Validating the management practice of strategic deployment of Blackleg major resistance gene groups in commercial canola fields on the Canadian prairies.

By

Justine Emily Jean Cornelsen

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Department of Plant Science

University of Manitoba

Winnipeg, Manitoba, Canada

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ABSTRACT

Cornelsen, Justine Emily Jean. M.Sc. The University of Manitoba, April 2021. Improving blackleg resistance durability through strategic deployment of major-gene resistance groups in commercial canola fields on the Canadian prairies. Advising Professor: Dr. W. G. Dilantha Fernando.

Blackleg, caused by the fungal pathogen *Leptosphaeria maculans*, is a serious threat to canola (*Brassica napus* L.*)* production in western Canada. In recent years, new pathogen races have reduced the effectiveness of some of the resistant cultivars deployed. Strategic deployment and rotation of major resistance genes in cultivars has been used in France and Australia to help increase the longevity of blackleg resistance. Canada introduced a grouping system in 2017 to identify blackleg resistance genes in canola cultivars. The goal of this research was to examine and validate the concept of major resistance gene deployment through monitoring the avirulence profile of *L. maculans* population and disease levels in commercial canola fields within the Canadian prairies. Blackleg disease incidence and severity was collected from 146 cultivars from 53 sites across Manitoba, Saskatchewan, and Alberta in 2018 and 2019, and the results varied significantly between resistance gene groups, which is influenced by the pathogen population. Isolates collected from spring and fall stubble residues were examined for the presence of *Avr* alleles *AvrLm1*, *AvrLm2*, *AvrLm3*, *AvrLm4*, *AvrLm5*, *AvrLm6*, *AvrLm7*, *AvrLm9*, *AvrLm10*, *AvrLm11*, *AvrLepR1*, *AvrLepR2*, *AvrLep3*, and *AvrLmS* using a set of differential host genotypes carrying known resistance genes or PCR based markers. The Simpson's evenness index was very low, due to two dominant *L. maculans* races (*AvrLm2-4-5-6-7-10-11* and *AvrLm2-5-6-7-10-11*) in representing 49% of the population, but diversity of the population was high from the 35 *L. maculans* races isolated in Manitoba. *AvrLm6* and *AvrLm11* were found in all 254 *L. maculans* isolates collected in Manitoba and over 90% of isolates collected in Alberta contained *AvrLm5*, *AvrLm6*, and *AvrLm7*. Knowledge of the blackleg disease levels in relation to the resistance genes deployed, along with the *L. maculans* avirulence profile helps to measure the effectiveness of genetic resistance and the use of this management practice.

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Introduction

Canola (*Brassica napus* L.*)* is an oilseed crop grown in Canada on approximately 8,093,700 hectares (20 million acres) each year. Canadian-grown canola contributes $29.9 billion to the Canadian economy each year and is the largest canola producer globally (LMC International, 2020). Blackleg, caused by the fungal pathogen *Leptosphaeria maculans,* is a serious threat to canola production in western Canada. Blackleg is one of the major plant diseases to infect canola in Canada. The disease causes constriction of the plants which minimizes moisture and nutrient up-take resulting in premature ripening or death. Blackleg can cause significant yield loss for canola farmers and is a trade conflict that could jeopardize the Canadian canola industry. To minimize yield loss and solidify trade, strategies to mitigate the disease have been put into action (Canola Council of Canada, 2020a).

One management option that canola farmers use to minimize blackleg on their farms is to grow a resistant cultivar. When the same resistant cultivars are grown repeatedly in high frequency canola cropping cycles the blackleg pathogen within the field adapts to overcome the resistant cultivars being deployed. Farmers with blackleg damage continue to grow canola as it is seen as a cash crop but struggle to choose a cultivar to successfully keep blackleg at bay. To provide more information to farmers to help make cultivar decisions the Canadian canola industry developed blackleg resistance gene groups based on the major resistance genes used within cultivars. My thesis research investigated the effectiveness of strategic deployment of canola cultivars based on their blackleg major gene resistance group to lower the blackleg incidence and severity within high canola cropping frequencies. It also provided a current update of the *L. maculans* pathogen avirulence profile across the Canadian prairies.

Some expected outcomes of this work were to validate the concept of deploying blackleg major resistance gene labelled cultivars at the field level to minimize the incidence and severity of the disease. This work provides canola farmers with information to make informed cultivar decisions to effectively manage blackleg on their farm. By understanding *L. maculans* avirulence profile, Canola breeders can use this information to create cultivars with blackleg major resistance genes that effectively work in the Canadian prairies by matching to *L. maculans* avirulence profile. Lowering the pressure of blackleg will keep the $29.9 billion canola industry open and growing within Canada.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Canola

2.1.1 Canola

Canola (*Brassica napus L.)* belongs to the genera *Brassica* from the *Brassicaceae or Cruciferae* (mustard) family, which is comprised of over 3,000 species. Many *Brassica* species have been cultivated as vegetable crops for centuries due to their edible plant organs, such as roots, stems, leaves, buds, flowers, and seeds. Within the latter half of the 20th century, *Brassica* species were used for their oil to fuel lights, then for human consumption, and recently as a source of biofuel (Downey, 1983). Canola or rapeseed is now the largest *Brassica* species cultivated worldwide. The seed is crushed for its oil content for both human consumption and biofuel. The remainder after the oil is extracted is referred to as meal and used in animal feed as it provides a source of protein. Meal consumption is being investigated for humans as a new source of protein.

Canola was developed through traditional plant breeding techniques from rapeseed by researchers from Agriculture and Agri-Food Canada (AAFC) and the University of Manitoba in the 1970s. Products must meet internationally recognized standards, low levels of erucic acid and glucosinolate, to be considered canola (Lin et al., 2013). Canola is defined as: "Seeds of the genus *Brassica* (*Brassica napus*, *Brassica rapa*, or *Brassica juncea*) from which the oil shall contain less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenyl glucosinolate, and 2-hydroxy- 4-pentenyl glucosinolate per gram of air-dry, oil-free solid" (Canola Council of Canada, 2020). Due to this development in Canada, the name canola comes from contraction of Canada and Latin word *ola*, meaning oil, Canadian oil.

The three main species of canola are *Brassica rapa* (Polish canola), *Brassica napus* (Argentine canola), and *Brassica juncea* (canola quality brown mustard). The species are closely related and their relationships are depicted in a *Brassica* triangle or U's Triangle (Nagaharu, 1935). The *Brassica* triangle consists of three diploid species, *Brassica rapa* L. (n=10, AA), *Brassica nigra* L. (n=8, BB), and *Brassica oleracea* L. (n=9, CC); and three amphidiploid species: *Brassica carinata* L. (n=17, BBCC), *Brassica juncea* L. (n=18, AABB), and *Brassica napus* L. (n=19, AACC). The most common of species grown is *Brassica napus*, which originated from the cross between *Brassica rapa* and *Brassica oleracea* (Nagaharu, 1935). *Brassica napus* production

increased in Canada once the introduction of a low erucic acid, low glucosinolate, and high yielding cultivar named Tower was released in 1974 (Stefansson & Kondra, 1975). *Brassica napus* is successfully grown worldwide because of its preference to cool growing environments.

2.1.2 Growth Stages of Canola

Canola is cultivated in several regions across the globe with cultivars designed for different production regions. Winter annuals are usually grown in Asia, Europe, and southern United States. Europe and Asia still refer to the crops as oilseed rape or rapeseed, even though they fit the definition of canola based on their oil profile. Spring/summer annuals of canola are grown in Canada, Australia, and northern regions of Europe due to shorter growing seasons and colder climates. In Canada depending on growing conditions and cultivar, *Brassica napus* takes from 74 – 130 days to fully mature (Morrison et al., 1989). Cultivars are positioned and sold based on days to maturity, through short, mid, and long season cultivars.

The Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (BBCH) decimal system is used to describe canola development through nine growth stages: 0 – germination; 1 – leaf development; 2 – tillering (not used in canola); 3 – stem elongation; 4 – vegetative (not used in canola); 5 – inflorescence emergence; 6 – flowering; 7 – development of seed; 8 – ripening; and 9 – senescence (Lancashire et al., 1991). Growth stages can also be summarized by two larger stages: the vegetative and the reproductive stages. Germination is seen as a pre growth stage and is the stage where the seed must take in water and oxygen to initiate embryo growth. Seed to soil contact along with optimal soil temperatures above 10 ℃ are required for development to begin (Christensen et al., 1985). The first true growth phase is leaf development where the cotyledons push up through the soil surface and unfold. Time from planting to emergence depends on factors such as: soil temperature and soil moisture. The hypocotyl or growing point is situated between the cotyledons above the soil surface. True leaves start to develop and establish a rosette with older leaves at the base and younger leaves developing in the center (Freyman et al., 1973). Stem elongation or bolting overlaps leaf development stages. The stem is an important photosynthetic structure during pod and seed growth. Inflorescence emergence starts with the development of buds near the base of the stem. From seeding to first flower are considered the vegetative stages.

The reproductive stage starts with flowering as the buds start to open to reveal tiny yellow petals. Most *Brassica napus* cultivars in Canada are self-pollinating, but is attractive to species of insect pollinators, allowing for cross-pollination to occur (Eisikowitch, 1981). The abortion of flowers and pods is normal for canola, as the plant produces extra buds (McGregor, 1981). Only around 50% of flowers develop productive pods (Tayo & Morgan, 1975). Through flowering, pods start to elongate starting with the lower pods on the stem. Seeds formation relies on the photosynthetic surface area of the stem and pod wall (Allen et al., 1971). The seed in early stages of development is translucent until the seed's embryo starts to form taking up space and hardening the seed coat. Seed number per pod and seed size depends on the availability of assimilates. Plants under stress during this stage may produce smaller pods and seed (Thurling, 1974). During the ripening stage, pods begin to change colour and become firm, while the seed turn hard and black in colour. Plants at this stage become fragile and can shatter, releasing their seed. Once seeds have changed colour, the plant has reached maturity it starts to lose moisture concentration in plant tissue.

2.1.3 Diseases of Canola

Canola or oilseed rape is susceptible to many pathogens that have the potential to reduce seed yield and therefore jeopardize production. In Canadian canola production, three predominant plant diseases are sclerotinia stem rot, clubroot, and blackleg. Sclerotinia stem rot is caused by the fungus *Sclerotinia sclerotiorum* which produces a white mold on the stems that can weaken stem strength and result in lodging and premature maturity (Rimmer, 2003). It is the most destructive plant disease due to its unpredictability, wide host range, and extreme seed yield loss if plants are left unprotected (Zheng et al., 2020). Disease pressure can be reduced by a timely foliar fungicide application during flowering as peak spores leading to infection are released during this time (Turkington & Morrall, 1993). Clubroot is caused by the species *Plasmodiophora brassicae* and is a relatively new disease found in canola producing regions. It is a soil-borne pathogen that spreads with soil movement. It causes swelling of roots which limits nutrient and moisture uptake of the plant (Tewari et al., 2005). The aggressiveness and shifts in clubroot pathotypes have made it difficult to manage the disease.

Blackleg caused by *Leptosphaeria maculans* is a stubble borne disease that causes plants to lodge or prematurely ripen due to crown cankering (constriction of the vascular tissue at the base

of plant stem) (Hammond et al., 1985). Widespread occurrences of the disease occurred in the early 1980s causing significant yield losses (Gugel & Petrie, 1992). After the introduction of resistant cultivars in the 1990s, blackleg disease outbreaks have lessened in Canada. Other plant diseases found in canola of less concern are alternaria black spot (*Alternaria brassicae*), aster yellow disease (*aster yellow phytoplasma*), root rot (*Rhizoctonia solani*, *Fusarium spp*.), downy mildew (*Peronospora parasitica*), and verticillium stripe (*Verticillium longisporum*). Changes in climate continue to alter pest species life cycles, incidence, and severity of diseases in canola across Canada.

2.1.4 Canola Production in Canada

Globally, 27.9 M tonnes of canola/rapeseed are annually produced. The vegetable oil market generated 203.5 M tonnes in 2018 – 2019, with palm oil the largest producer at 73.9 M tonnes followed by 55.7 M tonnes of soy (United States Department of Agriculture, 2020). Over a five-year trend, canola/rapeseed production has been consistent, whereas palm and soy production continue to increase. The top five rapeseed producing regions based on area sown are Canada, China, India, European Union, and Australia (*Latifunsit*, 2020). Canada leads in overall production and contributes to 28% of the world's total production.

Canola production in Canada is focused on the cultivation of mainly *Brassica napus* and a very small acreage of *Brassica rapa* and *Brassica juncea*. The total contribution of canola to the Canadian economy has been projected at $29.9 billion in 2016-2019 (LMC International, 2020). Most of the canola produced in Canada is in the prairie provinces of Alberta, Saskatchewan, and Manitoba, where canola is seen as a cash crop (high commercial value) for farmers. In 2018, farmers harvested 9,119,672 hectares (22,535,200 acres) of canola making it the largest field crop planted in Canada surpassing wheat in 2018 (Statistics Canada, 2018). In 2017 the largest canola crop was produced in Canada with over 21,458,100 M tonnes (Figure 2. 1). Ninety percent of canola production in Canada is exported, with the top four importers consisting of the United States, China, Japan, and Mexico.

The amount of canola produced in Canada has doubled over the last ten years, with a most recent five-year average yield of 40 bushels per acre. This is on track to reach the goal of the Canadian canola industry of 52 bushels per acre by the year 2025 to help meet the global demand for vegetable oil (Canola Coucnil of Canada, 2014). Increasing yields to reach the goal has been

broken down into five categories: genetic improvements, plant establishment, fertility management, integrated pest management, and harvest management. Integrated pest management is a diverse pillar where an additional two bushels of production is estimated to come from; it encompasses improved management of weeds, insects, and diseases.

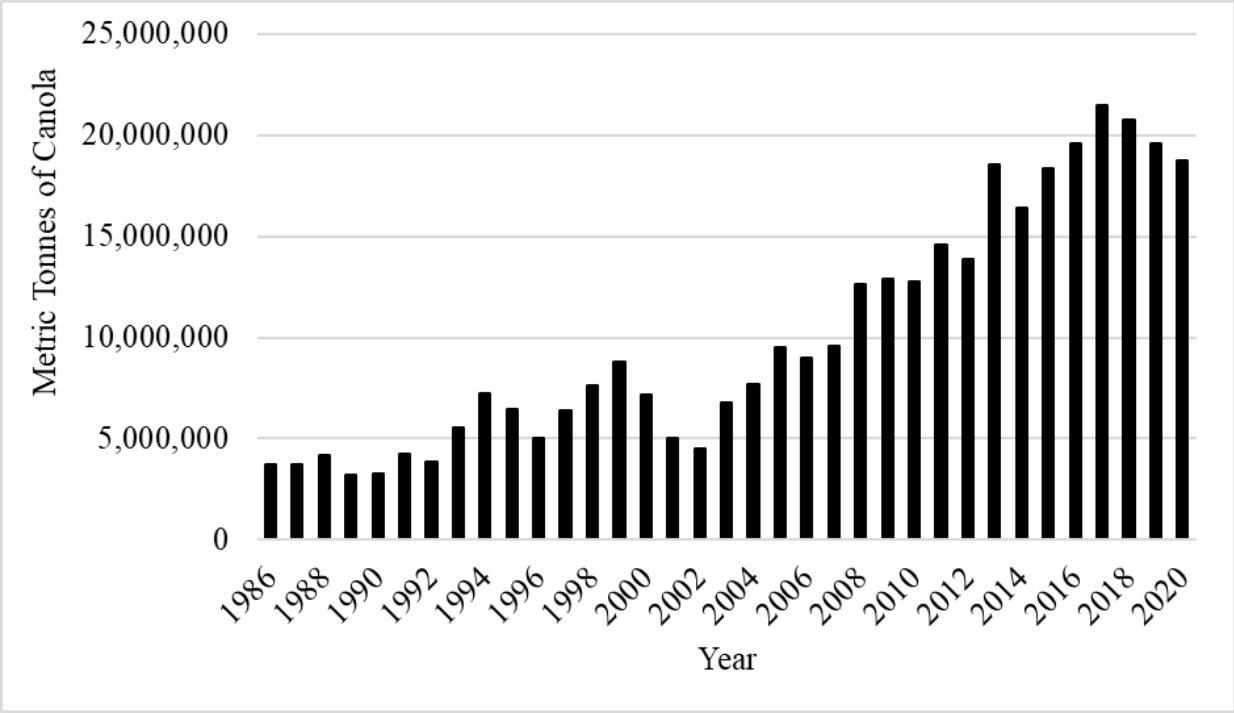

Figure 2. 1 Canadian canola production in metric tonnes from 1986 to 2020. Source: Statistics Canada; Table 32-10-0359-01.

2.2 Blackleg

2.2.1 Taxonomy & History

Blackleg is caused by two fungal species: *Leptosphaeria maculans* (Desmaz.) Ces. & De Not. and *Leptosphaeria biglobosa* (Shoemaker & Brun, 2001). *Leptosphaeria maculans* has been described as an aggressive or virulent form of the species, while *L. biglobosa* is referred to as a less aggressive form (Fitt et al., 2006). Prior to the identification of *L. biglobosa* and the distinction between species, they were referred to as highly and weakly virulent forms of *L. maculans* (Pétrie, 1988). They had also been referred to as A and B groups (West et al., 2001).

Leptosphaeria maculans has now become the dominant and damaging species in canola producing regions, including areas in Australia, Canada, and Europe.

Reports of visual blackleg symptoms on *Brassica* species have been identified over the 19th century. Early reports of the disease in canola in Canada were caused from *L. biglobosa* (McGee & Petrie, 1978). The aggressive, *L. maculans* species was not reported until 1978 in the province of Saskatchewan. *Leptosphaeria maculans* delayed presence in production areas such as western Canada and Poland suggest that it is slowly spreading and replacing *L. biglobosa* (T. Rouxel et al., 2004; West et al., 2002). The provinces of Manitoba and Alberta reported virulent blackleg during the early 80s. Increases in the number of fields, the disease incidence and severity, and yield losses were reported all across the prairies (Gugel & Petrie, 1992). The introduction of the virulent strain of the pathogen along with large regions of susceptible hosts, buildup of infested residues, and favourable climatic conditions, allowed the disease to spread rapidly.

2.2.2 Host Range

Leptosphaeria maculans and *L. biglobosa* are the casual species of blackleg that cause damage to a wide range of *Brassica* species. *Brassica napus* and *Brassica rapa* are suitable hosts for the fungal species, which can cause seedling death, lodging, and premature plant death (West et al., 2001). Blackleg has also been reported on other host genera *Alliaria* (garlic mustard), *Berteroa* (false madworts), *Cardamine* (bittercresses), *Erysimum* (wallflower), *Iberis* (candytuft), *Lepidium* (mustard/cabbage), *Lobularia* (alyssum), *Matthiola* (common stock), and *Raphanus* (radish) (Farr et al., 1989; Smith & Sutton, 1964). Once the pathogen has infected *Brassica* production areas, it is difficult to eliminate due to longevity on residue and the presence of susceptible hosts.

Some *Brassica* species show levels of resistance to *L. maculans* and *L. biglobosa*. Wild crucifers *Arabidopsis thaliana, Diplotaxis muralis, Diplotaxis tenuifolia,* and *Sisymbrium loeselii* all show high levels of resistance to *L. maculans*, whereas *Raphanus raphanistrum* was observed to be susceptible (Chen & Séguin-Swartz, 1999). *Brassica juncea, Brassica nigra,* and *Brassica carinata* have been identified as resistant to blackleg disease infection (Dolores Sacristan & Gerdemann, 1986; Sjödin & Glimelius, 1988). Within the B genome there are some species that are sensitive to blackleg but *Brassica nigra* (BB) shows the most resistance within the *Brassica* triangle (Chevre et al., 1996). Most of the resistance genes identified in *Brassica napus* have

been identified in the A genome (*Rlm1, 2, 3, 4, 7, 9, LepR1, LepR2, LepR3, RlmS*) (Hayward et al., 2012). With species showing levels of resistance to *L. maculans* there is still not complete immunity to the pathogen.

2.2.3 Significance of Blackleg

The fungal pathogen, *L. maculans,* causes blackleg, one of the most economically important diseases of canola (*Brassica napus* L.*)* worldwide. Billions of dollars are lost every year to blackleg, with severe yield losses occurring across North America, Europe, and Australia (Fitt et al., 2006). Blackleg is a worldwide threat to *Brassica* species, and some canola/oilseed rape production areas currently do not have the *L. maculans* species present. Trade concerns have been raised by China, *L. maculans* is unwanted to enter their production regions through seed shipments from countries with *L. maculans,* as it would rapidly spread (Fitt et al., 2008). For the Canadian canola industry, this is of concern due to the estimated $2 billion canola seed trade with China.

The first report of the highly virulent form of the blackleg disease in Canada was in 1975 in Saskatchewan, shortly after the introduction of canola to western Canada (McGee & Petrie, 1978). The disease was so severe, causing lodging and premature death of plants that canola production slowed in areas across Saskatchewan. Due to the severity of the disease, something else had to be done to allow farmers to grow canola successfully. Blackleg resistant cultivars were introduced in the early 1990s in Canada, which helped to minimize the disease pressure back to manageable levels. Provincial blackleg disease surveys capture the disease prevalence, the number of fields showing symptomatic plants; disease incidence, the percentage of symptomatic plants; and disease severity, using a 0-5 disease severity rating scale assessing the proportion of blackened tissue at the cross-section of the crown (base of the plant stem) (West et al., 2001). In 2019, blackleg basal cankers were reported in 68% of Manitoba surveyed fields with a mean disease incidence of 10% for all surveyed canola crops; 69% prevalence in Saskatchewan with 11% disease incidence; and 53% prevalence in Alberta with 10% disease incidence (Canadian Plant Disease Survey, 2020). Canadian canola production since 1998 showing the percentage of blackleg disease prevalence and incidence is presented in Figure 2. 2. Blackleg disease is prevalent in Canadian canola fields, but the number of plants infected within fields remains relatively low.

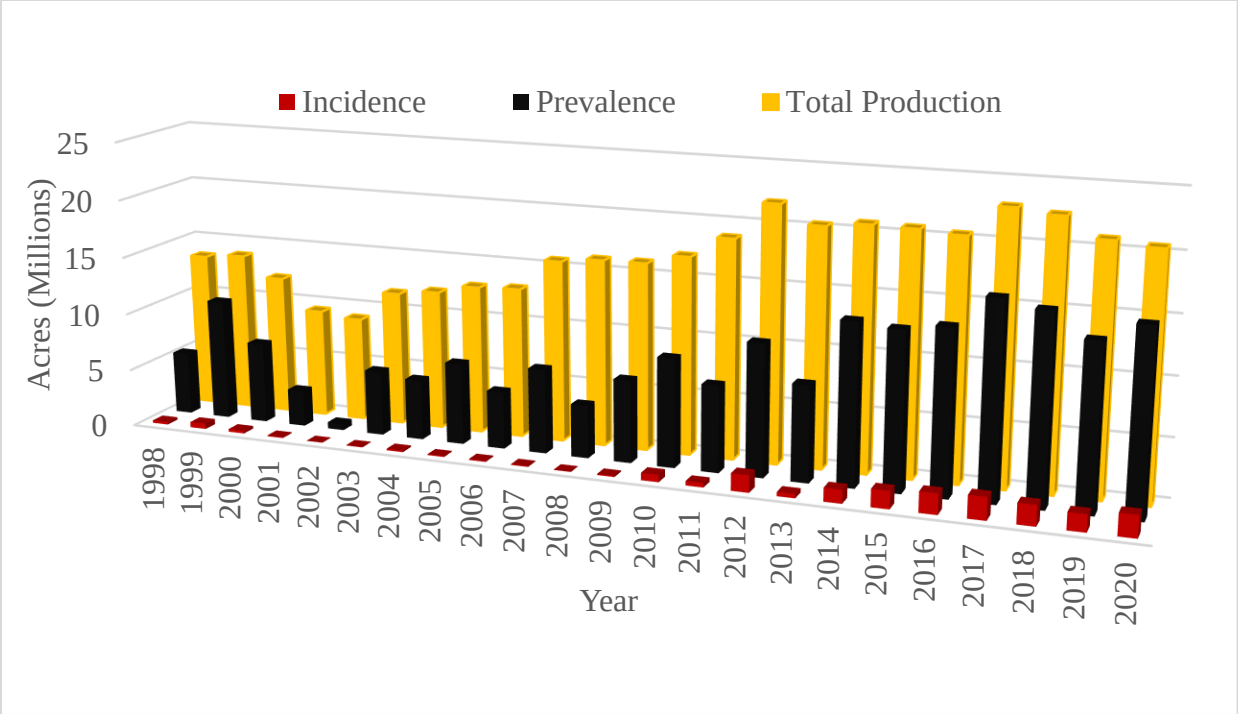

Figure 2. 2. Canadian canola production based on harvested acres per year along with the number of acres with confirmed blackleg symptoms within a field(prevalence) and acres that are infected with blackleg (incidence). Source: Statistics Canada. Table 32-10-0359-01 and Canadian Plant Disease Survey 1998-2020.

2.3 Blackleg Disease Epidemiology with *Brassica napus*

2.3.1 Disease Cycle & Symptoms

Leptosphaeria maculans is the aggressive virulent form of the blackleg disease which causes severe stem cankering that contributes to yield loss. *Leptosphaeria maculans*-infected canola residue has the ability to harbour the pathogen for five years (Petrie, 1995). Blackleg is considered a monocyclic disease by having one cycle of development per year (Hall, 1992; McGee & Petrie, 1978). Not allowing canola residue to break-down allows the pathogen to continue its life cycle when host crops are grown (Figure 2. 3). Under western Canada growing conditions, pycnidiospores are typically released, from old canola residue, in late May to September and act as the primary source of inoculum (Guo & Fernando, 2005). Airborne spores then adhere to stomatal pores and wounds on leaf tissue caused by flea beetle (*Coleoptera: Chrysomelidae)* feeding or environmental damage (Chen & Howlett, 1996). The initial site of infection develops a lesion that can range in appearance and size; lesion size does not correlate to

disease severity. Lesions are pale in colour and are small, irregular shaped in size, ranging under 1 cm in width (West et al., 2001). Lesions formed from ascospores typically have a distinctive dark margin and light coloured center with pycnidia forming (Toscano-Underwood et al., 2001). The tiny black pycnidia throughout the lesion can produce asexual pycnidiospores. This can introduce a secondary infection by rain splash or wind dispersal to neighbouring plants.

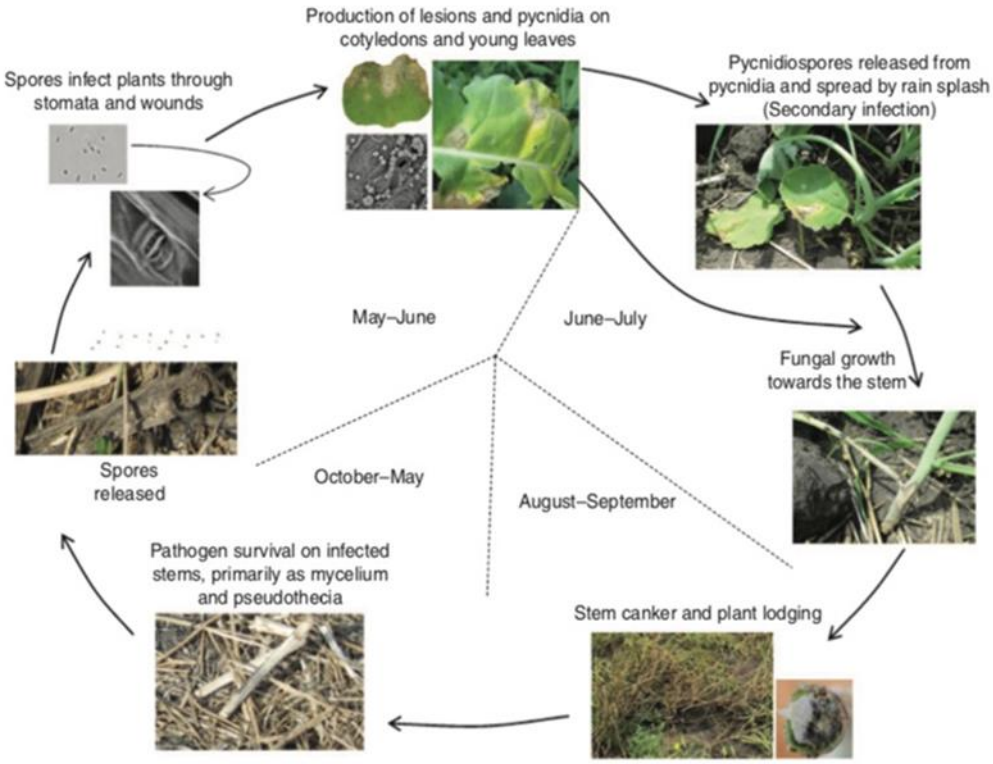

Figure 2. 3. Life cycle of *Leptosphaeria maculans* in canola (*Brassica napus* L.) in western Canada (Zhang & Fernando, 2018).

Once the pathogen has entered the plant tissue it travels through the lamina and petiole of the leaf down into the stem via the xylem (Hall, 1992). Infection occurring from cotyledon to 6-leaf stage (BBCH) can cause stem cankering at the end of the growing season. Leaf lesions are challenging to identify at the field level. There is no correlation between the number of leaf lesions in young plants and necrotic crowns in mature plants (Pierre et al., 1982). Stem cankering or crown necrosis causes stem girdling, which can constrict nutrient and moisture uptake that can

cause plants to lodge, prematurely ripen, or die before harvest. Infected residue and stubble then remain in the field after harvest, which can initiate the *L. maculans* life cycle to begin again.

2.3.2 Environmental Factors

Blackleg occurs across a wide rage of environments. Production of pycnidia is controlled by environmental factors such as air temperature, relative humidity, solar radiation, and duration of leaf wetness (Vanniasingham & Gilligan, 1989). Temperature and frequency of precipitation affects the sporulation of *L. maculans* during the Canadian growing season between the months of April to August (Petrie, 1995). Peak ascospore dispersal in Canada has been found after as little as 2 mm of rainfall, and release can occur for up to 3 days if the temperature is optimal between the temperatures of 13 to 18 ℃, when release of ascospores and pycnidiospores occurs (Guo & Fernando, 2005). Pseudothecial maturation is when the pseudothecia are viable to release ascospores but this is variable in length due to temperature and wetness (West et al., 1999). Leaf infection from ascospores germinating is correlated more with leaf wetness over temperature, as temperature can range between 4 to 28 ℃ (Hall, 1992). A leaf wetness duration of 48 hours is optimal for leaf lesions to form, and as leaf wetness duration decreases so does the number of lesions formed (Biddulph et al., 1999). Rain splash and wind can then spread pycnidiospores from leaf lesions to nearby plants. Ascospores from infected residue can travel several kilometers with the wind.

The presence of pseudothecia is dependent on climatic conditions. Cold summers and hot dry summers in Canada help to preserve infected crop residue for several years (Petrie, 1986). Mild, wet climates allow for rapid degradation of debris. Field wetness and warm soil temperatures help to degrade debris (West et al., 2001). Weather-based models have been developed to help predict the release of ascospores from infected residue to help determine if further management strategies need to be deployed (Salam et al., 2007). Changes in climate will influence the severity and spread of blackleg (Evans et al., 2008; Siebold & von Tiedemann, 2013). In Canada, the critical window for blackleg infection in canola coincides with environmental conditions that are conclusive for *L. maculans* spread and development. The time of ideal conditions for blackleg development may increase with these changes to environment occurring.

2.3.3 Yield Loss

Early records of blackleg yield loss were completed on a 0 – 4 scale by scoring the severity of internal necrosis of crown cankers. The incidence and severity of disease were negatively correlated with yield, seed weight, and vigor index (Rempel & Hall, 1995). Furthermore, through correlation analysis it was determined that incidence and severity of the disease were related to plant lodging. Rempel and Hall (1995) reported that seed yield was reduced by 18 % for each unit increase in disease severity. Field surveys in China found yield losses of 10 – 37 % from *L. biglobosa* and inoculated plants had losses of 29 – 56 % (Cai et al., 2018). Plants infected with blackleg can have a fewer number of branches, pods, and seeds compared to healthy plants.

Recent work with a blackleg susceptible cultivar, 'Westar', has shown a decrease in yield per plant by 17.2 % for every unit of increase in blackleg disease severity, using the 0 – 5 stem rating system (Hwang et al., 2016). There is a negative relationship between pod number and disease severity. Hwang et al., (2016) found that for each increase in disease severity index, seed yield declined by 13 %. The yield models were updated using moderately resistant cultivars to represent the losses seen from blackleg in producer's fields. Field trials have reported relationships between disease severity and the pod number and seed yield were best described by second-degree quadratic equations (Wang et al., 2020). Moderately resistant cultivars' yield losses ranged from 18 – 99 % when disease severity was between a rating of 2 – 5, compared to plants rating between 0 – 1. Blackleg disease yield loss on resistant cultivars is difficult to compare because cultivars will react differently to the *L. maculans* races used to inoculate plants. Fungicide applications are used to try to rescue yield losses experienced from high frequency canola cropping frequencies and high levels of blackleg disease pressure (Harker et al., 2015; Harker et al., 2017). Measuring yield loss allows for a better understanding of economic implications caused from the disease and shows the benefit of disease mitigation and agronomic practices for managing the disease.

2.4 Blackleg Disease Management

2.4.1 Cultural Management

Cultural or agronomic practices to minimize blackleg disease pressure consist of extending the frequency in which canola is grown on the same field and managing residue or stubble that acts

as inoculum for the pathogen. Extending crop frequency, with non-brassica host crops, to at least two years between canola crops is one of the most effective approaches to decrease inoculum levels (Harker et al., 2015). To further limit the risk of inoculum buildup, resistance breakdown, and yield loss, growing canola less frequently in rotations on the same field is recommended. Kutcher et al. (2013) emphasized that rotations needed to be greater than one canola crop in four years. Intensive canola growing regions are susceptible to blackleg epidemics (Hegewald et al., 2018). Not only does canola frequency in rotation become important to manage the disease spread but also spatial cropping must be considered.

Crop isolation is another method to minimize the inoculum pressure in new canola crops from known blackleg infected canola residue. Planting recommendations in Australia are to grow canola at least 500 m away (Marcroft et al., 2004). Canadian recommendation is for only 50-100 m away from known blackleg infected residue (Guo et al., 2005). Another alternative to managing blackleg infected residue is to destroy the source of inoculum. Guo et al. (2008) reported that the amount of ascospores and pycnidiospores dispersed was less from tilled plots in comparison to no-till plots, indicating that tillage may reduce inoculum levels. Spore dispersal is associated with the amount of infested canola residue on the soil surface and environmental conclusive conditions (Guo et al., 2008). Burning residue or tillage and burial of infested canola residue does not always decrease the risk of blackleg and that rotation interval, cultivar grown, and seasonal weather conditions have a much greater impact of managing blackleg inoculum than tillage (Kutcher & Malhi, 2010). Due to negative impacts on soil health of using routine tillage (Blackshaw et al., 2005), the practice is not commonly adopted in western Canada to manage blackleg infested residue.

2.4.2 Crop Protection Products

Canadian canola farmers have the option to use chemical formulations as crop protection products to manage blackleg. However, no biological controls are currently on the market. Some fungi and bacteria have been found to limit the growth or stop formation of *L. maculans*, which have been tested in a lab setting but once taken to the field are not effective (Ramarathnam et al., 2007, 2011). A foliar fungicide application applied during the 2 – 6 leaf stage is a recommended practice to protect young plants from a blackleg infection. Azoxystrobin, fluxapyroxad, propiconazole, and pyraclostrobin are active ingredients used in foliar fungicide protection

products that are registered in Canada to suppress blackleg (Manitoba Agriculture, 2018). Foliar fungicides applied between the 2 – 6 leaf stages typically do not increase canola yield on resistant canola cultivars when cultivar resistance is effective (Peng et al., 2020a). Increases in yield are seen on susceptible cultivars when foliar fungicides are applied early in the growing season prior to the 6-leaf stage. Penthiopyrad plus picoxystrobin and prothioconazole foliar fungicides both reduced blackleg severity but increases in yield were not seen during low disease pressure seasons (Sewell et al., 2016). Flusilazole plus carbendazim foliar fungicide was able to decrease the severity of stem cankering and frequency of *L. maculans* spread into pith tissues (Huang et al., 2011).

Globally, seed treatments and in-furrow fungicide applications are used in combination with foliar fungicides to manage blackleg. Fungicide resistance has already been identified in Australian canola production regions where *L. maculans* populations are showing sensitivity to fluquinconazole (Van De Wouw et al., 2017). Fluopyram seed treatment tested in Canada, inhibited cotyledon infection and substantially reduced the disease severity on a susceptible cultivar (Peng et al., 2020b). The same research tested fluopyram on a resistant cultivar, but disease severity remained low with or without fluopyram. Seed treatments are an effective tool when cultivar resistance is no longer effective or when growing a susceptible cultivar (Fraser et al., 2020). If cultivar resistance is effective, the benefit of fungicide application is negligible.

2.4.3 Genetic Resistance

Cultivars with resistance to blackleg were first deployed in the 1990s, which helped minimize impact of the disease. Resistance ratings to blackleg in Canada are determined prior to cultivar registration through the Western Canada Canola and Rapeseed Recommending Committee (WCCRRC). Four classes of blackleg resistance have been established: resistant (R; 0-29.9% of susceptible check cultivar), moderately resistant (MR; 30.0-49.9% of check), moderately susceptible (MS; 50.0-69.9% of check), and susceptible (S; 70.0-100% of check cultivar) (Canola Council of Canada, 2020). Once a cultivar is commercialized, assessments for blackleg are no longer continued. Resistance in R-rated cultivars can be overcome by shifts in pathogen populations, as they are not immune to all pathogen races. If cultivars are labelled R-rated at the time of registration, several cropping cycles of growing that cultivar may lead to the cultivar behaving like a susceptible cultivar, depending on the predominant blackleg races present in the

field (Liban et al., 2016; Zhang et al., 2016). Strategic deployment of resistant cultivars to maintain the effectiveness and longevity of resistance sources has become a research priority.

2.4.4 Rotational Systems

Rotating cultivar genetics in a field has been one management practice deployed in larger canola production regions. To ensure the lifespan of resistant cultivars, even on extended canola cropping systems, it is important to use major resistance genes that target the predominant *L. maculans* population and to rotate between resistance genes when virulent alleles start developing. The Grains Research and Development Corporation (GRDC) in Australia adopted a major resistance gene labelling system that places cultivars with the same gene into groups to help farmers manage blackleg by cultivar rotation (GRDC, 2018). This approach has worked as a predictor system to indicate which major genes are no longer going to be successful in specific cropping regions. An intensive cultivar monitoring trial network is used to help predict which major resistance genes are going to remain successful and which genes have been overcome by virulent populations (Marcroft et al., 2012).

In Australia, the environmental conditions are ideal for the increase in incidence and development of *L. maculans*, resulting in very severe disease symptoms. Cultivars have short lifespans within specific regions of Australia but can be re-introduced to an area after switching to a different major gene grouping. This monitoring approach in Australia has been able to predict resistance gene failure and avoid disasters from blackleg disease for farmers (Sprague et al., 2006). Being able to reuse blackleg resistance genes in areas where they previously were overcome has been a huge success to the cultivar rotation system in Australia. It also alleviates pressure on canola breeders to develop blackleg resistance cultivars with new novel traits.

In Canada, when canola farmers found increased levels of blackleg within their R-rated cultivar, the recommendation was to rotate to a different cultivar (Kutcher et al., 2011). Unknowingly, they could have been selecting a cultivar with the same major resistance genes that were not matching the predominant blackleg races within the field. Zhang et al (2016) identified the major resistance genes deployed in Canadian cultivars/germplasm. In 2017, the Canadian canola industry had adopted a new resistance labelling scheme to identify the major resistance genes deployed within a cultivar, allowing farmers to rotate cultivars based on major resistance gene groups (Table 2. 1). Cultivars continue to be labelled with the R/MR resistance rating, but plant

breeders and the seed industry now have the option to include a major resistance gene label: A, B, C, D, E_1, E_2, F, G, J, K, L, N, P, or X. Each group represents blackleg major resistance gene(s), while 'X' represents a labelled cultivar with an unknown resistance gene (CCC, 2020; Zhang & Fernando, 2018). Groups have been determined based on resistance gene interactions and similar responses to *L. maculans* avirulence genes. Van de Wouw and Howlett (2019) outline the differences in resistance gene groups between the Canadian and Australian systems. One main difference is that farmers in Canada know the exact gene that is being used within their cultivar, whereas Australian farmers only know what major resistance gene group the cultivar contains. Marcroft et al. (2012) cultivar rotation work indicates that Australian farmers have at least nine resistance genes available in commercial cultivars.

Table 2. 1. The Canadian Blackleg Major Resistance Gene labelling system which classifies *Brassica napus* cultivars' major resistance genes by lettered resistance gene groups (RG).

Resistance Gene Group (RG)	Major Resistance Genes
A	*Rlm1* or *LepR3*
B	*Rlm2*
C	*Rlm3*
D	*LepR1*
E_1	*Rlm4*
E_2	*Rlm7*
F	*Rlm9*
G	*RlmS or LepR2*
J	*Rlm5*
K	*Rlm6*
L	*Rlm8*
N	*Rlm11*
P	*LepR4*
X	Unknown

This approach may mitigate the risk of rapid resistance erosion and extend the life of cultivars grown in Canada Fernando et al., 2016; Harker et al., 2015; Liban et al., 2016). Similar strategies have been deployed in Australia and France with great success (Ansan-Melayah et al., 1998; Marcroft et al., 2012). A major resistance gene cultivar rotation system helps to preserve advanced genetics and takes the pressure off of development of new cultivars with novel sources of resistance (Van de Wouw et al., 2014). Identifying major resistance genes within

commercially available cultivars creates the opportunity for farmers to switch the major resistance gene deployed if experiencing high disease pressure. The rotation of resistance genes provides farmers with an additional tool to use in minimizing disease pressure.

2.4.5 Integrated Pest Management

Integrated pest management (IPM) is an agronomic approach that integrates varied management practices to control pests. IPM does not rely on one management practice for control but a combination and sequence of practices together to help minimize pest impacts, while also monitoring the pest population (Prokopy, 2003). Relying on only one practice is not an effective long term strategy for disease control (Kutcher et al., 2011). Blackleg management in Canada has focused greatly on scouting or the proper identification of the disease, the extension of canola crop frequency, and the use of blackleg resistant cultivars. All commercially available cultivars are resistant (R) or moderately resistant (MR) to blackleg (Canola Council of Canada, 2020). Successful rotation of resistance genes and extending cropping frequency both help to minimize the impact observed from the blackleg disease in Canada.

Crop scouting and proper disease identification are crucial to monitor the effectiveness of management practices deployed. There are no recovery practices to minimize disease symptoms found in season but information is beneficial for future planning and assessments of management strategies to control blackleg (Kutcher et al., 2011). Plant disease symptoms caused from *L. maculans* can easily be confused with other disease or plant damage symptoms such as grey stem (*Mycosphaerella capsellae*) or verticillium stripe (*Verticillium longisporum*) (Rimmer, 2003; Zhou et al., 2006). Misidentification of plant diseases can result in changes to management practices that do not address the main cause of plant symptoms. Farmers in Canada can submit their canola stubble samples for *L. maculans* identification through commercial labs in western Canada. The testing identifies predominant *L. maculans* races in the farmer's field, providing the field-level information to facilitate better decision making on effective resistant cultivars.

Some practices can have an input expense associated with them. Determining the cost of product or application compared to the yield increase is how farmers determine effectiveness. Use of fungicide seed treatments or foliar fungicides to control blackleg do not typically see an economic benefit when resistant cultivars are effective (Peng et al., 2020). Practices that look to control one specific pest need to be assessed fully to understand the implications to beneficial

organisms living in the same environment (Stanley et al., 2015). It is beneficial to properly assess and accurately identify the successful management practices in lowering disease pressure to the crop. Long term crop sustainability relies on a diversified cropping systems (Harker et al., 2015). Management of any pest needs to incorporate a holistic approach to preserve the available tools and technology (Canola Council of Canada, 2020b). A combination of cultural practices, chemical and biological controls, and genetic resistance should be used to best manage the spread, frequency, incidence, and severity of the blackleg disease.

2.5 *Leptosphaeria maculans - Brassica napus* Interaction

2.5.1 Host Resistance

Canola (*B. napus*) relies on two types of resistance: major gene resistance (also known as qualitative resistance) and minor gene (quantitative) resistance. Major gene resistance is race-specific and stops the pathogen from spreading at the initial site of infection (Rimmer & Van Den Berg, 1992). Quantitative resistance is non-race specific and has an additive effect which provides partial resistance to the pathogen (Hayward et al., 2012). When a cultivar only has quantitative resistance, it will be susceptible to infection by *L. maculans* early in plant development, but disease symptoms and yield impact will be significantly reduced by crop maturity. Quantitative resistance remains a crucial player in minimizing blackleg disease pressure but due to the complexity in measuring the effect it plays at reducing disease severity it remains difficult to properly quantify the role it plays (Van De Wouw et al., 2016). Major gene resistance is only effective against *L. maculans* races that contain the corresponding avirulence (*Avr*) genes (Flor, 1971). If the major resistance gene matches the avirulence allele within the *L. maculans* population, the plant will initiate a defense response or incompatible interaction, killing the cells around the infected cell and stop the pathogen from spreading any further (Rimmer, 2006). The optimal cultivar contains both major resistance genes and quantitative resistance genes to defend against a diversity of *L. maculans* races.

2.5.2 Major Resistance Genes

The *Leptosphaeria maculans-Brassica napus* pathosystem follows the gene-for-gene interaction model but with some exceptions to the model (Flor, 1971). In the *L. maculans-B. napus* coevolution, avirulence protein known as effectors, have been recognized by the *B. napus*

resistance (R) proteins (receptor) encoded by major resistance genes (Hayward et al., 2012). An Effector-Triggered-Immunity (ETI) occurs with the gene-for-gene relationship between an avirulence gene and its matched major resistance gene resulting in disease defense. Coevolution between the host and pathogen have allowed for diversifying selection operating to promote protein diversity in both partners (Rouxel & Balesdent, 2010). One exception to the gene-for-gene model is the dual specificity of the single avirulence gene *AvrLm1*, which is recognized by both *Rlm1* and *LepR3* (Larkan et al., 2013). Another exception is when an isolate is characterized to carry *AvrLm4-7* or *AvrLm7*, a "hide and seek" interaction occurs which renders *AvrLm3* and *AvrLm9* ineffective within the isolate (Ghanbarnia et al., 2018; Plissonneau et al., 2016). This phenomenon is also known as epistasis suppression. Understanding the resistance gene interactions between major genes and with the *L. maculans* avirulence alleles, allows for strategic deployment of resistance genes. Table 2. 2 identifies the 19 major resistance genes against *L. maculans* that have been identified out of *Brassica* species (Larkan et al., 2016). *LepR3*/*Rlm2* and *Rlm9* are currently the only host resistance genes that have been cloned (Larkan et al., 2015, 2020). Diversity of genes provides plant breeders with options to incorporate into Canadian *B. napus* germplasm. Major resistance genes are identified in cultivars by using differential sets by completing pathogenicity tests and identifying through Polymerase Chain Reaction (PCR) based markers.

Table 2. 2. Identified major resistance genes against *Leptosphaeria maculans* from *Brassica* species.

Species	**Major Resistance Gene**	**Reference**
B. napus	*Rlm1, Rlm2, Rlm3, Rlm4, Rlm7, Rlm9*	Ferreira, et al, 1995; Mayerhofer et al., 1997; Ansan-Melayah et al., 1998; Zhu & Rimmer, 2003; Rimmer, 2006; Delourme et al., 2006
B. rapa	*Rlm8, Rlm11*	Balesdent et al., 2002; Balesdent et al., 2013
B. juncea	*Rlm5, Rlm6*	Chèvre et al., 1997; Balesdent et al., 2002
B. nigra	*Rlm10*	Chevre et al., 1996; Eber et al., 2011
Re-synthesized *B. rapa* subspecies Sylvestris	*LepR1, LepR2, LepR3, LepR4, RlmS*	Yu et al., 2005, 2008, 2013; Van De Wouw et al., 2009
Surpass 400	*BlmR1, BlmR2*	Long et al., 2011

2.5.3 Avirulence Alleles of *L. maculans*

Leptosphaeria maculans shows variability in virulence, having avirulent and virulent strains towards *Brassica* species (Kutcher et al., 1993; Newman, 1984; Thurling & Venn, 1977). An avirulence gene or allele encodes a protein that is recognized by genotypes of the host plant species that has the matching resistance gene (Rouxel & Balesdent, 2010). Due to sexual recombination, *L. maculans* populations change rapidly due to selection pressure from *B. napus* resistance (Van de Wouw et al., 2010). This leads to virulent isolates that can cause host resistance to breakdown resulting in yield losses. A total of 14 avirulence genes have been identified in *L. maculans*, with only eight avirulence genes being cloned to date (Larkan et al., 2016; Van de Wouw et al., 2018) (Table 2. 3). The allele *AvrLm4-7* is recognized by both host resistance genes *Rlm4* and *Rlm7* (Parlange et al., 2009). A similar interaction has been documented with *AvrLm5-9* being recognized by host resistance genes *Rlm5* and *Rlm9* (Ghanbarnia et al., 2018). These interactions are valuable to understand when trying to determine the reasons behind changes in virulence.

Table 2. 3. Identified avirulence genes cloned in *Leptosphaeria maculans* by name and reference.

Leptosphaeria Maculans **Avirulence Gene**	**Reference**
AvrLm1	Gout et al., 2006
AvrLm2	Ghanbarnia et al., 2015
AvrLm3	Plissonneau et al., 2016
AvrLm4	Parlange et al. 2009
AvrLm5	Van de Wouw, et al., 2014
AvrLm6	Fudal et al., 2007
AvrLm7	Parlange et al., 2009
AvrLm11	Balesdent et al., 2013

Marker development for the detection of avirulence and virulent alleles allows for rapid testing to occur. One example is the development of a marker to discriminate the *AvrLm9* from *avrLm9* in *L. maculans* isolates (Liu et al., 2020). This allows for rapid identification of avirulence alleles within the *L. maculans* population. Through the cloning of avirulence alleles and development of specific markers for detection, testing of infected stubble can occur to determine *L. maculans* avirulence profile. Testing is available to farmers for predominant *L. maculans* races in their field through commercial labs using Kompetitive allele specific PCR (KASP) markers

(Cornelsen et al., 2019). This provides the field-level information to facilitate better decision making on effective resistant cultivar use.

Understanding *L. maculans* races and avirulence profile has become an industry focus in trying to manage the disease. Across western Canada two dominant *L. maculans* races were identified, *AvrLm2 – 4– 6 – 7* and *AvrLm2– 4 – 6 – 7 – S* and 55 less common races detected, indicating that diversity is high (Liban et al., 2016). Soomro et al. (2020) also found the same top two races in commercial and Westar trap crops in western Canada confirming the predominant isolate races have not changed. Isolates in that work were characterized for 10 avirulence alleles: *AvrLm1, AvLm2, AvrLm3, AvrLm4, AvrLm6, AvrLm7, AvrLm9, AvrLepR1, AvrLepR2* and *AvrLmS*. Monitoring blackleg races spatially and temporally creates the opportunity to study the impact of major resistance genes on the changes of avirulence genes within the population (Kutcher et al., 2007). Changes in the virulence profile of *L. maculans* can indicate the success of major resistance genes deployed, estimate the durability of resistance genes deployed, and predict predominant *L. maculans* races that will occur in the future (Van de Wouw et al., 2014). Liban et al. (2016) found that over 85 % of isolates contained *AvrLm6* and *AvrLm7,* whereas under 10 % of isolates contained *AvrLm3, AvrLm9,* and *AvrLepR2*. This work compared to previous studies identifies the changes in virulence over time. Knowing the *L. maculans* races at the field level allows for the opportunity for a strategic cultivar rotation system to be applied, where cultivars can be deployed to target specific *L. maculans* races.

2.5.4 Influence of Major Resistance Gene Deployment

Understanding the *L. maculans* avirulence profile within a field becomes a key component of learning how to effectively deploy resistance genes. Deploying the same major resistance gene repeatedly creates a virulent allele within the pathogen population (Kutcher et al., 2011). When major resistance genes are deployed broadly on many acres, strong selection pressure is put on the pathogen population resulting in an increase in the frequency of virulent individuals (Van de Wouw & Howlett, 2019). The virulent allele bypasses the major resistance gene mechanisms within the plant and the pathogen can cause infection. A well documented case of resistance breakdown was in the Eyre Peninsula of Australia, where cultivars with resistance gene *RlmS, B. rapa* subsp. *Sylvestris,* were released commercially in 2000. The cultivars had a high level of blackleg resistance and were grown widely until their breakdown in 2003, only three years after

commercialization (Sprague et al., 2006; Sprague et al., 2006). Since then, the avirulence frequency surveillance of *L. maculans* isolates has occurred to help predict future cultivar breakdowns (Van De Wouw et al., 2016). The shifts in virulence documented within the surveillance program in Australia has provided valuable information on cultivar selection to avoid resistance breakdown (Van de Wouw, et al., 2014).

Within Canadian canola growing regions, the *AvrLm3* avirulence allele has become scarce in the *L. maculans* population due to over use of *Rlm3* resistance gene in Canadian *B. napus* germplasm (Zhang et al., 2016). Rashid et al. (2020) found rapid loss of avirulence and shifts to virulence by *L. maculans* isolates in as little as one year in Canada. Isolates collected from commercial fields across the Canadian prairies found *AvrLm2*, *AvrLm4*, *AvrLm6*, and *AvrLm7* in most of the isolates and found low frequencies of *AvrLm1*, *AvrLm3*, *AvrLm9*, and *AvrLep2* (Soomro et al., 2020). Gaining knowledge of what major resistance genes are in Canadian *B. napus* germplasm and what the avirulence alleles are within the *L. maculans* population, creates the opportunity to strategically deploy resistance genes to match the predominant *L. maculans* races successfully.

2.5.5 Pyramiding Major Resistance Genes

The longevity of a resistance gene diminishes rapidly when a virulent population overcomes the avirulent population in the field (Lof & van der Werf, 2017). Gene stacking or pyramiding is when a host contains several major resistance genes (Mundt, 2018). The combination of a major resistance gene with quantitative resistance is also considered to be a gene stack; this has proven to be important for extending the effectives of major resistance genes (Pilet-Nayel et al., 2017). Pyramiding of major resistance genes is durable when the virulent population is low and works best when new genes that have not been previously deployed are incorporated (Fuchs, 2017). One concern of pyramiding major resistance genes is that the virulent races will develop that are able to defeat all the major genes deployed in the host (de Vallavieille-Pope et al., 2012). This is particularly true when moderate to high gene flow occurs. Gene stacks are used to provide broad spectrum and durable resistance, but not enough information is yet known about the longevity of stacked gene cultivars.

Leptosphaeria maculans is considered a recombining pathogen population which would easily be able to break the effectiveness of stacked major resistance gene cultivars (Marcroft et al.,

2012; McDonald & Linde, 2002). In Canada, lower rates of pathogen sexual recombination occur, minimizing the impact on avirulence frequency (Ghanbarnia et al., 2011). Based on the *L. maculans* race profile in Canada, Liban et al. (2016) suggested a *Rlm6* and *Rlm7* stacked cultivar would be effective against most *L. maculans* races. Screening of Canadian *B. napus* germplasm found a combination of major resistance genes with quantitative resistance; with only a few gene stacks identified (Zhang et al., 2016). The ideal resistance gene deployment approach depends on the virulence profile of the *L. maculans* population and race dynamics.

2.5.6 Strategic Major Resistance Gene Deployment

Further understanding of cultivar resistance genetics, fungal population, and disease dynamics in Canada is key to successful blackleg disease management (Zhang & Fernando, 2018). Surveillance of *L. maculans* avirulence frequency along with major resistance gene grouped cultivars have been successfully used in Australia for nearly two decades (Marcroft et al., 2012; Van de Wouw et al., 2021). The predictive abilities of the Australian program and the access to valuable information for cultivar selection has grabbed the attention of Canadian farmers. The development of the major resistance gene cultivar labels in Canada, creates the opportunity to assess this management practice of cultivar rotation based on major resistance gene groups. Will identified major resistance gene grouped cultivars help to stop the increase of blackleg incidence that has been documented over the past ten years across the Canadian prairies? Therefore, the main objectives of this work were:

1) To assess the concept of blackleg major resistance gene labelled cultivar deployment through monitoring the avirulence profile of *L. maculans* population and disease levels in commercial canola fields within the Canadian prairies;

2) To develop empirical data on changes of the *L. maculans* avirulence alleles in response to the deployment of specific major resistance genes in these fields.

The use of commercial fields in this study provides insight into how farmers have been influencing the *L. maculans* population on their fields, through the deployment of cultivars carrying specific major resistance genes. Knowledge gained helped validate the effectiveness of deploying cultivars carrying specific major resistance genes to match the *L. maculans* avirulence profile in farmer's fields allowing them to better manage blackleg. This study places a high

importance of knowing what the canola cultivar's blackleg major resistance genes are to make informed crop management decisions. Overall, this experiment looked at the combination of management strategies, by using crop rotation, major resistance gene rotation, and strategic deployment of resistant cultivars, to extend the longevity of crop production tool.

CHAPTER 3

3.0 VALIDATING THE STRATEGIC DEPLOYMENT OF BLACKLEG RESISTANCE GENE GROUPS IN COMMERCIAL CANOLA FIELDS ON THE CANADIAN PRAIRIES

3.1 Abstract

Blackleg, caused by the fungal pathogen *Leptosphaeria maculans*, is a serious threat to canola (*Brassica napus* L.) production in western Canada. Crop scouting and extended crop rotation, along with the use of effective genetic resistance, have been key management practices available to mitigate the impact of the disease. In recent years, new pathogen races have reduced the effectiveness of some of the resistant cultivars deployed. Strategic deployment and rotation of major resistance (R) genes in cultivars has been used in France and Australia to help increase the longevity of blackleg resistance. Canada also introduced a grouping system in 2017 to identify blackleg R genes in canola cultivars. The main objective of this study was to examine and validate the concept of R gene deployment through monitoring the avirulence (*Avr*) profile of *L. maculans* population and disease levels in commercial canola fields within the Canadian prairies. Blackleg disease incidence and severity was collected from 146 cultivars from 53 sites across Manitoba, Saskatchewan, and Alberta in 2018 and 2019, and the results varied significantly between gene groups, which is likely influenced by the pathogen population. Isolates collected from spring and fall stubble residues were examined for the presence of *Avr* alleles *AvrLm1, AvrLm2, AvrLm3, AvrLm4, AvrLm5, AvrLm6, AvrLm7, AvrLm9, AvrLm10, AvrLm11, AvrLepR1, AvrLepR2, AvrLep3,* and *AvrLmS* using a set of differential host genotypes carrying known resistance genes or PCR based markers. The Simpson's evenness index was very low, due to two dominant *L. maculans* races (*AvrLm2-4-5-6-7-10-11* and *AvrLm2-5-6-7-10-11*) in representing 49% of the population, but diversity of the population was high from the 35 *L. maculans* races isolated in Manitoba. *AvrLm6* and *AvrLm11* were found in all 254 *L. maculans* isolates collected in Manitoba. Knowledge of the blackleg disease levels in relation to the R genes deployed, along with the *L. maculans Avr* profile helps to measure the effectiveness of genetic resistance.

3.2 Introduction

Blackleg, caused by the fungal pathogen *Leptosphaeria maculans* (Desm.) Ces. & de Not, is an economically important disease of canola (*Brassica napus* L.) in many parts of the world, including western Canada, due to yield loss and trade conflicts (Fitt et al., 2006; Van De Wouw et al., 2016; Zhang & Fernando, 2018). Recommended practices to minimize disease impact consist of an extended crop rotation ensuring at least a two-year break between canola crops, crop scouting and proper pathogen identification, use of cultivars resistant to blackleg, rotation of blackleg resistance sources, and foliar or seed treatment fungicides (Canola Council of Canada, 2020). In Canada, blackleg resistance ratings are determined prior to cultivar registration using procedures defined by the Western Canada Canola and Rapeseed Recommending Committee (WCCRRC). When blackleg resistance cultivars were introduced in the late 1990s and early 2000s, incidence levels of the disease dropped well below 5% across the Canadian prairies (Kutcher et al., 2010). Over the last 10 years blackleg disease incidence levels have been slowly increasing, raising the question of what is happening to the resistant cultivars being deployed (Hwang et al., 2016). Exploration into stewarding resistant cultivars has become a priority for the Canadian canola industry.

Canada is the largest producer of canola globally and the canola industry is estimated to have a $29.9 billion economic impact to Canada each year (LMC International, 2020). In 2019, there was 8,571,700 hectares (21.2 million acres) seeded to canola in Canada, which produced 19.6 million metric tonnes (Statistics Canada, 2019). Seeded acres of canola have doubled since the early 2000s, bringing changes to the level of blackleg disease. Provincial governments in western Canada periodically conduct disease surveys for blackleg prevalence, the number of fields infected with the disease; the disease incidence, the number of plants within a field infected with the disease; and disease severity, severity of plants infected rated on a 0-5 disease severity scale. The prevalence of blackleg disease in Canada is around 70% of fields surveyed showing evidence of the disease. In 2019, the incidence levels were 10%, 11%, and 10% for Manitoba, Saskatchewan, and Alberta, respectively (Canadian Plant Disease Survey 2020). Blackleg disease incidence numbers have also doubled since the early 2000s and have increased due to intensified canola cropping frequencies and a shift in the *L. maculans* race profile (Harker et al., 2015; Zhang & Fernando, 2018). Changes in climate have also been noted as a factor for blackleg disease growth and development (Barnes et al., 2010; Siebold & von Tiedemann, 2013;

Zou et al., 2019). Management of the disease has relied heavily on proper identification of the disease or crop scouting, extending out the canola crop frequency, and the use of resistant cultivars to blackleg.

Shifts in *L. maculans* race profile can render blackleg resistant cultivars less or ineffective. Across western Canada two dominant *L. maculans* races were identified in 2010 and 2011 (*AvrLm2–4–6–7* and *AvrLm2–4–6–7–S*) and 55 less common races detected, indicating that diversity is high (Liban et al., 2016). Regional monitoring over time has revealed changes within the population due to the use of resistance genes in many canola cultivars (Fernando et al., 2018; Kutcher et al., 2007; Liban et al., 2016; Soomro et al., 2020); the avirulence gene *AvrLm3* had become scarce in the *L. maculans* population due to over use of *Rlm3* resistance gene in Canadian *B. napus* germplasm. The recent increase of *AvrLm7* and *AvrLm4-7* within the population (Liban et al., 2016; Soomro et al., 2020) also masked the effect of *AvrLm3* and *AvrLm9* (Ghanbarnia et al., 2018; Plissonneau et al., 2016; Zhang et al., 2016). Similar scenarios have occurred globally. For example, the commercial use of *Rlm1* in France resulted in a decrease of the proportion of isolates carrying *AvrLm1* (Rouxel et al., 2003) and in Australia, 'sylvestris' resistance was overcome within three years after commercial release (Sprague et al., 2006; van de Wouw et al., 2010). Kutcher et al. (2010) suggested that improved understanding of the genetic interactions between *B. napus* and *L. maculans* would help to deploy resistant cultivars in time and space to allow for durable resistance. Further knowledge gained on *B. napus - L. maculans* interactions could help alleviate selection pressure from deploying race specific resistance genes.

An approach that identifies resistance genes in a canola cultivar has been used to better steward with success reported in other canola production regions (Ansan-Melayah et al., 1998; Marcroft et al., 2012). In Australia, an intensive cultivar monitoring trial network is used to help predict which R genes may remain successful and which genes might have been overcome by virulent populations (Marcroft et al., 2012). This monitoring approach has been able to predict R gene failure fairly successfully, avoiding disasters from blackleg disease for farmers (Sprague et al., 2006; Van de Wouw et al., 2014). Being able to reuse R genes in areas where they were overcome previously has also been part of the success to the cultivar rotation system in Australia; it helps preserve advanced genetics and takes some pressure off for the development

of new cultivars with novel sources of resistance (Van de Wouw et al., 2014). Learning from the Australia experience, the Canadian canola industry developed its own R-gene labelling system in 2017 to support the cultivar resistance deployment by farmers.

The new resistance labelling scheme identifies the specific R genes deployed within a cultivar, allowing farmers to rotate cultivars based on major resistance gene groups (Van de Wouw & Howlett, 2019; Zhang & Fernando, 2018). Previously, if canola farmers were finding increased levels of blackleg within their R-rated cultivar, the recommendation was to rotate to a different cultivar (Kutcher et al., 2011). Now farmers have the option to pick a cultivar based on the R genes. The Canadian system places major resistance genes into groups based on their interactions with *L. maculans* avirulence genes (Zhang & Fernando, 2018), and canola plant breeders can now label known resistance genes into the groups A, B, C, D, E_1, E_2, F, G, J, K, L, N, P, or X. Each group represents R gene(s), while 'X' represents an unknown or unidentified R gene (CCC, 2020). Cultivars continue to be labelled with the resistant (R) or moderately resistance (MR) rating which rates cultivars based on blackleg severity in comparison to a susceptible check cultivar. Testing is available to farmers for predominant *L. maculans* races in their field through commercial labs in western Canada, providing the field-level information to facilitate better decision making on effective resistant cultivars.

The main objective of this study was to assess the concept of blackleg major resistance gene labelled cultivar deployment through monitoring the avirulence profile of *L. maculans* population and disease levels in commercial canola fields within the Canadian prairies. The study also intended to develop empirical data on changes of the *L. maculans* avirulence alleles in response to the development of specific resistance genes in these fields. Knowledge gained may help validate the effectiveness of deploying cultivars carrying specific major resistance genes in farmers' fields to manage blackleg disease in Canada. The use of commercial fields in this study provides insight into how farmers have been influencing the *L. maculans* population on their fields through the deployment of cultivars carrying different resistance genes.

3.3 Material and Methods

3.3.1 Comprehensive Survey Field Selection

Fields for this project were selected based on their crop history and blackleg major gene resistance group in the canola cultivar grown. Fields with high frequencies of canola were preferable having canola grown back-to-back or every second year. Crop rotation was not a factor in this study as all fields were chosen based on having canola two years prior to the crop surveyed. Fields used for the study in 2018 were seeded to canola in 2016, and fields in 2019 were seeded to canola in 2017. In 2018, ten fields were used from Manitoba, eight from Saskatchewan, and ten from Alberta for a combined total of 28 locations with 77 cultivar samples (Figure 3. 1). In 2019, 11 fields were used from Manitoba, nine from Saskatchewan, and five from Alberta for a combined total of 25 locations with 69 cultivar samples (Figure 3.1). Field sites were coded with a provincial designation of MB, SK, or AB to represent Manitoba, Saskatchewan, and Alberta, respectively and a number from 1 to 25 (example: MB5).

Only fields growing a cultivar with identified blackleg major genes were selected. Cultivars were identified by their blackleg major resistance gene groups which were established through the WCC/RRC (WCC/RRC, 2017). Within these fields, cultivar trials were established by life science companies to test cultivar performance. The trials were then surveyed for this project to compare blackleg resistance performance of canola cultivars carrying different resistance genes within the same field. Some trials did contain cultivars that carried unknown or undetermined major resistance genes (X).

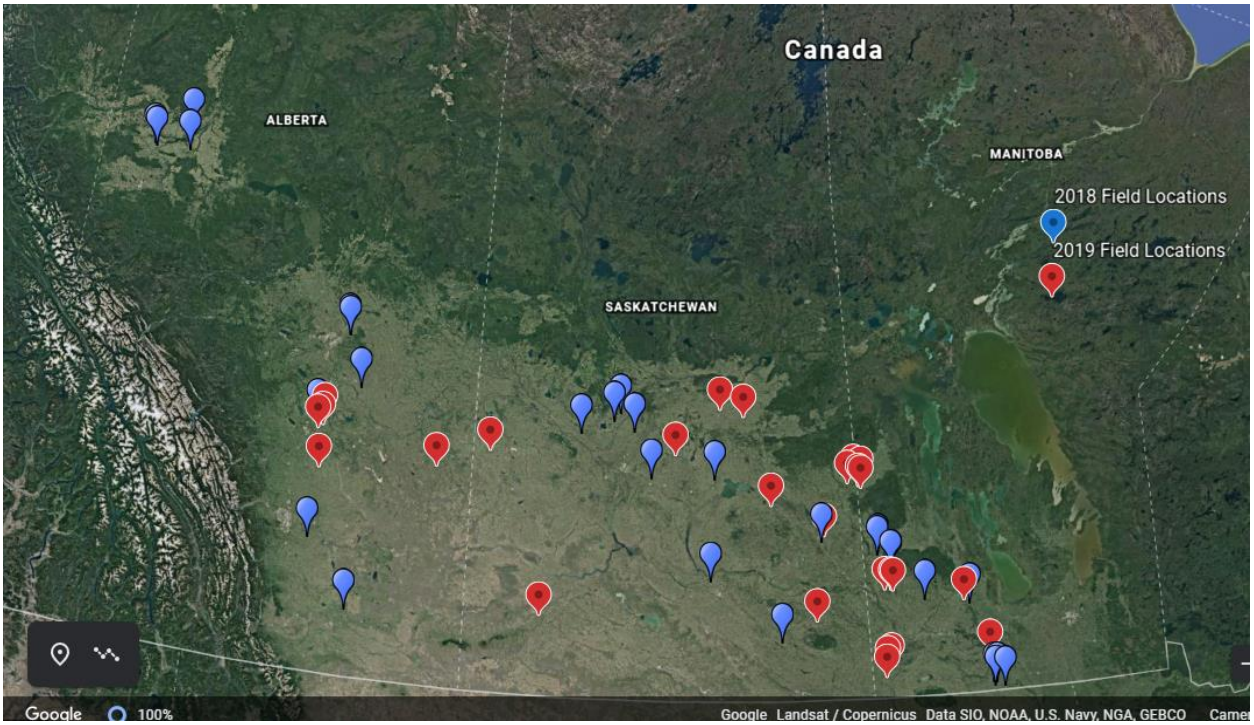

Figure 3. 1. Field locations across the prairies of Canada in Manitoba, Saskatchewan, and Alberta from 2018 and 2019. Blue pins locating 2018 surveyed fields and red pins 2019 surveyed fields.

3.3.2 Data Collection

Once fields were identified, overwintered canola residue was collected from each field in spring and *L. maculans* isolated to help determine the *Avr* profile within the field. Ten isolates were used to represent the pathogen population within a field. Isolates of *L. biglobosa* were common in the overwintered residues, complicating the efforts of getting enough *L. maculans* isolates. At canola plant growth stage of 60% seed colour change or prior to harvest, diseased canola plants were collected for the same purpose, with less interference from *L. biglobosa*, of determining the avirulence profile. The spring and fall sampling were intended to monitor changes in the *L. maculans* population influenced by different resistance genes deployed in the cultivars.

Just prior to swathing or at growth stage 5.2 (seed in lower pods green) to 5.3 (seeds in lower pods green-brown or green-yellow, molted), 50 plants were pulled from each cultivar (10 plants at 5 sites along a "w" pattern in the field) to assess the field for blackleg severity. Plants were rated for blackleg severity using a 0 – 5 disease severity rating scale assessing the proportion of blackened tissue at the cross-section of the crown (base of the plant stem) (West et al., 2001)

(**Figure 3. 2.** Blackleg disease severity rating scale based on basal cross-section infection (Canola Council of Canada, 2020).Figure 3. 2). Diseased stems were collected, and the pathogen isolated for analysis of *L. maculans* races in a field. Blackleg disease incidence, the percentage of symptomatic plants, was recorded for each cultivar assessed within the field. Over the two-year period the 150 cultivars were assessed across the prairie region of Canada. Supporting images of the methodology section are in Appendix B.

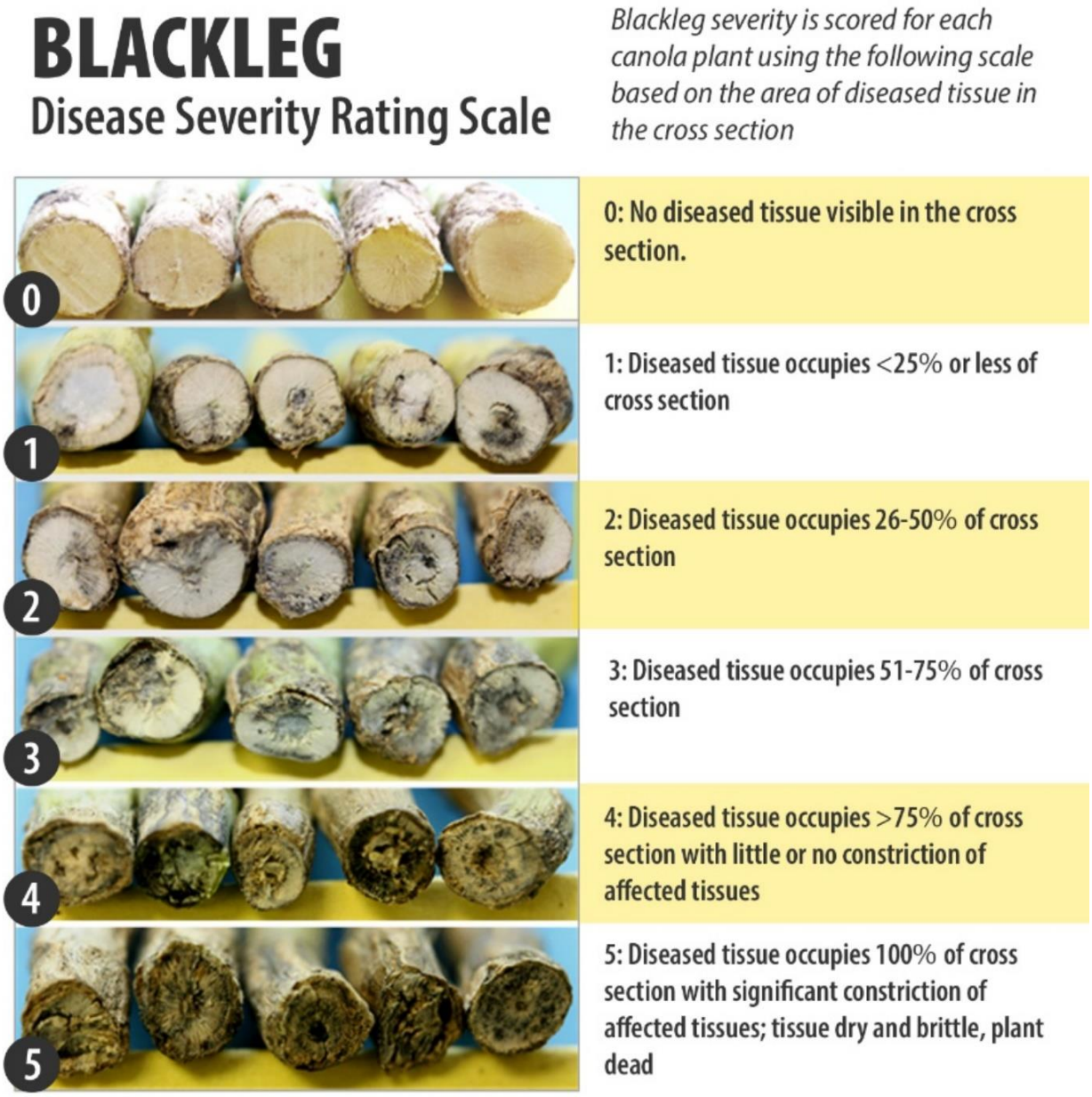

Figure 3. 2. Blackleg disease severity rating scale based on basal cross-section infection (Canola Council of Canada, 2020).

3.3.3 Fungal Isolation

The blackleg infected stubble pieces from the spring and fall field samples for each cultivar were cut into 2 mm pieces then surface sterilized in a 10 % bleach solution for two minutes. Once rinsed in sterile water the pieces were incubated on V8 juice agar [200 mL V8 juice (Campbells, Toronto, ON), 800 mL distilled water, 15 g Difco Agar Technical (BD Diagnostics Systems, Sparks, MD), 0.75 g calcium carbonate (Fisher Scientific, Fair Lawn, NJ), and 0.1 g streptomycin sulfate salt (Sigma-Aldrich, Saint Louis, MO)] amended with 10 mL of streptomycin sulfate. Two Petri dishes per stubble sample were placed on a light bench under cool white, fluorescent light at 22-24 ℃ for 4-7 days. Samples of 10 to 20 stems were plated per field sample to try to achieve the goal of 10 isolates per sample. Around five days post plating, a single pycnidia was picked from the conidial ooze using a fine wire under a dissecting microscope and plated onto a fresh V8 juice agar plate as a single spore isolate; this was duplicated to ensure isolates were gathered from each stem sample. The pycnidia samples grew for 5-12 days on a light bench under the same conditions as the previous step.

3.3.4 Preparation of Fungal Inoculum and DNA Samples

Pycnidiospores were harvested by flooding *L. maculans* and *L. biglobosa* cultures on the agar plate with sterile distilled water and scraping with a sterilized metal rod to dislodge spores. Spore suspensions were pipetted into two 50 mL sterile centrifuge tubes for DNA extraction (Fisher Scientific, Pittsburgh, PA). Small sterile filter paper disks were placed into the remaining mixture of hyphae, pycnidia, and spores still on the agar plates to capture spores to use for plant inoculation. The soaked disks were then dried and placed into 50 mL sterile centrifuge tubes then stored in the freezer at -20 ℃.

3.3.5 DNA Extraction and *Lm*/*Lb* Differentiation

The DNA samples extracted from fungal isolates were used to differentiate between *L. maculans* or *L. biglobosa*. When *L. maculans* was confirmed, PCR was used to determine the presence or absence of targeted avirulence genes. Genomic DNA of each isolate was extracted by the method described by Liban et al., 2016. A mixture of fungal pycnidia, conidia, and hyphae harvested from 8–12 day old cultures was kept in 1.5 mL micro-centrifuge tubes at -20 ℃, and DNA was extracted by using a modified procedure developed by Lee & Taylor (1990). Samples were

mixed with a lysis buffer (CTAB extraction buffer), lysed with mechanical beads at 5000 rpm for 30 s, incubated at 65 °C for 0.5 hours, extracted with phenol:chloroform:isoamyl alcohol (25:24:1), and precipitated with 95 % ethanol by adding 5 M NaCl. The pellet was washed with 70% ethanol twice. Following the final centrifugation, the DNA pellet was dissolved in 100 µL sterile distilled water. To determine if an isolate was *L. maculans* or *L. biglobosa* ITS-F (PN3): CCGTTGGTGAACCAGCGGAGGGATC and ITS-R (PN10): TCCGCTTATTGATATGCTTAAG primers were used (Mendes-Pereira et al., 2003). The primer set generates 555 to 560 bp fragment for *L. maculans* and a 580 to 588 bp fragment for *L. biglobosa*. With a 20 bp band difference between the two species, the agarose gel ran for 1 hour under 110V electrophoresis.

3.3.6 PCR Genotyping for Avirulence Alleles

Multiplex PCR developed by Cozijnsen & Howlett (2003) was used for mating types and avirulence allele characterization of *L. maculans* isolates. DNA samples from *L. maculans* isolates were used for *AvrLm1, AvrLm2, AvrLm3, AvrLm4-7, AvrLm6, AvrLmJ1/5/9, AvrLm10,* and *AvrLm11* using the appropriate primers (Table 3. 1). For *AvrLm4-7* or *AvrLm7* allele was identified by tetra primer ARMS-PCR (Zou et al., 2018). A marker and methods described by Liu et al. (2020) was used for *AvrLm5/9* to identify *AvrLm5avrLm9, AvrLm5AvrLm9,* and *avrLm5AvrLm9.* All other avirulence genes were identified by the presence or absence of their alleles. The PCR reaction included the following reagents: 100-200 ng DNA, 0.25 µL of each primer (10 pmol/ µL), 5 µL PCR buffer, 5 µL dNTPs, and 0.5 µL Taq polymerase, filled with water to total volume as 50 µL. PCR was performed with the following conditions: 3 min at 95 °C; 30 cycles of 30 s at 95 °C, 30 s at 50 °C, 1.5 min at 72 °C; and lastly, 5 min at 72 °C for extension. The PCR product was visualized after running in 1.5% agarose gel electrophoresis under the condition specified above.

Table 3. 1. Primer name, sequence, product size, and source of avirulence allele primers used in PCR analysis.

Primer name	Sequence (5'-3')	Product size (bp)	Reference
AvrLm1-F	CTATTTAGGCTAAGCGTATTCATAAG	1,123	Gout et al., 2006
AvrLm1-R	GCGCTGTAGGCTTCATTGTAC		
AvrLm2-F	CGTCATCAATGCGTTCGG	258	Ghanbarnia et al., 2015
AvrLm2-R	CTGGATCGTTTGCATGGA		
AvrLm3-F	GAGAGAACTAGTCTGTTAAATGCCTGCTGT	1,357	Plissonneau et al., 2016
AvrLm3-R	GAGAGACTCGAGCGCGCTTATGTTAGAATC		
AvrLm4-7-F	TATCGCATACCAAACATTAGGC	1,433	Parlange et al., 2009
AvrLm4-7-R	GATGGATCAACCGCTAACAA		
AvrLmJ1/5/9-F	ACAACCACTCTTCTTCACAGT	479	Van de Wouw et al., 2014
AvrLmJ1/5/9-R	TGGTTTGGGTAAAGTTGTCCT		
AvrLm6-F	TCAATTTGTCTGTTCAAGTTATGGA	774	Fudal et al., 2007
AvrLm6-R	CCAGTTTTGAACCGTAGTGGTAGCA		
AvrLm10A-F	TCAAAAAGCGGCCTTCTC	669	Petit-Houdenot et al., 2019
AvrLm10A-R	GAAGTTAAGAGAGCAGGTGAGG		
AvrLm10B-F	GCGACAGGAATCACAACCTT	288	
AvrLm10B-R	GCCTACGCCAATCTCCAATA		
AvrLm11-F	TGCGTTTCTTGCTTCCTATATTT	359	Balesdent et al., 2013
AvrLm11-R	CAAGTTGGATCTTTCTCATTCG		

3.3.7 Avirulence phenotyping through Cotyledon Inoculation Tests in Greenhouse

Leptosphaeria maculans isolates were used to inoculate a set of differential *Brassica* lines carrying known major resistance genes to observe the phenotypic reaction and identify the corresponding avirulence genes carried in the isolates (Table 3. 2). The presence of avirulence genes in *L. maculans* isolates was determined based on symptoms on cotyledons after inoculating. Inoculum concentration was adjusted to 2×10^7 spores mL^{-1} from the harvested cultures derived from single pycnidiospores cultured on V8 medium plate. Differential lines were seeded in Sunshine growth mix and put in a growth chamber at nighttime temperature of 16 ℃ and daytime temperature of 21 ℃, with 16-hour photoperiod (Rashid, Hausner, et al., 2018). For the inoculations, 10 µL of spore suspension (2×10^7spores mL^{-1}) was deposited on each lobe of 7-day-old seedlings which were wounded with a modified tweezer. Inoculated pots of cotyledon plants were fertilized on the second day after inoculation. Five- and ten-days post inoculation, true leaves were trimmed to delay the cotyledon senescence. Six plants were used for each line-isolate interaction, and each lobe of cotyledon was inoculated (four per plant). Westar was used as control to test the virulence of isolates as it is a susceptible cultivar to blackleg. Symptoms on cotyledons were scored 14 days post inoculation using a disease rating

scale of 0 – 9 (“0” indicating no infection, “9” indicating a large leaf lesion) based on lesion size, chlorosis or necrosis, and presence of pycnidia (Kutcher et al., 2007). A mean of 6.1 to 9.0 was considered a susceptible (S) reaction, 4.6 to 6.0 an intermediate (I) reaction, and less than or equal to 4.5 a resistant (R) reaction (Zhang et al., 2016). If an isolate was characterized to carry *AvrLm4-7* or *AvrLm7*, phenotyping for *AvrLm3* and *AvrLm9* would not be carried out for the isolate due to the “masking effect” (Ghanbarnia et al., 2018; Plissonneau et al., 2016). If an isolate did not carry *AvrLm4-7* or *AvrLm7* it was tested for the interaction on another two cultivars carrying *Rlm3* (01-22-2-1) and *Rlm9* (Goéland). Each isolate-host interaction was used to deduce the avirulence allele carried by the isolate.

Table 3. 2. Canola cultivars with corresponding resistance genotype used as differentials to identify avirulence genotypes of *Leptosphaeria maculans* isolates. Cultivars used from Agriculture and Agri-Food Canada Saskatoon are identified as AAFC-SK.

Cultivar	Resistance genotype	Reference
01-23-2-1	*Rlm7*	Dilmaghani et al., 2009
Surpass 400	*Rlm1, RlmS*	Van De Wouw et al., 2009
1065	*LepR1*	Kutcher et al., 2010
1135	*LepR2*	Kutcher et al., 2010
Jet Neuf	*Rlm4*	Gout et al., 2006
Westar	*No R gene*	Balesdent et al., 2002
TopasRlm1	*Rlm1*	AAFC-SK
TopasRlm2	*Rlm2*	AAFC-SK
Forge (*B. juncea*)	*Rlm6*	Rashid et al., 2018
02-22-2-1	*Rlm3*	Gout et al., 2006
Goéland	*Rlm9*	Balesdent et al., 2006

3.3.8 Statistical Analysis

Data recording, preliminary analysis, and the preparation of graphs was done in Microsoft Excel for Microsoft 365. Analysis of variance (ANOVA) was used to compare means as an initial statistical analysis tool using the PROC MIXED procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) (Littell, 2006). Disease incidence (DI) was transformed using an arcsine root square transformation and a log transformation was performed for disease severity (DS) (Ghanbarnia et al., 2011; Rashid et al., 2020) to improve the normality of data distribution. When ANOVA was significant ($P<0.05$) for DI and DS among resistance gene groups, the means were separated using Tukey-Kramer test. The Tukey-Kramer test with a probability level for significance of

0.05 was used due to unequal sample sizes (Day & Quinn, 1989). Resistance gene group, year, and province were considered fixed effects.

Diversity and evenness of the *L. maculans* population were calculated using Simpson's index of diversity (IOD) and index of evenness (IOE), respectively (Simpson, 1949). The IOD is calculated by weighing the number of *L. maculans* races relative to the total number of *L. maculans* races tested. An index of 1 is considered a random or diverse population, whereas an index of 0 would consist of a single race. The IOE is a measure of the relative abundance of different *L. maculans* races in the population, whereas an index of 1 indicates even representation of all races and an index of 0 indicates unequal representation of races.

3.4 Results

3.4.1 Disease Incidence and Severity by Major Resistance Gene Group

A total of 146 cultivars over two years were surveyed for disease incidence and severity. The mean disease incidence, severity, and severity of infected plants was summarized by cultivar's resistance gene group (Table 3. 3). The resistance gene groups are based on the Canadian blackleg major resistance gene labelling system that was introduced in 2017 (Canola Council of Canada, 2020). Four blackleg major resistance genes were commercially available during the study, which resulted in six different resistance gene group combinations; AC (*LepR3, Rlm3*), ACG (*LepR3, Rlm3, RlmS*), C (*Rlm3*), CE_1 (*Rlm3, Rlm4*), CG (*LepR3, RlmS*), and X (unknown or not commercially identified major resistance gene). The use of X in this study meant that the cultivar did not have its major resistance genes identified or labelled.

Table 3. 3. Blackleg disease incidence, severity, and severity of only infected plants from field sites in Manitoba, Saskatchewan, and Alberta in 2018 and 2019 based on cultivars' major resistance gene groups (RG).

Resistance Gene Group (RG)	Incidence	Severity[a]	Severity of Infected Only
AC	0.57	0.96	1.65
ACG	0.47	0.79	1.36
C	0.36	0.57	1.36
CE_1	0.24	0.33	1.19
CG	0.25	0.31	1.17
X	0.43	0.62	1.30

[a] Blackleg disease severity rated on a 0 – 5 severity rating scale (West et al., 2001).

Blackleg disease incidence and severity were both significantly different among resistance gene groups ($P < 0.05$; Table 3. 4). Interaction between resistance gene groups between the years were found to not have a difference on disease incidence or severity. With no difference between the years in this study, it is an indication that disease pressure was consistent between the two growing seasons. Crop rotation was not a factor in this study as all fields were chosen based on having canola two years prior to the crop surveyed. Fields used for the study in 2018 were seeded to canola in 2016, and fields in 2019 were seeded to canola in 2017. The significant differences in disease incidence and severity between resistance gene groups demonstrates the value of strategic deployment of resistance genes.

Table 3. 4. Type III ANOVA test for fixed effects for blackleg disease incidence and severity for field sites from Manitoba, Saskatchewan, and Alberta in 2018 and 2019 based on major resistance gene group (RG).

Variable	Source of Variation	df	F	Pr > F
Disease Incidence	RG	5	4.26	0.0013
	Year	1	0.02	0.8848
	RG*Year	5	0.68	0.6414
Disease Severity	RG	5	4.09	0.0017
	Year	1	0.04	0.849
	RG*Year	5	0.74	0.5955

The only significant difference for disease incidence was between resistance gene group CE_1 and the unknown group X ($P = 0.0062$). There were significant differences between cultivars in resistance gene group AC compared to resistance gene group CE_1 cultivars ($P = 0.0326$).

Additionally, among cultivars in the resistance gene group ACG compared to resistance gene group CE_1 cultivars (P= 0.0459) and between cultivars in the resistance gene group CE_1 compared to cultivars in unknown group X (P = 0.0306). These differences are from all sites surveyed over the two years of this study; with significant differences observed within a field site (example shown in

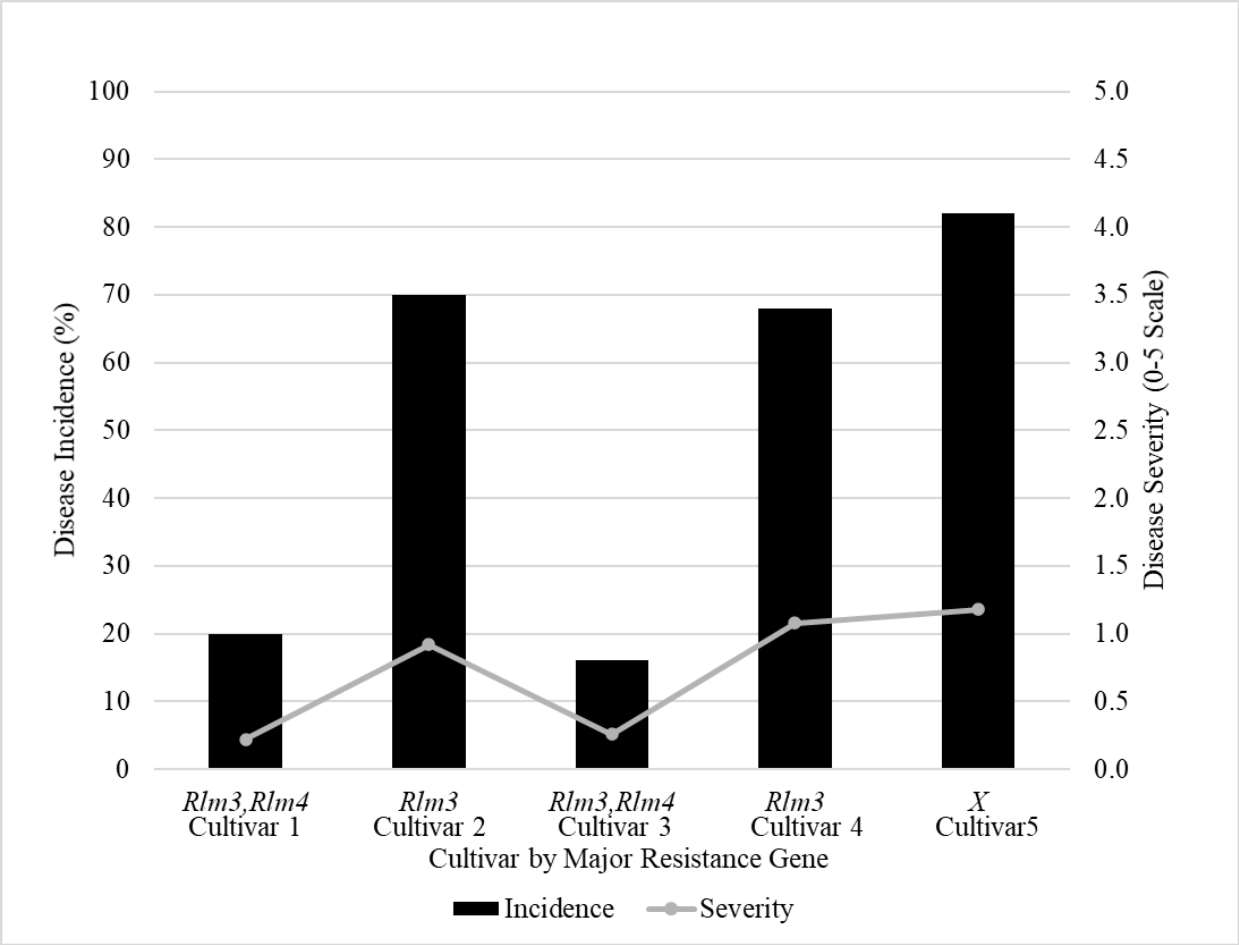

Figure **3. 3**). The cultivar comparison experiments were where differences can be seen for disease incidence and severity between resistance gene groups. A field site in 2018 from Saskatchewan (SK1) shows five cultivars grown in the field identified by their major resistance gene profile (

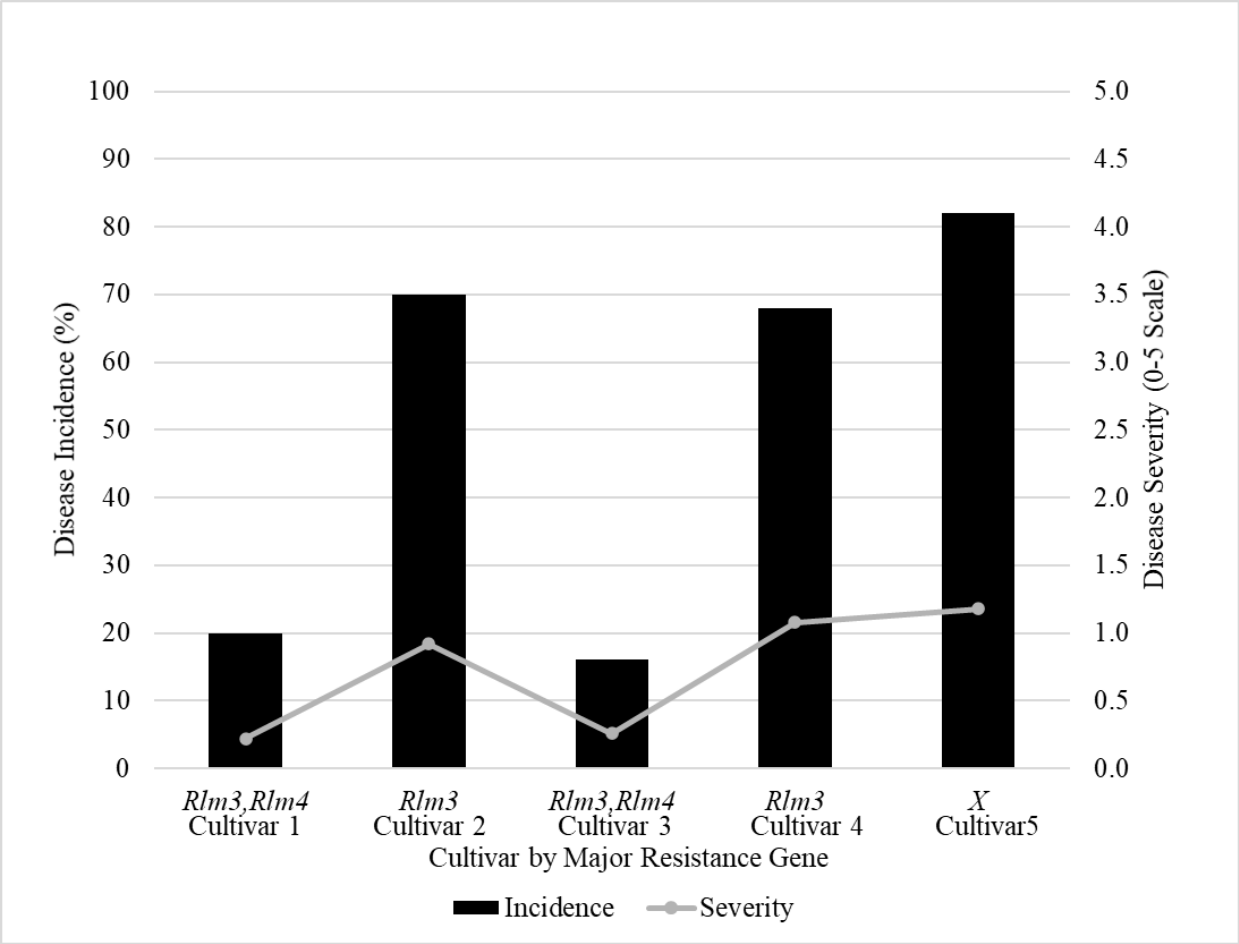

Figure **3. 3**). Two cultivars were labelled with resistance gene group C (*Rlm3*), two cultivars with resistance gene group CE_1 (*Rlm3, Rlm4*), and one cultivar as 'X' as its major resistance gene was not labelled for a total of five cultivars. The mean disease incidence was 51 % lower in the CE_1 resistance gene group cultivars compared to the C resistance gene group cultivars. The mean disease severity rating was 0.24 in the resistance gene group CE_1 cultivars and a rating of 1 for the resistance gene group C cultivars. The difference between groups is the addition of *Rlm4* in the CE_1 resistance gene group. Low disease severity ratings of under 1.18 in both the resistance groups C and CE_1 indicates that quantitative resistance is contributing by slowing the rate of infection, or that the environmental conditions were not conclusive for further disease development (Appendix C).

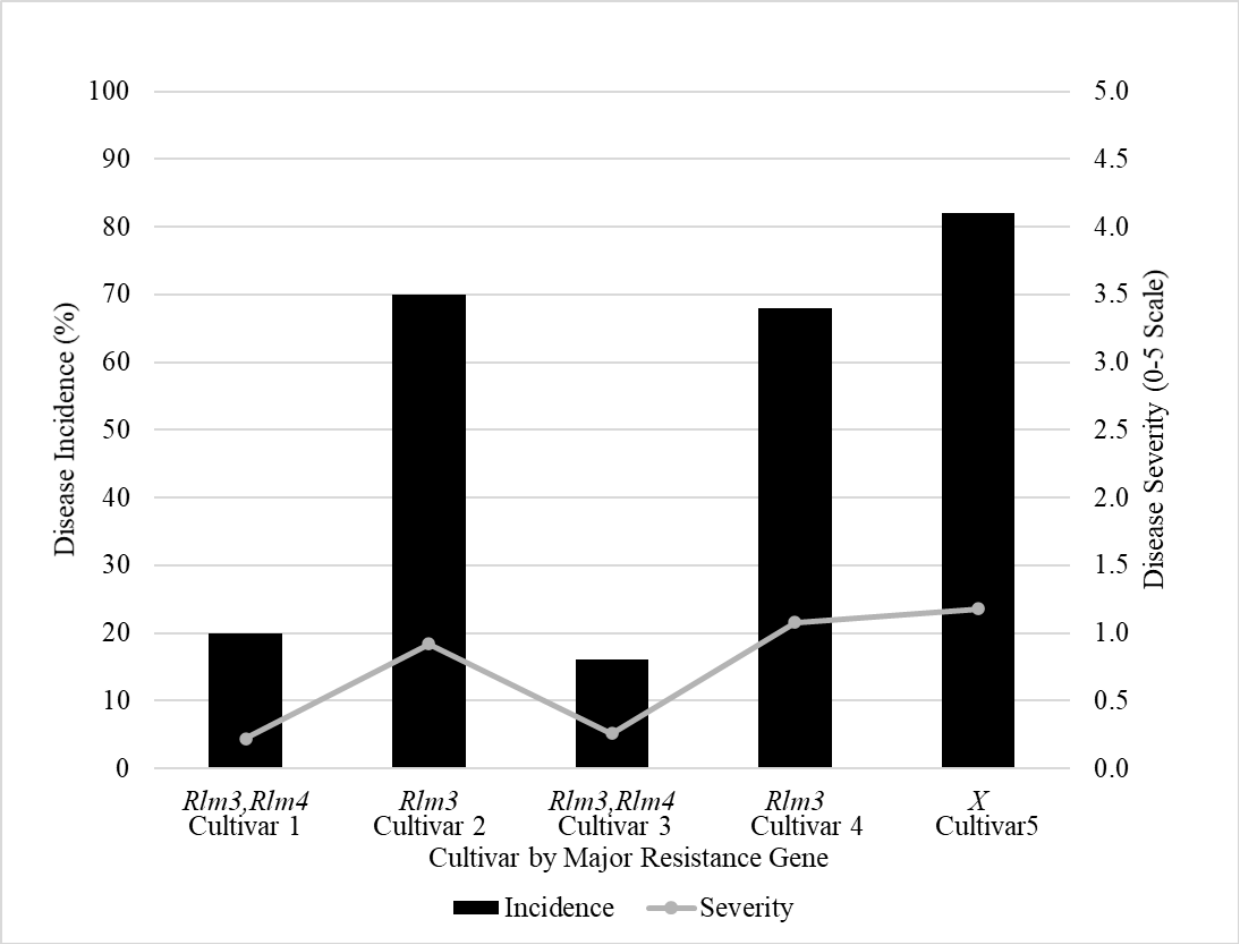

Figure 3. 3. The blackleg disease incidence and severity for field site SK1 from 2018 in Saskatchewan. Field site had five cultivars grown to compare the effectiveness of different major resistance genes.

Isolates collected in the spring prior to the five cultivars being grown identifies what the *L. maculans* avirulence profile was in the field. Out of the 22 isolates collected from SK1, 73% had the *L. maculans* race profile of *AvrLm2-4-5-6-7-11*. The remaining 27 % was made up of four *L. maculans* races, *AvrLm2-5-6-7-11*, *AvrLm2-5-6-7-11-LepR1*, *AvrLm2-4-5-6-7-11-LepR1*, and *AvrLm2-4-5-6-7-11-(s)*. Figure 3. 4 depicts the frequency of *Avr* genes in *L. maculans* population. *AvrLm3* and *AvrLm9* are masked by the presence of *AvrLm7* in the *L. maculans* avirulence gene profile (Ghanbarnia et al., 2018; Plissonneau et al., 2016). This would explain the greater disease incidence in the C resistance gene group cultivars as they rely on the use of major resistance gene *Rlm3*. The frequency of *AvrLm4* in the population was 82 %. The use of

the major resistance gene *Rlm4* in the two cultivars to *AvrLm4* in the *L. maculans* population inferred the defense response within the plants to initiate disease resistance.

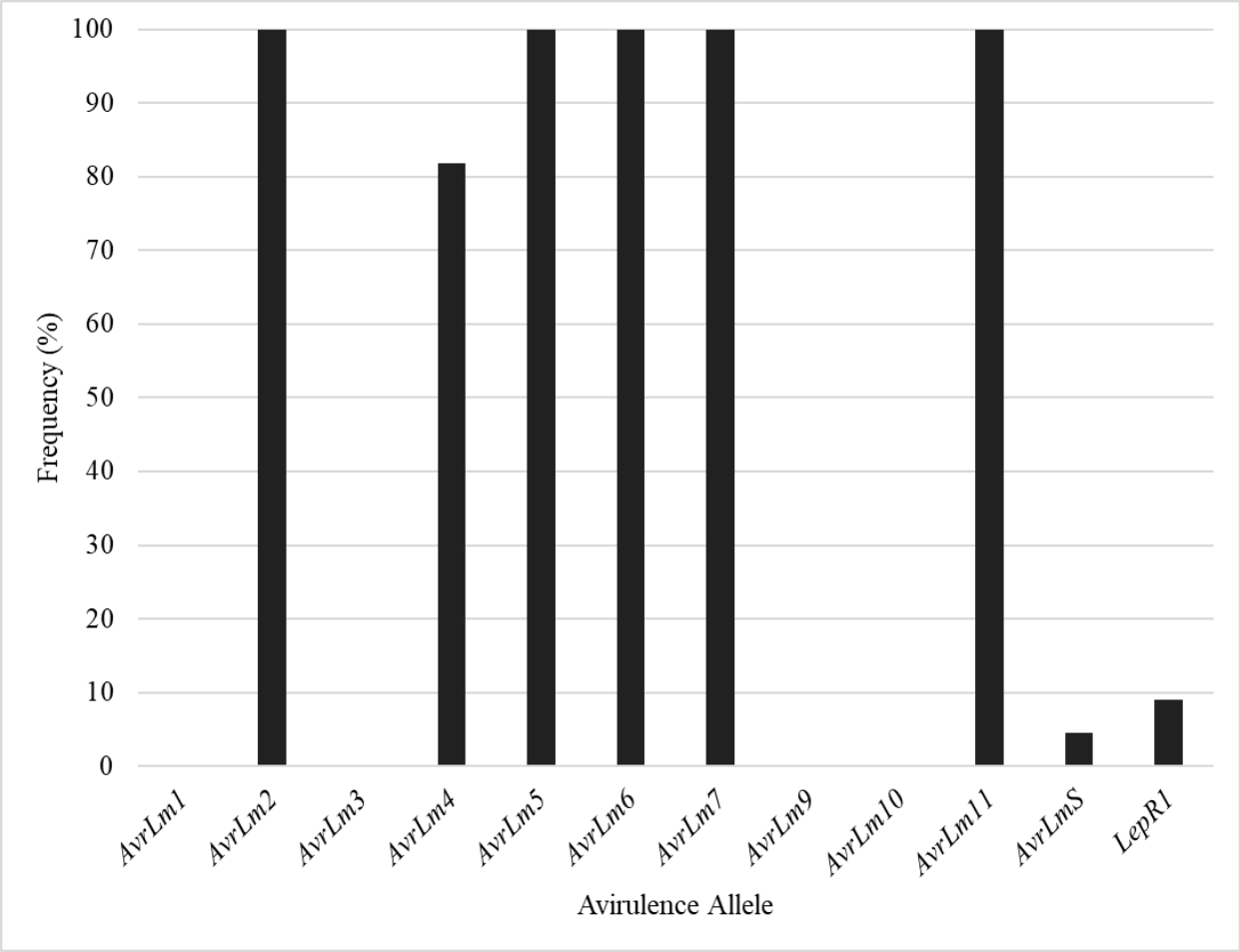

Figure 3. 4. The frequency of avirulence alleles in the *Leptosphaeria maculans* population from 22 isolates collected in the spring of 2018, from 2016 canola residue, prior to seeding Saskatchewan field SK1 in 2018.

3.4.2 Disease Incidence and Severity by Geographic Environment

The mean blackleg disease incidence from the 53 locations across the prairies in 2018 and 2019 was 39 %, 27 %, and 49 % for Alberta, Saskatchewan, and Manitoba, respectively. This is greater in comparison to provincial blackleg disease survey data because fields were chosen for this study based on high canola frequency fields which would increase disease pressure within the field (Canadian Plant Disease Survey, 2020). Sampling error must be considered, as provincial disease surveys are completed by many surveyors. The mean blackleg disease severity was 0.61, 0.34, and 0.81 for Alberta, Saskatchewan, and Manitoba, respectively. Disease severity

for all plants surveyed typically falls below a disease severity rating of 1 on the 0 – 5 blackleg disease rating scale.

Six resistance gene group combinations were surveyed in Alberta and Manitoba, and only five surveyed in Saskatchewan. Disease incidence and severity by resistance gene group was only significantly different in Saskatchewan ($P < 0.05$; Table 3. 5). Disease incidence differences between years was only reported in Alberta but is due to dry conditions experienced in 2019, which led to low disease establishment. Disease severity differences were seen in Manitoba for the resistance gene groups compared between years.

Table 3. 5. Type III test for fixed effects ANOVA for blackleg disease incidence and severity for field sites based on resistance gene group (RG) from Manitoba (MB), Saskatchewan (SK), and Alberta (AB) in 2018 and 2019.

Variable	Province	df	F	Pr > F
Disease Incidence	MB	5	0.54	0.7435
	SK	4	3.59	0.0111
	AB	5	1.39	0.2510
Disease Severity	MB	5	0.81	0.5499
	SK	4	2.91	0.0291
	AB	5	1.23	0.3144

3.4.3 Deployment of Single Gene Cultivars vs. Multiple Gene Cultivars

Single gene cultivars were compared against all multiple gene cultivar combinations in this study from all the sites in 2018 and 2019. The cultivars labelled with an X were omitted to complete the analysis as they may be composed of varying number of genes and combinations. Resistance gene group C was the only single gene resistance group, while the multiple gene cultivars consisted of four resistance gene group combinations: AC, CG, ACG, and CE1. There was no significant difference between single gene and multiple gene cultivars ($P < 0.05$; Table 3. 6). There was also no relationship between year or from the interaction of the multiple gene cultivars with the year. This highlights the importance of matching a resistance gene in the cultivar to the dominant avirulence gene in the *L. maculans* population.

Only when you analyze the data by cultivars consisting of two resistance genes versus cultivars consisting of three resistance genes is there a significant difference in disease severity ($P = 0.045$; $P < 0.05$). Differences are from the comparison of cultivars in the CE_1 (*Rlm3, Rlm4*) classification and in the ACG (*LepR2, Rlm3, RlmS*) classification where the differences are

found as *AvrLm4* is more frequent in the *L. maculans* population than *AvrLepR2* and *AvrLmS*. The result is higher disease severity in the three gene cultivars as they would only match up to a very small population of *L. maculans* races found across the prairies.

Table 3. 6. Type III test for fixed effects ANOVA for blackleg disease incidence and severity for all field sites comparing single resistance gene cultivars to multiple resistance gene cultivars in 2018 and 2019.

Variable	Source of Variation	df	F	Pr > F
Disease Incidence	Multiple Gene	1	1.01	0.316
	Year	1	2.15	0.146
	Multiple Gene*Year	1	1.17	0.283
Disease Severity	Multiple Gene	1	1.06	0.305
	Year	1	0.16	0.690
	Multiple Gene *Year	1	1	0.319

3.4.4 Frequency of *L. maculans* Avirulence Alleles in Alberta

A total of 245 isolates were characterized for the presence of *AvrLm1, Avlm2, AvrLm3, AvrLm4, AvrLm5, AvrLm6, AvrLm7, AvrLm9,* and *AvrLm11*. Both spring and fall collected isolates were used to determine the *L. maculans* races. Figure 3. 6 illustrates the frequency and number of isolates for each *L. maculans* race found in Alberta. The isolate collection consisted of 38 *L. biglobosa* species. There were 19 unique *L. maculans* races found over the two-year study in Alberta. The top two *L. maculans* races only differ by the presence or absence of *AvrLm2*. The most common *L. maculans* race in Alberta was *AvrLm5-6-7-11* at 40 %, followed by *AvrLm2-5-6-7-11* at 38 % and *AvrLm5-6-7* at 6 %. Avirulence alleles, *AvrLm5, AvrLm6,* and *AvrLm7,* were found in over 90 % of the *L. maculans* isolates collected. Avirulence race profiles are shown phenotypically with the removal of *AvrLm3* and *AvrLm9* due to the 'hide and seek' interaction with the presence of either *AvrLm4-7* or *AvrLm7* (Ghanbarnia et al., 2018; Plissonneau et al., 2016).

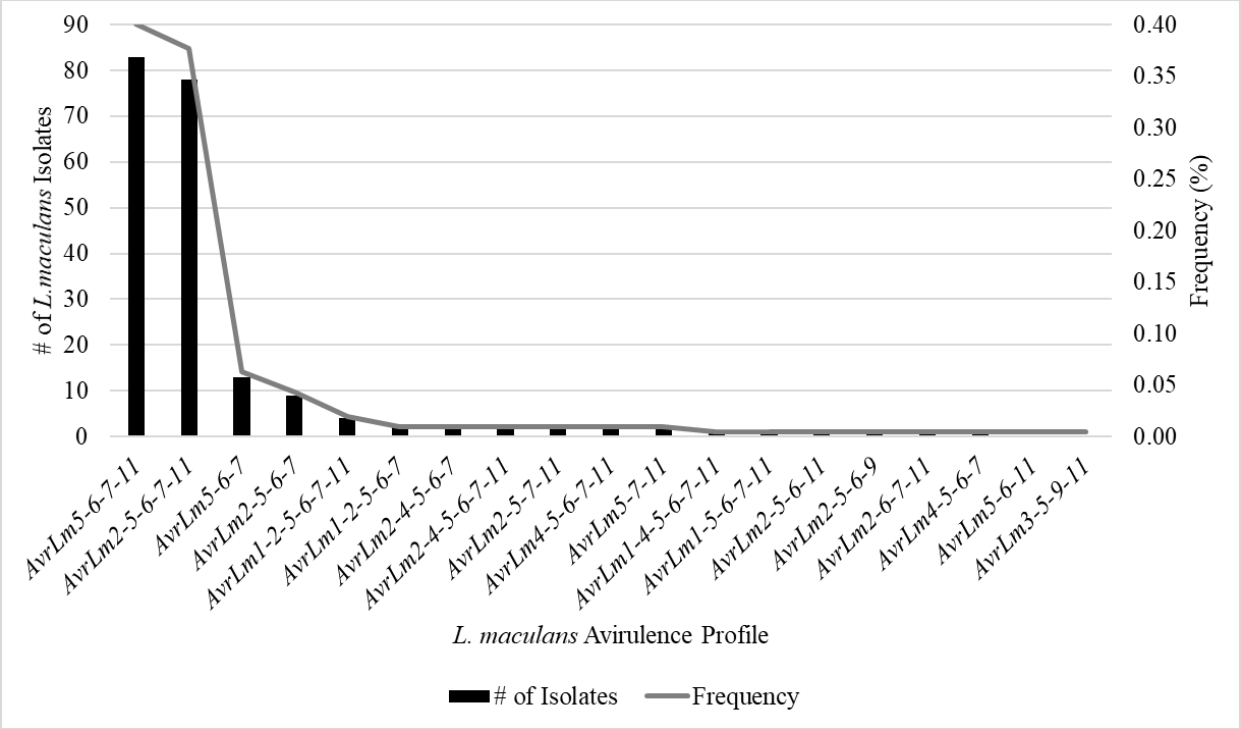

Figure 3. 5. Frequency of 19 *Leptosphaeria maculans* races based on the 14 avirulence alleles characterized in this study. A total of 207 isolates were examined on the nine avirulence alleles, 38 of the isolates were *L. biglobosa.*

3.4.5 Frequency of *L. maculans* Avirulence Alleles in Manitoba

A total of 359 isolates were characterized for the presence of *AvrLm1, Avlm2, AvrLm3, AvrLm4, AvrLm5, AvrLm6, AvrLm7, AvrLm9, AvrLm10, AvrLm11, AvrLepR1, AvrLepR2, AvrLepR3,* and *RlmS*. Both spring and fall collected isolates were used to determine the *L. maculans* races. Figure 3. 6 illustrates the frequency and number of isolates for each *L. maculans* race found in Manitoba. The isolate collection consisted of 105 *L. biglobosa* species. There were 35 unique *L. maculans* races found over the two-year study in Manitoba. The top two *L. maculans* races only differ by the presence or absence of *AvrLm4*. The most common *L. maculans* race in Manitoba was *AvrLm2-4-5-6-7-10-11* at 38 %, followed by *AvrLm2-5-6-7-10-11* at 11 % and *AvrLm2-4-5-6-7-10-11-LepR1* at 6 %. These three races are the most frequent for both the spring and fall collected samples. The next top races from the spring collected isolates were *AvrLm2-4-5-6-7-10-11-(s)* and *AvrLm4-5-6-7-10-11-(s).* The following top races from the fall collected isolates were *AvrLm2-4-5-6-7-10-11-LepR3-(s)* and *AvrLm4-5-6-7-10-11-LepR3-(s).*

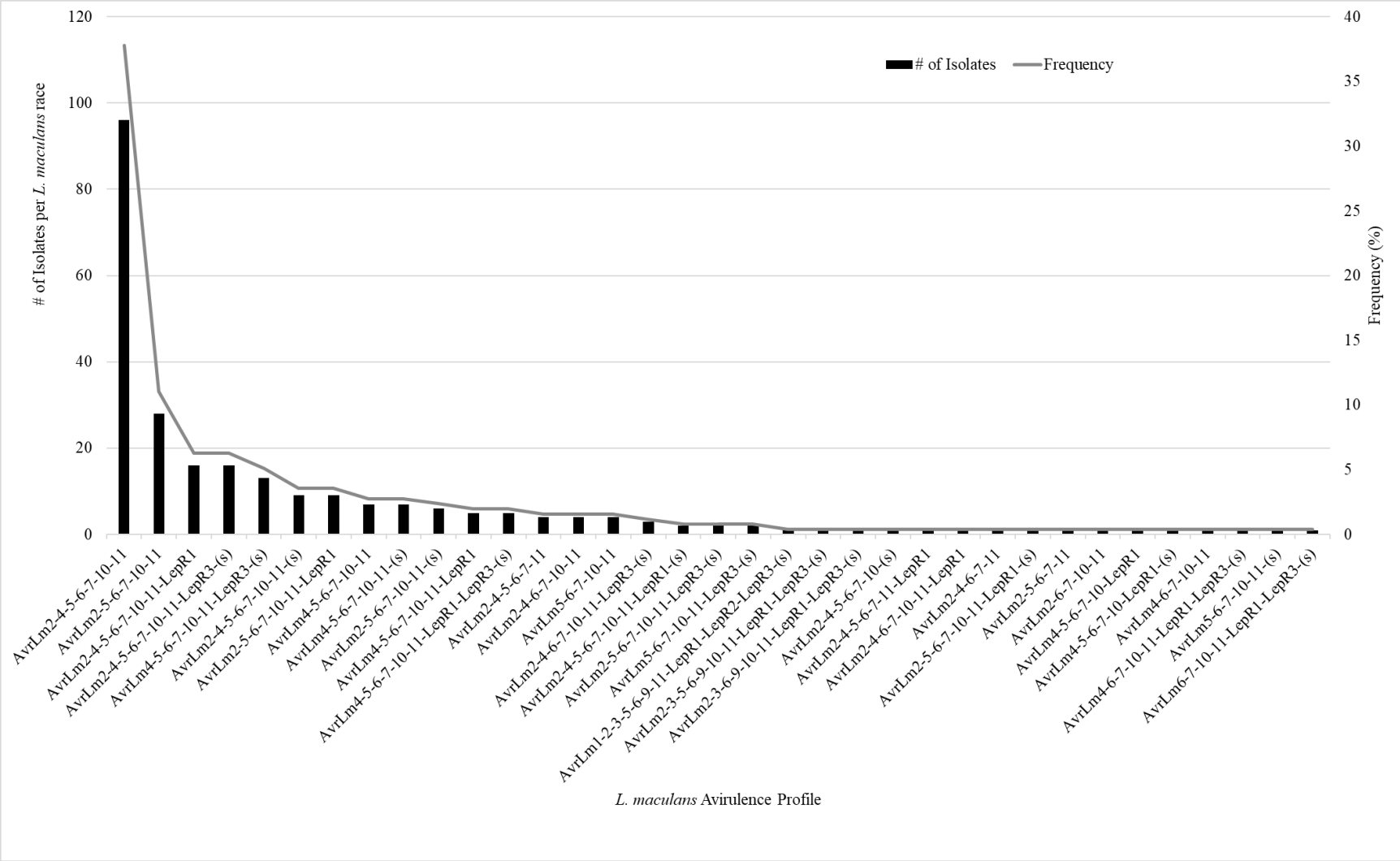

Figure 3. 6. Frequency of 35 *Leptosphaeria maculans* races based on the 14 avirulence alleles characterized in this study. A total of 359 isolates were examined the avirulence alleles, 105 of the isolates were *L. biglobosa.*

The *AvrLepR3* gene was only found in fall collected isolates, it was not present in any of the spring collected isolates. The *L. maculans* population evaluated by terms of complexity is the number of avirulence genes carried per isolate. Figure 3. 7 depicts the *L. maculans* race complexity by presenting the frequency for isolates collected in Manitoba by the number of avirulence alleles present. Of the 254 *L. maculans* isolates collected 51% had seven avirulence genes, 18% had six, and 18% had eight. There were no isolates containing one, two, three, five or 12 avirulence alleles. Avirulence race profiles are shown after the removal of *AvrLm3* and *AvrLm9* due to the 'hide and seek' interaction with the presence of either *AvrLm4-7* or *AvrLm7* (Ghanbarnia et al., 2018; Plissonneau et al., 2016). The *L. maculans* complexity provides many options to match resistance genes to avirulence genes within the population.

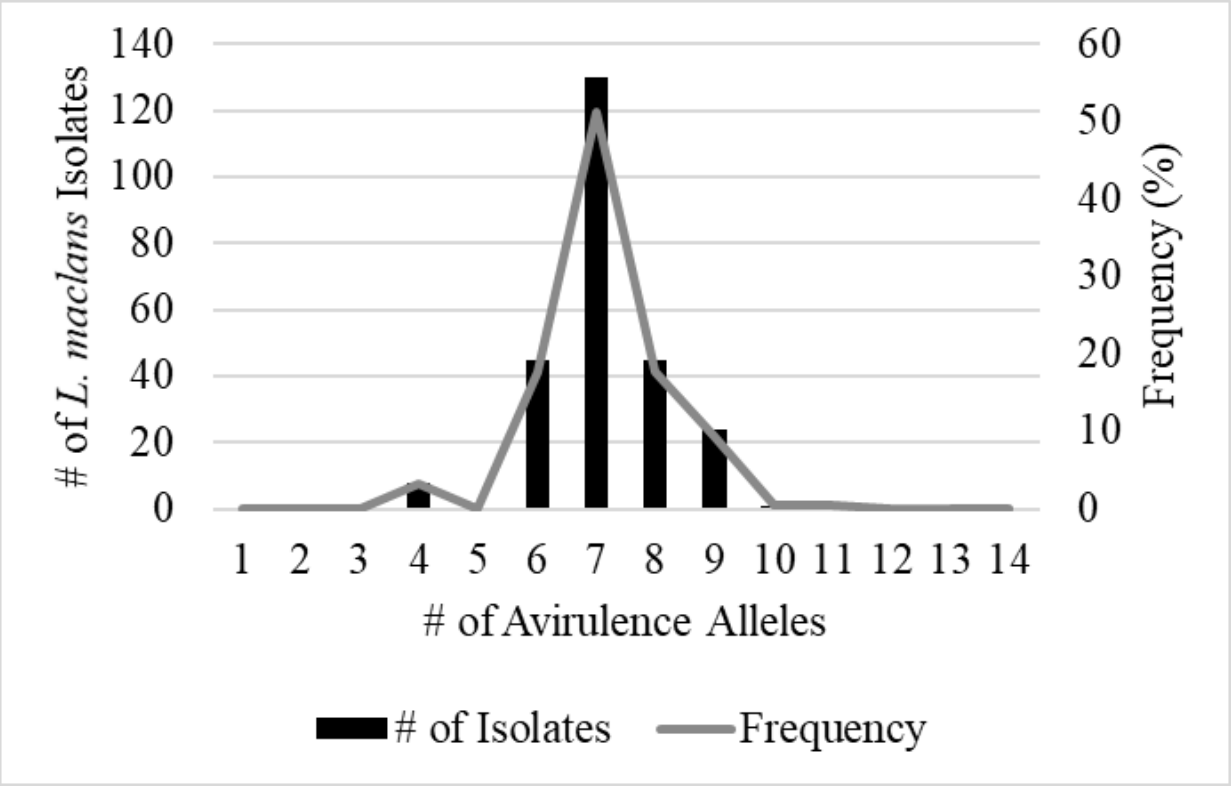

Figure 3. 7. *Leptosphaeria maculans* race complexity based on a total of 254 isolates from Manitoba in 2018-19 assessed at 14 avirulence alleles.

AvrLm6 and *AvrLm11* were found in all 254 *L. maculans* isolates collected in Manitoba in 2018 and 2019 (Figure 3.8). Of the 92 *L. maculans* isolates collected in the spring *AvrLm5, AvrLm6, AvrLm7, AvrLm10,* and *AvrLm11* were found in over 98% of the isolates. Similar results were seen in the fall isolate collection except for lower levels of *AvrLm5*. Three isolates collected in

the fall did not have *AvrLm7* so *AvrLm3* and *AvrLm9* were unmasked. Low frequencies of *AvrLm1*, *AvrLmS*, *LepR1*, *LepR2*, and *LepR3* were detected.

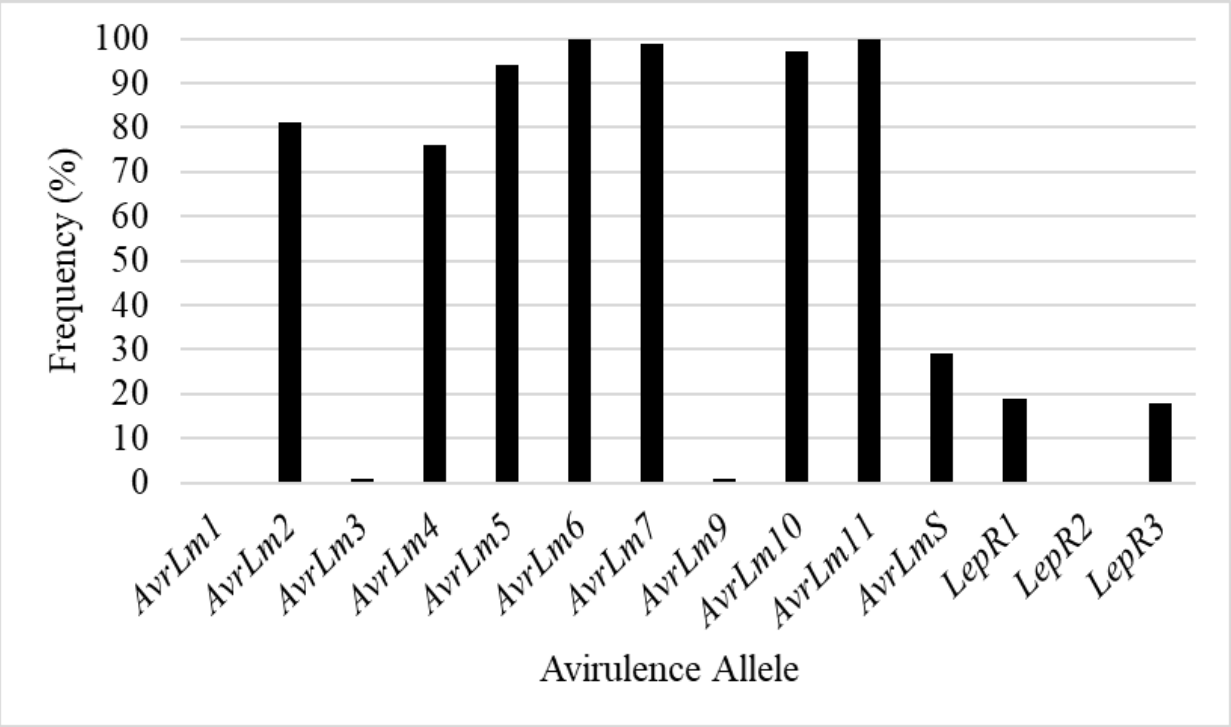

Figure 3. 8. Frequency of avirulence alleles in 254 *Leptosphaeria maculans* isolates collected in Manitoba from 2018-2019; assessed for 14 avirulence alleles from spring and fall isolate collections.

3.4.6 Diversity and Evenness of the *L. maculans* population in Manitoba

The Simpson index of diversity (IOD) weighs the number of races relative to the total number of samples, and was calculated to be 0.85, where an index of 1 is a random or diverse population and an index of 0 is 1 race (Simpson, 1949). The Simpson's index of diversity indicated that the *L. maculans* population appears genetically diverse (Table 3. 7). The Simpson index of evenness (IOE) measures the relative abundance of different races and was calculated to be 0.02, indicating low evenness in the population. The low evenness in the population is likely due to four dominant races that make up 61% of the population. The IOE remained low between the fall and spring collected samples for both years. The IOE did not change significantly between the years with an index of 0.03 in 2018 and an index of 0.04 in 2019.

Table 3. 7. Simpson's Index of Diversity (IOD) and Evenness (IOE) for 254 *Leptosphaeria maculans* isolates collected from commercial canola fields in Manitoba in 2018 and 2019.

	Year		Years
Index	2018	2019	Combined
# of Races	26	20	35
IOD	0.836	0.805	0.853
IOE	0.032	0.040	0.021

3.4.7 Spring Isolate vs. Fall Isolate Collection

Isolates were collected in the spring prior to canola seeding by collecting preceding canola residue to help determine the predominant races in the field. Isolates were also collected in the fall at the canola plant growth stage of 60 % seed colour change or prior to harvest. The comparison of isolates between the spring and fall helps to identify the impact of resistance gene deployment. In the collection of isolates from Manitoba field locations in 2018 and 2019, 46 % of spring collected isolates were identified as *L. biglobosa,* where only 14 % of the fall collected isolates were *L. biglobosa*.

Field site coded as MB5 had 20 isolates collected between the spring and fall sampling in 2018. The frequency of avirulence genes for MB5 is depicted in Figure 3.9. The 2018 canola cultivar grown in MB5 belonged to the resistance gene group ACG which contains resistance genes *LepR3, Rlm3,* and *RlmS*. The blackleg disease incidence for this field was calculated to be 64 % and disease severity rating was 1.36. The high levels of disease incidence can be explained by the major resistance genes not matching up to the *L. maculans* avirulence alleles. *AvrLepR3* and *AvrLm3* are not found in the spring population, and only 38% of the races contained *AvrLmS*. The resistance gene *RlmS* would have only been able to initiate a defense response against a small percentage of attacking *L. maculans* races. *AvrLmS* is not recognized in the fall isolate population suggesting a change in virulence. The *AvrLm2, AvrLm4,* and *AvrLm11* avirulence alleles all increased in frequency from the spring to fall collected isolates.

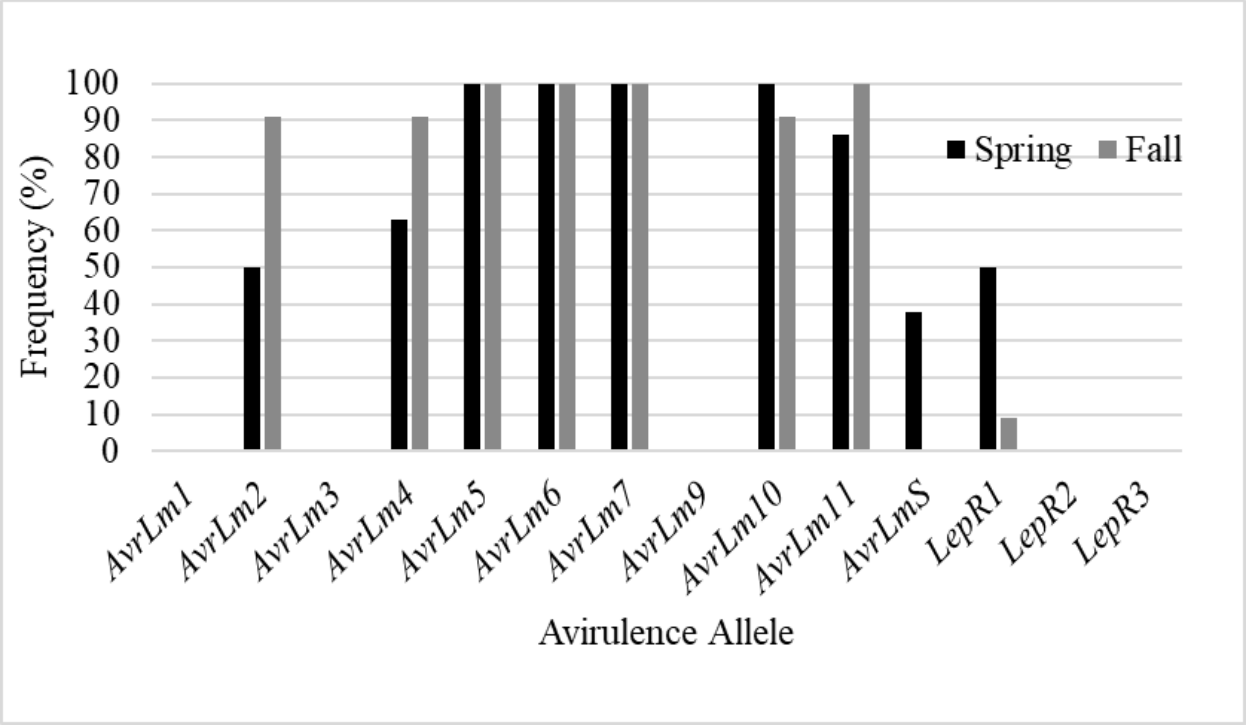

Figure 3. 9. The frequency of avirulence alleles in the *Leptosphaeria maculans* population from 20 isolates collected in the spring and fall from field site MB5 in Manitoba from 2018.

Field site MB6 from Manitoba in 2018 is an additional example where spring and fall isolates were compared based on the resistant cultivars grown. The difference in isolates collected from each resistance gene group (C, CE_1) in the fall are compared to the spring isolates collected for the field (Figure 3.10). The blackleg disease incidence for the resistance gene group C was 64% and disease severity rating was 1.22, where resistance gene group CE_1 had a disease incidence of 42% and disease severity rating of 0.76. The higher disease incidence and severity in reported in resistance gene group C would be from no *AvrLm3* in the population.

The spring isolate population had a *AvrLm4* frequency of 58 %. This would explain why the disease incidence and severity was less in the resistance gene group CE_1 cultivar. The addition of the *Rlm4* gene in resistance gene group CE_1 would allow for the defence response in the plants to be initiated. However, it is interesting that the *AvrLm4* avirulence gene decreased in frequency between the use of resistance gene group C and CE_1 by 37 %. This would suggest a shift in virulence occurring where the CE_1 cultivar was deployed.

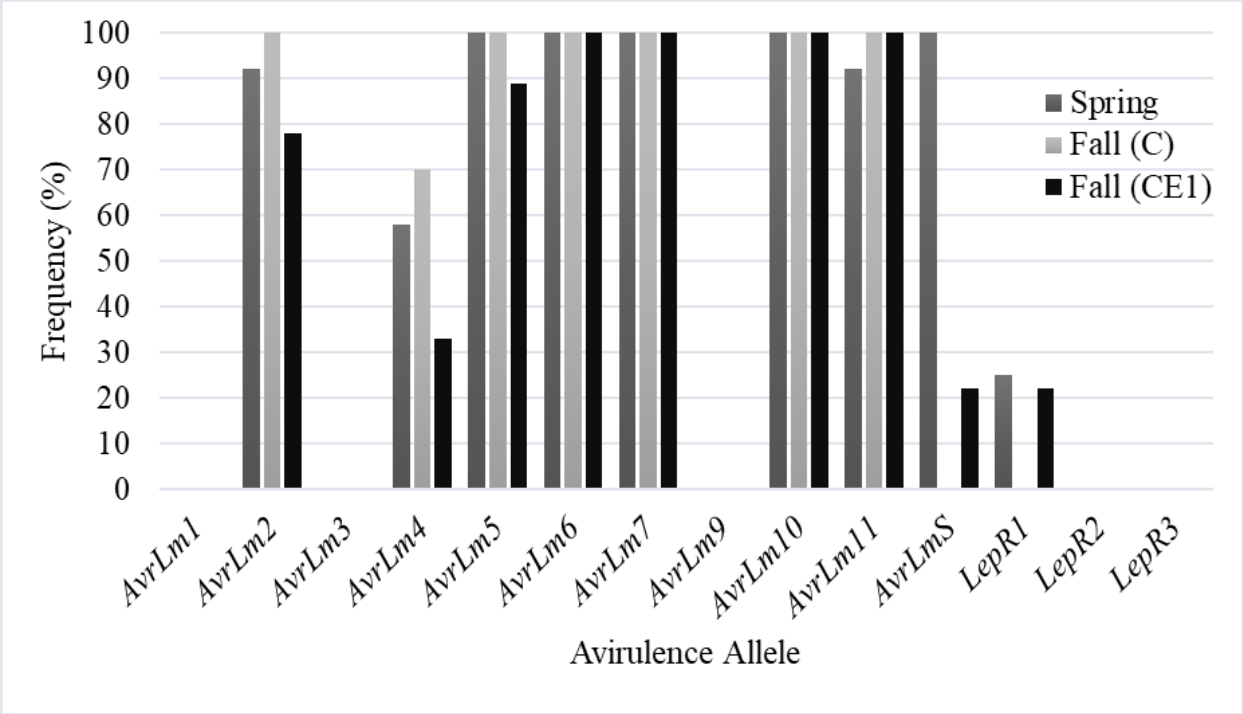

Figure 3. 10. The frequency of avirulence alleles in the *Leptosphaeria maculans* population from 30 isolates collected in the spring and fall from field site MB6 in Manitoba from 2018. The field site had two cultivars grown with different resistance gene groups (RG) identified in the fall samples.

3.5 Discussion

The current study validated the significance of deploying different blackleg resistance gene groups in commercial canola fields in the prairie region of Canada by analyzing differences in disease levels between resistance gene groups. Blackleg disease incidence and severity were significantly different between resistance gene groups. The importance of knowing what blackleg major resistance gene is deployed in the canola cultivar and the frequency of avirulence genes in the *L. maculans* population helps to better steward blackleg resistance sources. The most common *L. maculans* races in Alberta were *AvrLm5-6-7-11* and *AvrLm2-5-6-7-11*, with 19 unique races being identified. The two most common *L. maculans* races in Manitoba were *AvrLm2-4-5-6-7-10-11* and *AvrLm2-5-6-7-10-11*, with 35 unique races being identified. *AvrLm6* and *AvrLm11* were found in all 254 *L. maculans* isolates collected in Manitoba. This study provides an updated *L. maculans* race identification, frequency of races, and avirulence genes found in commercial canola fields. Knowing the blackleg major resistance gene deployed, the blackleg disease incidence and severity, along with the *L. maculans* avirulence profile causing

the disease helps to measure the success of management practices and strengthen disease management recommendations.

To date, Canada only has four major resistance genes that are identified in commercially available cultivars. The four major resistance genes result in six different resistance gene group combinations: AC (*LepR3, Rlm3*), ACG (*LepR3, Rlm3, RlmS*), C (*Rlm3*), CE_1 (*Rlm3, Rlm4*), CG (*LepR3, RlmS*), and X for unknown or not commercially identified major resistance gene (CCC, 2020). In 2016, reported *Rlm3* to be the most common deployed resistance gene in Canada, as it was found in over 55 % of *B. napus* accessions (Zhang et al., 2016). The high frequency of resistance gene *Rlm3* use today is most likely due from its early introduction into Canadian canola breeding programs (Gugel & Petrie, 1992). Therefore, it has been deployed in all resistant cultivars and paired with other major resistance genes. All commercially available resistance gene combinations were used in this study to provide relevant information to the farmer field level.

Both blackleg disease incidence and severity were significantly different between resistance gene groups over the two-year study period ($P < 0.05$; Table 3. 4). The mean disease incidence of the resistance gene groups ranged from 24 % to 57 % and disease severity ratings between 0.31 and 0.96. Overall, disease severity ratings were relatively low, as yield losses are not typically seen from blackleg until a disease severity rating of 2 is reached (Hwang et al., 2016; Wang et al., 2020). The study however did rely on natural inoculum to cause blackleg disease symptoms, so it is expected to be less than inoculated experiments. Under natural conditions Kutcher et al. (2013) reported disease severity levels of less than 0.5. Experiments that are inoculated in Canada can still experience low disease severity levels of less than 1.0 (Rashid et al., 2020). In comparison to provincial blackleg disease survey data, this study had higher disease incidence (Canadian Plant Disease Survey, 2020). This could be explained by choosing fields with high canola cropping frequency where the provincial blackleg disease survey captures fields with varying crop rotation lengths. The blackleg disease severity rating scale is subjective based on the surveyor's perception of the level of infection. Provincial disease surveys are completed by many surveyors, whereas this project only had one individual complete the ratings. This is still noted as a potential source of error within this study.

Crop rotation is a major influence of blackleg disease incidence and severity (Harker et al., 2015; Kutcher et al., 2013; Marcroft et al., 2012; Rimmer, 2003). Crop rotation was a fixed effect, as all fields surveyed for this study were on a high canola frequency by having canola two years prior to the surveyed canola crop. High canola frequency rotations were chosen for the project as it well represents the current cropping sequence in the prairie region of Canada (Statistics Canada, 2019; Harker et al., 2015). The crop in between canola crops did vary, but for the most part consisted of a cereal crop in rotation. High frequency canola rotations were chosen to ensure blackleg disease pressure would be present to test the effectiveness of major resistance gene groups deployed. The durability of resistance genes can be increased through lengthened crop rotations (Cook, 2006; Kutcher et al., 2013). Canola is a "cash crop", meaning high return on investment, in Canada and continues to be grown on short rotations that allow for pest pressure to build.

Identifying the avirulence alleles present in the *L. maculans* population in this study paves the way for a better understanding of blackleg disease pressure. The resistant gene group CE_1 cultivars containing *Rlm3* and *Rlm4* were different than several other resistance gene group combinations. This is due to the addition of the *Rlm4* gene that matches up to the *AvrLm4* avirulence gene which is frequent in the *L. maculans* population (Fernando et al., 2018; Liban et al., 2016; Soomro et al., 2020; Zhang et al., 2016). *AvrLm3* and *AvrLm9* frequencies remained low or non-existent due to the 'hide and seek' interaction with the presence of either *AvrLm4-7* or *AvrLm7* (Ghanbarnia et al., 2018; Plissonneau et al., 2016). The masking of *AvrLm3*, the deployment of *Rlm3* is ineffective, this further explains the differences seen in disease incidence and severity between resistance gene groups. Zhang et al. (2016) reported a breakdown of *Rlm3* resistance, demonstrating the high evolutionary potential of *L. maculans* populations in western Canada and the overuse of the resistance gene in Canadian *B. napus* cultivars. Cultivars in this study used *Rlm3* alone or in combination with other genes, emphasizing the overuse of this gene still in Canada.

The blackleg disease has been described as 'boom and bust' in nature, because of the changes it can have in virulence (Marcroft et al., 2012). In 2003, Southern Australia experienced the breakdown of 'Sylvestris' resistance, which consisted of *LepR3*, just three years after the commercial release of cultivars harbouring it (Sprague et al., 2006). France saw increases in the

frequency of virulent *avrLm1* isolates due to increased adoption of cultivars harbouring *Rlm1* (Rouxel et al., 2003). These two examples with the Canadian *Rlm3* breakdown example show the impact major resistance genes can have on the *L. maculans* avirulence profile (Zhang et al., 2016). Rashid et al. (2020) found rapid loss of avirulence and shifts to virulence by *L. maculans* isolates in as little as one year in Canada. Identifying the blackleg major resistance genes within a cultivar becomes valuable to help properly steward and increase the longevity of the resistance genes (Van de Wouw & Howlett, 2019). Validating the concept of strategic deployment of blackleg major resistance genes was the key objective of this study.

There are only a few labelled major resistance gene cultivars available in Canada, with some life science companies choosing not to identify the resistance genes in their cultivars. Five resistance gene group combinations were used for this study from commercially available cultivars. There were no significant differences between single gene and stacked gene cultivars in disease incidence and severity. Resistance gene group C, consisting of *Rlm3,* was the only single gene resistance group with consistent resistance as the stacked cultivars. The erosion of *Rlm3* effectiveness in Canada may be why there was not any differences between single and stacked cultivars (Zhang et al., 2016). Recommendations from Liban et al. (2016) suggested a *Rlm6* and *Rlm7* stacked cultivar would be effective against most *L. maculans* races found in Canada, but also looked at the possibility of rotating resistant genes. In Australia, rotating different blackleg resistance genes is effective in field trials (Marcroft et al., 2012). The discussion between the use of single resistance gene and stacked cultivars remains an important topic when working towards a disease management strategy.

Leptosphaeria maculans is considered a recombining pathogen population, that can easily break the effectiveness of a pyramided, stacked, or multiple major gene resistant cultivar (Marcroft et al., 2012; McDonald & Linde, 2002). Stacked major gene cultivars have the potential to create races that are virulent towards several resistance genes (Rouxel et al., 2003; Sprague et al., 2006; Van de Wouw et al., 2018; Zhang et al., 2016). One concern of pyramiding major resistance genes is that virulent races will develop and will defeat all the major genes deployed (de Vallavieille-Pope et al., 2012). There however has been a surge to develop resistant crops carrying multiple resistance conferring gene sequences through further investigation and understanding of host-pathogen interactions (Fuchs, 2017). Lots of questions remain in regards

to gene stacks such as the mechanisms that contribute to stacks durability, durability of stacks with different resistance classifications, predicting the most durable stacks, the durability of stacks for pathogens with differing life strategies, and population biology parameters for predicting pathogen evolution towards stacked cultivars (Mundt, 2018). Another limitation to stacked cultivars is the time it takes plant breeders to develop a successful stacked cultivar for commercialization (Bourguet et al., 2016). This further explains the limited major resistance gene stacked cultivars available in Canada.

Rotating major resistance genes deployed is an option when managing blackleg over time and to preserve the longevity of genetics. The Australian National Blackleg Management Guide defines which cultivars belong to which resistance gene group, and identifies risk level of rotating cultivars together (Grains Research & Development Corporation, 2018). This guide is updated annually as cultivars that were once resistant may show increased blackleg diseases incidence and appear more susceptible over time (Marcroft et al., 2012). Field sites across canola production regions in Australia are used to assess the levels of blackleg disease on cultivars. In France, a similar system is used that requires assignment of resistance genes before the commercial release of cultivars (Terres Inovia Institut, 2021). By learning from the successes and failures of these resistant gene systems the Canadian canola industry formed their own system. The avirulence profiles and major resistance genes that are deployed in Canada are different from Australia and France. The Canadian system groups major resistance genes that interact with the same avirulence allele together and highlights potential ways to strategically deploy resistance genes for effectiveness (Zhang & Fernando, 2018). There remains knowledge gaps on how to properly rotate resistance genes, and whether different resistance genes will have more impact than others (Van De Wouw et al., 2016). One project underway in Canada is exploring options to strategically rotate resistance gene groups to minimize the shifts in virulence seen in the pathogen due to the overuse of specific major resistance genes (Zou et al. unpublished). This study has helped to validate differences seen by deploying different resistance gene groups in the western Canada and opens the door to additional research questions and initiatives.

The two most common *L. maculans* races in Manitoba were *AvrLm2-4-5-6-7-10-11* and *AvrLm2-5-6-7-10-11* which only differ by the presence or absence of *AvrLm4*. The results identified 35 *L.*

maculans races in Manitoba from commercial fields in 2018 and 2019. *AvrLm6* and *AvrLm11* were found in all 254 *L. maculans* isolates collected in Manitoba. Liban et al., (2016) found 55 *L. maculans* races across western Canada with *AvrLm2-4-6-7* and *AvrLm2-4-6-7-(s)* being the most frequent races. Soomro et al. (2020) also found the same top two races in commercial and Westar trap crops in western Canada to well represent the naturally occurring *L. maculans* races within the population. Isolates in this study were characterized for 10 avirulence alleles: *AvrLm1*, *AvLm2*, *AvrLm3*, *AvrLm4*, *AvrLm6*, *AvrLm7*, *AvrLm9*, *AvrLepR1*, *AvrLepR2* and *AvrLmS*. This study characterized isolates for 14 avirulence alleles which explain some of the differences seen in race profiles such as the presence of *AvrLm5*, *AvrLm10* and *AvrLm11*. These studies ranged in time with isolates collected for Liban's work in 2010-11, Soomro's work in 2012-13, and the current study in 2018-19.

A more recent study exploring isolates from one site in Manitoba from 2010 to 2015 found similar isolates to this study: *AvrLm-2-4-5- 6-7-11*, *AvrLm-2-4-5-6-7-11-(s)*, and *Avr-1-4-5-6-7-11-(s)* (Fernando et al., 2018). The presence of 14 avirulence alleles was done by using differential canola cultivars and/or PCR assays which identified 170 races from 956 isolates that were collected. Only 254 *L. maculans* isolates were collected from commercial fields in Manitoba from 2018 to 2019 in this study resulting in 35 races. Research from 2010 found 16 races of *L. maculans* from only 96 isolates collected across western Canada (Kutcher et al., 2010). One conclusion from all the *L. maculans* isolate collection studies would be that the higher number of isolates collected the higher number of *L. maculans* races identified. This adds to the growing amount of literature referencing the great diversity of *L. maculans* races found in western Canada (Liban et al., 2016; Soomro et al., 2020).

The Simpson's diversity index indicated that diversity was high in the overall population due to the 35 *L. maculans* races isolated. The Simpson's evenness index was very low, due to two dominant *L. maculans* races representing 49% of the population. Avirulence frequency data is helpful in the development of resistance management strategies and also why there has been a heavy focus on further understanding the frequency and diversity of *L. maculans* avirulence alleles in western Canada (Kutcher et al., 2010). The *L. maculans* population in western Canada is genetically diverse and includes avirulence alleles that are uncommon in other canola producing regions (Dilmaghani et al., 2009). A diverse population with many avirulence alleles

to match with provides options to introduce corresponding resistance genes within canola cultivars.

Only two avirulence alleles, *AvrLm6* and *AvrLm11*, were found in all 254 *L. maculans* isolates collected in Manitoba of this project. This is different to the results in Alberta where *AvrLm5*, *AvrLm6*, and *AvrLm7* were seen in over 90 % of isolates collected but no avirulence allele found in all of the isolates. Liu et al. (2020) used 1229 *L. maculans* isolates from Manitoba to determine avirulence allele frequency. *AvrLm4, AvrLm5, AvrLm6, AvrLm7,* and *AvrLm9* were all found in over 70 % of the isolates collected from 2014 to 2016. Identifying and knowing the high frequency *L. maculans* races or the predominant avirulence alleles within the population will aid in determining which resistance genes should be considered in canola breeding programs.

A unique component of this work was assessing avirulence gene changes after the deployment of labelled blackleg major resistance gene cultivars. Shifts of virulence occurred in the *L. maculans* population after one growing season; the use of specific major resistance genes should be influencing the shifts (Rashid et al., 2020). Therefore, isolates were collected in the spring, around seeding, and in the fall prior to harvest. One methodology limitation was the ability to isolate *L. maculans* from two-year-old canola residue in the field. The study was designed to have a clear indication of what *L. maculans* races were viable when the study canola crop began to grow. Stubble samples were chosen based on the presence of pycnidia and pseudothecia; however, samples also contained many non-targeted pathogens, and the saprophytic *L. biglobosa* was easier to isolate and culture than *L. maculans*. *Leptosphaeria biglobosa* was easily isolated being identified in 46% of the spring collected isolates. Fall collected samples were cleaner with less saprophytic activity occurring on the stubble pieces which made isolation of *L. maculans* easier.

The MB5 field location had the gene stacked cultivar of ACG which contains major resistance genes *LepR3, Rlm3,* and *RlmS*. The spring *L. maculans* isolates collected from the field had a low frequency of *AvrLmS* present but no *AvrLepR3* and *AvrLm3* within the races. However, in the fall isolates *AvrLmS* was not present, this is an example of the *L. maculans* population changing in virulence to overcome the deployed *RlmS* resistance gene. The field did have a disease incidence of 64% of plants surveyed showing disease symptoms. Avirulence alleles *AvrLm2*, *AvrLm4*, and *AvrLm11* all increased in frequency from the spring to fall collected isolates which

could also be linked to the selection pressure caused by the ACG cultivar grown. This study links back to the discussion on successful stacked gene cultivar deployment and the risk of development of virulent races towards all deployed resistance genes.

Learning how to successfully deploy resistant cultivars to manage or mitigate blackleg disease in Canada is a priority not only for market access, but for the associated production losses it can cause. This study only looked at one component of blackleg resistance, the major resistance genes: the other component being quantitative resistance. Quantitative resistance remains a crucial tool in minimizing blackleg disease pressure, but due to the complexity in measuring the effect it has on reducing disease severity, its effect remains difficult to properly quantify (Van de Wouw et al., 2016). To improve resistance durability, both major gene resistance and quantitative resistance must be combined to provide optimal blackleg management (Brun et al., 2010; Delourme et al., 2006). Resistant cultivars can become increasingly durable when combined with other disease management tactics (Bourguet et al., 2016). The durability and longevity of crop protection products, such as resistance cultivars and fungicides, relies on using an integrated management approach to disease management.

The validation of deployment of blackleg resistance gene groups in commercial canola fields on the Canadian prairies adds to the credibility of this management tactic, already proven to be effective in managing blackleg disease in other canola producing regions. The applied component of this research can be incorporated into best management practices and provide farmers with information to help when choosing cultivars to effectively manage blackleg on their farms. Updated avirulence race profiles of *L. maculans* will provide plant breeders with information they need to help select resistance in their respective canola breeding programs. This information must be used as a foundation on how to strategically select major resistance genes and how to effectively use them in rotation to maintain effectiveness and durability.

CHAPTER 4

4.0 GENERAL DISCUSSION

4.1 Contributions to the Scientific Community

Strategic deployment of resistant cultivars is not a new idea. For blackleg management in Canada, we now just have the information and systems in place to do so. Major resistance gene cultivar deployment has been successful in Australia and France. The differences between resistance gene groups within a field location were expected, which this work indicated. Having significantly different disease levels between major resistance gene groups across western Canada, validated the concept of importance around strategic deployment of resistant cultivars. Overall, this experiment had relatively low disease severity amongst major resistance gene groups. This indicates that the environmental conditions or use of quantitative resistance are playing a much larger role in disease severity than the use of major resistance gene.

The comparison between cultivars with a single major resistance gene or one with multiple major resistance genes is a widely debated topic for plant breeders. The argument is that with only one major resistance gene it only had one option for matching an avirulence allele to initiate the defence response, whereas multiple genes increase the potential to infer resistance. The other side of the argument is that virulent *L. maculans* isolates could be selective for that can now overcome all resistance genes used in the multi-gene cultivar, especially when pathogen isolates carry more than one avirulence allele. This work showed that there was no significant difference between single-gene or multi-gene cultivars, and that plant breeders should focus on integrating major resistance genes that match the avirulence frequency seen in western Canada.

The selection pressure created when deploying major resistance gene cultivars was shown by the differences in avirulence frequencies between spring- and fall-collected isolates. Through one cropping cycle, major resistance gene cultivars were having an impact on the *L. maculans* races isolated in the field. This places high importance on finding ways to successfully rotate between major resistance gene cultivars to minimize changes of *L. maculans* virulence.

This work informs the scientific community of what *L. maculans* isolates to be using in small plot, growth chamber, and greenhouse experiments by identifying what *L. maculans* races are naturally occurring in western Canada. This is a valuable component of this research as it identifies *L. maculans* races that have been selected for based on crop management by farmers. Knowing what is occurring at the farmer field level helps to develop cultivars that will be effective on their farms. Avirulence frequency differences were seen between field locations and

provinces. In Manitoba, *AvrLm2, AvrLm5, AvrLm6, AvrLm7, AvrLm10,* and *AvrLm11* were identified in over 80 % of *L. maculans* isolates providing more options than what was found in Alberta with only *AvrLm5, AvrLm6,* and *AvrLm7* being identified in 90% of isolates. Canola plant breeders can use this information and isolates collected to screen their *B. napus* germplasm, ensuring that their newest cultivars contain resistance genes to correspond with the avirulence allele frequency seen in the canola producing regions of Canada.

The question around how many isolates need to be collected comes up frequently in the scientific community when trying to determine the *L. maculans* race profile for an experiment. This work had a Simpson's index of diversity which indicated that the *L. maculans* population appears genetically diverse, while the Simpson index of evenness indicated the population had a low evenness in Manitoba. This work along with previous studies show that the more isolates collected the more diverse the population becomes; however, the evenness remains low. In Manitoba, *AvrLm2-4-5-6-7-10-11*and *AvrLm2-5-6-7-10-11* made up 49 % of the 254 *L. maculans* isolates collected. *AvrLm5-6-7-11* and *AvrLm2-5-6-7-11* made up 78 % of the 207 *L. maculans* isolates collected in Alberta. This work indicates that there are typically two predominant *L. maculans* races found at the field level as well. This information can be used to help strengthen the methodology used for commercial testing for blackleg race identification.

The scientific community continues to look for ways to effectively reduce the risk posed by blackleg in canola while staying ahead of changes in the *L. maculans* population. This work contributes blackleg pathogen population data in western Canada by providing distribution of *L. maculans* races and avirulence frequencies. It also validates the concept of strategically deploying resistant cultivars as a blackleg disease management strategy. This information can be used by plant breeders and pathologists, canola farmers, and regulators to better manage blackleg in canola.

4.2 Benefit to Farmers

Blackleg in canola has been a production issue for farmers in Canada for decades. The introduction of blackleg resistant cultivars in the late 1990s minimized the impact of the disease and lowered the awareness around potential disease impacts. The *L. maculans* pathogen has

adapted to the resistant cultivars deployed and clarity needs to be brought forth on how to select a cultivar to best manage the pathogen at the field level. The purpose of this work was to validate a management practice to help farmers make an informed decision on cultivar selection to manage blackleg.

Blackleg major resistance gene identification and labelling is a complex system but the benefits of knowing a cultivar's major resistance gene were identified in this work. Through the strategic deployment of major resistance gene cultivar's, farmers were able to lower their blackleg disease incidence and severity within the field. Validation of strategically deploying resistant cultivars in western Canada provides farmers with the confidence that this approach will work on their own farm. This work should encourage seed companies to be transparent around blackleg major resistance genes to provide farmers with information they need to strategically deploy resistant cultivars. If farmers can use an integrated pest management approach to blackleg, cultivar resistance durability will be increased relieving pressure off seed companies to introduce new resistant cultivars. First part is knowing the cultivar's major resistance gene, and the second part is determining what the avirulence frequency of *L. maculans* is within the field.

The research of determining the predominant *L. maculans* races, along with the frequency of avirulence alleles provides updated data for farmers to make cultivar decisions. Similar research has been conducted but every time it is updated changes in *L. maculans* races and avirulence frequency have been reported. From Manitoba, this work identified *AvrLm2-4-5-6-7-10-11* and *AvrLm2-5-6-7-10-11* to be the predominant *L. maculans* races, with *AvrLm2, AvrLm5*, *AvrLm6*, *AvrLm7*, *AvrLm10*, and *AvrLm11* identified in over 80 % of isolates collected. In Alberta, *AvrLm5-6-7-11* and *AvrLm2-5-6-7-11* were the most common *L. maculans* races with *AvrLm5*, *AvrLm6*, and *AvrLm7* identified in over 90 % of the *L. maculans* isolates collected. Phenotypic data is what farmers need, as it considers the avirulence allele interactions that allow for some alleles not to be expressed. Major resistance gene groups have been created to summarize the interactions seen between resistance genes to help make minimize confusion on the host side. The findings from this work can be applied directly to farmer's cultivar selections by looking at provincial avirulence data.

Canola farmers are faced with the decision of using a cultivar with a single major resistance gene or one with multiple major resistance genes. A result from this work was that there was no

significant difference between the use of a single or stacked cultivar. The important piece for farmers to incorporate is that it is more effective to have one major resistance matching the highest frequency avirulence allele of *L. maculans* in the population. The use of multiple major resistance genes that do not match the avirulence profile of *L. maculans* is not as effective of having just one major resistance gene matching the avirulence profile. More genes are not always better. Multi-gene cultivars do exist commercially, and farmers should look to select cultivars that have at least one major resistance gene group to match the *L. maculans* avirulence profile.

Although the *L. maculans – B. napus* pathosystem may be complex, this work does validate the importance of knowing the pathogen avirulence profile and host resistance genes. Next steps are taking this research and finding ways of clarifying existing extension resources and developing new resources to help farmers manage blackleg.

4.3 Future Research Direction

From my work many questions arise but its valuable to take a step back to look at the bigger picture of blackleg management in Canada. Having a clear set of research priorities helps to drive innovation and change, while steering the agricultural sector forward to a sustainable future. Van de Wouw et al. (2021) completed a review of changes in farming practices to manage blackleg in Australia. In the review, a list of research priorities for long-term management of blackleg were included; one priority focused on development of a rotation plan for major resistance genes. This work can be used as a base for future major gene rotation research in Canada to help meet global research priorities on blackleg management.

Another component of this Canadian Agriculture Partnership (CAP) project is a small plot experiment looking at the rotation of major resistance genes. This work validated the basic concept of strategic cultivar deployment. The small plot experiment will identify how to then successfully rotate major resistance genes to minimize blackleg levels while protecting cultivar durability. A small plot experiment allows for specific major genes to be tested in a predetermined cropping rotation. Changes in *L. maculans* virulence will be used to determine the effectiveness of rotations along with blackleg disease incidence and severity.

Spring sampling for *L. maculans* isolates was a challenge in this project due to other pathogens colonizing the old canola stubble. *Leptosphaeria biglobosa* is seen as a saprophytic pathogen in Canada making isolation of *L. maculans* from old canola residue challenging. The project should be repeated looking at other ways of determining the predominant *L. maculans* races infected the new canola crop. This could be done using spore collecting technology such as a Burkard spore trap at the time of peak pycnidiospores production, early in the growing season. It would also be beneficial to find ways to test between *L. biglobosa* and *L. maculans* within the field; maybe one day, having the capabilities to take real time PCR tests in the field to differentiate between species.

A research priority in the Canadian canola industry is to further understand the role of quantitative resistance in blackleg management. This research project could be redesigned to look at quantitative resistance by using virulent isolates to get past the major resistance gene, which is the first line of host defence. Canola farmers are dealing with *L. maculans* races able to overcome the major resistance gene in their cultivars already. Quantitative resistance performs differently under environmental conditions so testing this in the end-use environment would help to validate the effectiveness. Regional monitoring sites, similar to those used in Australia, have been discussed but are dismissed as an option in Canada for monitoring the effectiveness of major genes due to using *L. maculans* isolates that could be virulent towards one major gene but not another. However, monitoring sites could be used to test cultivars' quantitative resistance in the environment and supplement ongoing work on quantitative resistance in growth chambers.

Commercially available cultivars were used in this work which consisted of single resistance gene group cultivars but also combinations of groups in a cultivar. This work should be repeated using cultivars that consist of only one major resistance gene to test the durability of the major resistance gene on its own. Since the start of this project two additional resistance genes, *Rlm2* and *Rlm7*, have been identified in commercial cultivars in Canada. These recently labelled genes in Canada should be used in future experiments, especially *Rlm7* as there is a very high frequency of *AvrLm7* found in western Canada. *Rlm7* is a resistance gene not known to be deployed in Canada previously. Monitoring of the changes in virulence of *AvrLm7* within the *L. maculans* population will be crucial to understanding the durability of cultivars using *Rlm7*.

Regional or provincial blackleg disease surveying should include *L. maculans* avirulence frequency data. A research project could look at identifying *L. maculans* races yearly and be designed to survey cultivars with different major resistance genes. This information needs to be produced shortly after harvest to provide canola farmers the time to review and use data prior to cultivar selection.

Research projects looking to develop new disease management practices need to be validated in the environment they are planned for. The value of using farmers' fields to accurately assess the *L. maculans* avirulence profile in western Canada provides the latest information the canola industry needs to stay ahead of blackleg.

4.4 Recommendations

From this research a list of recommendations for the canola industry in Canada have been generated. These should be used within extension resources for farmers and agronomists to educate on the latest findings in blackleg management.

- Through the strategic deployment of major resistance gene cultivars, farmers can lower their blackleg disease incidence and severity within the field.
- Differences in disease levels amongst major resistance gene groups deployed across western Canada validates the concept of strategic deployment of resistant cultivars based on *L. maculans* avirulence frequencies.
- Major resistance genes can change the *L. maculans* avirulence frequency after one cropping season.
- The encouragement of seed companies to be transparent with blackleg major resistance genes, to provide farmers with information they need to strategically deploy resistant cultivars.
- Regional and provincial *L. maculans* avirulence data should be updated yearly for farmers to have while making cultivar selections. Distribution of *L. maculans* races and avirulence frequencies should be available to the wider canola industry.
- There is no significant difference between the use of a single or stacked gene cultivars, cultivar selection should be focused on matching at least one resistance gene to the *L. maculans* avirulence frequency.

- Best time to collect canola stubble samples to determine *L. maculans* race profile is at harvest timing or shortly after to get clean samples for isolations. Samples that have overwintered and that are collected in the spring are difficult to isolate *L. maculans* successfully from.

CHAPTER 5

5.0 LITERATURE

5.1 LITERATURE CITED

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6.0 APPENDICES

6.1 APPENDIX A

Table A. 1. Defined abbreviations used in thesis.

Abbreviation	Definition
AAFC	Agriculture and Agri-Food Canada
AB	Alberta
ANOVA	Analysis of Variance
Avr	Avirulence / avirulent
avr	Virulent
BBCH	Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie
CAP	Canadian Agriculture Partnership
IOD	Simpson's Index of Diversity
IOE	Simpson's Index of Evenness
KASP	Kompetitive allele specific PCR KASP
MB	Manitoba
R-gene	Resistance Gene
RG	Resistance Gene Group
SK	Saskatchewan
WCC/RRC	Western Canada Canola and Rapeseed Recommending Committee

6.2 APPENDIX B

Figure B. 1. *Leptosphaeria maculans* fruiting bodies (pycnidia) visible on two-year-old canola (*Brassica napus*) stubble. Field samples collected in the spring consisted of stubble showing pycnidia to determine the avirulence profile of *L. maculans*. Photo credit: J. Cornelsen

Figure B. 2. Lodging of canola (*Brassica napus*) plants and premature ripening caused by Blackleg with visual differences seen between cultivars of different resistance gene groups at one of the trial locations. Photo credit: J. Cornelsen

Figure B. 3. Blackleg disease incidence and severity ratings completed on basal stem cuttings identifying internal blackening of tissue (A). Blackleg severity is rated on a 0 – 5 scale with '0' indicating a plant with no internal blackening on left side, then increasing in severity moving to the right, resulting in a dead plant with a disease severity rating of '5' (B). Photo credit: A: Canola Council of Canada; B: J. Cornelsen

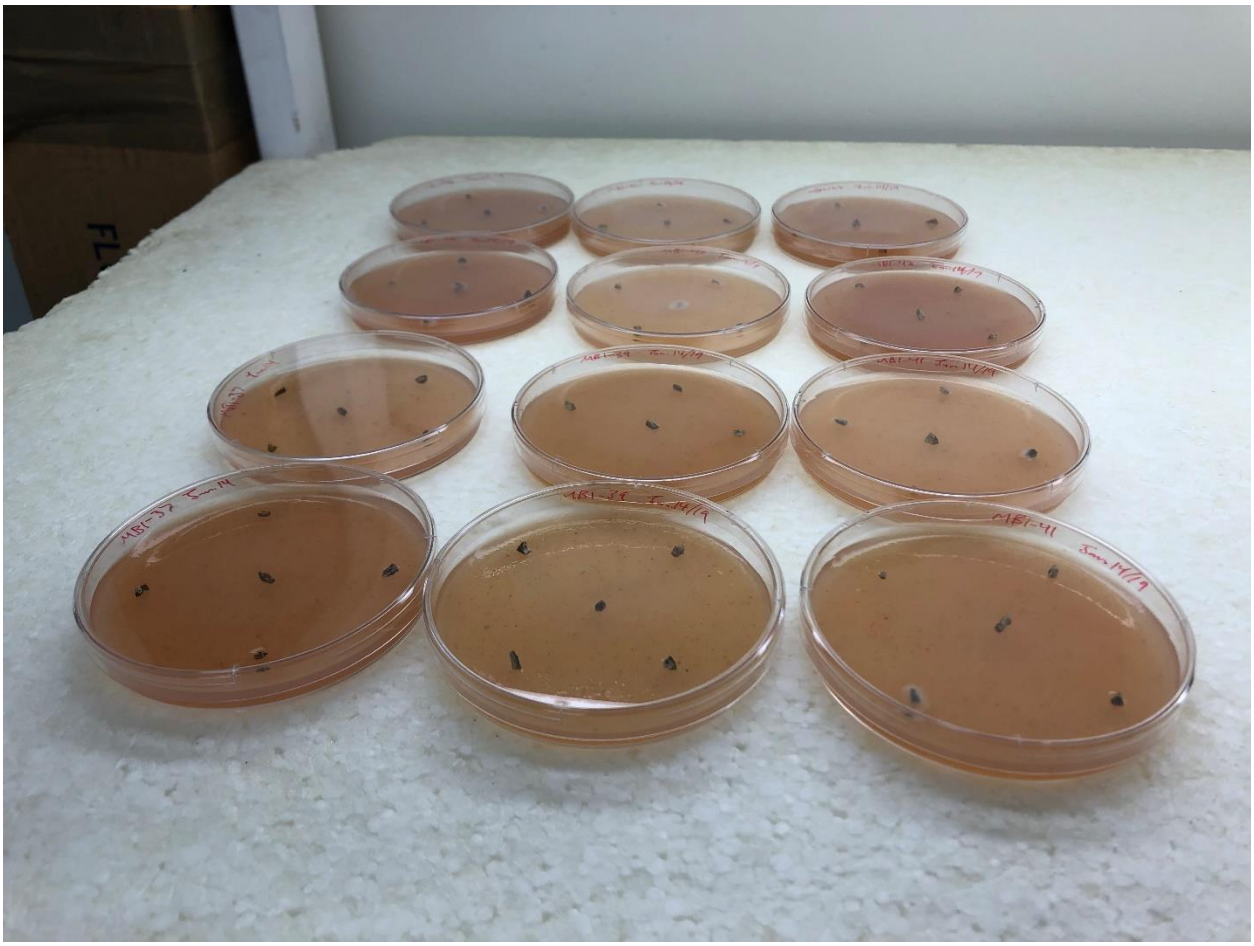

Figure B. 4. Canola (*Brassica napus*) plant stem samples surface sterilized with bleach, plated onto V8 agar media, and placed on a light bench under cool white, fluorescent light at 22-24 ℃ for four to seven days. Photo credit: J. Cornelsen

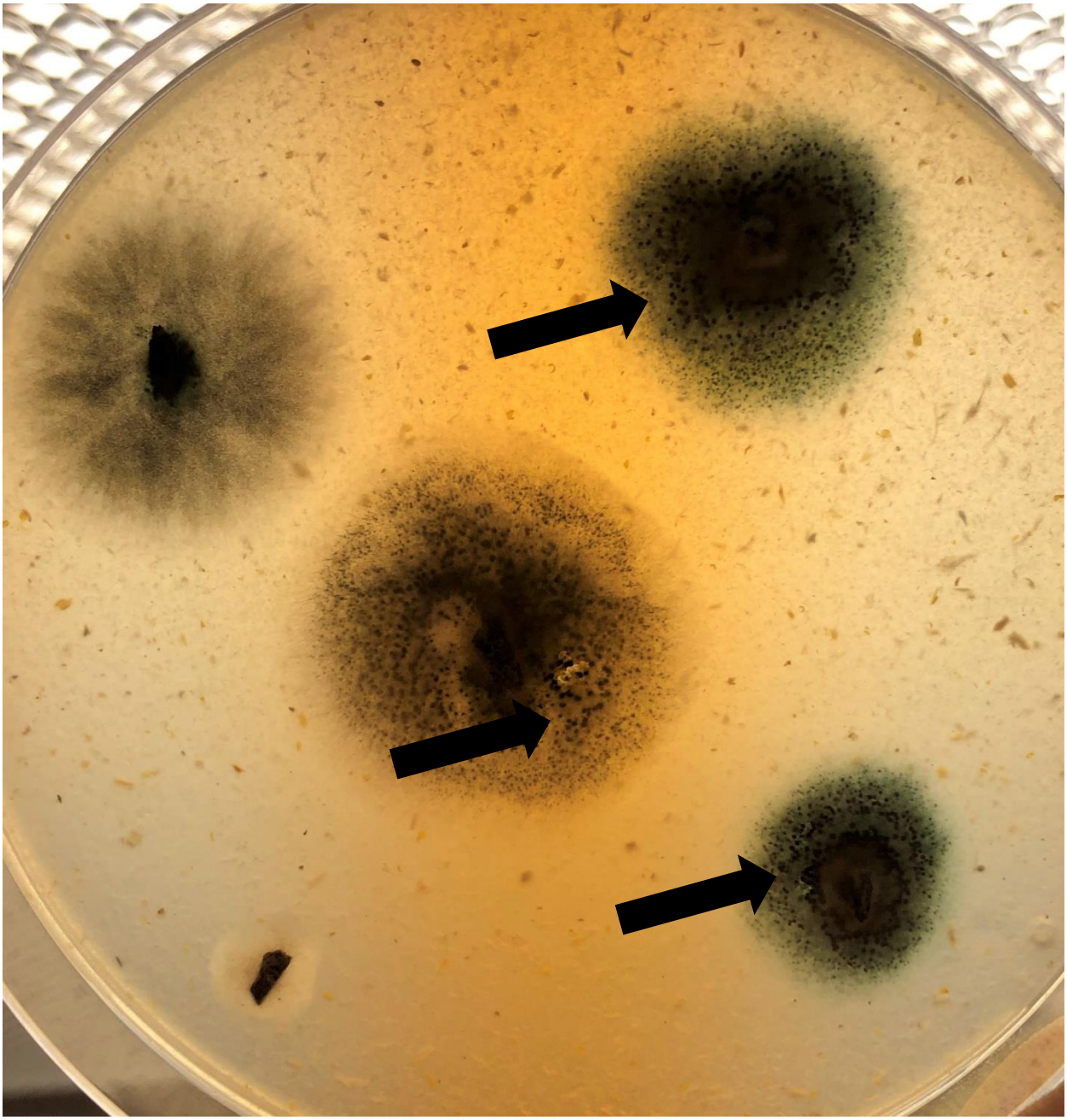

Figure B. 5. V8 agar media plate showing *Leptosphaeria maculans* cultures indicated by the arrows. Other pathogen species are seen growing on the plate. Photo credit: J. Cornelsen

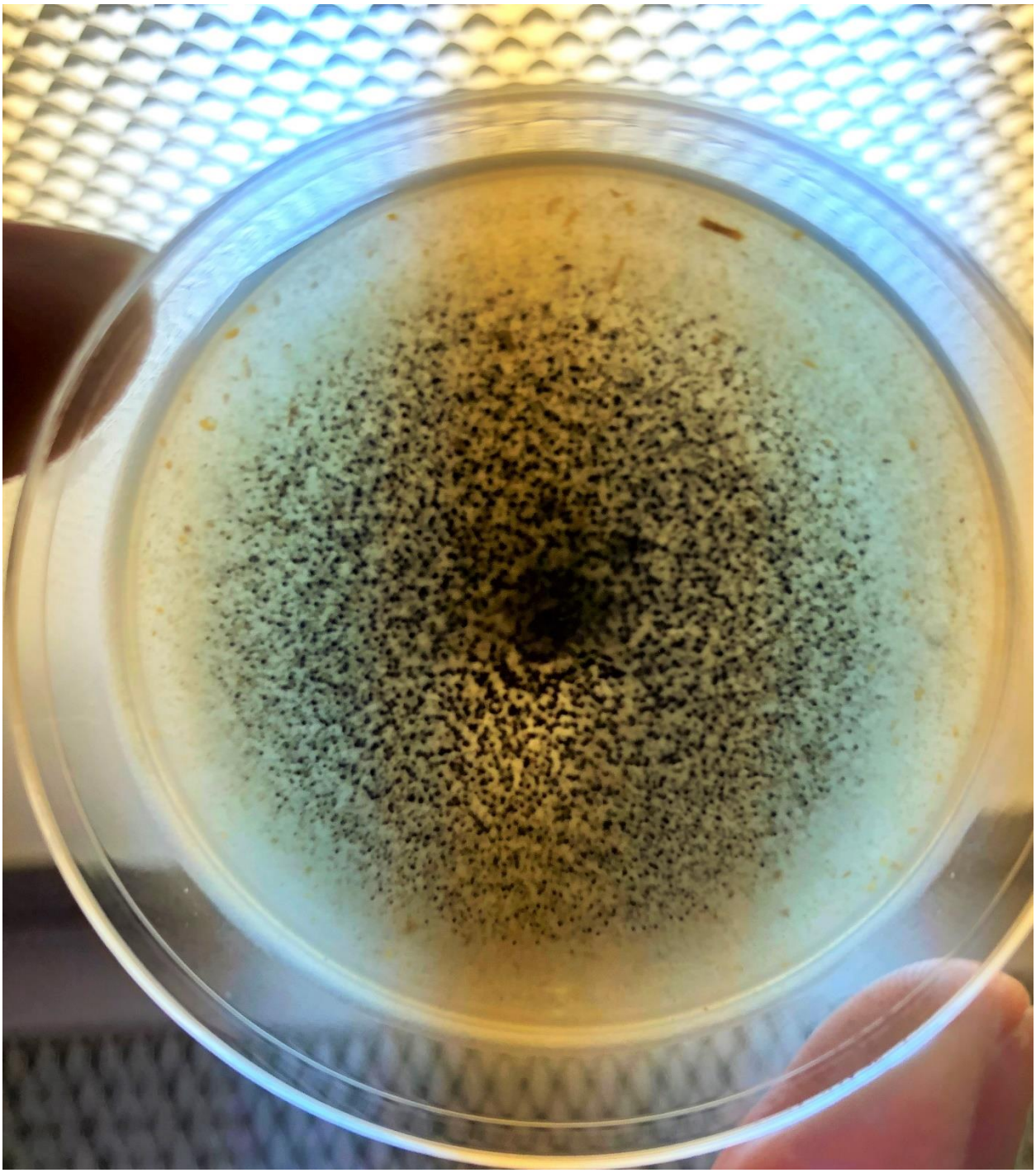

Figure B. 6. Single spore *Leptosphaeria maculans* culture produced by selecting pycnidiospores from a single pycnidia from cultures from canola plant stem samples. Photo credit: J. Cornelsen

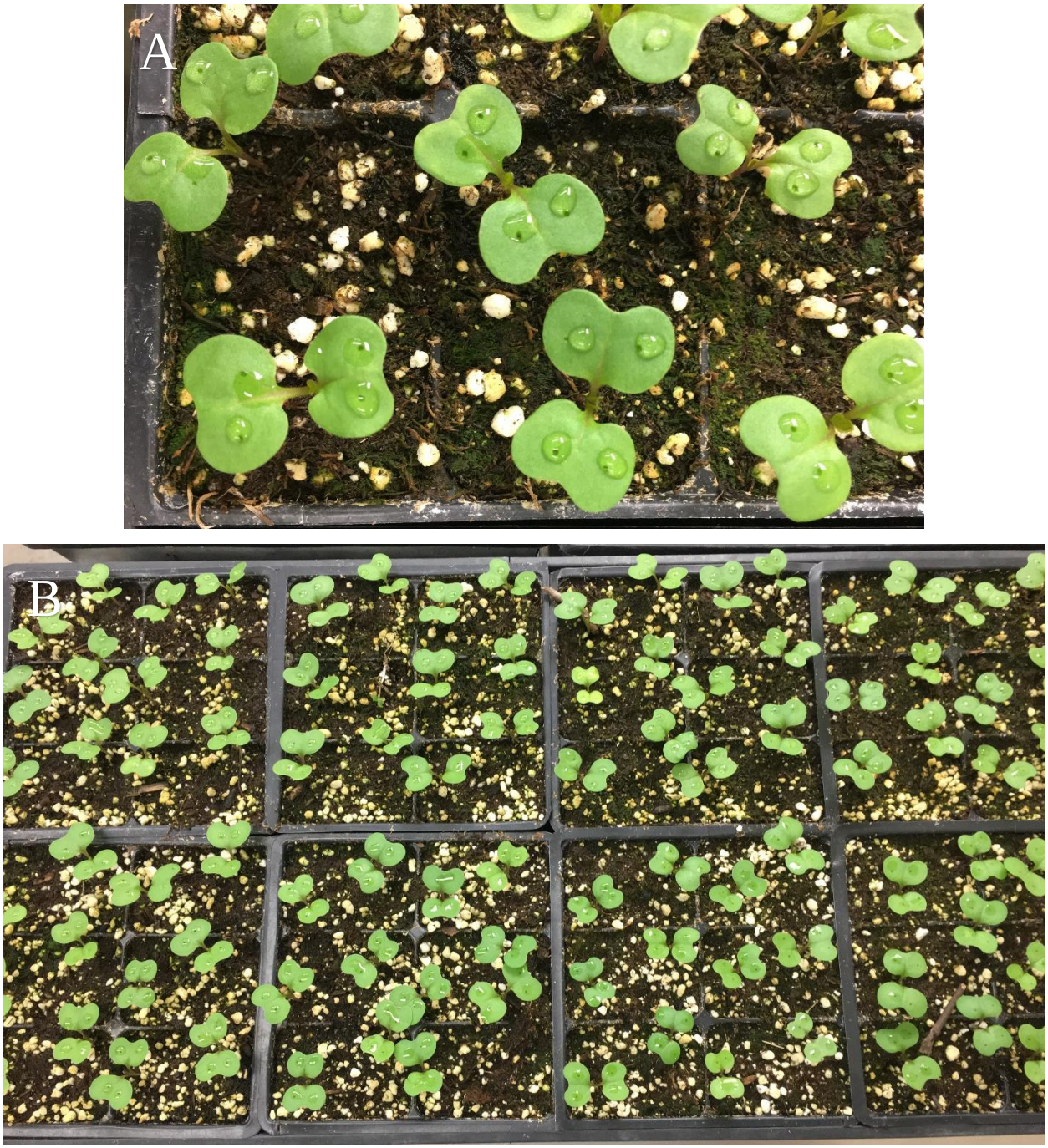

Figure B. 7. Seven-day old canola (*Brassica napus*) cotyledons inoculated with 10ul droplets of *Leptosphaeria maculans* pycnidiospores (A). Differential *Brassica* lines carrying known major resistance genes to observe the phenotypic reaction (B). Photo credit: Dr. Zhongwei Zou

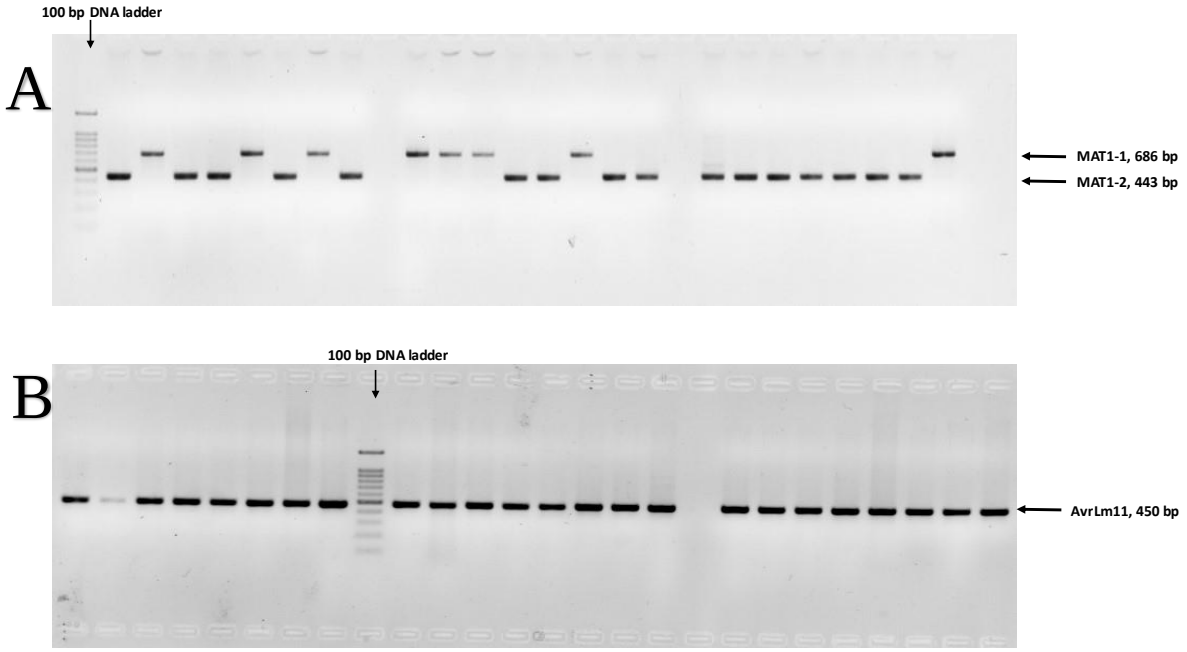

Figure B. 8. Photos taken from gel electrophoresis to visualize PCR results of *Leptosphaeria maculans* mating type (Cozijnsen & Howlett, 2003) (A) and of avirulence allele *AvrLm11* (Balesdent et al., 2013) (B). Photo credit: Dr. Zhongwei Zou

6.3 APPENDIX C

Table C. 1. Regional maximum temperatures (°C) used to generalize weather conditions seen at field locations across western Canada. Source: Government of Canada Historical Climate Data.

					April			May			June			July			August			September			October		
Year	Province	Sample ID	Nearest town with weather station	Station used for normal data	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal
2018	Alberta	AB4	Peace River A	Peace River A	4.8	10.7	44.9	22.9	17.6	130.1	23.5	21.8	107.8	25.2	25.1	100.4	26.1	24.2	107.9	14.6	18.0	81.1	7.7	10.1	76.2
2018	Alberta	AB1	Camrose	Camrose	6.9	11.2	61.6	22.8	16.3	139.9	24.9	19.8	125.8	25.9	23.2	111.6	26.3	22.8	115.4	15.5	17.8	87.1	6.8	11.7	58.1
2018	Alberta	AB3	Strathmore AGDM	Calgary	10.7	10.7	100.0	23.8	17.0	140.0	24.1	20.4	118.1	26.3	22.9	114.8	25.5	22.3	114.3	15.2	16.7	91.0	9.0	10.2	88.2
2018	Alberta	AB9	Fort Sask	Fort Sask	7.4	9.6	77.1	20.9	17.4	120.1	22.1	22.1	100.0	24.0	25.3	94.9	23.8	24.4	97.5	10.9	17.8	61.2	10.4	10.0	104.0
2018	Alberta	AB5	Peace River A	Peace River A	7.2	10.1	71.3	23.2	16.8	138.1	25.0	20.5	122.0	24.4	22.6	108.0	24.0	21.7	110.6	12.1	16.6	72.9	11.1	9.4	118.1
2018	Alberta	AB2	Lacombe CDA 2	Red Deer	2.8	10.7	26.2	21.8	17.8	122.5	23.7	22.9	103.5	24.1	25.2	95.6	23.3	25.0	93.2	11.9	19.4	61.3	7.6	10.6	71.7
2018	Alberta	AB7	Grand Prairie A	Grand Prairie A	3.7	9.8	37.8	21.5	16.6	129.5	23.7	20.5	115.6	24.3	22.6	107.5	24.2	21.5	112.6	14.0	15.9	88.1	6.0	8.2	73.2
2018	Alberta	AB8	Vauxhall CDA CS	Vauxhall North	3.7	12.1	30.6	21.5	19.1	112.6	23.7	23.6	100.4	24.3	26.7	91.0	24.2	26.6	91.0	14.0	20.2	69.3	6.0	11.6	51.7
2018	Manitoba	MB10	Roblin	Gilbert Plains	5.0	8.2	61.0	21.8	17.2	126.7	20.8	22.3	93.3	22.5	23.6	95.3	22.2	23.5	94.5	10.1	16.8	60.1	8.2	8.3	98.8
2018	Manitoba	MB6	Carberry	Neepawa Southwest	3.6	10.7	33.6	21.1	18.0	117.2	23.1	21.9	105.5	23.4	24.2	96.7	23.2	23.9	97.1	13.1	17.7	74.0	5.8	9.7	59.8
2018	Manitoba	MB1	Shoal Lake CS	Minnedosa	6.0	12.2	49.2	23.0	18.2	126.4	25.0	22.1	113.1	26.3	26.1	100.8	25.0	25.6	97.7	12.9	18.8	68.6	9.4	11.1	84.7
2018	Manitoba	MB5	Pilot Mound	Pilot Mound 2	6.0	9.8	61.2	23.0	16.6	138.6	25.0	20.5	122.0	26.3	22.6	116.4	25.0	21.5	116.3	12.9	15.9	81.1	9.4	8.2	114.6
2018	Manitoba	MB7	Shoal Lake CS	Minnedosa	8.3	9.8	84.7	22.9	17.8	128.7	23.7	21.9	108.2	27.5	24.2	113.6	26.9	24.0	112.1	15.5	17.6	88.1	12.9	9.4	137.2
2018	Saskatchewan	SK8	Saskatoon RCS	Saskatoon SRC	9.2	10.7	86.0	21.2	17.6	120.5	22.5	21.8	103.2	24.7	25.1	98.4	23.4	24.2	96.7	10.6	18.0	58.9	9.4	10.1	93.1
2018	Saskatchewan	SK4	Regina RCS	Regina INT'L A	4.8	11.3	42.5	21.0	17.9	117.3	21.4	21.2	100.9	22.6	23.3	97.0	21.6	22.4	96.4	9.9	17.2	57.6	9.2	10.3	89.3
2018	Saskatchewan	SK5	Broadview	Broadview	5.0	8.2	61.0	21.8	17.2	126.7	20.8	22.3	93.3	22.5	23.6	95.3	22.2	23.5	94.5	10.1	16.8	60.1	8.2	8.3	98.8
2018	Saskatchewan	SK2	Pilger	Pilger	6.6	10.7	61.7	22.3	17.8	125.3	24.4	22.9	106.6	25.4	25.2	100.8	26.0	25.0	104.0	15.7	19.4	80.9	7.3	10.6	68.9
2018	Saskatchewan	SK6	North Battleford	North Battleford A	6.1	10.7	57.0	23.4	18.0	130.0	24.8	21.9	113.2	27.1	24.2	112.0	27.3	23.9	114.2	15.2	17.7	85.9	9.3	9.7	95.9
2018	Saskatchewan	SK3	Saskatoon RCS	Saskatoon SRC	5.3	9.8	54.1	22.2	17.8	124.7	21.7	21.9	99.1	24.7	24.2	102.1	24.3	24.0	101.3	11.9	17.6	67.6	10.2	9.4	108.5
2018	Saskatchewan	SK1	Yorkton	Yorkton A	4.4	9.8	44.9	22.7	18.2	124.7	23.9	22.8	104.8	24.6	24.9	98.8	25.8	24.8	104.0	13.8	19.0	72.6	7.8	10.1	77.2
2019	Alberta	AB13	Red Deer REGIONAL A	Red Deer	10.6	11.5	92.2	16.0	18.5	86.5	19.0	22.6	84.1	21.9	25.7	85.2	20.9	25.2	82.9	16.5	18.4	89.7	8.4	10.3	81.6
2019	Alberta	AB11	Three Hills	Trochu Town	12.3	9.3	132.3	16.9	17.3	97.7	20.7	21.7	95.4	22.4	24.3	92.2	22.1	23.9	92.5	17.8	17.5	101.7	9.4	9.5	98.9
2019	Alberta	AB12	Lacombe CDA 2	Red Deer	10.9	11.6	94.0	16.5	18.5	89.2	18.7	22.8	82.0	21.2	25.8	82.2	20.3	25.5	79.6	16.2	19.1	84.8	9.1	11.0	82.7
2019	Alberta	AB15	Consort AGDM	Oyen Cappon	12.2	8.8	138.6	17.1	17.0	100.6	21.2	21.7	97.7	23.6	24.0	98.3	22.4	24.0	93.3	17.2	17.1	100.6	7.8	9.0	86.7
2019	Manitoba	MB13	Swan River RCS	Swan River	9.8	9.8	100.0	16.4	18.2	90.1	23.5	22.8	103.1	25.2	24.9	101.2	23.4	24.8	94.4	18.2	19.0	95.8	7.0	10.1	69.3
2019	Manitoba	MB14	Shoal Lake CS	Minnedosa	9.4	11.3	83.2	16.3	16.8	97.0	22.2	20.5	108.3	24.2	23.1	104.8	22.1	22.5	98.2	16.7	17.3	96.5	5.3	11.2	47.3
2019	Manitoba	MB19	Melita	Pierson	11.9	11.6	102.6	17.9	17.6	101.7	24.2	21.3	113.6	26.1	23.7	110.1	24.9	23.8	104.6	18.9	17.8	106.2	7.6	10.9	69.7
2019	Manitoba	MB17	Pilot Mound (AUT)	Pilot Mound 2	10.0	11.3	88.5	16.3	16.8	97.0	23.5	20.5	114.6	24.8	23.1	107.4	23.8	22.5	105.8	17.6	17.3	101.7	5.9	11.2	52.7
2019	Manitoba	MB18	Carberry CS	Neepawa Southwest	10.6	13.7	77.4	16.8	19.0	88.4	23.9	22.3	107.2	25.6	25.5	100.4	23.8	25.8	92.2	17.5	20.7	84.5	6.4	13.4	47.8
2019	Saskatchewan	SK15	Yorkton	Yorkton A	12.8	11.3	113.3	18.2	16.8	108.3	23.0	20.5	112.2	24.4	23.1	105.6	23.4	22.5	104.0	18.5	17.3	106.9	7.1	11.2	63.4
2019	Saskatchewan	SK11	Saskatoon RCS	Pilger	10.5	9.3	112.9	15.8	17.3	91.3	23.1	21.7	106.5	24.8	24.3	102.1	22.7	23.9	95.0	17.4	17.5	99.4	6.7	9.5	70.5
2019	Saskatchewan	SK14	Wynyard (AUT)	Wynyard	10.0	8.8	113.6	16.0	17.0	94.1	21.9	21.7	100.9	23.4	24.0	97.5	21.4	24.0	89.2	16.9	17.1	98.8	6.5	9.0	72.2
2019	Saskatchewan	SK13	Melfort	Melfort CDA	8.8	9.3	94.6	16.1	17.0	94.7	21.7	21.4	101.4	22.9	23.9	95.8	21.3	23.9	89.1	16.5	17.1	96.5	6.4	9.1	70.3
2019	Saskatchewan	SK20	North Battleford	North Battleford A	10.9	8.8	123.9	17.4	17.0	102.4	22.3	21.7	102.8	23.8	24.0	99.2	21.8	24.0	90.8	17.5	17.1	102.3	7.6	9.0	84.4
2019	Saskatchewan	SK19	Eastend CYPRESS (AUT)	Swift Current A	11.1	11.5	96.5	15.0	18.5	81.1	21.3	22.6	94.2	24.6	25.7	95.7	23.6	25.2	93.7	17.5	18.4	95.1	6.5	10.3	63.1
2019	Saskatchewan	SK18	Broadview	Broadview	10.8	11.8	91.5	17.1	17.6	97.2	22.9	21.7	105.5	24.6	25.1	98.0	22.7	24.6	92.3	17.3	18.1	95.6	6.4	11.0	58.2
2019	Saskatchewan	SK12	Melfort	Melfort CDA	8.8	9.1	96.7	16.1	17.0	94.7	21.7	21.9	99.1	22.9	24.3	94.2	21.3	23.7	89.9	16.5	17.5	94.3	6.4	9.5	67.4

*M indicates weather stations that were missing weather data for over 50 % for that month.

Table C. 2. Regional minimum temperatures (°C) used to generalize weather conditions seen at field locations across western Canada. Source: Government of Canada Historical Climate Data.

					April			May			June			July			August			September			October		
Year	Province	Sample ID	Nearest town with weather station	Station used for Normal Data	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal
2018	Alberta	AB1	Camrose	Camrose	-3.1	-1.7	182.4	6.2	4.0	155.0	7.9	8.5	92.9	9.5	10.7	88.8	8.3	9.3	89.2	1.0	4.0	25.0	-3.1	-2.0	155.0
2018	Alberta	AB9	Fort Sask	Fort Sask	-3.0	-1.5	200.0	7.9	4.3	183.7	9.9	8.8	112.5	12.0	10.8	111.1	11.2	9.4	119.1	5.9	4.5	131.1	-3.6	-1.6	225.0
2018	Alberta	AB7	Grand Prairie A	Grand Prairie A	-7.7	-2.0	385.0	6.2	3.6	172.2	8.0	8.1	98.8	10.0	9.8	102.0	9.0	8.3	108.4	0.4	3.8	10.5	-3.3	-2.1	157.1
2018	Alberta	AB2	Lacombe CDA 2	Red Deer	-7.0	-2.0	350.0	5.9	4.1	143.9	7.1	9.5	74.7	8.7	11.9	73.1	7.6	10.7	71.0	1.0	4.6	21.7	-3.6	-2.4	150.0
2018	Alberta	AB4	Peace River A	Peace River A	-7.6	-2.7	281.5	5.8	3.7	156.8	8.6	9.1	94.5	9.8	11.4	86.0	8.0	10.3	77.7	-1.6	4.6	-34.8	-4.1	-2.0	205.0
2018	Alberta	AB5	Peace River A	Peace River A	-7.6	-2.7	281.5	5.8	3.7	156.8	8.6	9.1	94.5	9.8	11.4	86.0	8.0	10.3	77.7	-1.6	4.6	-34.8	-4.1	-2.0	205.0
2018	Alberta	AB3	Strathmore AGDM	Calgary	-5.3	-2.0	265.0	7.4	3.1	238.7	8.6	7.5	114.7	10.4	9.8	106.1	9.6	8.8	109.1	2.5	4.1	61.0	-1.2	-1.4	85.7
2018	Alberta	AB8	Vauxhall CDA CS	Vauxhall North	-4.2	-1.6	262.5	7.1	3.8	186.8	10.0	8.6	116.3	10.4	10.0	104.0	8.9	8.9	100.0	3.9	4.1	95.1	-1.9	-1.9	100.0
2018	Manitoba	MB6	Carberry	Neepawa Southwest	-6.4	-2.2	290.9	5.5	3.9	141.0	12.7	9.0	141.1	12.1	11.0	110.0	9.7	9.9	98.0	3.9	4.1	95.1	-3.5	-2.5	140.0
2018	Manitoba	MB5	Pilot Mound	Pilot Mound 2	-6.6	-1.7	388.2	5.5	3.7	148.6	12.4	8.4	147.6	11.6	10.5	110.5	9.5	9.2	103.3	4.4	4.3	102.3	-3.1	-1.3	238.5
2018	Manitoba	MB10	Roblin	Gilbert Plains	-8.3	-3.6	230.6	6.0	3.1	193.5	11.4	9.0	126.7	11.5	11.6	99.1	8.7	9.9	87.9	1.6	4.3	37.2	-4.2	-2.1	200.0
2018	Manitoba	MB1	Shoal Lake CS	Minnedosa	-10.2	-2.3	443.5	5.7	4.2	135.7	11.7	10.4	112.5	11.1	12.4	89.5	8.2	11.2	73.2	1.9	6.0	31.7	-4.3	-0.9	477.8
2018	Manitoba	MB7	Shoal Lake CS	Minnedosa	-10.2	-2.3	443.5	5.7	4.2	135.7	11.7	10.4	112.5	11.1	12.4	89.5	8.2	11.2	73.2	1.9	6.0	31.7	-4.3	-0.9	477.8
2018	Saskatchewan	SK5	Broadview	Broadview	-8.6	-2.5	344.0	5.1	3.7	137.8	9.7	8.8	110.2	10.5	11.0	95.5	8.4	9.7	86.6	1.4	4.0	35.0	-5.1	-2.1	242.9
2018	Saskatchewan	SK6	North Battleford	North Battleford A	-7.3	-2.3	317.4	4.6	3.3	139.4	10.0	8.1	123.5	11.0	10.0	110.0	9.2	8.3	110.8	1.7	3.2	53.1	-4.6	-2.7	170.4
2018	Saskatchewan	Sk2	Pilger	Pilger	-9.7	-1.6	606.3	5.9	5.4	109.3	10.8	11.1	97.3	11.4	13.4	85.1	9.3	12.3	75.6	1.5	7.0	21.4	-4.6	-0.3	1533.3
2018	Saskatchewan	SK4	Regina RCS	Regina INT'L A	-7.3	-4.2	173.8	6.7	2.7	248.1	10.0	8.5	117.6	10.1	10.6	95.3	9.2	9.5	96.8	1.8	3.7	48.6	-4.7	-2.7	174.1
2018	Saskatchewan	SK8	Saskatoon RCS	Saskatoon SRC	-7.3	-1.2	608.3	5.7	5.1	111.8	9.6	9.6	100.0	11.0	12.3	89.4	9.2	11.1	82.9	2.0	5.5	36.4	-4.4	-1.4	314.3
2018	Saskatchewan	SK3	Saskatoon RCS	Saskatoon SRC	-7.3	-1.2	608.3	5.7	5.1	111.8	9.6	9.6	100.0	11.0	12.3	89.4	9.2	11.1	82.9	2.0	5.5	36.4	-4.4	-1.4	314.3
2018	Saskatchewan	SK1	Yorkton	Yorkton A	-8.3	-2.9	286.2	5.2	3.5	148.6	11.4	9.2	123.9	11.9	11.5	103.5	10.3	10.2	101.0	2.2	4.7	46.8	-4.1	-2.2	186.4
2019	Alberta	AB13	Red Deer REGIONAL A	Red Deer	-3.5	-4.2	83.3	2.5	2.7	92.6	6.8	8.5	80.0	8.3	10.6	78.3	7.9	9.5	83.2	4.1	3.7	110.8	-6.6	-2.7	244.4
2019	Alberta	AB11	Three Hills	Trochu Town	-5.0	-2.0	250.0	0.9	3.7	24.3	6.5	8.1	80.2	8.4	9.9	84.8	7.2	9.1	79.1	3.1	4.0	77.5	-7.8	-2.1	371.4
2019	Alberta	AB15	Consort AGDM	Oyen Cappon	-2.2	-1.6	137.5	2.5	4.7	53.2	8.0	10.1	79.2	10.4	12.3	84.6	8.4	11.2	75.0	5.7	5.6	101.8	-5.0	-1.4	357.1
2019	Alberta	AB12	Lacombe CDA 2	Red Deer	-3.0	-4.2	71.4	3.1	2.7	114.8	6.9	8.5	81.2	9.2	10.6	86.8	8.0	9.5	84.2	4.9	3.7	132.4	-5.2	-2.7	192.6
2019	Manitoba	MB19	Melita	Pierson	-1.1	-1.6	68.8	1.4	5.4	25.9	9.7	11.1	87.4	13.0	13.4	97.0	10.4	12.3	84.6	7.9	7.0	112.9	-1.7	-0.3	566.7
2019	Manitoba	MB14	Shoal Lake CS	Minnedosa	-2.6	-2.2	118.2	1.0	3.9	25.6	8.6	9.0	95.6	11.6	11.0	105.5	8.4	9.9	84.8	6.2	4.1	151.2	-3.2	-2.5	128.0
2019	Manitoba	MB13	Swan River RCS	Swan River	-3.0	-1.3	230.8	0.3	4.0	7.5	8.5	8.9	95.5	11.9	11.2	106.3	8.8	10.5	83.8	5.9	4.9	120.4	-2.9	-1.4	207.1
2019	Manitoba	MB18	Carberry CS	Neepawa Southwest	-1.5	-1.3	115.4	1.5	4.3	34.9	9.6	8.9	107.9	12.5	11.4	109.6	9.8	10.4	94.2	7.1	5.2	136.5	-1.5	-1.0	150.0
2019	Manitoba	MB17	Pilot Mound (AUT)	Pilot Mound 2	-1.7	-1.7	100.0	0.6	3.7	16.2	9.3	8.4	110.7	12.1	10.5	115.2	9.8	9.2	106.5	6.7	4.3	155.8	-1.8	-1.3	138.5
2019	Saskatchewan	SK11	Saskatoon RCS	Pilger	-3.2	-1.7	188.2	1.2	3.7	32.4	9.0	8.4	107.1	11.3	10.5	107.6	7.5	9.2	81.5	6.1	4.3	141.9	-5.5	-1.3	423.1
2019	Saskatchewan	SK15	Yorkton	Yorkton A	-2.7	-2.9	93.1	-0.1	3.5	-2.9	9.2	9.2	100.0	11.6	11.5	100.9	9.1	10.2	89.2	6.6	4.7	140.4	-3.2	-2.2	145.5
2019	Saskatchewan	SK13	Melfort	Melfort CDA	-2.8	-2.6	107.7	1.5	4.1	36.6	8.8	9.4	93.6	11.0	11.3	97.3	8.6	10.0	86.0	5.9	4.7	125.5	-4.4	-1.7	258.8
2019	Saskatchewan	SK14	Wynyard (AUT)	Wynyard	-2.7	-2.2	122.7	1.0	4.1	24.4	8.9	9.6	92.7	11.9	11.8	100.8	9.2	10.9	84.4	7.0	5.1	137.3	-3.5	-1.6	218.8
2019	Saskatchewan	SK18	Broadview	Broadview	-2.6	-2.5	104.0	-0.3	3.7	-8.1	8.4	8.8	95.5	10.8	11.0	98.2	8.3	9.7	85.6	6.2	4.0	155.0	-3.9	-2.1	185.7
2019	Saskatchewan	SK19	Eastend CYPRESS (AUT)	Swift Current A	-2.2	-3.0	73.3	2.2	3.6	61.1	7.5	9.0	83.3	9.7	11.7	82.9	10.0	10.3	97.1	5.4	5.2	103.8	-5.6	-1.2	466.7
2019	Saskatchewan	SK12	Melfort	Melfort CDA	-2.8	-2.6	107.7	1.5	4.1	36.6	8.8	9.4	93.6	11.0	11.3	97.3	8.6	10.0	86.0	5.9	4.7	125.5	-4.4	-1.7	258.8
2019	Saskatchewan	SK20	North Battleford	North Battleford A	-3.2	-2.3	139.1	1.5	3.3	45.5	8.4	8.1	103.7	10.5	10.0	105.0	8.1	8.3	97.6	6.1	3.2	190.6	-5.4	-2.7	200.0

*M indicates weather stations that were missing weather data for over 50 % for that month.

Table C. 3. Regional temperature averages (°C) used to generalize weather conditions seen at field locations across western Canada. Source: Government of Canada Historical Climate Data.

					April			May			June			July			August			September			October		
Year	Province	Sample ID	Nearest town w/ weather station	Station used for Normal Data	Site Yr	Norm	% of Normal	Site Yr	Norm	% of Normal	Site Yr	Norm	% of Normal	Site Yr	Norm	% of Normal	Site Yr	Norm	% of Normal	Site Yr	Norm	% of Normal	Site Yr	Norm	% of Normal
2018	Alberta	AB1	Camrose	Camrose	3.3	4.5	73.3	13.7	10.5	130.5	15.2	14.5	104.8	17.1	16.8	101.8	15.8	15.8	100.0	5.8	10.4	55.8	3.2	4.1	78.0
2018	Alberta	AB9	Fort Sask	Fort Sask	3.9	4.9	79.6	15.8	11.1	142.3	16.8	15	112.0	19.2	17.1	112.3	18.4	15.9	115.7	11.3	10.9	103.7	3.3	4.4	75.0
2018	Alberta	AB7	Grand Prairie A	Grand Prairie A	-1.4	4.1	(34.1)	13.7	10.2	134.3	14.7	14.3	102.8	16.3	16.2	100.6	15.3	15.1	101.3	5.2	10.2	51.0	3	3.6	83.3
2018	Alberta	AB2	Lacombe CDA 2	Red Deer	0.5	4.8	10.4	13.4	11.3	118.6	14.7	16.2	90.7	16.3	18.9	86.2	15.7	18.1	86.7	5.9	11.8	50.0	3.4	4.3	79.1
2018	Alberta	AB4	Peace River A	Peace River A	-1.3	3.6	(36.1)	13.8	10.8	127.8	14.7	15.5	94.8	16.1	17.8	90.4	15.1	17.1	88.3	4.2	11.1	37.8	2.1	3.7	56.8
2018	Alberta	AB5	Peace River A	Peace River A	-1.3	3.6	(36.1)	13.8	10.8	127.8	14.7	15.5	94.8	16.1	17.8	90.4	15.1	17.1	88.3	4.2	11.1	37.8	2.1	3.7	56.8
2018	Alberta	AB3	Strathmore AGDM	Calgary	0.1	4.6	2.2	14.8	9.7	152.6	15.1	13.7	110.2	17.6	16.5	106.7	17	15.8	107.6	7.2	11	65.5	4.5	5.2	86.5
2018	Alberta	AB8	Vauxhall CDA CS	Vauxhall North	2.3	6	38.3	15	11.4	131.6	16.8	15.4	109.1	19	17.7	107.3	17.9	17.4	102.9	9.6	12.4	77.4	5.5	5.7	96.5
2018	Manitoba	MB6	Carberry	Neepawa Southwest	0.3	4.3	7.0	14.2	11	129.1	18.8	15.5	121.3	19	17.6	108.0	18	16.9	106.5	9.7	10.9	89.0	1.6	3.6	44.4
2018	Manitoba	MB5	Pilot Mound	Pilot Mound 2	0	4.8	-	14	10.3	135.9	18.4	14.5	126.9	18.6	16.8	110.7	17.7	15.9	111.3	10	10.8	92.6	2.1	5	42.0
2018	Manitoba	MB10	Roblin	Gilbert Plains	-2.4	3	(80.0)	13.6	10.3	132.0	17.3	15.6	110.9	17.5	18.5	94.6	16	17.2	93.0	7.3	11.1	65.8	0.8	4	20.0
2018	Manitoba	MB1	Shoal Lake CS	Minnedosa	-3.2	4.2	(76.2)	13.6	11	123.6	17.7	16.7	106.0	17.7	18.8	94.1	16.2	18.1	89.5	7.9	12.7	62.2	0.9	4.9	18.4
2018	Manitoba	MB7	Shoal Lake CS	Minnedosa	-3.2	4.2	(76.2)	13.6	11	123.6	17.7	16.7	106.0	17.7	18.8	94.1	16.2	18.1	89.5	7.9	12.7	62.2	0.9	4.9	18.4
2018	Saskatchewan	SK5	Broadview	Broadview	-1.9	4.1	(46.3)	14	10.7	130.8	16.6	15.4	107.8	17.9	18.1	98.9	17.3	16.9	102.4	8	11	72.7	1.3	4	32.5
2018	Saskatchewan	SK6	North Battleford	North Battleford A	-0.1	3.8	(2.6)	13.9	10	139.0	17.4	14.3	121.7	17.8	16.3	109.2	16.7	14.9	112.1	6.9	9.6	71.9	3.1	2.8	110.7
2018	Saskatchewan	SK2	Pilger	Pilger	-3.6	4.1	(87.8)	13.9	11.8	117.8	17.2	17	101.2	17.7	19.2	92.2	16.3	18.6	87.6	6.7	13.1	51.1	1.5	4.9	30.6
2018	Saskatchewan	SK4	Regina RCS	Regina INT'L A	-0.6	2.3	(26.1)	15.1	9.8	154.1	17.3	15.1	114.6	18.6	17.3	107.5	18.3	16.8	108.9	8.5	10.5	81.0	2.3	3.2	71.9
2018	Saskatchewan	SK8	Saskatoon RCS	Saskatoon SRC	-0.7	5.2	(13.5)	14.3	11.8	121.2	17.3	16.1	107.5	18.7	19	98.4	17.1	18.2	94.0	7.4	12	61.7	2.5	4.4	56.8
2018	Saskatchewan	SK3	Saskatoon RCS	Saskatoon SRC	-0.7	5.2	(13.5)	14.3	11.8	121.2	17.3	16.1	107.5	18.7	19	98.4	17.1	18.2	94.0	7.4	12	61.7	2.5	4.4	56.8
2018	Saskatchewan	SK1	Yorkton	Yorkton A	-1.9	3.2	(59.4)	14	10.4	134.6	17.7	15.5	114.2	18.3	17.9	102.2	18.1	17.1	105.8	8	11.1	72.1	1.9	3.7	51.4
2019	Alberta	AB12	Lacombe CDA 2	Red Deer	3.9	2.3	169.6	9.8	9.8	100.0	12.8	15.1	84.8	15.2	17.3	87.9	14.2	16.8	84.5	10.5	10.5	100.0	1.9	3.2	59.4
2019	Alberta	AB13	Red Deer REGIONAL A	Red Deer	3.5	2.3	152.2	9.3	9.8	94.9	12.9	15.1	85.4	15.1	17.3	87.3	14.4	16.8	85.7	10.3	10.5	98.1	0.9	3.2	28.1
2019	Alberta	AB11	Three Hills	Trochu Town	3.7	4.8	77.1	8.9	10.6	84.0	13.6	14.7	92.5	15.4	16.9	91.1	14.6	16.5	88.5	10.4	10.9	95.4	0.8	4.4	18.2
2019	Alberta	AB15	Consort AGDM	Oyen Cappon	5	5.3	94.3	9.8	11.9	82.4	14.6	16.8	86.9	17	19.6	86.7	15.4	18.9	81.5	11.5	12.9	89.1	1.4	5.1	27.5
2019	Manitoba	MB19	Melita	Pierson	5.4	4.1	131.7	9.7	11.8	82.2	16.9	17	99.4	19.5	19.2	101.6	17.6	18.6	94.6	13.4	13.1	102.3	2.9	4.9	59.2
2019	Manitoba	MB17	Pilot Mound (AUT)	Pilot Mound 2	4.2	4.8	87.5	8.5	10.3	82.5	16.4	14.5	113.1	18.4	16.8	109.5	16.8	15.9	105.7	12.1	10.8	112.0	2.1	5	42.0
2019	Manitoba	MB14	Shoal Lake CS	Minnedosa	3.4	4.3	79.1	8.6	11	78.2	15.4	15.5	99.4	17.9	17.6	101.7	15.2	16.9	89.9	11.4	10.9	104.6	1.1	3.6	30.6
2019	Manitoba	MB13	Swan River RCS	Swan River	3.4	5.3	64.2	8.4	10.9	77.1	16	15.3	104.6	18.6	18.2	102.2	16.1	17.6	91.5	12.1	11.5	105.2	2.1	4.9	42.9
2019	Manitoba	MB18	Carberry CS	Neepawa Southwest	4.6	5.5	83.6	9.2	11.3	81.4	16.7	15.5	107.7	19.1	18.8	101.6	16.8	18	93.3	12.3	12	102.5	2.5	5.1	49.0
2019	Saskatchewan	SK18	Broadview	Broadview	4.1	4.1	100.0	8.4	10.7	78.5	15.7	15.4	101.9	17.7	18.1	97.8	15.5	16.9	91.7	11.7	11	106.4	1.2	4	30.0
2019	Saskatchewan	SK19	Eastend CYPRESS (AUT)	Swift Current A	4.5	3.1	145.2	8.6	10.3	83.5	14.4	15.5	92.9	17.1	18.1	94.5	16.8	17.1	98.2	11.4	11.3	100.9	0.4	4.2	9.5
2019	Saskatchewan	SK12	Melfort	Melfort CDA	3	2.8	107.1	8.8	10.7	82.2	15.3	15.9	96.2	16.9	17.5	96.6	14.9	16.8	88.7	11.2	10.8	103.7	1	3.3	30.3
2019	Saskatchewan	SK13	Melfort	Melfort CDA	3	2.8	107.1	8.8	10.7	82.2	15.3	15.9	96.2	16.9	17.5	96.6	14.9	16.8	88.7	11.2	10.8	103.7	1	3.3	30.3
2019	Saskatchewan	SK20	North Battleford	North Battleford A	3.9	3.8	102.6	9.5	10	95.0	15.3	14.3	107.0	17.2	16.3	105.5	15	14.9	100.7	11.8	9.6	122.9	1.1	2.8	39.3
2019	Saskatchewan	SK11	Saskatoon RCS	Pilger	4.8	4.8	100.0	9.7	10.3	94.2	16	14.5	110.3	17.8	16.8	106.0	15.4	15.9	96.9	12.3	10.8	113.9	0.8	5	16.0
2019	Saskatchewan	SK14	Wynyard (AUT)	Wynyard	3.7	3.6	102.8	8.5	10.6	80.2	15.4	15.5	99.4	17.7	17.9	98.9	15.3	17.4	87.9	11.9	11.1	107.2	1.5	3.7	40.5
2019	Saskatchewan	SK15	Yorkton	Yorkton A	3.9	3.2	121.9	7.8	10.4	75.0	16.1	15.5	103.9	18.2	17.9	101.7	15.9	17.1	93.0	12	11.1	108.1	1.7	3.7	45.9

*M indicates weather stations that were missing weather data for over 50 % for that month.

Table C. 4. Regional precipitation (mm) used to generalize weather conditions seen at field testing sites across western Canada. Source: Government of Canada Historical Climate Data.

					April			May			June			July			August			September			October		
Year	Province	Sample ID	Nearest town w/ weather station	Station used for Normal Data	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal	Site Year	Normal	% of Normal
2018	Alberta	AB1	Camrose	Camrose	11.7	28.9	40.5	41.9	41.2	101.7	33.3	74.4	44.8	70.8	85.8	82.5	30.4	51.5	59.0	51.7	39.9	129.6	17.5	23.5	74.5
2018	Alberta	AB9	Fort Sask	Fort Sask	2.0	24.3	8.2	12.2	43.1	28.3	13.1	80.0	16.4	46.2	92.0	50.2	14.3	55.4	25.8	46.8	40.8	114.7	4.0	20.6	19.4
2018	Alberta	AB7	Grand Prairie A	Grand Prairie A	16.4	19.8	82.8	5.8	41.0	14.1	103.2	75.9	136.0	127.2	76.1	167.1	70.6	55.8	126.5	52.0	43.0	120.9	33.6	26.0	129.2
2018	Alberta	AB2	Lacombe CDA 2	Red Deer	21.9	24.1	90.9	25.8	51.4	50.2	53.6	70.9	75.6	69.7	66.9	104.2	36.7	44.8	81.9	61.0	32.8	186.0	17.1	24.5	69.8
2018	Alberta	AB4	Peace River A	Peace River A	9.2	25.7	35.8	6.4	47.1	13.6	75.6	76.9	98.3	81.4	77.8	104.6	21.6	58.7	36.8	24.0	47.0	51.1	5.2	26.3	19.8
2018	Alberta	AB5	Peace River A	Peace River A	9.2	25.7	35.8	6.4	47.1	13.6	75.6	76.9	98.3	81.4	77.8	104.6	21.6	58.7	36.8	24.0	47.0	51.1	5.2	26.3	19.8
2018	Alberta	AB3	Strathmore AGDM	Calgary	16.5	25.2	65.5	9.8	56.8	17.3	48.8	94.0	51.9	23.8	65.5	36.3	28.5	57.0	50.0	22.2	45.1	49.2	18.8	15.3	122.9
2018	Alberta	AB8	Vauxhall CDA CS	Vauxhall North	M	25.1	0.0	M	40.0	0.0	M	72.9	0.0	M	32.9	0.0	16.8	32.9	51.1	21.8	34.9	62.5	10.7	14.4	74.3
2018	Manitoba	MB6	Carberry	Neepawa Southwest	5.5	25.8	21.3	27.4	32.8	83.5	96.6	65.0	148.6	70.9	74.1	95.7	28.4	57.9	49.1	75.1	34.1	220.2	36.9	16.1	229.2
2018	Manitoba	MB5	Pilot Mound	Pilot Mound 2	7.0	21.5	32.6	53.7	55.8	96.2	98.0	89.3	109.7	30.9	96.6	32.0	37.5	63.1	59.4	42.5	51.1	83.2	42.6	20.7	205.8
2018	Manitoba	MB10	Roblin	Gilbert Plains	6.7	28.1	23.8	77.7	58.9	131.9	180.2	85.6	210.5	101.2	76.4	132.5	19.7	70.2	28.1	40.2	53.3	75.4	27.3	36.4	75.0
2018	Manitoba	MB1	Shoal Lake CS	Minnedosa	4.3	25.4	16.9	14.8	68.4	21.6	93.5	80.3	116.4	50.2	83.7	60.0	25.1	61.9	40.5	19.3	45.3	42.6	16.9	30.2	56.0
2018	Manitoba	MB7	Shoal Lake CS	Minnedosa	4.3	25.4	16.9	14.8	68.4	21.6	93.5	80.3	116.4	50.2	83.7	60.0	25.1	61.9	40.5	19.3	45.3	42.6	16.9	30.2	56.0
2018	Saskatchewan	SK5	Broadview	Broadview	10.2	23.1	44.2	35.5	55.9	63.5	168.7	76.9	219.4	33.6	57.3	58.6	6.9	62.5	11.0	30.3	41.5	73.0	28.5	22.0	129.5
2018	Saskatchewan	SK6	North Battleford	North Battleford A	0.5	18.1	2.8	10.5	40.2	26.1	41.4	66.4	62.3	71.6	63.4	112.9	38.8	45.4	85.5	43.9	39.2	112.0	0.7	23.5	3.0
2018	Saskatchewan	SK2	Pilger	Pilger	8.4	25.6	32.8	24.3	75.0	32.4	37.7	92.9	40.6	14.3	82.1	17.4	17.1	72.5	23.6	35.8	44.8	79.9	5.2	35.9	14.5
2018	Saskatchewan	SK4	Regina RCS	Regina INT'L A	5.1	29.5	17.3	25.4	59.0	43.1	43.9	76.7	57.2	19.5	81.5	23.9	17.4	69.6	25.0	27.6	47.8	57.7	22.6	34.3	65.9
2018	Saskatchewan	SK3	Saskatoon RCS	Saskatoon SRC	9.1	21.8	41.7	35.0	36.5	95.9	19.9	63.6	31.3	31.1	53.8	57.8	17.2	44.4	38.7	37.1	38.1	97.4	8.0	18.8	42.6
2018	Saskatchewan	SK8	Saskatoon RCS	Saskatoon SRC	9.1	21.8	41.7	35.0	36.5	95.9	19.9	63.6	31.3	31.1	53.8	57.8	17.2	44.4	38.7	37.1	38.1	97.4	8.0	18.8	42.6
2018	Saskatchewan	SK1	Yorkton	Yorkton A	1.2	21.6	5.6	0.8	51.3	1.6	120.1	80.1	149.9	53.8	78.2	68.8	21.1	62.2	33.9	48.9	44.9	108.9	2.2	26.5	8.3
2019	Alberta	AB12	Lacombe CDA 2	Red Deer	9.7	29.5	32.9	25.6	59.0	43.4	80.7	76.7	105.2	91.5	81.5	112.3	36.7	69.6	52.7	34.2	47.8	71.5	7.4	34.3	21.6
2019	Alberta	AB13	Red Deer REGIONAL A	Red Deer	27.2	29.5	92.2	30.4	59.0	51.5	74.5	76.7	97.1	44.4	81.5	54.5	29.2	69.6	42.0	36.5	47.8	76.4	11.1	34.3	32.4
2019	Alberta	AB11	Three Hills	Trochu Town	11.3	19.7	57.4	49.7	50.5	98.4	131.4	78.8	166.8	64.8	77.0	84.2	39.9	60.9	65.5	39.0	45.1	86.5	6.1	13.4	45.5
2019	Alberta	AB15	Consort AGDM	Oyen Cappon	3.7	27.5	13.5	22.3	55.1	40.5	57.8	77.7	74.4	43.4	70.4	61.6	23.5	51.6	45.5	38.3	37.3	102.7	8.6	33.2	25.9
2019	Manitoba	MB18	Carberry CS	Neepawa Southwest	17.1	16.9	101.2	34.6	41.2	84.0	55.0	74.7	73.6	60.2	51.3	117.3	104.7	34.9	300.0	202.4	32.0	632.5	37.0	13.4	276.1
2019	Manitoba	MB19	Melita	Pierson	18.1	25.6	70.7	15.6	75.0	20.8	84.6	92.9	91.1	74.1	82.1	90.3	100.5	72.5	138.6	137.3	44.8	306.5	18.7	35.9	52.1
2019	Manitoba	MB14	Shoal Lake CS	Minnedosa	16.9	25.8	65.5	12.0	32.8	36.6	61.4	65.0	94.5	57.6	74.1	77.7	61.0	57.9	105.4	81.5	34.1	239.0	11.5	16.1	71.4
2019	Manitoba	MB13	Swan River RCS	Swan River	10.0	19.1	52.4	25.4	51.2	49.6	26.1	77.1	33.9	59.3	60.1	98.7	51.8	47.4	109.3	48.7	36.0	135.3	13.0	18.9	68.8
2019	Manitoba	MB17	Pilot Mound (AUT)	Pilot Mound 2	M	21.5	0.0	M	55.8	0.0	66.3	89.3	74.2	76.2	96.6	78.9	62.8	63.1	99.5	123.1	51.1	240.9	55.8	20.7	269.6
2019	Saskatchewan	SK18	Broadview	Broadview	24.1	23.1	104.3	10.9	55.9	19.5	97.9	76.9	127.3	79.4	57.3	138.6	56.8	62.5	90.9	98.8	41.5	238.1	18.6	22.0	84.5
2019	Saskatchewan	SK19	Eastend CYPRESS (AUT)	Swift Current A	28.9	34.7	83.3	25.2	54.1	46.6	85.9	85.4	100.6	17.5	95.6	18.3	46.9	76.8	61.1	54.7	53.4	102.4	12.1	33.7	35.9
2019	Saskatchewan	SK12	Melfort	Melfort CDA	4.1	26.7	15.4	18.8	42.9	43.8	87.4	54.3	161.0	72.7	76.7	94.8	30.7	52.4	58.6	43.0	38.7	111.1	11.9	27.9	42.7
2019	Saskatchewan	SK13	Melfort	Melfort CDA	4.1	26.7	15.4	18.8	42.9	43.8	87.4	54.3	161.0	72.7	76.7	94.8	30.7	52.4	58.6	43.0	38.7	111.1	11.9	27.9	42.7
2019	Saskatchewan	SK20	North Battleford	North Battleford A	9.8	18.1	54.1	11.2	40.2	27.9	66.2	66.4	99.7	70.8	63.4	111.7	25.1	45.4	55.3	42.4	39.2	108.2	4.5	23.5	19.1
2019	Saskatchewan	SK11	Saskatoon RCS	Pilger	0.4	21.5	1.9	4.4	55.8	7.9	84.8	89.3	95.0	67.6	96.6	70.0	20.3	63.1	32.2	39.5	51.1	77.3	11.2	20.7	54.1
2019	Saskatchewan	SK14	Wynyard (AUT)	Wynyard	9.3	20.4	45.6	11.7	46.2	25.3	109.6	71.9	152.4	121.9	70.4	173.2	28.9	57.5	50.3	57.2	40.7	140.5	10.3	26.9	38.3
2019	Saskatchewan	SK15	Yorkton	Yorkton A	17.6	21.6	81.5	11.3	51.3	22.0	75.6	80.1	94.4	49.9	78.2	63.8	31.0	62.2	49.8	53.6	44.9	119.4	9.1	26.5	34.3

*M indicates weather stations that were missing weather data for over 50 % for that month.