	36.3 ab	33.3 b
Time			
Unsprayed	31.6 bc	34.7 [ns]	32.5 b
Fall	30.5 c	35.3	32.5 b
Spring	33.1 ab	36.4	35.8 a
Both	33.3 a	36.5	35.1 a

[a] Treatments with different letters indicate significant differences among fungicide treatment and/or cultivars according to the Tukey-Kramer test, $p = 0.05$.
"ns" = non-significant difference among means.

Table 3.16 The *p* values and correlation coefficients (r) from correlation analysis between thousand kernel weight (TKW) and stripe rust severity, and TKW and leaf spot severity of winter wheat at each site-year.

	Variables	Stripe rust severity			Leaf spot severity		
Site	Year	n	*p* value	r	n	*p* value	r
Saskatoon	2013/2014	64	0.0009***	-0.407	64	0.0004***	-0.427
	2014/2015	64	ns	-	64	ns	-
	2015/2016	64	0.0005***	-0.421	64	0.0081**	-0.329
	2016/2017	64	<.0001***	-0.556	64	ns	-
Indian Head	2015/2016	63	ns	-	63	ns	-
Lethbridge	2014/2015	64	<.0001***	-0.682	54	ns	-
	2015/2016	64	<.0001***	-0.483			
	2016/2017	64	<.0001***	-0.905			
Lacombe	2014/2015						
	2015/2016	63	0.0084**	-0.330	64	<.0001***	-0.568
	2016/2017	64	<.0001***	-0.645	63	<.0001***	-0.554

* = $0.01 \leq p < 0.05$, ** = $0.001 \leq p < 0.01$, *** = $p < 0.001$
"ns" = non-significant ($P > 0.05$).

Table 3.17 Protein content (%) of winter wheat at Lethbridge in 2016/2017 with an interaction between cultivar and fungicide application timing.

		2016/2017
Cultivar	Time	Lethbridge
AC Bellatrix	Check	12.6 a[a]
	Fall	11.6 ab
	Spring	10.5 ab
	Both	10.2 b
CDC Osprey	Check	11.9 ab
	Fall	11.4 ab
	Spring	11.3 ab
	Both	11.0 ab
Radiant	Check	12.2 ab
	Fall	12.1 ab
	Spring	11.0 ab
	Both	11.1 ab
Moats	Check	11.9 ab
	Fall	11.5 ab
	Spring	11.3 ab
	Both	11.7 ab

[a] Treatments with different letters indicate significant differences among fungicide treatment for each winter wheat cultivar according to the Tukey-Kramer test, $p = 0.05$.

Table 3.18 Protein content (%) of winter wheat at site-years with effects of cultivar and/or fungicide application timing.

	2013/2014	2014/2015		2015/2016			
Cultivar	Saskatoon	Saskatoon	Lethbridge	Saskatoon	Lethbridge	Indian Head	Lacombe
AC Bellatrix	10.7 ab	11.9 a	13.1 b	10.2 c	10.3 c	9.4 ab	9.3 b
CDC Osprey	10.3 b	11.5 b	12.7 b	10.2 bc	11.2 ab	9.3 b	9.4 b
Radiant	10.7 ab	11.6 a	11.7 c	10.6 ab	10.4 bc	9.8 a	9.7 ab
Moats	11.0 a	11.4 b	13.6 a	10.7 ab	11.7 a	9.6 ab	10.1 a
Time							
Check	10.7 [ns]	11.7 b	12.5 [ns]	10.3 bc	10.6 [ns]	9.5 [ns]	9.8 [ns]
Fall	10.5	11.2 ab	12.6	10.2 c	10.4	9.7	9.6
Spring	10.6	11.3 ab	12.7	10.6 a	10.1	9.4	9.6
Both	10.9	12.0 a	13.2	10.6 ab	10.3	9.5	9.6

	2016/2017	
Cultivar	Saskatoon	Lacombe
AC Bellatrix	9.7 c	10.3 ab
CDC Osprey	9.9 bc	9.9 b
Radiant	10.6 ab	10.4 ab
Moats	11.3 a	11.0 a
Time		
Check	10.6 [ns]	10.8 [ns]
Fall	10.3	10.2
Spring	10.3	10.3
Both	10.3	10.3

[a] Treatments with different letters indicate significant differences among fungicide treatment and/or cultivars according to the Tukey-Kramer test, $p = 0.05$.
"ns" = non-significant difference among means.

Table 3.19 The *p* values and correlation coefficients (r) from correlation analysis between protein content and stripe rust severity and between protein content and leaf spot severity of winter wheat at each site-year.

	Variables	Stripe rust severity			Leaf spot severity		
Site	Year	n	*p* value	r	n	*p* value	r
Saskatoon	2013/2014	64	ns	-	64	ns	-
	2014/2015	64	ns	-	64	ns	-
	2015/2016	64	<.0001***	-0.590	64	0.0291*	-0.273
	2016/2017	64	0.0463*	-0.250	64	ns	-
Indian Head	2015/2016	64	ns	-	64	ns	-
Lethbridge	2014/2015	64	0.0007***	-0.412	54	ns	-
	2015/2016	64	ns	-			
	2016/2017	64	0.0022**	0.376			
Lacombe	2014/2015						
	2015/2016	63	ns	-	64	ns	-
	2016/2017	64	ns	-	63	ns	-

* = $0.01 \leq p < 0.05$, ** = $0.001 \leq p < 0.01$, *** = $p \leq 0.001$.

3.4 Discussion

Fungicide application has been a major stripe rust control method to protect wheat from yield loss (Chen 2005). After deciding on which fungicide to apply for stripe rust, the timing of application is another critical factor that can have an impact on the effectiveness of disease control and yield protection (Chen 2007). In the current study, reduction in stripe rust severity by spring fungicide application at the flag leaf/booting stage, with or without fall fungicide application, was effective on disease susceptible cultivars such as 'AC Bellatrix', 'CDC Osprey' and 'Radiant'. A statistically significant reduction was observed even when stripe rust severity of the unsprayed susceptible cultivar was low. These results correspond with past studies, in which fungicide applications at similar timings reduced foliar disease severity and protected yield potential (Wegulo et al. 2012; Fernandez et al. 2013; Gomes et al. 2016; Bhatta et al. 2018; Sylvester & Kleczewski 2018). Yield improvement with fungicide application at the flag leaf emergence and booting stages can be attributed to the reduction of foliar disease severity and subsequent retention of green leaf area of flag leaves (Cook et al. 1999). Therefore, the lack of effect at the soft-dough stage with a single fall fungicide application was expected because fungicide application at this stage did not provide protection of flag leaves later in the growing season.

Benefits associated with a fall fungicide application were observed by Turkington et al. (2016), who speculated that the reduction in stripe rust severity on seedlings improved winter survival rates and led to yield improvement. Stripe rust management of winter wheat at the seedling stage has been studied and proven to be an effective practice to protect yield in different parts of the world where stripe rust epidemics are severe during the autumn. Seed treatment with triadimefon is often used on winter wheat to reduce early season stripe rust severity, protect yield

and avoid a green bridge in the areas where fall stripe rust infection is severe and overwintering of *Pst* is common, such as in wheat growing regions in China (Wan et al. 2007; Chen & Kang 2017). Greenhouse experiments on the efficacy of seed treatment for early tan spot and stripe rust control found that stripe rust severity was reduced 20 days after inoculation with *Pst* urediniospores at the seedling stage (Bugingo 2018). A field experiment in South Dakota, USA from the same study also indicated that seed treatment of winter wheat improved yield. In Australia, where stripe rust is a constant threat throughout the growing season, seed treatment lowered stripe rust severity in the early growth stage and maintained yield potential of susceptible or moderately resistant cultivars (Brown 1987). Fall fungicide application could function similar to seed treatment, by reducing early season stripe rust severity and improving yield if a constant threat of severe stripe rust epidemics is present.

In the current study, the winter survival rate of seedlings over the three growing seasons at Saskatoon was not affected by fall fungicide application (data not shown). Also, the lack of yield improvement by a single fall fungicide application at any site-year indicated that the fall fungicide treatment had no effect on factors such as plant vigour or winter survival of seedlings that would have influenced yield. Widespread incidence and high stripe rust severity was reported in western Canada in 2010/2011 and 2011/2012 (Puchalski et al. 2013). These years with severe stripe rust epidemics coincided with the years of the study by Turkington et al. (2016). Stripe rust severity in fall 2013 to 2017 was not as high as in 2011/2012 even at Saskatoon where the plots were artificially inoculated in both fall and spring. Seedling stripe rust severity in fall ranged from trace at Saskatoon and trace to moderate at Lethbridge and Lacombe. Even when fall stripe rust severity was substantial at Lethbridge and Lacombe, a single fall fungicide application did not affect yield. It is possible that fall fungicide application was

beneficial in the previous study because study stripe rust severity was higher than usual in western Canada during that study. We observed little to no benefit from fall fungicide application because stripe rust severity during the years of this study were not as high as in 2010/2011 or 2011/2012.

Another possible benefit of fall fungicide application is that it may reduce the amount of *Pst* inoculum overwintering on winter wheat. Overwintering of *Pst* in winter wheat has been observed sporadically in southern Alberta and southern Manitoba in Canada (Kumar et al. 2013; Puchalski et al. 2013; Gaudet et al. 2016; Aboukhaddour et al. 2017; Ms. Holly Derksen, personal communication). Winter wheat acts as a "green bridge" for *Pst* and can cause early stripe rust infection of winter wheat as well as spring wheat seeded adjacent to an infected winter wheat field. This is a serious threat to the production of both spring and winter wheat since stripe rust thrives under the cool wet conditions in spring and early summer, resulting in greater production of urediniospores and higher stripe rust severity at the grain filling stage. Although the extremely cold winter conditions in western Canada is less than ideal for *Pst* to overwinter, the pathogen is capable of surviving as mycelia inside winter wheat tissues for 48 hours at -10℃ and survive better in more winter-hardy winter wheat cultivars (Ma et al. 2015). Improved survival of winter wheat seedlings under deeper and continuous snow cover and in warmer winters make *Pst* overwintering in western Canada a potentially serious problem. As stripe rust of wheat has become more prevalent and widespread in the recent past in western Canada, the risk of *Pst* overwintering in winter wheat has increased and requires close monitoring. In the current study, however, there was no sign of pathogen overwintering at any site-year. The lack of widespread overwintering in the past indicates that under the present conditions, the

overwintering of *Pst* may still be rare and fall fungicide application is not warranted in western Canada.

At all site-years of this study, 'AC Bellatrix' and 'CDC Osprey' were susceptible and 'Moats' proved to be the resistant cultivar against stripe rust. The transition of 'Radiant' from moderately resistant to susceptible against stripe rust gives some insight into the difference among the *Pst* populations that overcame the *Yr10* gene in western Canada. The *Pst* isolates virulent to the cultivars and genotypes with *Yr10* was first detected in the United States in 1970 (Liu et al. 2017) and in Canada in 1994 (Su et al. 2003). The frequency of the virulence to *Yr10* in the *Pst* populations of the United States was extremely low until 2004, but it rapidly increased in 2005 and became predominant in the Pacific Northwest (Chen et al. 2010). The stripe rust epidemic in winter wheat in southern Alberta in 2010/2011 was partially attributed to the breakdown of seedling resistance conditioned by *Yr10* in 'Radiant' (Puchalski & Gaudet 2011). When the virulence profile of *Pst* populations was evaluated post 2010, the frequency of *Pst* isolates with virulence to *Yr10* was low (15-17%) (Xi et al. 2015; Brar & Kutcher 2016). The low frequency of virulent isolates in natural inoculum, the distance for urediniospores to travel and the environmental conditions could have contributed to the differences in the reaction of 'Radiant' among site-years. Xi et al. (2015) suggested that because Lacombe is in central Alberta, it is further away from the source of *Pst* inoculum (the Pacific Northwest) than sites in southern Alberta; this results in relatively late arrival of urediniospores in the growing season. As a result, there are fewer urediniospores and lower severity in Lacombe than in southern Alberta. The same is true for Saskatoon as decreased stripe rust severity was observed in Saskatchewan compared to southern Alberta (Brar & Kutcher 2016). Artificial inoculation in fall and early spring was conducted in Saskatoon every year during the experiment; however, the inoculum

consisted of the *Pst* isolates that were not virulent to *Yr10* and this may explain why 'Radiant' was resistant at Saskatoon. At Lethbridge, irrigation is a common practice in commercial fields and the experimental plots were regularly irrigated to encourage high disease severity, which, along with the higher load of natural inoculum arriving from the Pacific Northwest of the United States, created conditions more suitable for stripe rust establishment and spread in Lethbridge compared to the other sites. The susceptibility of 'Radiant' at Lacombe and Saskatoon over time indicates increased virulence frequency to *Yr10* in the natural *Pst* population, which is consistent with the virulence frequency observed in the United States from 2014 to 2017 (Annual Stripe Rust Race Data Report 2017).

Leaf spot diseases

Leaf spot severity was low at most site-years, high at one site-year and data unavailable at two. The low leaf spot severity at most site-years was consistent with survey reports from Saskatchewan from 2013/2014 to 2016/2017 (Fernandez et al. 2015; 2016; 2017; 2018). The effects of fungicide treatment on leaf spot severity were similar to that observed on stripe rust. A single spring and dual fungicide applications lowered leaf spot severity, while a single fall fungicide application did not. As indicated by the lower coefficients of determination for leaf spot severity compared to stripe rust severity, leaf spot severity had less effect on yield. There was no strong cultivar resistance against leaf spot diseases, which were inconsistent, although cultivars differed in disease severity among site-years. There was no difference among cultivars when leaf spot severity was high at Lacombe in 2015/2016. The profile of leaf spot pathogens changed dramatically over time at Saskatoon. *Pyrenophora tritici-repentis* and *S. tritici* were the two predominant pathogens, and *St. nodorum* are *S. avenae* were not isolated in 2013/2014 and 2014/2015. *Stagonospora nodorum* became the predominant pathogen and *S. avenae* was

isolated at low frequency in 2015/2016 and 2016/2017. *Pyrenophora tritici-repentis* was not isolated and *S. tritici* was isolated infrequently in the last two years. The pathogen profile of leaf spot diseases can change from year to year depending on environmental factors such as rainfall and temperature, particularly within 14 days of rating as reported by Gilbert et al. (1998). They observed that *St. nodorum* tended to be prevalent in the years with higher daily temperature and rainfall during the 14 days before disease rating. At Saskatoon in 2016, there was higher rainfall compared to three other years within the 14-day period and this could explain the higher prevalence of this pathogen in the 2015/2016 growing season. The higher prevalence of *P. tritici-repentis* in the drier growing season of 2013/2014 and 2014/2015 corresponded with the negative correlation found between rainfall and pathogen prevalence found in the previous study. However, these trends did not explain the pathogen profile in 2016/2017, which had weather conditions similar to 2013/2014 during the 14 days before rating and still had low *P. tritici-repentis* and high *St. nodorum* prevalence. The location of each experiment was moved every year at all sites and this change may have caused the change in leaf spot pathogen profile among the years.

Yield and grain quality

Yield was inversely correlated with stripe rust severity, and to a lesser extent with leaf spot severity; however, the reduction in disease severity was one of many factors that affected yield. Reduced stripe rust severity by fungicide application in spring and both fall and spring did not affect yield at Indian Head in 2014/2015. This could have been due to the extremely low stripe rust and leaf spot severity at that site-year and the limited influence of stripe rust on yield as a result. At Lethbridge in 2014/2015, stripe rust severity was high and spring fungicide application reduced severity to a level comparable with Lethbridge in 2016/2017. However, yield

in 2014/2015 did not improve with the reduced stripe rust severity while it did in 2016/2017, even though yield tended to be higher with spring fungicide application in 2014/2015. This indicated the strong influence of other environmental factors, such as amount and timing of precipitation and the variation of these factors among growing seasons. One factor that made Lethbridge unique was irrigation; the experimental plots were irrigated three times a week in spring and summer to promote disease development. Irrigation would have positively affected grain filling and protected yield potential for all treatments, thus obscuring yield differences among treatments.

Although yield and grain quality were never improved by a single fall fungicide application at any site-year, there were a few site-years where a single spring application did not improve yield or grain quality compared to the unsprayed check, but dual fungicide applications did. These increases in yield and grain quality were rare. The susceptible cultivar tended to benefit from the dual fungicide treatment, but it was not consistent over site-years. The benefit of the dual fungicide application treatment was not high or consistent enough to be of practical benefit. These results correspond well with the reports from another study where dual or triple fungicide applications were reported not to be more effective than single fungicide application on grain quality improvement (Jarroudi et al. 2015). In another study on the effect of early fungicide applications on durum wheat, double fungicide application at the stem elongation or flag leaf emergence stages, and at the mid-anthesis stages were not more effective to protect yield potential than the single application at the mid-anthesis stage (Fernandez et al. 2014b). Based on the lack of benefit of dual fungicide application over single spring application in the current study, dual application (in fall and spring) is not recommended in western Canada,

especially considering the cost of fungicide, labour and time required to apply fungicide in the fall within a short period of time between emergence and snow fall.

Both TW and TKW were improved by fungicide application in spring and dual fungicide treatment at some site-years, especially on the stripe rust susceptible cultivars ‘AC Bellatrix’ and ‘CDC Osprey’. ‘Radiant’ was affected by the treatments when *Pst* inoculum was virulent to the cultivar. This agrees with the results from other studies that investigated the effect of fungicide treatment on yield and grain quality (Bhatta et al. 2018). At most site-years, a single spring and the dual fungicide treatments improved grain quality of one or more cultivars. A single fall fungicide application never improved grain quality (either TKW or TW), and this agrees with what was observed for yield with the same treatment. No fungicide treatment influenced TW at Saskatoon in 2014/2015, which could be attributed to the low stripe rust severity. The correlation between TW and stripe rust severity was positive at this site-year; this indicates that TW was affected by factors other than disease severity. Similar to yield, TW at Lethbridge in 2014/2015 was not affected by fungicide treatment. Again, this may indicate that factors other than stripe rust influenced yield and TW. The positive correlation for TW with leaf spot severity at the same site-year could be due to low leaf spot severity and the multiple missing data points for leaf spot severity at this site-year. At Lethbridge in 2015/2016, TW decreased with a single spring or dual fungicide applications and TKW was not affected by fungicide treatment. This indicated the high variability of fungicide application on grain quality and the effect of factors other than fungicide application. A single fall fungicide application was consistently ineffective in improving grain quality.

Protein content was affected more by differences among cultivars and by environmental conditions than disease control. An important threshold of protein content for Canada West Red

Winter (CWRW) is 11%; higher than this may improve grain grades (No.1 and No.2) with possible premiums (Canada Grain Commission 2018). When stripe rust severity was exceptionally high during the summer of 2017 at Lethbridge, the protein content of 'AC Bellatrix' decreased with dual fungicide application compared to the unsprayed check. This could be explained by the loss in kernel mass and moisture due to high disease severity during the grain-filling period.

In summary, single fall fungicide treatment was not beneficial in terms of stripe rust control or grain yield and quality improvement under the common climatic conditions in western Canada. A single spring and the dual fungicide treatment were beneficial to improve yield of stripe rust susceptible cultivars; this was attributed to effective stripe rust control on the flag leaf through grain filling stages. The results should help growers to make practical decisions on when to apply fungicide to manage stripe rust of winter wheat in western Canada. The difference in distribution of natural *Pst* populations that is virulent to *Yr10* in 'Radiant' among sites provided insight into how a specific virulence in the natural *Pst* population is distributed in western Canada. The lack of overwintering of *Pst* in winter wheat in the current study suggested that it is not a widespread issue in western Canada. However, the change in virulence in the *Pst* population and the amount of inoculum coming into Canada from the United States need to be closely monitored for any possibility of severe future epidemics like in 2010/2011. With climate change and the rapid shift in stripe rust virulence and distribution in North America, more studies need to be conducted to better understand stripe rust epidemics and control in winter wheat.

CHAPTER 4

4. DETECTION AND EVALUATION OF RESIDUAL EFFECTS OF DEFEATED RESISTANCE GENES, *Yr10, Yr26* and *Yr32,* BY GROWTH CHAMBER AND FIELD EXPERIMENTATION

4.1 Introduction

Defeated resistance genes are defined as major race-specific resistance genes of a host plant that have been overcome by virulent pathogen races or strains. There are many stripe rust resistance genes in wheat that condition seedling resistance (all-stage resistance, ASR). These may be highly effective, but are often not durable due to the strong selection pressure that results from the widespread use. The constant evolution of the pathogen results in virulent *Pst* races that overcome the resistance in as short a time-frame as a few years after introduction in commercial cultivars (Line & Qayoum 1991; Line & Chen 1995; Chen 2007). Defeated resistance genes have been speculated to possess residual effects as partial, race-nonspecific resistance that could be pyramided into a host genotype and result in quantitative resistance against virulent pathogen strains (Nelson et al. 1970; Nelson 1978). Residual effects of defeated qualitative resistance genes have been reported for leaf rust of poplars (*Melampsora larici-populina*) (Dowkiw & Bastien 2007), powdery mildew of wheat (*Erysiphe graminis* DC. f. sp. *tritici* E. Marchal) (Nass et al. 1981; Royer et al. 1984), stem rust of wheat (*Puccinia graminis* Pers. f. sp. *tritici*) (Brondy et al. 1986), and leaf rust (*Puccinia triticina* Eriks.) of wheat (Basandrai et al. 1998) to list a few. Some of the issues with past research on the residual effects of defeated genes are the difficulty of differentiating a residual effect from partial resistance expressed by unknown resistance genes

in the genetic background, inconsistency of partially resistant reactions among gene combinations and the lack of diversity in the pathogen isolates used.

Precise identification of the host genotypes is crucial to study residual effects as the effect of defeated R genes need to be compared to other lines of identical genotype except for the presence or number of the R genes. Great advancements in technology for molecular genotyping has been made over the last few decades (He et al. 2014), which was not available when early studies on residual effects were conducted. This provides the ability to select host genotypes that differ only for the R gene to be assessed and to analyze the effect of genotypes for residual effects. Single nucleotide polymorphism (SNP) markers, polymorphisms characterized by single base differences in a DNA sequence, are a widely used and highly effective type of molecular marker due to their abundance in plant genomes (Rafalski 2002) and applicability to a high through-put process (He et al. 2014). With progress in sequencing of the wheat genome (Lukaszewski et al. 2014) and expressed sequence tags (Rudd 2003), more SNP markers are discovered from those reference sequences and are utilized in plant breeding. Detection and quantification of residual resistance conditioned by defeated resistance genes in wheat against *Pst* may be important to create wheat cultivars with durable stripe rust resistance.

4.2 Materials and methods

4.2.1 Plant materials

Near isogenic lines (NILs) in the cultivar 'Avocet-S' (hereafter 'Avocet') background with single stripe rust resistance genes (Wellings et al. 2004) were crossed to create progeny with two resistance genes. The NILs with resistance genes *Yr10, Yr26*, and *Yr32* were intercrossed in the combinations: *Yr26/Yr10, Yr32/Yr10*, and *Yr26/Yr32*. Individual F_1 kernels were grown, self-pollinated and F_2 kernels were harvested. Kernels from the F_2 generation were randomly selected as individual lines and increased by single seed descent for five generations. The F_5 generation

was harvested in 2017. Leaf tissue was sampled from the parental lines, 12 F_5 plants of each line selected by phenotypic screening, and cultivars 'Moro' and 'Radiant' at the two-leaf stage and flash frozen in liquid nitrogen upon sampling. Frozen tissue of each plant was freeze-ground individually in 1.5-mL microcentrifuge tubes with a 3-mm tungsten bead by a cell lyser (2010 Geno/Grinder®, SPEX SamplePrep NJ, USA) at 1100 rpm for 1 minute. Genomic DNA was extracted from the ground tissue by using the 10% sodium dodecyl sulfate (SDS) DNA extraction method (Department of Plant Science, personal communication). Extracted DNA was suspended in sterile distilled water and the DNA concentrations of 23 random samples measured using a NanoDrop™ 8000 Spectrophotometer (Thermo Scientific™, MA, USA). The DNA concentrations of samples ranged from 83 to 1198 ng μL^{-1} with acceptable quality. The DNA samples were stored at -80°C until analysis.

4.2.2 *Puccinia striiformis* f. sp. *tritici* isolates

Isolates of *Pst* collected in Saskatchewan (Brar 2015), Alberta and BC (Kumar et al. 2012) were used for phenotypic screening and residual effect evaluation. For screening, the isolates were selected based on virulent and avirulent reactions to the three *Yr* genes of interest to identify the genotypes homozygous for these resistance genes (Table 4.1). After the preliminary experiments, it was discovered that all isolates identified to be avirulent to either *Yr10* or *Yr26* were avirulent to both *Yr* genes and no isolate was available to phenotypically identify double homozygous lines from the *Yr26/Yr10* cross. The F_5 plants of this combination were first screened by an isolate avirulent to both genes to eliminate the lines with no *Yr* gene (Table 4.2) and the rest were screened genotypically using the KASP assay with SNP markers for *Yr10* and *Yr26* genes developed for this study. The F_5 plants from *Yr32/Yr10* and *Yr32/Yr26* genotypes were first screened for one of two *Yr* genes with the *Pst* isolates and the genotypes selected from

the first screening were screened a second time with another *Pst* isolate for the second *Yr* gene based on the resistance phenotypes (Table 4.2).

Table 4.1 List of *Puccinia striiformis* f. sp. *tritici* isolates used to screen and evaluate defeated resistance genes, *Yr10, Yr26* and *Yr32* collected from Saskatchewan (Brar 2015), Alberta and BC (Kumar et al. 2012).

Isolates	*Yr10*	*Yr26*	*Yr32*	Origin
T030	-	+	-	Edmonton, AB
T022	-	+	+	Lacombe, AB
W031	-	+	-	Saskatoon, SK
W050	+	-	+	Saskatoon, SK
W052	+	-	+	Saskatoon, SK
W057	+	-	+	Saskatoon, SK
T034	+	+	-	Creston, BC
W015	+	+	+	Lethbridge, AB
W020	+	+	+	Landis, SK
W034	+	+	+	Bussano, AB
W048	+	+	+	Fairfield, AB
W049	+	+	+	Fairfield, AB

+: virulent, -: avirulent.

Table 4.2 'Avocet' NILs with *Yr* gene combinations and *Pst* isolates used to screen each genotype with specific virulence and avirulence to *Yr10, Yr26* and *Yr32* genes.

	Cross	Number of	*Pst* isolates	
Host genotypes	Group[a]	Lines tested	1st screen	2nd screen
Yr26/Yr10	1	48	W050 (AvrYr10, AvrYr26)	-
Yr26/Yr10	2	48	W050 (AvrYr10, AvrYr26)	-
Yr32/Yr10	1	48	T022 (AvrYr10)	T034 (AvrYr32)
Yr32/Yr10	2	48	T022 (AvrYr10)	T034 (AvrYr32)
Yr32/Yr26	1	48	T034 (AvrYr32)	W050 (AvrYr26)
Yr32/Yr26	2	48	T034 (AvrYr32)	W050 (AvrYr26)

[a] Plants groups descended from different F_1 seeds of the same crossing combination.

4.2.3 SNP marker design and validation by KASP assay

The cloned genomic sequence of the *Yr10* gene (Accession # AF149112) (Liu et al. 2014) was aligned with the gene sequences of wheat cultivars: 'Chinese Spring', 'CDC Landmark' and 'CDC Stanley', at the location of the locus on chromosome 1BS to identify the susceptible sequence with BLAST (basic local alignment search tool). The sequences were also aligned to homeologous sequences as well to identify and design the SNP markers specific for *Yr10* on chromosome 1BS. For *Yr26*, SNP markers were designed with iSelect 90K SNPs with two flanking EST markers, CON4 and CON12 (Zhang et al. 2013), as guides and the primers for the KASP assay were designed using PolyMarker (Ramirez-Gonzalez et al. 2015). Two forward primers were designed for the SNPs to distinguish presence or absence of the *Yr* gene and differentiate it as having FAM^{TM} or HEX^{TM} fluorescent dye attachment at the 3' or 5' end of the primer. One common reverse primer was designed to replicate the reverse end of the sequence with or without the target *Yr* gene. The assay mix (10X) was comprised of 46 µL of sterile ddH_2O (double distilled water), 30 µL of common reverse primer (100 µM), and 12 µL each of FAM and HEX fluorescence primers (100 µM). The assay was performed on 384-well trays and each well had 7 µL of the master mix comprised of 1 µL of DNA (ranged 80-250 ng μL^{-1}), 2 µL of sterile ddH_2O, 4 µL of 2X KASP master mixture and 0.11 µL of the assay mix. The real-time PCR protocol was set to 94°C for 15 min, followed by 10 touchdown cycles (94°C for 20 seconds, touchdown at 65°C for 1 min, which decreased by 0.8°C every cycle). This was followed by 35 additional cycles of 94°C for 20 sec and 57°C for 1 min and the same cycle was repeated up to 47 cycles. The data was recorded and analyzed using BioRad data analysis software (Bio-Rad Laboratories Ltd., ON, Canada). The relative fluorescent unit (RFU) was calculated as the strength of the fluorescent signal specific for each fluorescent label by BioRad and used to differentiate genotype as homozygous, heterozygous or no *Yr* gene. The RFU data

from empty wells ("Empty") and no template cell (NTC) (master mix without DNA template) were used as a negative control and to standardize RFU data points for analysis.

The SNP markers were validated by genotyping the DNA sample from wheat cultivars of 'Chinese Spring', 'CDC Landmark', 'CDC Stanley', 'Waskada', 'Domain', 'Conquer', 'Lillian' and the wheat stripe rust differential lines each with single *Yr* genes including the NILs with *Yr10* and *Yr26* by the KASP assay with the putative SNP markers. After the initial validation, the successful SNP markers were again validated with the parental NILs used for phenotype screening, and two commercial cultivars that carry the *Yr10* gene, 'Moro' and 'Radiant'. The DNA of eight to 11 F_5 plants for each genotype screened from the *Yr26/Yr10* cross were evaluated with the KASP assay to identify F_4 genotypes that were homozygous for both *Yr10* and *Yr26*.

Table 4.3. List of primer sequences used in the KASP assay.

Target gene	Primer names	Primer sequences 5' to 3'
Yr10	Yr10_Res_HF[1]	GAAGGTCGGAGTCAACGGATTTGGTACACCTTGTACCAATAA
	Yr10_Sus_FF[2]	GAAGGTGACCAAGTTCATGCTTKGTACACCTTGKACCAATAT
	Yr10_KR2	GTTAGTGGTGTTTATCAGCTT
Yr26	wsnp_Ex_c3057_5636572_HF	GAAGGTCGGAGTCAACGGATTACCCAGAAATTTGCCCCAA
	wsnp_Ex_c3057_5636572_FF	GAAGGTGACCAAGTTCATGCTACCCAGAAATTTGCCCCAG
	wsnp_Ex_c3057_5636572_R	CACGAGTAGCGCCCTGTG

[1]HF = HEX FRET (fluorescent resonant energy transfer),
[2]FF = FAM FRET

4.2.4 Growth chamber experiment

From the double homozygous genotypes identified through screening, two genotypes per cross combination were selected based on seed availability for the growth chamber experiment. A total of 10 genotypes ('Avocet', Avo-Yr10, Avo-Yr26, Avo-Yr32 and six double homozygous genotypes) were tested with four *Pst* isolates (W020, W049, mix of T034 and W052) in mineral

oil (Bayol® 35) and a control inoculation with no spores in each experiment. The experiment was designed as a split plot arranged in four blocks with the four inocula as the main plot factors and the 10 wheat genotypes as the sub plot factors. For each genotype, 16 seedlings were grown in a 4-inch pot filled with propagation mix #3 (Sun Gro Horticulture, Agawam, MA) for 160 plants in total for one experiment. On the 14th day after seeding, seedlings were placed in random order inside one of 16 clear plastic boxes, so each plastic box had one of the 10 genotypes. Sixteen plastic boxes were then separated into four groups as four replicates of each experimental unit. In one experimental unit, four boxes were inoculated with one of four inocula and placed randomly in one of four corners inside a growth chamber. The experiment was repeated three times. Inoculum was prepared with fresh (not previously frozen) urediniospores harvested at the time of inoculation. The second leaves of 10 seedlings were inoculated by spraying them with 560 µL of inoculum at a spore concentration of 11 mg mL^{-1} in Bayol with an air compressor. The concentration and volume of inoculum was kept constant through out the inoculation processes, so every plant was inoculated with a similar number of spores. Inoculated plants were then incubated as described earlier, covered by plastic lids at the end of incubation and transferred to a growth chamber. Starting at 7 days post inoculation (dpi), the plants were examined for the first signs of sporulation as visible urediniospores on the leaf surface and the time was recorded at 24-h intervals. When sporulation was observed, the nearest 12-h interval time was recorded as the latent period (LP). Once sporulation began, infection type (IT) was recorded on a 0-9 scale (McNeal et al. 1971) and infection area (IA) recorded daily as the percent of the leaf area covered by sporulating pustules up to 14 dpi.

4.2.5 Field nursery experiment

In the spring of 2018, 15 to 30 kernels of the double homozygous genotypes with all three *Yr* gene combinations, the three parental NILs, and cv. ‘Avocet’ were seeded in hill plots in

a field stripe rust nursery, in randomized order with 10 replications at Saskatoon, SK. The nursery was seeded on May 5th of 2018 and surrounded by spreader rows of 'AC Bellatrix', a stripe rust susceptible winter wheat cultivar seeded the previous fall. The nursery was artificially inoculated three times in May with the same technique described in Chapter 3. The same seeds were sown in rows, in randomized order with 10 replicates in a disease nursery at Lethbridge, AB in May. The nursery was inoculated by natural inoculum and irrigated three times a week every week from July 3rd to August 3rd. Stripe rust incidence and severity, based on the modified Cobb's scale, were recorded at the soft dough stage, which was in late July at Saskatoon and early August at Lethbridge.

4.2.6 Statistical analysis

The mean values of LP and IA of the double homozygous genotypes from the growth chamber experiments were analyzed by analysis of variance (ANOVA) and genotypes differentiated from 'Avocet' and parental genotypes by Dunnett's test ($p < 0.05$). Data analysis was performed with PROC GLIMMIX using SAS 9.4 software (SAS Institute Inc., Cary, NC, USA) with genotypes and inoculum as fixed effects and experiments and replicates as random effects. The data was tested for normality using PROC UNIVARIATE and for homoscedasticity of variance using Levene's test in PROC GLM. When the assumption of normality and homoscedasticity of variance was not satisfied, a model that best fit the data distribution was selected and used with PROC GLIMMIX based on the values of model fit estimation. The IT data set was analyzed nonparametrically with inoculum as the whole plot factor and genotype as the subplot factor using the F1_LD_F1 macro in SAS 9.4. After the initial ANOVA on IT, the IT values were ranked using PROC RANK and analyzed using Dunnett's test. Stripe rust incidence and severity data from field nurseries were analyzed separately for each site with ANOVA and

Dunnett's test using PROC GLIMMIX with wheat genotype as a fixed effect and experiment as a random effect.

Correlation analysis of the relationships between LP, IT and IA was conducted using PROC CORR with LP as independent variable and IT or IA as dependent variables. The correlation of the number of *Yr* genes in NILs to the three variables was also analyzed using PROC CORR.

4.3 Results

4.3.1 Phenotypic screening

The *Pst* isolates, T022, T030 and W031 were profiled to be virulent to *Yr26* and avirulent to *Yr10* and vice versa for the isolates W050, W052 and W057; however, all six isolates were found to be avirulent to both *Yr* genes during the multiple preliminary tests for this study. As a result, phenotypic screening of genotypes with *Yr26/Yr10* was impossible. Instead, the screening of this genotype relied on genotypic analysis after the first screening with the isolate W050 to eliminate the genotype s with no *Yr* genes. The phenotypic screenings for *Yr32/Yr10* and *Yr32/Yr26* were conducted successfully with *Pst* isolate T034, avirulent to *Yr32* and virulent to both *Yr10* and *Yr26*; T022, virulent to *Yr32* and avirulent to *Yr10*; and W050, virulent to *Yr32* and avirulent to *Yr26*, and selected four genotypes homozygous for the *Yr32/Yr10* gene combination and five genotypes for *Yr32/Yr26*.

4.3.2 Genotypic screening

The PCR primers designed with SNP markers for *Yr10* successfully differentiated Avo-Yr10 among the stripe rust differential lines from other single *Yr* genes including *Yr26*, and stripe rust resistant genotypes without *Yr10* (Fig. 4.1). In the first denaturing and annealing cycle of the rtPCR, the primers with a SNP marker specific to the presence of *Yr10* (YR10-Res) attached to the target gene sequence of the wheat genotypes with *Yr10* (Serial 13 Yr10 = Av-Yr10) and created new DNA strands with the additional tail ends that corresponded to the HEX fluorescent dye oligo. In the second denature and annealing cycle, the fluorescent resonance energy transfer (FRET) cassette with the HEX fluorescent dye oligo in the KASP master mix denatured and the HEX-labeled oligo bonded to the corresponding tail end of amplified strands with *Yr10*. Upon annealing, the fluorescent signal from the HEX dye was emitted at a specific wavelength (Absorbance max: 538 nm) from the amplified product with *Yr10*. The same process

occurred with the second forward primer, Yr10-Sus, FRET with FAM fluorescent dye oligo (Absorbance max: 495 nm), and the genotypes without *Yr10* during the PCR cycles. The fluorescence signal emission was detected and measured in relative fluorescent units (RFU) by subtracting the average emission of the empty cell from the emission of each other cell. In Figure 4.1, the NILs with *Yr10* emitted the HEX fluorescent signal but no FAM fluorescent signal as the data points are located high on the y-axis (HEX signal RFU) and at zero on the x-axis (FAM signal RFU). The DNA samples without *Yr10* were clustered high on the X-axis and at zero on the Y-axis. The negative controls (empty, NTC) were located around zero on both the X- and Y-axes. No DNA samples were found to be heterozygous for the *Yr10* gene as was expected from the inbred wheat cultivars and genotypes tested in this evaluation process. The second KASP assay with the stripe rust differential lines, 'Radiant' and 'Moro' again successfully identified Avo-Yr10, 'Radiant' and 'Moro' as the only genotypes homozygous for *Yr10* (data not shown). Of the seven SNP markers for *Yr26* in the KASP assays, four successfully identified genotypes with *Yr26* and *Yr24* (Fig. 4.2). The subsequent KASP assay with DNA from parental genotypes with *Yr10* and *Yr26*, 'Radiant' and 'Moro', in addition to the stripe rust differential lines, demonstrated that all four SNP markers successfully differentiated genotypes homozygous for *Yr26* from the rest. Of the four markers, *wsnp_Ex_c3057_5636572* was selected for future screening for its relatively clearer results as the data points within clusters were closer together compared to the results with other markers.

The genotypic evaluation of the F_5 plants of cross *Yr26/Yr10* revealed that 11 genotypes were homozygous for *Yr10* (Fig. 4.3) and 21 genotypes were homozygous for *Yr26* (Fig. 4.4). Three genotypes were identified to be homozygous for both *Yr10* and *Yr26*. The KASP assay with selected genotypes from crosses *Yr32/Yr10* and *Yr32/Yr26* corresponded to the phenotypic

screening results (Fig. 4.5). Upon completion of screening, 12 genotypes, three *Yr26/Yr10* NILs, four *Yr32/Yr10* NILs and five *Yr32/Yr26* NILs, were identified.

4.3.3 Growth chamber experiment

Since two NILs with double homozygous *Yr* genes behaved similarly for all parameters, the NILs data for each double homozygous *Yr* genotype was combined for each genotype and analyzed as three genotypes. Analysis of variance for latent period (LP), infection area (IA) and infection type (IT) indicated that the interactions of inoculum and genotype were highly significant for all parameters (Table 4.4). The interaction between genotypes and isolates for all parameters were attributed to the difference in reaction by wheat genotypes to the different isolate/isolate combinations. When compared, there was no difference in LP, IA or IT of 'Avocet' among the three *Pst* isolate/isolate mixtures (data no shown).

Eight plants of *Yr32/Yr10* genotype and four plants of *Yr32/Yr26* genotype inoculated with T034/W052 never sporulated at 14 dpi; this data was not included in the analysis of LP for those two genotypes. With T034/W052, the LP of all genotypes with one or two *Yr* genes was longer (ranging from 9.7 to 11.6 dpi) compared to 'Avocet' (8.4 dpi) (Table 4.5). With the same inoculum, *Yr32/Yr10* and *Yr32/Yr26* genotypes had longer LP than NILs with single *Yr* genes, while the NIL with the *Yr26/Yr10* genotype did not. When the plants were inoculated with W020, the LP of *Yr32/Yr26* was longer (9.0 dpi) than 'Avocet' (8.3 dpi), but not different from other NILs with single or double *Yr* genes. With isolate W049, the LP was longer with *Yr10* (9.3 dpi), *Yr26* (9.0 dpi), *Yr32/Yr10* (9.1 dpi) and *Yr32/Yr26* genotypes (9.3 dpi) than 'Avocet' (8.3 dpi). The LP of *Yr10* was longer than NILs with *Yr32* (8.5 dpi) or *Yr26/Yr10* (8.7 dpi), but not different from *Yr26*, *Yr32/Yr10* and *Yr32/Yr26* genotypes with the same isolate. There was no difference in LP when all genotypes were compared to the NIL with the single *Yr26* gene. When the NILs were compared to *Yr32*, the *Yr32/Yr10* and *Yr32/Yr26* genotypes had longer LP.

Differences in IA among NILs was similar to LP patterns for the *Yr32/Yr10* and *Yr32/Yr26* genotypes. The IA with T034/W052 inoculum was smaller for NILs with *Yr32/Yr10* (8.9%) and *Yr32/Yr26* genotypes (6.8%) compared to 'Avocet' (58.1%) and all NILs with a single *Yr* gene (ranged from 38.3% to 55.2%) (Table 4.6). With the isolate W020, only the *Yr32/Yr26* genotype (41.0%) had a smaller IA compared to 'Avocet' (68.8%). There was no difference in IA between single and double *Yr* genotypes. When inoculated with the isolate W049, no NILs had smaller IAs than 'Avocet' (54.3%), however the IA of the *Yr32/Yr26* genotype (36.5%) was smaller than the *Yr32* genotype (58.5%), which had the highest IA of all genotypes. When the genotypes were compared to the single *Yr10* or *Yr26* genotypes, there was no difference among genotypes.

The mean ranks of IT were used to compare the genotypes to a control genotype in Dunnett's analysis (Table 4.7). With the isolate mixture T034/W052, the IT rank means of *Yr10* (216.8), *Yr32/Yr10* (51.5) and *Yr32/Yr26* genotypes (39.0) were lower than that of 'Avocet' (336.8). Also, the *Yr32/Yr10* and *Yr32/Yr26* genotypes had lower IT rank means compared to all single *Yr* genotypes (ranging from 216.8 to 325.5). When compared to the *Yr10* genotype, the *Yr32* genotype had a higher IT rank mean (325.5). The difference in IT rank means with isolate W020 was that the rank means of *Yr26* (251.8), *Yr26/Yr10* (285.9), *Yr32/Yr10* (286.6) and *Yr32/Yr26* (227.4) genotypes were lower than 'Avocet' (378.4). When inoculated with isolate W049, the *Yr32* genotype had the highest IT rank mean (355.7), although it was not significantly higher than 'Avocet' (304.3). The rank means were lower with *Yr10* (200.0) and *Yr32/Yr26* (196.4) genotypes compared to 'Avocet'. The rank means of *Yr26/Yr10* (245.1), *Yr32/Yr10* (261.3) and *Yr32/Yr26* (196.4) genotypes were lower compared to the *Yr32* genotype.

Correlation analysis of the relationship between IA and LP, and IT and LP detected a highly significant correlation of LP with both IA ($p < 0.0001$, r = -0.667) and IT ($p < 0.0001$, r =

-0.765) (Table 4.8; Fig. 4.6). Both IA and IT were inversely correlated to LP suggesting that LP is a good parameter to measure partial resistance and a longer LP contributes to residual resistance of defeated *Yr* genes.

When the correlations of LP, IT, and IA combined for all isolate/isolate mixtures to the number of *Yr* genes were analyzed, all parameters had significant correlations with the increasing number of *Yr* genes (Table 4.9). The correlation between the *Yr* number and IA (r = -0.382) was inverse and slightly stronger than IT (r = -0.338), and LP was positively correlated with the *Yr* number (r = 0.299). The values of IA and IT were lower and LP was longer when the *Yr* number was increased from zero to two. The strength of the correlations differed among the *Pst* isolate/isolates mixtures but the relationship (positive or negative correlation) did not change when data were analyzed separately. The strength of the correlations for all three parameters were higher with isolate mixture T034/W052 than for the two other isolates. The correlation was strongest with IA (r = -0.600) compared to LP (r = 0.576) and IT (r = -0.537). The LP had no correlation with the *Yr* frequency when inoculated with isolate W020, while the *Yr* frequency was significantly correlated with IT (r = -0.312) and IA (r = -0.337). With isolate W049, all parameters were significantly correlated with the *Yr* frequency. The LP had a stronger correlation (r = 0.295) than with W020, but the correlation coefficients for both IT (r = -0.193) and IA (r = -0.238) were weaker than the rest of the *Pst* isolate/isolate mixtures.

4.3.4 Field nursery experiment

Stripe rust incidence was high at Saskatoon (100% incidence in 'Avocet') (Table 4.10) and Lethbridge (data not shown) in 2018. Since all plants at Lethbridge had 100% incidence, only stripe rust severity was analyzed for this site (Tables 4.11 and 4.12). Also, the individual NILs were compared separately due to the difference among the genotypes with supposedly identical resistance gene composition.

At Saskatoon, when the stripe rust incidence was compared, it was lower with the NILs with *Yr10* (6.1% incidence) than 'Avocet' (100%), but not with *Yr26* (48.0% incidence) or *Yr32* (70.0% incidence) (Table 4.10). The NILs with *Yr32/Yr10* (ranged from 2.2 to 19.1%), two NILs with *Yr32/Yr26* (15.7 and 11.4%) and one genotype with *Yr26/Yr10* (21.8%) had lower stripe rust incidence than 'Avocet'. When compared to the NIL with *Yr10*, the rest of the NILs had similar or higher incidence. Similar to the comparison with 'Avocet', all NILs with *Yr32/Yr10* and two of the *Yr32/Yr26* genotype had lower incidence than the NIL with the single *Yr26* gene. The same two NILs of the *Yr32/Yr26* genotype and three NILs of the *Yr32/Yr10* genotype had lower incidence compared to the *Yr32* NIL.

All parental and double homozygous genotypes had lower stripe rust severity compared to 'Avocet' (91.5%) (Table 4.11). The NIL with a single *Yr10* gene was resistant against the natural inoculum and had very low stripe rust severity (2.8%), which was the second lowest severity among all NILs. The NIL with a single *Yr26* gene was also resistant but the severity was higher (10.5%) than it was for the *Yr10* gene. Two NILs of the *Yr32/Yr10* genotype (3.1% and 1.9%) and one NIL of the *Yr32/Yr26* genotype (2.8%) had lower severity compared to the NIL with a single *Yr26* gene. All *Yr32/Yr10* NILs (ranging from 1.9 to 7.2%) and two *Yr32/Yr26* (2.8 and 3.7%) NILs had lower severity than the NIL with a single *Yr32* gene (44.4%). None of the NILs of the *Yr26/Yr10* genotype (ranging from 12.0 to 17.6%) had stripe rust severity lower than any of NILs with single *Yr* genes.

At Lethbridge, stripe rust severity was higher (ranging from 50.0 to 96.8%) than it was at Saskatoon; however, the susceptibility reaction of the NILs to the disease was comparable (Table 4.12). 'Avocet' had the highest severity (96.8%) and the NILs with single *Yr10* and *Yr26* genes, one NIL of the *Yr26/Yr10* genotype (69.9%), three NILs of the *Yr32/Yr10* genotype (ranging from 50.5 to 57.5%) and three NILs of the Yr32/Yr26 genotype (ranging from 56.5 to 64.0%)

had lower severity compared to ‘Avocet’. There was no NIL with severity lower than that of the NIL with a single *Yr10* gene (50.6%), as some races in the natural *Pst* populations at Lethbridge appeared to be avirulent to *Yr10* like it was at Saskatoon. However, unlike Saskatoon, the NIL with the *Yr26* gene was equally resistant to the natural inoculum as the NIL with the *Yr10* gene was at Lethbridge and there were no NILs with two *Yr* genes that had lower severity. The NILs with a single *Yr32* gene were as susceptible to stripe rust as ‘Avocet’. The same NILs of the *Yr32/Yr10* and *Yr32/Yr26* genotypes had lower severity than ‘Avocet’ and NILs with *Yr32*, but no NIL of the *Yr26/Yr10* genotype had lower severity than the NILs with *Yr32*.

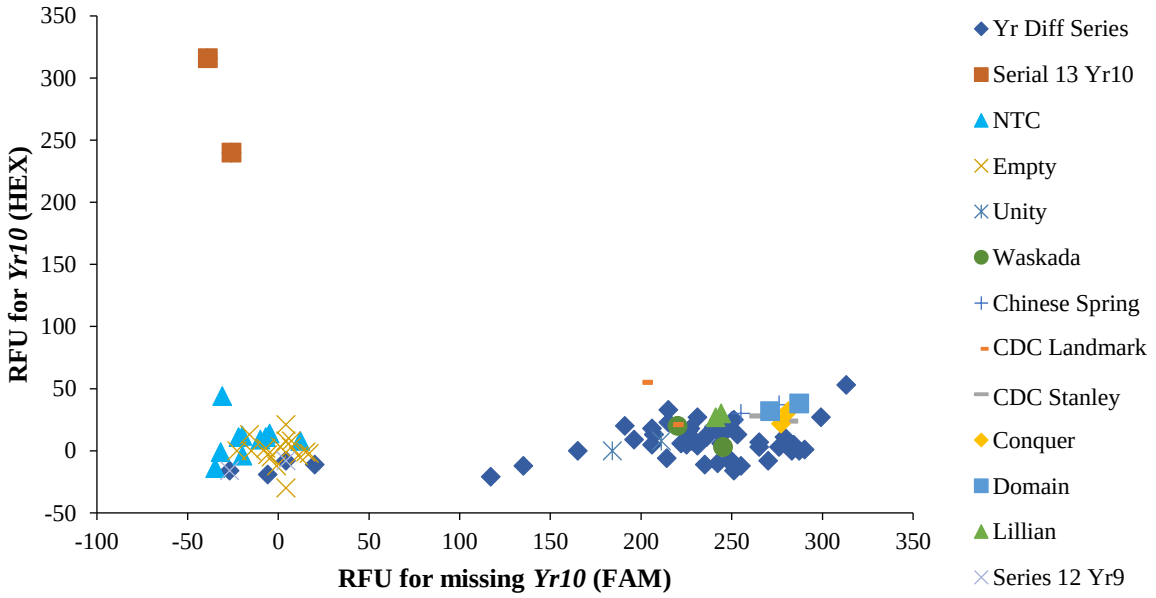

Figure 4.1 KASP assay results with SNP markers (Yr10-Res, Yr10-Sus) for stripe rust resistance gene *Yr10* showing the clustering of wheat genotypes near either the X-(FAM) or Y-(HEX) axes.
* (■) *Yr10*-NIL.
† Yr Diff Series = stripe rust differential lines. NTC = no template control. RFU = relative fluorescent unit. FAM and HEX = fluorescent dyes.
KASP = "Kompetitive" allele specific PCR.

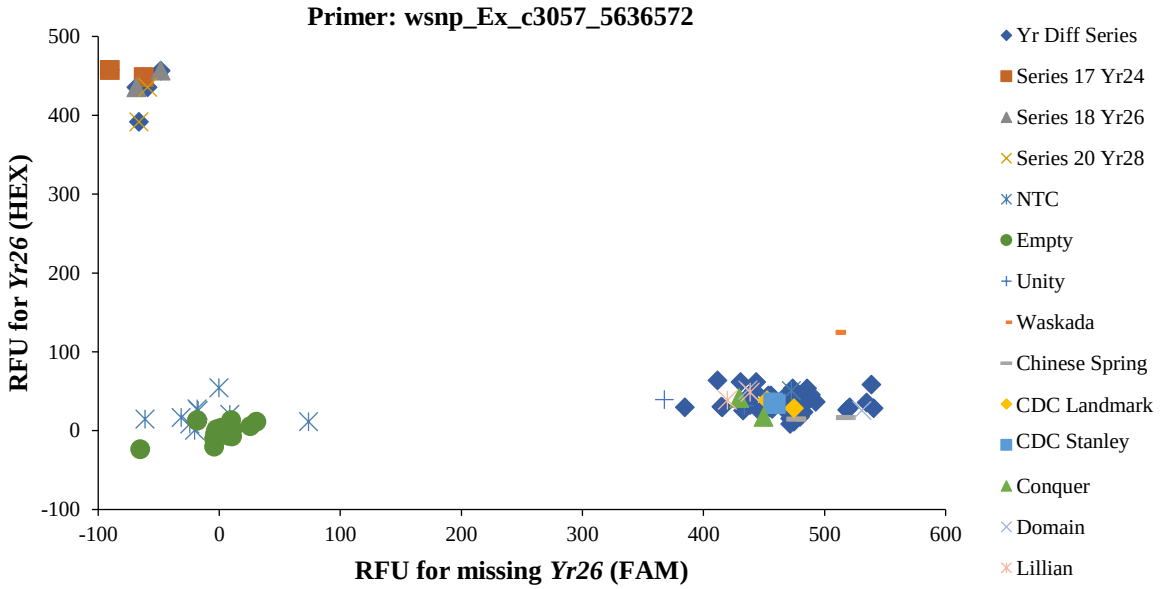

Figure 4.2 KASP assay results with a SNP marker (wsnp_Ex_c3057_5636572) for the stripe rust resistance gene *Yr26* showing clustering of wheat genotypes near either X-(FAM) or Y-(HEX) axes.
* (▲) *Yr26*-NIL, (■) *Yr24*-NIL.
† Yr Diff Series = stripe rust differential lines. NTC = no template control. RFU = relative fluorescent unit. FAM and HEX = fluorescent dyes.
KASP = "Kompetitive" allele specific PCR.

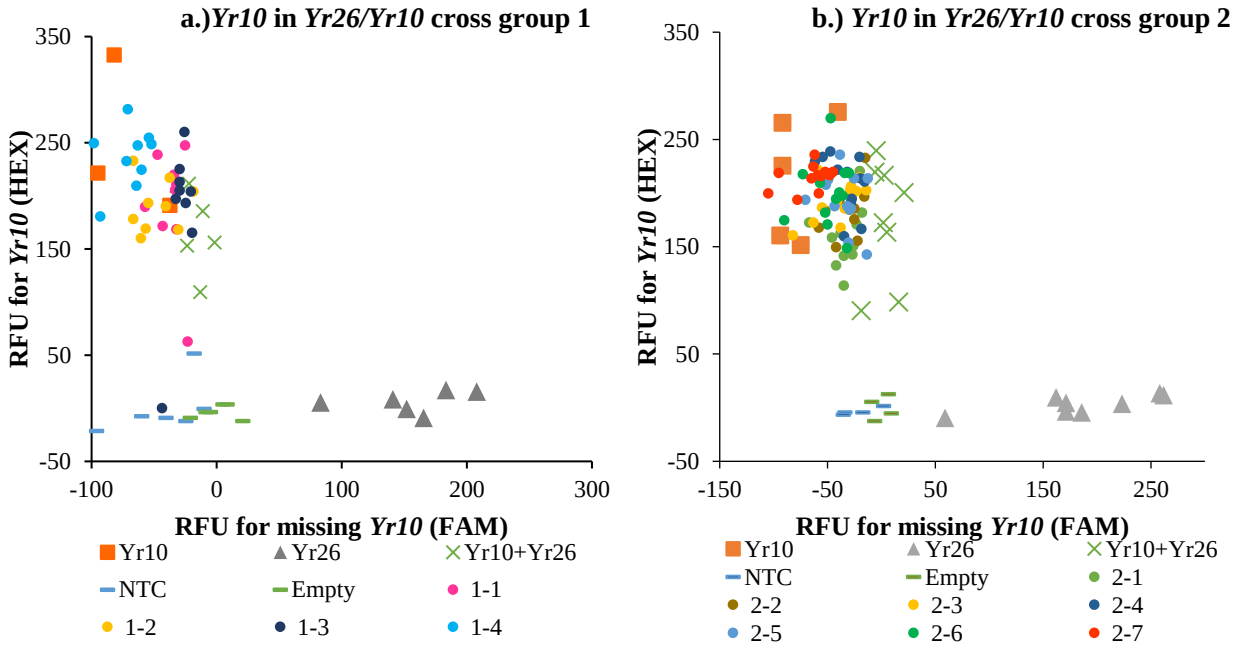

Figure 4.3 KASP assay with primers to detect the *Yr10* gene on F_5 seedlings of selected genotypes from a.) group 1, and b.) group 2 of the *Yr26/Yr10* cross. The genotypes homozygous for the *Yr10* gene were indicated by data points clustering near positive control DNA samples for *Yr10* (■).
† NTC = no template control. RFU = relative fluorescent unit. FAM and HEX = fluorescent dyes. KASP = "Kompetitive" allele specific PCR.

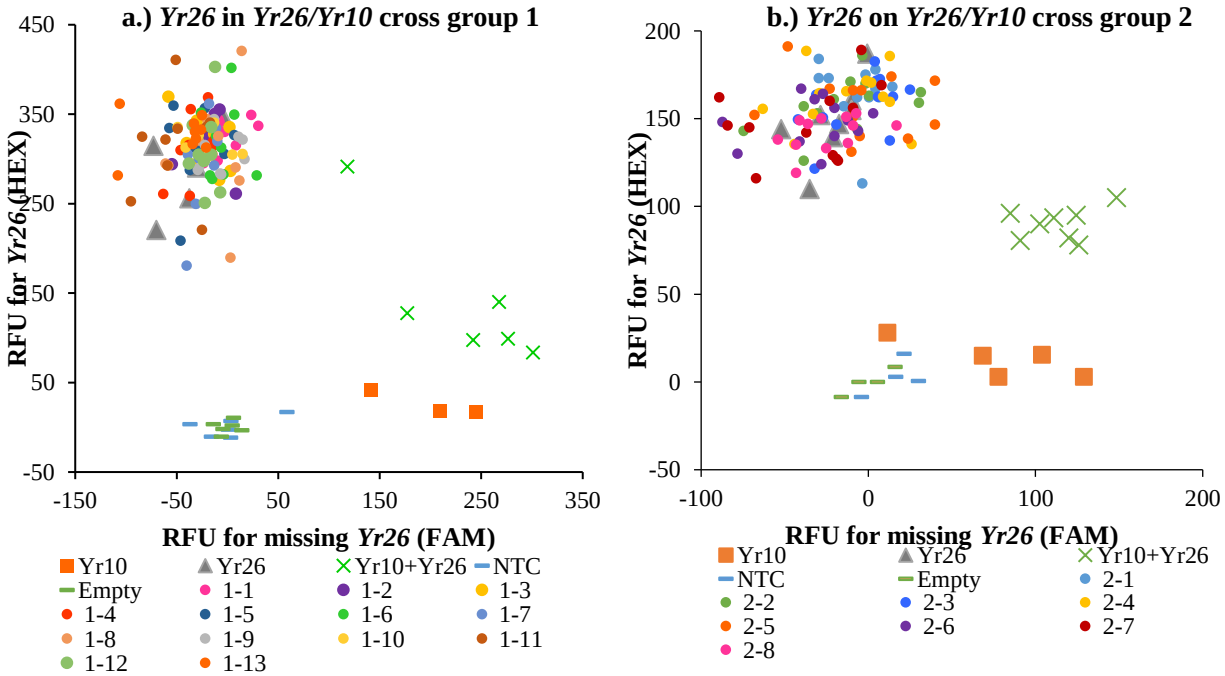

Figure 4.4 KASP assay with primers for detecting *Yr26* on F_5 seedlings of selected genotypes from: a.) group 1 and b.) group 2 of the *Yr26/Yr10* cross. The genotypes homozygous for the *Yr26* gene are indicated by data points clustering near positive control DNA samples for *Yr26* (▲).
† NTC = no template control, RFU = relative fluorescent unit. FAM and HEX = fluorescent dyes. KASP = "Kompetitive" allele specific PCR.

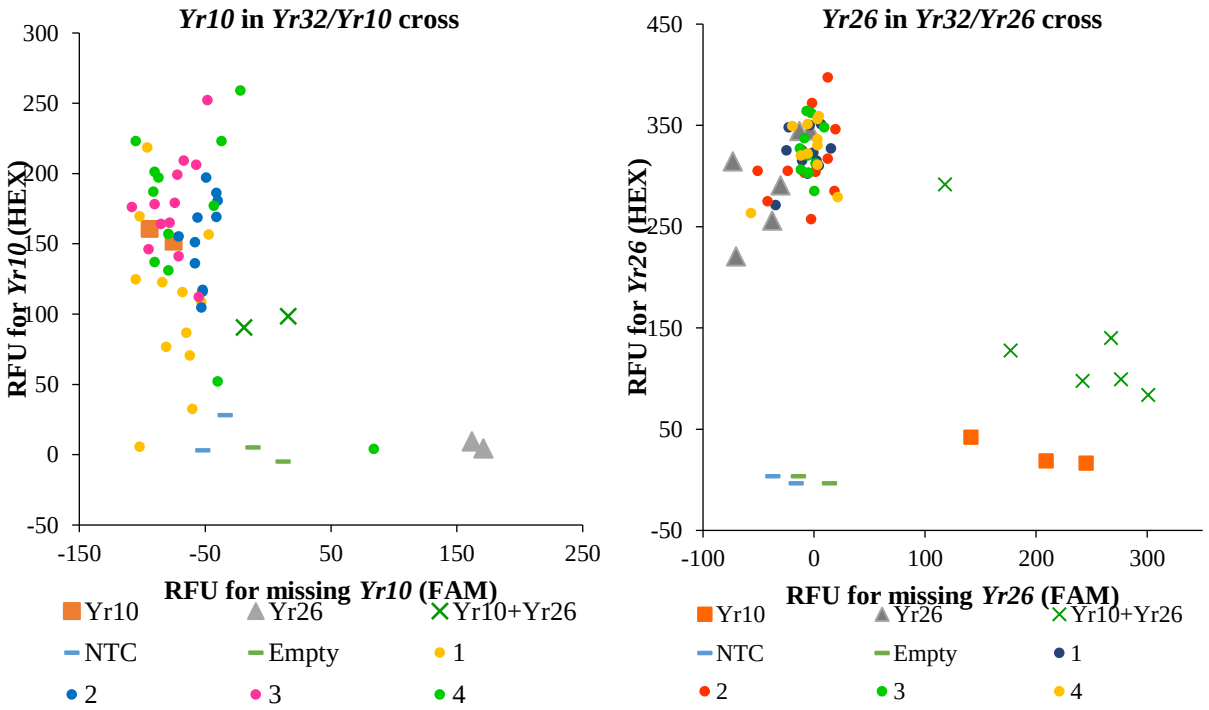

Figure 4.5 KASP assay on F_5 seedlings of selected genotypes from the *Yr32/Yr10* cross with primers for the *Yr10* gene and the *Yr32/Yr26* cross with primers for the *Yr26* gene. The genotypes homozygous for one of *Yr* genes are indicated by data points clustering near positive control DNA samples for *Yr10* (■) or *Yr26* (▲).
† NTC = no template control, RFU = relative fluorescent unit. FAM and HEX = fluorescent dyes. KASP = "Kompetitive" allele specific PCR.

Table 4.4 The main and interaction effects of inoculum and wheat genotypes on latent period (LP), infection area (IA) and infection type (IT) in the growth chamber experiment.

		ANOVA				ANOVA-type statistic (ATS)			
		LP		IA		IT			
Source	df	*F* value	*p* value	*F* value	*p* value	df_N	df_D	*F* value	*p* value
inoculum (I)	2	87.1	<.0001	36.9	<.0001	2.4	42.407	277.42	<.0001
genotype (G)	6	49.1	<.0001	50.5	<.0001	4.9	∞	26.96	<.0001
I x G	12	14.6	<.0001	31.2	<.0001	10.6	∞	10.11	<.0001

Table 4.5 Latent period (LP) with standard errors (SE) and multiple comparison of genotypes to a control genotype within each *Pst* isolate/isolate mixture by Dunnett's analysis.

Isolate	Genotypes	LP (dpi)		Dunnett's analysis			
				Control genotype			
		n	Mean (±SE)	'Avocet'	*Yr10*	*Yr26*	*Yr32*
T034/W052	'Avocet'	16	8.4 (±0.2)	-			
	Yr10	16	9.7 (±0.1)	***	-		
	Yr26	16	9.8 (±0.1)	***	ns	-	
	Yr32	16	9.3 (±0.1)	***	ns	ns	-
	Yr26/Yr10	32	9.5 (±0.1)	***	ns	ns	ns
	Yr32/Yr10	24	11.6 (±0.3)	***	***	***	***
	Yr32/Yr26	28	11.6 (±0.2)	***	***	***	***
W020	'Avocet'	16	8.3 (±0.2)	-			
	Yr10	16	8.8 (±0.1)	ns	-		
	Yr26	15	8.9 (±0.2)	ns	ns	-	
	Yr32	16	8.8 (±0.1)	ns	ns	ns	-
	Yr26/Yr10	32	8.6 (±0.1)	ns	ns	ns	ns
	Yr32/Yr10	32	8.6 (±0.1)	ns	ns	ns	ns
	Yr32/Yr26	32	9.0 (±0.1)	**	ns	ns	ns
W049	'Avocet'	16	8.3 (±0.2)	-			
	Yr10	16	9.3 (±0.2)	***	-		
	Yr26	16	9.0 (±0.1)	**	ns	-	
	Yr32	15	8.5 (±0.1)	ns	**	ns	-
	Yr26/Yr10	32	8.7 (±0.1)	ns	*	ns	ns
	Yr32/Yr10	32	9.1 (±0.1)	***	ns	ns	*
	Yr32/Yr26	32	9.3 (±0.1)	***	ns	ns	***

* = $0.01 \leq p < 0.05$, ** = $0.001 \leq p < 0.01$, *** = $p \leq 0.001$.
"ns" = non-significant.

Table 4.6 Infection area (IA) with standard error (SE) and multiple comparison of genotypes to a control genotype within each isolate/isolate mixture by Dunnett's analysis.

Isolate	Genotype	IA (%)		Dunnett's analysis			
				Control genotype			
		n	Mean (±SE)	'Avocet'	*Yr10*	*Yr26*	*Yr32*
T034/W052	'Avocet'	16	58.1 (±4.0)	-			
	Yr10	16	38.3 (±2.9)	ns	-		
	Yr26	16	46.6 (±4.3)	ns	ns	-	
	Yr32	16	55.2 (±2.7)	ns	ns	ns	-
	Yr26/Yr10	32	45.5 (±3.0)	ns	ns	ns	ns
	Yr32/Yr10	32	8.9 (±1.5)	***	***	***	***
	Yr32/Yr26	32	6.8 (±1.1)	***	***	***	***
W020	'Avocet'	16	68.8 (±3.2)	-			
	Yr10	16	49.3 (±3.5)	ns	-		
	Yr26	15	45.7 (±4.6)	ns	ns	-	
	Yr32	16	53.4 (±3.4)	ns	ns	ns	-
	Yr26/Yr10	32	50.5 (±2.3)	ns	ns	ns	ns
	Yr32/Yr10	32	51.4 (±2.0)	ns	ns	ns	ns
	Yr32/Yr26	32	41.0 (±2.6)	**	ns	ns	ns
W049	'Avocet'	16	54.3 (±4.0)	-			
	Yr10	16	37.9 (±4.5)	ns	-		
	Yr26	16	43.8 (±2.9)	ns	ns	-	
	Yr32	15	58.5 (±3.3)	ns	ns	ns	-
	Yr26/Yr10	32	44.3 (±2.7)	ns	ns	ns	ns
	Yr32/Yr10	32	44.7 (±2.7)	ns	ns	ns	ns
	Yr32/Yr26	32	36.5 (±2.6)	ns	ns	ns	*

* = $0.01 \leq p < 0.05$, ** = $0.001 \leq p < 0.01$, *** = $p \leq 0.001$.
"ns" = non-significant.

Table 4.7 Median and rank mean of infection type (IT) and multiple comparison to a control genotype within each isolate/isolate mixture by Dunnett's analysis.

Isolate	Genotype	IT (0-9)			Dunnett's analysis			
					Control genotype			
		n	Median	Rank mean	'Avocet'	*Yr10*	*Yr26*	*Yr32*
T034/W052	'Avocet'	16	7.0	336.8	-			
	Yr10	16	6.0	216.8	**	-		
	Yr26	16	6.0	262.0	ns	ns	-	
	Yr32	16	6.5	325.5	ns	*	ns	-
	Yr26/Yr10	32	6.0	265.1	ns	ns	ns	ns
	Yr32/Yr10	32	4.5	51.5	***	***	***	***
	Yr32/Yr26	32	4.0	39.0	***	***	***	***
W020	'Avocet'	16	7.0	378.4	-			
	Yr10	16	6.0	304.3	ns	-		
	Yr26	15	6.0	251.8	**	ns	-	
	Yr32	16	6.0	302.9	ns	ns	ns	-
	Yr26/Yr10	32	6.0	285.9	*	ns	ns	ns
	Yr32/Yr10	32	6.0	286.6	*	ns	ns	ns
	Yr32/Yr26	32	6.0	227.4	***	ns	ns	ns
W049	'Avocet'	16	6.0	304.3	-			
	Yr10	16	6.0	200.0	*	-		
	Yr26	16	6.0	237.9	ns	ns	-	
	Yr32	15	7.0	355.7	ns	***	***	-
	Yr26/Yr10	32	6.0	245.1	ns	ns	ns	**
	Yr32/Yr10	32	6.0	261.3	ns	ns	ns	*
	Yr32/Yr26	32	6.0	196.4	**	ns	ns	***

* = $0.01 \leq p < 0.05$, ** = $0.001 \leq p < 0.01$, *** = $p \leq 0.001$.
"ns" = non-significant.

Table 4.8 The *F* and *p* values from ANOVA and correlation coefficient (r) from the correlation analysis between latent period (LP) and infection type (IT) and between LP and infection area (IA).

Variables		ANOVA		
Dependent	Independent	F value	*p* value	r
IT	LP	672.88	<.0001	-0.765
IA	LP	381.10	<.0001	-0.667

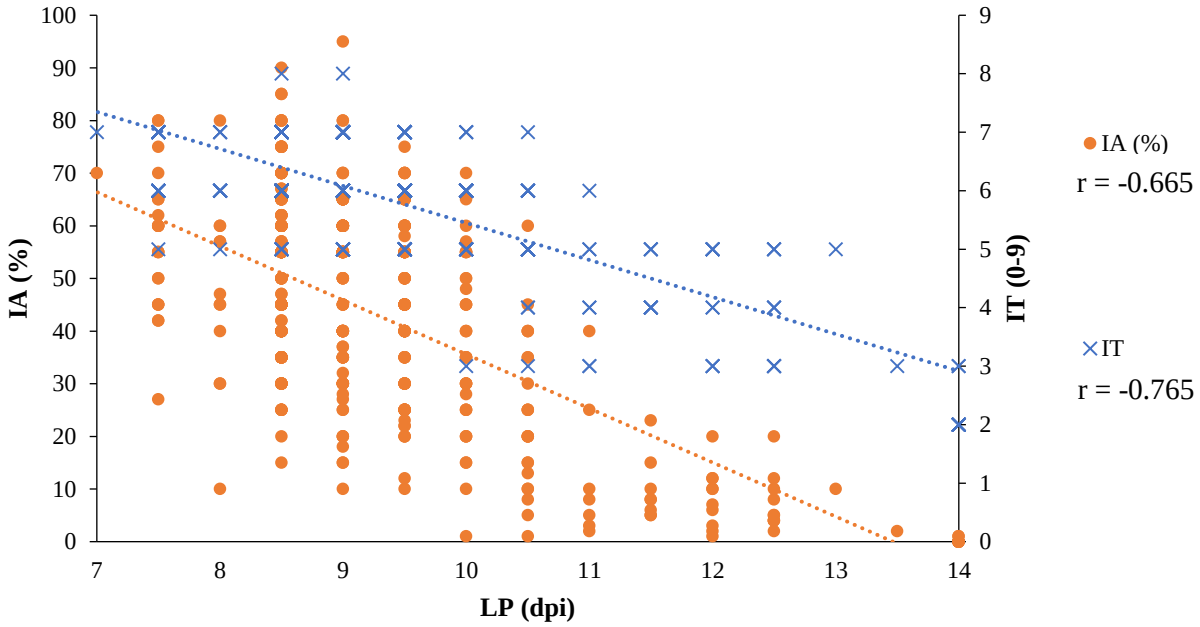

Figure 4.6 Scatter plot with trend lines and coefficient for correlation between infection area (IA) and latent period (LP), and between infection type (IT) and LP.
The trend lines: (·····) for IA and LP. (·····) for IT and LP.
"r" = correlation coefficient.

Table 4.9 The *p* values and correlation coefficient (r) for the relationship of the number of *Yr* gene in NILs to latent period (LP), infection type (IT) and infection area (IA) for all isolate/isolate combinations combined and each of three isolate/isolate combinations.

Variable	LP			IT			IA		
Isolate	n	*p*	r	n	*p*	r	n	*p*	r
All	466	<.0001	**0.299**	478	<.0001	**-0.338**	478	<.0001	**-0.382**
T034/W052	148	<.0001	**0.576**	160	<.0001	**-0.537**	160	<.0001	**-0.600**
W020	159	0.0616	0.149	159	<.0001	**-0.312**	159	<.0001	**-0.337**
W049	159	0.0002	**0.295**	159	0.0148	**-0.193**	159	0.0025	**-0.238**

*The r values in bold are statistically significant (α = 0.05).

Table 4.10 Stripe rust incidence at Saskatoon, SK in 2018 and the *p* values from each Dunnett's analysis with 'Avocet', *Yr10*, *Yr26* or *Yr32* as the control.

Stripe rust incidence (%)		Dunnett's analysis (adjusted *p* values)			
		Control			
Genotype	Mean (±SE)	'Avocet'	*Yr10*	*Yr26*	*Yr32*
'Avocet'	100.0 (±0.0)	---	---	---	---
Yr10	6.1 (±5.2)	<.0001***	---	---	---
Yr26	48.0 (±9.0)	ns	<.0001***	---	---
Yr32	70.0 (±14.0)	ns	<.0001***	ns	---
Yr26/Yr10-1	68.5 (±10.2)	ns	<.0001***	ns	ns
Yr26/Yr10-2	21.8 (±10.9)	0.0001***	ns	ns	ns
Yr26/Yr10-3	81.7 (±7.1)	ns	<.0001***	ns	ns
Yr32/Yr10-1	7.8 (±4.1)	<.0001***	ns	0.0006***	0.0024**
Yr32/Yr10-2	6.2 (±3.4)	<.0001***	ns	<.0001***	0.0004***
Yr32/Yr10-3	2.2 (±1.3)	<.0001***	ns	<.0001***	<.0001***
Yr32/Yr10-4	19.1 (±9.8)	<.0001***	ns	0.0207*	ns
Yr32/Yr26-1	93.5 (±3.0)	ns	<.0001***	ns	ns
Yr32/Yr26-2	15.7 (±7.4)	<.0001***	ns	0.0108*	0.0316*
Yr32/Yr26-3	81.0 (±8.2)	ns	<.0001***	ns	ns
Yr32/Yr26-4	11.4 (±7.9)	<.0001***	ns	0.0006***	0.0025**
Yr32/Yr26-5	45.1 (±11.7)	ns	0.0002***	ns	ns

* = $0.01 \leq p < 0.05$, ** = $0.001 \leq p < 0.01$, *** = $p \leq 0.001$ by Dunnett's test.
"ns" = non-significant.

Table 4.11 Stripe rust severity at Saskatoon, SK in 2018 and the *p* values from each Dunnett's analysis with 'Avocet', *Yr10*, *Yr26* or *Yr32* as the control.

Stripe rust severity (%)		Dunnett's analysis (adjusted *p* values)			
		Control			
Genotype	Mean (±SE)	Avocet	*Yr10*	*Yr26*	*Yr32*
'Avocet'	91.5 (±4.8)	-	-	-	-
Yr10	2.8 (±1.6)	<.0001***	-	-	-
Yr26	10.5 (±2.4)	<.0001***	0.0063**	-	-
Yr32	44.4 (±12.5)	0.0032**	<.0001***	ns	-
Yr26/Yr10-1	12.0 (±2.5)	<.0001***	0.0023**	ns	ns
Yr26/Yr10-2	17.6 (±9.4)	<.0001***	0.0447*	ns	ns
Yr26/Yr10-3	15.0 (±1.6)	0.0007***	0.0002***	ns	ns
Yr32/Yr10-1	3.2 (±1.0)	<.0001***	ns	ns	0.0008***
Yr32/Yr10-2	3.1 (±1.5)	<.0001***	ns	0.013*	<.0001***
Yr32/Yr10-3	1.9 (±1.0)	<.0001***	ns	0.0016**	<.0001***
Yr32/Yr10-4	7.2 (±4.8)	<.0001***	ns	ns	0.0031**
Yr32/Yr26-1	23.0 (±3.6)	0.0071**	<.0001***	ns	ns
Yr32/Yr26-2	3.7 (±1.2)	<.0001***	ns	ns	0.0011**
Yr32/Yr26-3	19.0 (±4.0)	0.0007***	<.0001***	ns	ns
Yr32/Yr26-4	2.8 (±1.3)	<.0001***	ns	0.0114*	<.0001***
Yr32/Yr26-5	6.8 (±1.6)	<.0001***	ns	ns	ns

Table 4.12 Stripe rust severity at Lethbridge, AB in 2018 and the *p* values from each Dunnett's analysis with 'Avocet', *Yr10, Yr26* or *Yr32* genotypes as the control.

Stripe rust severity (%)		Dunnett's analysis (adjusted *p* values)			
		Control			
Genotype	Mean (±SE)	'Avocet'	*Yr10*	*Yr26*	*Yr32*
'Avocet'	96.8 (±1.3)	-	-	-	-
Yr10	50.6 (±5.5)	<.0001***	-	-	-
Yr26	59.0 (±4.8)	<.0001***	ns	-	-
Yr32	88.9 (±2.5)	ns	<.0001***	0.0011**	-
Yr26/Yr10-1	84.0 (±3.1)	ns	<.0001***	0.0031**	ns
Yr26/Yr10-2	69.9 (±4.6)	0.0095**	0.0103*	ns	ns
Yr26/Yr10-3	83.4 (±3.8)	ns	<.0001***	0.0045**	ns
Yr32/Yr10-1	74.0 (±3.8)	ns	0.0013	ns	ns
Yr32/Yr10-2	53.0 (±4.7)	<.0001***	ns	ns	<.0001***
Yr32/Yr10-3	57.5 (±5.0)	<.0001***	ns	ns	0.0004***
Yr32/Yr10-4	50.5 (±6.4)	<.0001***	ns	ns	<.0001***
Yr32/Yr26-1	86.0 (±2.6)	ns	<.0001***	0.0012**	ns
Yr32/Yr26-2	64.0 (±4.8)	0.0003***	ns	ns	0.0187*
Yr32/Yr26-3	86.0 (±1.8)	ns	<.0001***	0.0011**	ns
Yr32/Yr26-4	56.5 (±7.4)	<.0001***	ns	ns	<.0001***
Yr32/Yr26-5	59.0 (±5.7)	<.0001***	ns	ns	0.0006***

* = $0.01 \leq p < 0.05$, ** = $0.001 \leq p < 0.01$, *** = $p \leq 0.001$ by Dunnett's test.
"ns" = non-significant.

4.4 Discussion

Nelson (1978) was the first to speculate that defeated race-specific resistance genes, or major genes that regulate vertical resistance in host plants, can have partial resistance against virulent pathogen races and act like horizontal resistance, which he named “residual effects”. He suggested that the residual effect of defeated resistance genes can be pyramided into a host plant to construct resistance that is highly effective against a broader range of pathogen races than using only a few race-specific resistance genes. The ability to use known resistance genes in commercial cultivars with desirable traits is another potential benefit of utilizing residual effects. However, identifying and evaluating residual effects has been extremely difficult due to the complexity of host-pathogen interactions from the genetic to the population level. The possibility of unknown resistance genes with partial resistance and/or epistatic effects in combination with another gene was pointed out to be a possible alternative explanation for residual effects (Johnson 1984). The use of molecular markers to determine the presence/absence of a resistance gene and creation of near isogenic wheat lines with a single resistance gene in a common background could make the study of residual effects more precise and robust.

The comparison of latent period (LP), infection area (IA) and infection type (IT) of the susceptible cultivar ‘Avocet’ among isolate/isolate mixtures indicated no difference in aggressiveness among the isolate/isolate mixtures (data not shown). A comparison of isolate/isolate mixtures for LP found more pairs of genes with statistically significant differences than for IA or IT. The significant inverse correlation between IT and IA with LP suggested that longer LP is associated with greater resistance and more sensitive to the quantitative difference than IT or IA. Although differences due to the presence of single or double *Yr* genes was not always significant, the significant inverse correlations of the number of *Yr* genes for IT and IA for all three *Pst* isolate/isolate mixtures suggested a beneficial effect of pyramiding defeated

resistance genes to reduce stripe rust severity. This result corresponded with other studies on wheat cultivars used in Germany and Canada, in which it was found that lines with more *Yr* genes tended to have lower stripe rust severity in the field even after the gene was confirmed to be defeated by natural *Pst* populations (Zheng et al. 2017; Zetzsche et al. 2019). How single *Yr* genes, combinations of genes and the *Pst* isolates interacted in the current study was not straightforward or simple and there are multiple factors that could explain the effects.

Defeated genes acting as QTL

The lack of reduction in IA and IT in NILs with either one or two defeated genes against virulent isolates was similar to the finding that colony formation of powdery mildew was not restricted on wheat (Nass et al. 1981) or that infection type of stem rust was not reduced (Brodny et al. 1986) by defeated genes. The residual effects of defeated genes are expected to be expressed as race-nonspecific, partial and additive resistance reaction. In the current study, NILs with single or double *Yr* genes were expressed as partial or almost fully resistant and the reactions varied depending on the isolate/isolate mixture. This indicated that the residual effects depend on both host genotype and pathogen race. With the T034/W052 isolate mixture, a partially resistant reaction was apparent as extended LP and reduced IA and IT compared to 'Avocet' in single and two gene *Yr* genotypes. In addition, the NILs with two *Yr* genes had longer LP than single *Yr* NILs. This was similar to the partial and additive resistance of defeated genes measured as reduced pustule size and sporulation amount in wheat lines with *Sr6*, *Sr8* or *Sr9a* genes, or combinations of two or all three genes (Brodny et al. 1986). It was also similar to the reduced number of sporulating colonies of powdery mildew on wheat lines with *Pm3c*, *Pm4* or *MA* genes when inoculated with virulent pathogen races (Nass et al. 1981). When *Yr5* and *Yr15* were pyramided into one wheat line and inoculated with a mixture of isolates virulent to each *Yr* gene, the pyramided line had a completely resistant reaction (Klymiuk et al. 2018). The

study by Klymiuk et al. (2018) suggested that these genes complemented each other, which is the definition of epistasis; therefore, this may explain the effect of *Yr32/Yr10* and *Yr32/Yr26*.

In the current study, NILs with *Yr32*, when in combination with *Yr10* or *Yr26*, tended to have longer LP and lower IA and IT compared to 'Avocet' or single *Yr* NILs. When deployed by itself, *Yr32* did not condition a partially resistant reaction to isolates W020 or W049. The *Yr32* gene is unique as an ASR gene that is expressed as slow rusting, partial resistance and found to complement another *Yr* gene when it was first identified in 'Carstens V' (Chen & Line 1993; Calonnec et al. 2002; Eriksen et al. 2004). Together with another partial resistance *Yr* gene in 'Carstens V', *Yr32* acted as an additive resistance gene and conditioned complete resistance against avirulent *Pst* isolates. The *Yr32* gene appears to be epistatic with other *Yr* genes and could have complemented the partial resistance exerted by *Yr10* and *Yr26* against the virulent isolates.

It has been found in several studies that ASR or APR genes with partial resistance complement other resistance genes when pyramided into a line. For example, when researchers investigated durable stem rust resistance of wheat cultivar 'Thatcher' with *Lr34*, two studies found two race-specific ASR genes complemented *Lr34* and had higher stem rust resistance than when the genes were deployed individually (Kolmer et al. 2011; Hiebert et al. 2016). Kolmer et al. (2011) observed a QTL that enhanced stem rust APR in combination with *Lr34* at the adult plant stage was located where ASR gene *Sr9g* was when wheat lines were inoculated with virulent races of *Puccinia graminis* f. sp. *tritici*. At the seedling stage, wheat lines with either *Sr12* (ASR) or *Lr34* expressed partial resistance against virulent races. The stronger resistance against virulent pathogen races indicated an additive genetic effect when *Sr12* and *Lr34* were combined.

This pattern of additive effect between partial slow rusting resistance is very similar to the additive effect observed by combining APR genes *Yr18* and *Yr30* (Randhawa et al. 2018). A similar observation was that the resistance gene that showed an additive effect also had a partially resistant reaction when deployed by itself. In studies on bacterial blight of rice, Li et al. (1999) and Zhou et al. (2012) reported that an allele $Xa4^T$ at the *Xa4* locus acted as a dominant race-specific resistance gene against an avirulent race of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) when deployed by itself. However, the same region where the gene is located was reported to be a recessive resistance QTL to a virulent race. Further studies on the resistance genes in rice against *Xoo* revealed that there were location overlaps between some major resistance genes for complete resistance and QTL associated with partial resistance on the rice genome (Li et al. 2006; Zhou et al. 2012). Li et al. (2006) found that both complete and partial resistance were race specific and the race-specificity in partial resistance may occur at a polygenic level, which means that multiple resistance genes with different race specificity generate the race specificity of one host plant. They concluded that the resistant reaction in rice was regulated by a genetic network, which means major and minor R genes interact with each other with epistatic effects and express resistant reactions.

Partial resistance gene *Yr32*, acting as an additive gene, follows the behaviour of other partial resistance genes. Complete resistance genes, *Yr10* and *Yr26*, could both be pleiotropic and have partial resistance against virulent *Pst* isolates. Epistasis between *Yr32* and two other *Yr* genes could explain the residual effects of defeated genes observed with *Yr32/Yr10* and *Yr32/Yr26* as additive partial resistance expressed by the qualitative resistance genes. This could also mean that major *Yr* genes may act as minor resistance genes, possibly in combination with other genes, after being defeated by virulent races and their accumulation could provide durable resistance against a wide range of *Pst* races. The possibility of a genetic network expressing

broad race resistance or residual effects could explain the difference in the resistance expression of *Yr* genes when they were deployed in various genetic backgrounds (Zheng et al. 2017). The residual effects observed with NILs could change when the same genes are pyramided into different genetic backgrounds, therefore use of residual effects of defeated genes would need to be carefully evaluated in plant breeding programs.

While pleiotropic and epistatic effects could explain residual effects, the residual effect as the partial resistance reaction from unknown minor R genes closely linked to a defeated major R gene could also explain the effects. Hiebert et al. (2016) could not determine whether the gene that complemented *Lr34* was an unknown gene that was closely linked and co-segregated with *Sr12*, or *Sr12* acted as a minor gene for APR with *Lr34* against stem rust. The possibility that residual effects could be expressed by an unknown gene or genes closely linked to the defeated R gene has been a constant issue when evaluating residual effects. The same issue is present in the current study as well even though the lines used were NILs and lines were screened with molecular markers for *Yr10* and *Yr26* genes. Recently it was found that some of the stripe rust differential lines with single *Yr* genes in the 'Avocet' background unintentionally carried *Yr18*, and the NIL with *Yr10* in the current study is one of those lines that co-segregated with *Yr18* (McIntosh et al. 2018). As an APR gene, *Yr18* should not affect the results of seedling stage resistant reactions; however, the results from the field trial were compromised. As this incidence demonstrated, the residual effects need to be evaluated carefully to rule out the possible interaction from unknown minor R genes. More molecular genetic research needs to be done to sequence the wheat genome to identify the exact genes for ASR and determine if the genotype carries other R genes.

Absence of residual effect

When inoculated with isolates W020 and W049, there were fewer genotypes with residual effects and the effects were weaker than those observed from the T034/W052 isolate mixture. For example, genotype *Yr26/Yr10* was less likely to show residual effects and when the effects were present, they were weaker than those expressed by *Yr32/Yr10* or *Yr32/Yr26*. Studies showed that not all defeated genes had residual effects when multiple R genes were tested (Chantret et al. 1999) and the residual effects expressed as partially resistant reactions could vary among isolates (Royer et al. 1984). When multiple combinations of two *Yr* genes in wheat against stripe rust were compared for the resistant reactions, several combinations were found to have lower resistance while others improved resistance (Zheng et al. 2017). The combinations that improved resistance often included genes with partial resistance (*Yr17*, *Yr18*, *Yr30* and *Yr46*); therefore, it was recommended that a major race-specific *Yr* gene be pyramided with a minor gene, often APR genes (Li et al. 2006; Mundt 2018). Although *Yr32* expresses race-specific ASR, its reaction is characterized as partial resistance; improved resistance in *Yr32/Yr10* and *Yr32/Yr26* combinations is consistent with these findings in the past. It is highly possible that the resistance mechanisms expressed by *Yr10* and *Yr26* are different from the *Yr32* gene and the two genes may lack pleiotropic gene expression or epistasis. This demonstrates that *Yr* genes have different pathways or mechanisms to regulate resistance reactions and need to be carefully selected to be pyramided into a cultivar to maximize the additive and residual effects of those genes.

Race-specificity of residual effect

The residual effect of a defeated gene was thought to be race-nonspecific when it was first described because a resistant reaction was effective against a virulent race. However, the studies on residual effects often lacked tests against multiple virulent pathogen races and whether

the residual effect is completely race-nonspecific or not is yet to be proven. Studies have shown that resistance genes for partial resistance, both as ASR and APR, can be race-specific (McIntosh et al. 1995; Milus et al. 2015) and this could also apply to the residual effects. It is possible that virulent races defined by race-specific ASR genes are different from the virulent races defined by APR or the partial resistance of ASR genes. The accumulation of *Yr* genes with partial resistance with different race-specificity would condition the resistance against a wider range of *Pst* races than monogenic resistance genotypes. This can be characterised as race-nonspecific if a virulent pathogen race was missed in the screening trial. The presence or absence of race-specificity of residual effects needs to be determined by testing with multiple pathogen races. Testing with multiple pathogen races is required for every combination of pyramided genes with residual effects to confirm non race-specific resistance or the range of race specificity.

Differences among pathogen isolates

The expression of residual effects differed greatly among the three pathogen races. The isolate mixture T034/W052 had a different virulence profile than W020 and W049, while W020 and W049 shared a similar profile with a virulence/avirulence difference on one differential wheat genotype (Brar 2015). Residual resistance effects were greater on all wheat genotypes with single or double *Yr* genes when challenged with the T034/W052 mixture than when the wheat genotypes were challenged with the two other isolates. This may indicate that the T034/W052 mixture was less aggressive than the two other isolates. However, the comparison of LP, IT and IA on 'Avocet' among three isolate/isolate mixtures showed no difference in the level of aggressiveness. Another explanation for the difference may be that the inoculum mixture T034/W052 contained only half the spores of each isolate compared to inoculum of only W020 or only W049. The lower spore concentration of each isolate virulent to different *Yr* genes could

have resulted in lower stripe rust severity with longer LP and lower IT and IA with the T034/W052 mixture than with W020 or W049.

Another possible explanation for the residual effect and its variability is the difference in pathogen races. The four isolates belonged to four different *Pst* races based on screening with 21 single *Yr* wheat lines and 10 supplemental wheat genotypes by Brar (2015) and with 18 single *Yr* wheat lines by Kumar et al. (2012). Royer et al. (1984) concluded that the expression of partial resistance that was regulated by the same R genes could vary among the different genotypes of virulent pathogen isolates. In the gene-for-gene theory, the product of an R gene recognizes the pathogen race through direct or indirect interaction (Jones & Dangl 2006). Many R genes have been identified as cell surface or intercellular receptor proteins that directly or indirectly recognize effectors of specific pathogen races (Kourelis & van der Hoorn 2018). The recognition of ligands (effectors) from an avirulent pathogen race by a receptor expressed by an R gene leads to the hypersensitive reaction (HR) and often complete resistance. A wheat plant with *Yr* genes for race-specific ASR expressed the HR earlier and at a higher rate against avirulent *Pst* isolates than susceptible lines without the genes (Bozkurt et al. 2010; Wang et al. 2014; Sørensen et al. 2017; Klymiuk et al. 2018). A study of *Puccinia graminis* f. sp. *tritici* (*Pgt*) isolates avirulent to *Sr50* found that the isolates were heterozygous for the avirulent gene, *avrSr50*, and its avirulence was lost when heterozygosity was lost by an insertion into the *avrSr50* gene (Chen & Kang 2017). The avirulence gene was expressed as a single dominant gene in heterozygous dikaryotic urediniospores. In another study, a *Pst* isolate with virulence to *Yr* genes was selfed on barberry leaves and the segregating isolates were tested for virulence using differential lines (Yuan et al. 2017). The study showed that the virulence genes could be regulated by single dominant genes, two dominant genes, or two complementary dominant genes. Genetic studies on the avirulence/virulence in pathogen isolates revealed that avirulence/virulence genes can be

recessive, dominant or complementary genes (Chen & Kang 2017; Yuan et al. 2017). This complicates the interaction between host resistance/susceptibility and pathogen avirulence/virulence of stripe rust of wheat even more. If the avirulence/virulence genes in pathogen isolates are regulated by two complementary genes, an isolate with one of the complementary genes could still infect the host with the corresponding ASR *Yr* gene with intermediate severity. This may appear as if the *Yr* gene has broken down and has partial resistance to the "residual effect", which is not the case.

The hypersensitive reaction (HR), which includes host cell death, is a crucial part of ASR that is triggered by recognition of pathogen effectors by the host receptors (Jones & Dangl 2006). Virulent rust races could avoid the onset of the HR not only by avoiding recognition by losing or changing its effectors, but also by supressing the HR after the host receptor detects the pathogen effectors. Ramachandran et al. (2017) examined two *Puccinia* species (*Pst* and *Pgt*) with potential effectors on susceptible wheat cultivars and found several pathogen effectors that suppressed host cell death. Some effectors could suppress the HR itself. The timing of gene expression differed among effectors and the success or failure of HR suppression depended on the different R-gene product and effector combinations. This suggested that the effectors have different mechanisms to interfere with host cell death and the HR. These findings indicate that virulence mechanisms in pathogen races can vary from loss of effector recognition to manipulation of HR expression after the HR is triggered. If host plants possess mechanisms to counteract the suppression of host defence responses, it may be possible to explain the quantitative response of the host.

Effect of the pathogen population structure on the host resistance reactions in the field

While there is little research to evaluate the mechanisms of residual effects of defeated resistance genes, there are several field observations that suggest multiple resistance genes tend

to lower disease severity even after virulence to the resistance genes was confirmed. Higher numbers of *Yr* genes in wheat cultivars was correlated with lower IT of stripe rust in the field (Zheng et al. 2017; Zetzsche et al. 2019). As discussed, not all combinations of pyramided R genes suppress disease severity. Inverse correlations between increased numbers of R genes and reduced disease severity (Zheng et al. 2017; Zetzsche et al. 2019) may be due to residual effects, but possibly other factors. One explanation may be that there is a benefit to extra race-specific R genes against the natural pathogen population that is a mix of virulent and avirulent races. Even though selection pressure would favor the races with virulence to existing race-specific R genes, the avirulent races could exist at lower frequency and plants with the R gene might benefit from resistance against avirulent races. The pathogen population structure could vary by year, location and with the resistant genotypes of crop cultivars grown most frequently.

Lower IT on NILs with the single genes *Yr10* or *Yr26* in the stripe rust nursery at Saskatoon and Lethbridge in 2018 demonstrated that the low frequency of virulent races could reduce stripe rust severity and mask any residual effect if present. The *Yr10* or *Yr26* NILs were highly resistant at Saskatoon, which was not the characteristic partial resistance expected from residual effects. Although Lethbridge had the highest stripe rust incidence (100% on all lines), the severity of NILs with two *Yr* genes of any combination was similar or higher than single *Yr10* or *Yr26* NILs. This indicated that the *Pst* population in Lethbridge consisted partially of races avirulent to *Yr10* or *Yr26* and having two *Yr* genes did not improve the resistance in the field, or that race-specific resistance could mask the partial resistance of residual effects. This demonstrated the benefit of having multiple race-specific R genes pyramided into one plant, as this would provide an advantage against avirulent races in the pathogen populations; however, it also showed how difficult it was to evaluate the presence and effectiveness of residual effects in the field.

The second factor is that the genotypes with defeated race-specific R genes could carry another unknown partial resistance gene. Similar to the controlled environment experiment for residual effects, hosts with defeated resistance genes could have partial quantitative resistance from unknown genes that could not be differentiated from residual effects until all the resistance genes in a host genotype were identified. It is highly likely that the correlation resulted from the mixed effects of these factors including residual effects. Whether the benefit of carrying multiple R genes in a host arise from residual effects or other factors, these field observations justify further research into R gene pyramiding in crop breeding for better disease resistance.

Residual effects in plant-pathogen coevolution

The theory of residual resistance gene effects was first explained as horizontal resistance reactions due to the accumulation of minor race-specific resistance genes, which leads to a more stable state of equilibrium in the natural ecosystem (Parlevliet & Zadoks 1977). However, as discussed earlier, the interaction between R genes in the host and effectors in the pathogen was far more complicated than the gene-for-gene theory described. The R genes can not simply be characterized as horizontal/vertical or quantitative/qualitative binary systems. Considering the complexity of interactions between host plants and pathogens, and the great variability that occurs on both sides, the origin of quantitative resistance genes would likely not only be the accumulation of defeated qualitative resistance genes. nelson function of race-specific R genes in host plants in natural ecosystems was discussed by Burdon et al. (1996), who suggested that pathogen and host populations in natural ecosystems are likely to exist in geographical patches with smaller population sizes and often experience local extinction. When a pathogen is re-introduced from another pathogen population and re-establishes, the pathogen genotypes would likely be unrelated to the old pathogen population. The local extinction and re-introduction of a pathogen population likely occurs more frequently in the natural ecosystem than in agro-

ecosystems. Race-specific host resistance could be overcome by simple genetic mutation in a pathogen population; however, the virulent pathogen population could become extinct due to reasons other than environmental factors and may not remain stable in natural ecosystems. In this case, there is a chance that race-specific resistance in the host population could be effective against the new pathogen population. This initial defence response could prevent the rapid spread of the new pathogen population. Therefore, race-specific R genes could remain beneficial with greater longevity in a dynamic natural ecosystem than in a large-scale, near-monoculture agro-ecosystem. This theory does not explain how the minor genes for quantitative disease resistance originated in the host; however, it provides a perspective on how race-specific R genes could behave, especially in combination with other resistance genes, which helps to better understand and utilize them in plant breeding programs.

Summary

The current study revealed the presence of residual effects of *Yr* genes in wheat against *Pst*. Despite the limited number of *Yr* genes and *Pst* isolates tested in this study, there were clear differences in the expression of residual effects among the genes and isolates. The expression of residual effects may exist in the wheat-stripe rust pathosystem, but the mechanisms and effectiveness may vary depending on the resistance genes, the *Pst* isolate genotypes and the genetic background of the host plant. The highly complicated mechanisms of residual effects may hinder their use in breeding programs; however, further research into residual effects could benefit the study of plant-pathogen interactions and the search for durable resistance against stripe rust. More *Yr* genes need to be evaluated for potential residual effects and advancing molecular technology in recent years will be essential for future studies. When selecting *Yr* genes for evaluation, the characteristics of the resistant reaction, partial or complete, should be considered to choose genes with greater potential to express residual effects based on the

findings that APR genes and ASR genes with partial resistant reactions tended to have additive effects with other R genes. Our study of genes *Yr10*, *Yr26* and *Yr32* will continue and a NIL with all three *Yr* genes will be created. The difference in stripe rust resistance among the NILs with multiple *Yr* genes will be evaluated under controlled and field conditions.

CHAPTER 5

5. DISCUSSION AND FUTURE PERSPECTIVES

5.1 General Discussion

Fall fungicide application on winter wheat

The control of stripe rust of winter wheat was not previously a high priority in western Canada, except southern Alberta, due to the lower production of winter wheat compared to spring wheat and the low frequency of stripe rust epidemics. However, some researchers reported that leaf and stem rust affected seedling vigour and ultimately reduced the winter survival rate of winter wheat in North America in the past (Chester 1946). Furthermore, from recent research, positive outcomes of fall fungicide application to winter wheat (Turkington et al. 2016) proved its potential benefit. The current study was designed to evaluate the benefit of fall fungicide application on winter wheat.

Stripe rust severity was reduced by a single fungicide application in spring and double applications in fall and spring on wheat cultivars that were susceptible to stripe rust, 'AC Bellatrix' and 'CDC Osprey'. Cultivar 'Moats' was resistant to stripe rust at all site-years and none of the fungicide treatments benefited this cultivar. The results were consistent with the fact that fungicide application in fall had no effect on the stripe rust severity the following summer because the application timing was too early. Improved seedling survival over winter due to fall fungicide application was not detected at Saskatoon from 2015 to 2017. Fall fungicide application can be beneficial in the regions where high stripe rust severity in fall could frequently

affect the winter survival rate of wheat and cause early development of stripe rust in spring. The stipe rust epidemic with exceptionally high severity was reported in 2010/2011 and this timing overlapped with the past study by Turkington et al. (2016). However, a stripe rust epidemic of a similar scale has not been reported since. It is uncommon for western Canada to experience such high stripe rust severity and fungicide application in fall is not recommended based on the current climate.

An unexpected finding from the current study was that cultivar 'Radiant' varied for reaction to stripe rust, ranging from resistant to moderately susceptible among site-years. Cultivar "Radiant" carries race specific ASR gene *Yr10* and was resistant to stripe rust until the virulent *Pst* races became prevalent in the natural inoculum in North America. The variation in stripe rust reaction of 'Radiant' demonstrated the different virulence profiles of the *Pst* population among locations within western Canada. The change from resistant to moderately susceptible reactions over time at Saskatoon and Lacombe could be explained by increased frequency of *Pst* races virulent to *Yr10* in the natural *Pst* population.

Similarly, leaf spot severity was not affected by a single fall fungicide application but was reduced by single spring or double fall and spring applications. Leaf spot severity remained low throughout all site-years except at Lacombe in 2015/2016. The low leaf spot severity likely did not affect the winter survival rate of winter wheat seedlings and the fall fungicide application had no benefit in terms of leaf spot disease control. Cultivars did not vary for leaf spot disease severity.

Yield protection was apparent with the stripe rust susceptible cultivars when stripe rust severity was reduced by single spring and double fall and spring fungicide applications. Test weight (TW) and thousand kernel weight (TKW) were often improved by a single spring and dual (fall and spring) fungicide applications on stripe rust susceptible cultivars as well. Protein

content was affected more often by cultivar than by fungicide treatment. When fungicide application timing had a statistically significant effect on protein content, the difference was subtle. The protection of the flag and penultimate leaves from stripe rust with fungicide application at the booting to flag leaf stages led to the protection of yield and improved grain quality of the stripe rust susceptible cultivars (Cook et al. 1999). Since a single fall fungicide application had no effect on winter survival rate or stripe rust severity at the grain filling stage, it did not have any positive effect on yield or grain quality.

In conclusion, the current study indicated that fall fungicide application was not beneficial when stripe rust severity did not occur in the fall with high severity. This finding provide information on when fall fungicide application should be considered on winter wheat. Considering the cost of fungicide, fuel and labour and the narrow application timing between seeding and first snow-fall, fall fungicide application would not be recommended to winter wheat growers under the current stripe rust conditions in western Canada.

Residual effects of defeated *Yr* genes

Stripe rust resistance genes, *Yr*, have been extensively utilized in wheat breeding programs to mitigate stripe rust epidemics and protect yield (Johnson 1992; Chen 2005). At the same time, resistance gene breakdown by virulent *Pst* races has been a constant and widespread issue for stripe rust control worldwide (Line & Qayoum 1992; Line & Chen 1995). The lack of durable and effective stripe rust resistance demands more research to find new sources of genetic disease resistance. It has been theorized that residual effect of defeated disease resistance genes may possess partial resistance after the race-specific qualitative resistance has been overcome by virulent pathogen races (Nelson 1978). The resistant reactions due to residual effects of defeated genes are also expected to be non race-specific and quantitative. The defeated genes may act as

additive resistance genes to express stronger resistant reaction when combined with other defeated resistance genes.

The current study is the first to report the residual effects of *Yr* genes under a controlled environment. The latent period (LP), infection area (IA) and infection type (IT) were useful parameters to detect the residual effects of *Yr* genes, while LP was more sensitive. The residual effects of *Yr* genes were highly variable among *Yr* gene combinations and among *Pst* isolate/isolate mixtures. Lines with single *Yr* genes were less likely to have residual effects, and the lines with two *Yr* genes had more frequent and stronger residual effects. These findings were consistent with the reports of additive effects of R genes with partial resistant reactions (Brodny et al. 1986).

The range of residual effects among *Yr* genotypes and *Pst* isolate/isolate mixtures indicated that the mechanism of residual effects is likely not simple but consists of complicated interactions between the host and pathogen genotypes. One potential explanation for residual effects is the partial resistance reaction of additive effects from the defeated R genes. Several researchers have found that some R genes with partial resistant reactions act complementarily with other resistance genes (Kolmer et al. 2011; Hiebert et al. 2016; Randhawa et al. 2018). Defeated *Yr* genes with partial resistant reactions could behave similarly to partial resistance genes with additive effects. Thus, lines with double *Yr* genes had stronger resistant reactions than the lines with a single *Yr* gene.

Although the single *Yr32* gene did not have a residual effect, double *Yr* genotypes *Yr32/Yr10* and *Yr32/Yr26* expressed residual effects more often than *Yr26/Yr10*. The ASR gene *Yr32* was originally identified in cultivar ‘Carsten V’ as a stripe rust resistance gene with a race-specific partial resistant reaction, which complemented another *Yr* gene in the same cultivar (Chen & Line 1993; Calonnec et al. 2002; Eriksen et al. 2004). The complementary

characteristic of *Yr32* to other *Yr* genes could explain the additive resistant reaction observed with *Yr32/Yr10* and *Yr32/Yr26*.

Near isogenic lines (NILs) were used in the current study so that the only genotype difference among the lines was the number of *Yr* genes they carried. However, a possibility that unknown minor R genes closely linked to *Yr* genes could express partial resistance cannot completely be dismissed. Each R gene with residual effect needs to be examined closely and analysed with accurate molecular markers to eliminate or confirm this possibility.

Besides host genotypes, pathogen genotypes could have an influence on the residual effects observed in the current study. The pathogen genes for the effectors that interact directly or indirectly with the receptors of host cells could be expressed as recessive, dominant or complementary genes (Chen & Kang 2017; Yuan et al. 2017). If virulence to a race-specific R gene was regulated by complementary genes, isolates heterozygous with the virulence gene could cause disease symptoms with intermediate severity. This could appear as if the defeated R gene expressed residual effects on the surface, which may not be the case. Some virulent pathogen races overcome host resistance by manipulating the hypersensitive reaction (HR) after a pathogen effector is recognized by a host receptor (Ramachandran et al. 2017). The differences in the mechanisms of virulence expression among pathogen isolates could explain why the R genes showing residual effect to some pathogen isolates lack the residual effect to others.

In the field, wheat lines with more *Yr* genes are observed to have better resistance to stripe rust even after the *Yr* genes are overcome by virulent *Pst* isolates in natural inoculum (Zheng et al. 2017; Zetzsche et al. 2019). The results of the growth chamber experiment from the current study showed the same correlation between the number of *Yr* genes and resistant reactions to stripe rust. However, the evaluation of residual effects in the field poses more difficulty as there is limited control to make sure the pathogen population is fully virulent to the

Yr genes. The results from the field stripe rust nurseries in Saskatoon and Lethbridge did not provide a clear conclusion of whether a residual effect was present at the adult plant stage in the field because of *Pst* isolates avirulent to *Yr10* and *Yr26* in the natural inoculum. Although pyramiding of the R genes could be beneficial due to the presence of avirulent pathogen isolates in natural inoculum, this masks the residual effects and makes it hard to evaluate the true effects from the defeated R genes. Additionally, it was confirmed that the NIL with the *Yr10* gene also carries *Yr18* (McIntosh et al. 2018) and any resistant reactions observed in NILs with the *Yr10* gene at the adult stage could not be evaluated for residual effects.

Although residual effects were present with *Yr* genes, there was a lack of consistent effects of each *Yr* gene. The variability due to the host genetic background and the *Pst* isolate genotypes potentially had an influence on residual effects and posed more questions about how residual effects are expressed. This variability makes it difficult to predict the outcome of residual effects and utilize defeated genes in breeding programs to create wheat cultivars with durable resistance to stripe rust. However, more research on residual effects could lead to a better understanding of how major and minor *Yr* genes function with other *Yr* genes. The presence of residual effects with at least some *Yr* genes is promising for future breeding and advancement of understanding the plant-pathogen interaction for stripe rust of wheat.

5.2 Future perspectives

The process of creating NILs with all three *Yr* genes is currently in progress. In future study, the same experiment under a controlled environment could be performed to compare the residual reaction among the NILs with zero, single, double and triple *Yr* genes.

The T034/W052 isolate mixture comprised a 50% concentration of these two isolates compared with isolates W020 or W049. The lower spore concentration of each isolate virulent to different *Yr* genes could have resulted in lower stripe rust severity with longer LP and lower IT

and IA with T034/W052 than with W020 and W049. In future controlled environment studies, the isolate mixture needs to be tested to determine whether half or full concentration of isolates T034 and W052 affect the resistant reactions.

A lack of virulence to *Yr10* and *Yr26* genes in the natural *Pst* inoculum was an issue at both Saskatoon and Lethbridge. The artificial inoculum with the *Pst* isolates that are virulent to all three *Yr* genes in early spring could be used to mitigate the problem. The NILs could also be grown to the adult plant stage, inoculated with virulent *Pst* isolates and rated in growth chambers to test the residual effects at the adult plant stage. Ultimately, all NILs with zero, double and triple *Yr* genes need to be grown in plots to test yield and grain quality and any positive or negative effect of pyramiding multiple *Yr* genes on those parameters.

The results from KASP assays with the primers for *Yr10* did not clearly distinguish between *Yr10* homozygous plants and heterozygous plants. The clustering of control DNA samples for heterozygosity located close to the *Yr10* homozygous control cluster indicated less amplification of DNA without *Yr10* than with *Yr10* in the heterozygous control sample. Another possible cause was that the primer for the genotype without *Yr10* annealed to homeologous sequences in the control DNA and could not amplify the target allele. Also, the samples tested dispersed loosely around the data points of the positive control for *Yr10*. This could have been caused by insufficient DNA concentration in the samples tested, although all measurement of randomly selected test samples showed all DNA concentrations were higher than required and were of sufficient quality. Adjusting the annealing temperature to increase the specificity of the primer or redesigning the primer for the allele without *Yr10* are possible solutions that should be explored for the future application of KASP to genotypically select the lines with molecular markers.

The NILs that are confirmed to have *Yr10* will need to be screened for *Yr18* and the lines that possess *Yr10* and lack *Yr18* should be selected for further study. If the link between *Yr10* and *Yr18* cannot be broken, future data analysis could be adjusted to include *Yr18* as the fourth *Yr* gene in the study of residual effects.

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APPENDIX

Table. A1 Summary of monthly average temperature and monthly cumulated precipitation during the winter wheat growing months at a.) Saskatoon SK (2013 - 2017), b.) Indian Head, SK (2015 – 2016), c.) Lethbridge, AB (2014 –2017) and d.) Lacombe, AB (2014 –2017). The 30-year average monthly temperature and precipitation for each site from Environment Canada weather stations (1981-2010) were used as the long term normal (LTN).

a.) Saskatoon	Year1				Year2							
Average temperature (°C)	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
2013/2014	15.2	3.3	-8.8	-19.9	-15.0	-19.2	-10.1	1.7	10.1	14.1	18.3	17.9
2014/2015	12.4	6.7	-9.7	-9.4	-11.8	-17.4	-2.4	5.6	10.1	17.2	19.4	17.4
2015/2016	11.9	6.7	-3.0	-9.3	-12.9	-7.9	-1.5	5.5	13.7	17.4	18.7	16.9
2016/2017	11.8	2.1	1.9	-13.7	-13.0	-9.3	-5.2	4.3	12.1	16.1	19.6	17.8
LTN	**12.0**	**4.4**	**-5.2**	**-12.4**	**-13.9**	**-11.4**	**-4.9**	**5.2**	**11.8**	**16.1**	**19.0**	**18.2**
Precipitation (mm)												
2013/2014	15.4	6.2	20.5	12.4	0.2	2.1	5.8	74.2	61.1	94.8	44.5	18.5
2014/2015	10.7	14.1	30.5	2.5	5.8	16.5	5.1	21.1	0.4	13.6	84.3	45.2
2015/2016	50.0	33.9	14.0	2.5	17.3	7.0	13.9	3.0	41.6	49.7	58.6	70.2
2016/2017	24.1	40.8	9.2	9.7	7.4	9.1	11.3	18.4	46.3	30.9	25.5	25.2
LTN	**38.1**	**18.8**	**12.4**	**12.8**	**14.6**	**9.1**	**14.5**	**21.8**	**36.5**	**63.6**	**53.8**	**44.4**

b.) Indian Head	Year1				Year2							
Average temperature (°C)	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
2015/2016	12.2	6.4	-2.2	-6.5	-11.3	-5.5	-1.2	3.6	12.0	16.9	17.1	16.9
LTN	**11.5**	**4.0**	**-5.3**	**-12.0**	**-15.0**	**-12.0**	**-5.4**	**4.2**	**10.8**	**15.8**	**18.2**	**17.4**
Precipitation (mm)												
2015/2016	67.8	39.0	16.0	7.0	11.4	7.4	18.1	14.9	74.7	50.2	107.9	21.9
LTN	**35.3**	**24.9**	**19.4**	**24.4**	**19.2**	**14.3**	**24.3**	**22.6**	**51.7**	**77.4**	**63.8**	**51.2**

c.) Lethbridge	Year1				Year2							
Average temperature (°C)	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
2014/2015	13.3	10.3	-4.2	-2.5	-3.2	3.6	5.9	9.4	16.9	18.3	18.2	12.2
2015/2016	12.2	8.7	-1.5	-5.2	-6.2	2.6	3.9	8.0	10.8	16.4	18.3	17.6
2016/2017	13.5	6.1	5.5	-11.9	-5.0	-2.5	0.5	6.1	12.7	16.1	20.4	18.7
LTN	**12.6**	**6.6**	**-1.2**	**-5.4**	**-6.0**	**-4.2**	**-0.1**	**6.0**	**11.1**	**15.2**	**18.2**	**17.7**
Precipitation (mm)												
2014/2015	51.1	10.1	10.1	0.0	13.0	2.8	22.7	17.0	40.4	16.7	34.7	11.1
2015/2016	39.5	7.1	9.0	5.1	4.3	0.6	21.3	13.8	67.5	12.8	32.4	30.1
2016/2017	19.4	14.2	0.0	3.6	0.0	2.2	9.0	26.8	41.1	28.3	7.3	10.8
LTN	**41.4**	**20.1**	**17.8**	**12.9**	**13.5**	**12.0**	**22.8**	**28.0**	**49.9**	**82.0**	**42.6**	**37.3**

d.) Lacombe	Year1				Year2							
Average temperature (°C)	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
2014/2015	10.8	6.3	-7.0	-6.8	-8.3	-9.0	0.3	4.8	9.7	15.1	17.0	15.7
2015/2016	9.8	6.0	-3.8	-9.8	-10.1	-3.8	1.3	7.6	10.0	15.2	16.4	15.4
2016/2017	10.1	1.5	0.7	-13.5	-10.0	-9.0	-4.8	3.3	12.3	14.8	16.9	16.0
LTN	**10.0**	**4.1**	**-4.6**	**-10.1**	**-10.9**	**-8.7**	**-3.5**	**4.2**	**9.7**	**13.7**	**15.8**	**15.0**
Precipitation (mm)												
2014/2015	40.4	10.2	28.6	6.6	25.4	18.5	21.3	11.6	23.3	71.2	103.7	48.9
2015/2016	47.4	6.6	8.7	12.4	13.9	9.1	16.8	15.1	79.1	26.7	119.9	66.8
2016/2017	31.4	35.1	16.0	16.9	7.1	17.8	15.2	24.7	45.2	69.7	39.7	27.7
LTN	**41.6**	**21.4**	**14.8**	**11.7**	**14.7**	**9.9**	**14.9**	**22.7**	**54.9**	**78.4**	**94.9**	**61.1**