Monitoring Airborne Inoculum of *Sclerotinia sclerotiorum* at Canola Flowering and Relationships to Weather Conditions and Disease Incidence and Severity

by

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Abstract

Sclerotinia stem rot, caused by *Sclerotinia sclerotiorum*, is an important disease of canola (*Brassica napus*). Disease development is highly dependent on weather conditions and is initiated by infection of the petals by airborne ascospores, followed by mycelial progression into leaf and stem tissues. Improved stem rot forecasts would facilitate improved fungicide application decisions, reducing costs and enhancing disease control. Various methods to monitor airborne inoculum of *S. sclerotiorum* were compared and related to stem rot incidence and severity. These included a passive spore trap (Spornado) and petal test kits from private seed testing laboratories, as well as a GRIPST-2009 rotation impact sampler (rotorod). Canola petal samples were also tested for *S. sclerotiorum* infection by the Plant Health Lab, Alberta Agriculture, Forestry and Rural Economic Development. Petal infestation levels (coupled with weather variables) provided the strongest linear relationship to disease incidence (R^2 = 86%) and severity (R^2 = 87%) during early-mid flowering, when fungicide application decisions are made. However, the strength of the relationship varied during the flowering period. The airborne spore traps did not show as strong of a relationship to stem rot levels as the petal tests, with the Spornado accounting for 48% and 40% of the variation in disease incidence and severity, respectively, and the rotorod accounting for 52% and 50% of the variation. Significance testing of petal, Spornado and rotorod samples taken from five different locations within a field did not show different population means, suggesting that one sample per field may be sufficient for monitoring purposes; however, more testing is required, especially across fields of different sizes. Quantification of *S. sclerotiorum* DNA on petal and rotorod samples by quantitative PCR indicated that 1.0×10^{-4} ng DNA per canola petal or per cubic meter of air per hour during early flowering would result in a disease incidence >15%, the level at which fungicide application is generally recommended. Given the wide range of variables affecting Sclerotinia stem rot development, an integrated disease forecasting approach, which includes monitoring of ambient weather conditions and an inoculum detection method, should be employed to determine the optimal timing of fungicide applications.

Preface

This thesis is an original work conducted by Eleanor McBain. Ms. McBain handled all of the experiments and wrote the first drafts of all chapters. These drafts were inspected by Ms. McBain's supervisors, Dr. Thomas Kelly Turkington and Dr. Stephen Strelkov, who provided editorial revisions and recommendations for each chapter. Revisions were subsequently incorporated by Ms. McBain.

Agronomists with Nutrien, at their Fort Saskatchewan, Alberta, retail location assisted in the selection of fields near Edmonton, Alberta. Fungicide-free check strips were left by co-operating farmers for all locations near Edmonton. Ms. McBain, with assistance from fellow graduate students from the University of Alberta Plant Pathology Lab, summer students from Agriculture and Agri-Food Canada (AAFC) set up, removed, and disinfected all field equipment. The University of Alberta graduate students also assisted with petal and ascospore collections. All AAFC trial work was conducted by Dr. Turkington and collaborating AAFC and Alberta Agriculture, Forestry and Rural Economic Development (AAFRED) staff at participating locations. All quantitative PCR work was conducted by Dr. Jie Feng and associates at the AAFRED Plant Health Lab in Edmonton.

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1 Introduction

1.1 Stem Rot of Canola

Sclerotinia sclerotiorum (Lib.) de Bary is a fungal pathogen with a host range of over 400 different plant species, including economically important crops like canola (*Brassica napus*), alfalfa (*Medicago sativa*), pulses, and lettuce (*Lactuga sativa*) (Boland and Hall 1994). On canola, *S. sclerotiorum* causes Sclerotinia stem rot (SSR), an economically important disease across western Canada and other regions where the crop is grown. The formation of long-lived survival structures (sclerotia) by the pathogen, its wide host range, lack of host resistance, and the production of windborne ascospores contribute to the widespread occurrence of SSR and make its management particularly challenging.

The SSR pathogen overwinters as sclerotia, and when these structures are exposed to optimal soil temperature and moisture, they can germinate either myceliogenically or carpogenically (Willetts and Wong 1980). Myceliogenic germination results in vegetative mycelium, while carpogenic germination produces ascospores via sexual recombination in cup-like apothecia (Willetts and Wong 1980). Ascospores are released in response to changes in environmental conditions and are forcibly ejected from the apothecium once spores are mature; the slightest breeze then picks them up (Bolton et al. 2006). Upon landing on a susceptible host, free moisture and an exogenous source of nutrients are required for infection to occur (Willetts and Wong 1980). In canola, the colonized, senescing inflorescence is the primary source of nutrients for disease progression (Jamaux et al. 1995), and plants are most susceptible to infection when the flowers are fully open but before they start to abscise (Turkington et al. 1991). Canola leaves, particularly senescing lower leaves, or other flower parts such as the stamens can also serve as alternative routes for infection (Almquist and Wallenhammar 2015; Huang and Kokko 1992; Jiang 2001). Infection and further disease development are highly dependent on weather conditions, with the most important being relative humidity and temperature. Although relative humidity and temperature can favor SSR when both are in the optimal range for the pathogen, they can also limit or halt disease progression when outside of the optimal range (Clarkson et al. 2003; Willetts and Wong 1980; Young et al. 2004).

Management options for SSR are limited, given the variable nature of the disease, the absence of fully resistant crop varieties, persistent sclerotia, its extensive host range, and the

windborne dispersal of ascospores to adjacent fields. Consequently, farmers rely on the use of fungicide during flowering to try to limit the impact of SSR. However, the variable occurrence of the disease and fluctuations in ascospore levels during flowering can lead to inconsistent fungicide responses, which can be cost-ineffective (Turkington 1991). This is because the cost of the management method (fungicide) is greater than the increased economic returns from improved yields resulting from application (Ficke et al. 2018). Another significant concern with the routine application of fungicides is the potential for selection of fungicide resistant *S. sclerotiorum* populations, some of which have already been found in Canada (Gossen et al. 2001) and China (Duan et al. 2014; Zhou et al. 2014).

Given the variable nature of SSR, assessing disease risk and the need for fungicide application in canola can be challenging. Consequently, a range of forecasting methods for SSR that focus on different aspects of the disease cycle have been developed and utilized. In Canada, the Canola Council of Canada historically recommended an easily accessible and easy to use risk point table developed in the 1980s by Thomas (1984), although more recently, the risk point table has been based on a similar risk assessment scheme developed in Sweden (Twengstrom 1998). These risk tables or checklists evaluate all components of the disease triangle and assign risk values depending on how important these are for SSR development. Over the years, weather-based forecasting systems have been used in several countries. In western Canada, a weather-based system was available from the mid-1990s to the early-mid 2000s and this system focused on soil moisture and its impact on the potential for germination of sclerotia (McLaren et al. 2004). Similar approaches were developed for North Dakota in the early 2000s (Lamey et al. 2003; McLaren et al. 2004) and have been further refined over the last 15-20 years, based primarily on rainfall and temperature conditions as well as factors related to crop and disease history (North Dakota State University 2009; Shahoveisi et al. 2020). In the United Kingdom, Young et al. (2020) reported a 26% reduction in unnecessary fungicide applications with the use of their live Sclerotinia risk reporting system. This system monitors forecasted weather for optimal conditions for SSR development during crop flowering, although it tended to be risk-averse in situations where a high amount of disease incidence was predicted (Young et al. 2020).

A focus on scouting for the presence of apothecia of *S. sclerotiorum* in canola and bean crops has been suggested as a method of assessing SSR risk (Boland 1984; Boland and Hall 1988; Gugel 1985; Gugel and Morrall 1986). However, the identification of apothecia in the field by

farmers and crop scouts can be cumbersome and problematic; moreover, significant levels of disease can occur in the absence of apothecia given potential clustering of sclerotia and apothecia within a field, the wind-borne nature of the ascospores and ability to travel to adjacent fields (Gugel and Morrall 1986). Subsequent research demonstrated the utility of agar plate assessments of the level of petal infestation by *S. sclerotiorum* (Gugel and Morrall 1986; Morrall and Thomson 1991; Turkington et al. 1991; Turkington and Morrall 1993). While agar plate testing is available, it has had limited adoption by growers due to the time required for the collection and processing of samples and delays in getting results; it can take up to 7 days for adequate growth of *S. sclerotiorum* to occur for subsequent identification. Over the last 20 years, further refinements in relation to petal testing have occurred with the development to DNA-based testing methodologies for detection of *S. sclerotiorum* from canola petals (Almquist and Wallenhammar 2015; Freeman et al. 2002; Parker et al. 2014; Rogers et al. 2008; Yin et al. 2009; Ziesman et al. 2016).

1.2 Hypothesis and Objectives

The main objectives of my research were as follows:

1) To develop a better understanding of how environmental conditions (i.e., relative humidity, temperature, and rainfall) influence changes in *S. sclerotiorum* inoculum load over the flowering period of canola and how these affect final SSR incidence and severity
2) To investigate the potential utilization of spore traps and DNA-based petal testing to assess airborne inoculum load as a means of forecasting SSR risk and determine if a quantitative measure of risk is required for accurate predictions

I hypothesize that: (1) there will be variability between years and regions in relation to ascospore load; (2) inoculum load and petal infestation will change over the flowering period of canola, which will influence SSR risk; and (3) spore traps and DNA-based petal testing can be utilized as field-specific forecasting tools, although environmental conditions will also have to be monitored to predict SSR development risk accurately.

2 Literature Review

2.1 Introduction

Globally, over 27 million metric tonnes of canola (rapeseed; *Brassica napus* L.) were produced in 2019-2020, with Canada, the European Union, and China leading in production (Statista 2021). In Canada, canola is second only to wheat in terms of production, with just under 19 million metric tonnes grown in 2020 (Statistics Canada 2021). Canola acreage and production have increased since the development of this crop in the 1970s, due to improved genetics, herbicide resistance technology, higher yield potential and commodity prices, its unique nutritional properties, and its multiple uses as a high-quality edible oil, biodiesel, and animal feed (Raymer 2002). As canola acreage has increased, so has disease pressure due to *Sclerotinia sclerotiorum* (Lib.) de Bary, an economically important fungal pathogen that occurs in every canola/rapeseed growing region worldwide (Zheng et al. 2020). Shorter rotations with non-host crops, the persistence of its resting structures (sclerotia), and its wide host range have resulted in *S. sclerotiorum* epidemics that occur with increasing frequency. Infection by *S. sclerotiorum* has the potential to reduce yield by 0.5% for every 1% increase in disease incidence (Del Rio et al. 2007). Practical predictions of the risk of Sclerotinia stem rot (SSR) have been notoriously difficult to achieve because environmental conditions heavily influence disease progression, and disease levels vary between years, regions, and fields (Morrall and Dueck 1982; Willetts and Wong 1980).

Given variability in the incidence and severity of SSR in different years, effective risk assessment and appropriate fungicide use decisions can be difficult. The reliance of farmers on fungicide application reflects the limitations in other cultural and host-based management options, which are not particularly effective (Boland and Hall 1987; Jurke 2003; Williams and Stelfox 1980). Nonetheless, fungicide application is only cost-effective when yield increases justify control costs (Ficke et al. 2018). Consequently, researchers have attempted to develop forecasting methods to predict the risk of *S. sclerotiorum*, thereby providing farmers with potentially more reliable indicators of stem rot risk on which to base their management decisions.

Initial forecasting methods in the Prairie region of Canada focused on a stem rot checklist developed by Thomas (1984), which is based on pathogen characteristics, cropping variables,

and environmental conditions, i.e., components of the disease triangle. Initially, this checklist was the basis for the risk point system recommended for use in Canada, but it has since been modified by incorporating aspects of a Swedish risk assessment tool (Twengstrom 1998), as well as by including other stem rot-related factors (Canola Council of Canada 2020a). Other methods include forecasts that focus solely on weather-based factors and use logistic regression to predict disease incidence (Clarkson et al. 2014; Harikrishnan and Rio 2008; McLaren et al. 2004; North Dakota State University 2009; Sharma et al. 2015; Young et al. 2020). More recently, methods have been developed that integrate various technologies, including the pairing of petal testing and spore traps with quantitative polymerase chain reaction (qPCR) analysis, to quantify the amount of *S. sclerotiorum* DNA present during flowering (Almquist and Wallenhammar 2015; Parker et al. 2014; Rogers et al. 2009; Yin et al. 2009; Young et al. 2020; Ziesman et al. 2016).

2.2 History and Importance

The impact of *Sclerotinia sclerotiorum* on canola production is significant. Undetected early infections can cause lodging and premature ripening, both of which directly damage yield and seed quality (Kamal et al. 2016). Lodging can result from the colonization of the stem and pith tissues by the pathogen, whereby cell-wall degrading enzymes produced by *S. sclerotiorum* result in significant weakening of canola stems, causing the plant to bend and snap at the site of lesions (Bolton et al. 2006). Extensive stem lesion development also can cause girdling of the stem and disruption of the integrity of the vascular tissue, which leads to fewer and smaller seeds, reduced yield, premature ripening of seed pods that shatter during harvest, and contamination of seeds with sclerotia (Adams and Ayers 1979; Ficke et al. 2018; Morrall and Dueck 1982). In contrast, late-stage SSR infections typically result in very little yield loss, with only a few pods affected (Bolton et al. 2006). Other impacts are related to production losses, where SSR risk is underestimated or as the result of negative net returns due to a lack of yield responses that do not cover the cost of fungicide, labour, and fuel required for application (Kamal et al. 2016). Poorly applied fungicides may also not be effective in reducing the incidence of SSR, resulting in a reduction in yield on top of the cost of the control measures (Kamal et al. 2016). Del Rio et al. (2007) estimated that a 17% disease incidence is approximately equal to the cost of fungicide, which is similar to values reported by Turkington

(1991) and Turkington et al. (1993). Surveys conducted since 2016 suggest a decline in the incidence of SSR in Alberta (Anonymous 2021; Harding et al. 2020) and Manitoba (Anonymous 2021), perhaps because the summer of 2016 was one of the 10 wettest on the Prairies since 1948 (Government of Canada 2017) and hence very favorable for disease development. In contrast, in Saskatchewan, a steady increase in the incidence of SSR has been reported since 2017 (Anonymous 2021; Peru et al. 2020). Despite the fluctuations in stem rot on a regional level, the disease remains one of the most important for Canadian canola growers (Zheng et al. 2020).

2.3 Biology

2.3.1 *Sclerotinia Sclerotiorum*

2.3.1.1 Distribution, Taxonomy and Host Range

Belonging to the family Sclerotiniaceae in the phylum Ascomycota, *S. sclerotiorum* is an important fungal pathogen causing similar diseases known by many different names, including white mould, stem rot of canola/oilseed rape and blossom blight, among others (Bolton et al. 2006). While it is especially favoured in temperate climates like Canada, Northern Europe, and the United Kingdom, the pathogen is found in almost every region of the world, including drier, warmer regions of the United States of America, South Africa, Southern Europe, India, and Australia (Adams and Ayers 1979; Purdy 1979; Rothmann and McLaren 2018; Sharma et al. 2016; Willetts and Wong 1980). Genetically diverse populations of *S. sclerotiorum* exist in different geographic areas and within the same field (Kohli et al. 1992). This is because *S. sclerotiorum* is homothallic, allowing sexual recombination to occur during apothecial production (Bolton et al. 2006). Isolates of *S. sclerotiorum* from different geographical regions have been shown to have different temperature requirements for carpogenic germination, depending on the temperature at which they were formed, causing some isolates to require a cool conditioning period for germination if they originated from regions with warmer climates (Huang and Kozub 1991; Nepal and del Río Mendoza 2012; Uloth et al. 2015; Willetts and Wong 1980; Wu and Subbarao 2008).

Three major species have been described within the family Sclerotiniaceae: *Sclerotinia sclerotiorum* (Lib.) de Bary*, Sclerotinia minor* (Jagger), and *Sclerotinia trifoliorum* (Erikss) (Purdy 1979). Characteristics common to these species include the development of long-lived

hyphal aggregate resting structures called sclerotia, the production of apothecia, and the lack of a conidial stage (Willetts and Wong 1980). Willets and Wong (1980) summarized the morphology of each species and concluded that they do in fact represent three separate species, which can be distinguished based on sclerotium size, ascospore size and ascus size, despite suggestions that there is too much variation within these characteristicsfor species identification (Morrall et al. 1972). While host range had previously been suggested as a method of species identification, the host range of all three species is large and mostly non-specific, so it is not adequate for this purpose (Willetts and Wong 1980). To aid in the identification of the three main species within the *Sclerotinia* genus, Ekins et al. (2005) developed restriction fragment length polymorphism (RFLP) probes that could distinguish between *S. sclerotiorum*, *S. minor* and *S. trifoliorum*. The authors noted that although molecular identification was consistent with the findings of Willetts and Wong (1980), i.e. sclerotial size and host can be sufficient for identification in the field (Ekins et al. 2005).

The stem rot pathogen was first described in 1837 by Madame M. A. Libert as *Peziza sclerotiorum*, but was later renamed *Sclerotinia libertiana* by L. Fuckel in 1870 in a tribute to Madame Libert (Purdy 1979). This nomenclature was used until 1934, when it was found to conflict with the International Rules of Botanical Nomenclature. Thus, *S. libertiana* was changed to *Sclerotinia sclerotiorum* (Lib.) de Bary, as Anton de Bary had used the binomial in 1884 (Purdy 1979).

An index of susceptible hosts of *S. sclerotiorum* was published by Boland and Hall (1994) and included over 400 species across various genera and families. Of these, most are herbaceous plants within the Dicotyledonae subclass, including the families Brassicaceae, Asteraceae and Fabaceae, although a few woody plants have also been reported as hosts. At the time of compilation of this index, there were also 25 hosts reported in the Monocotyledonae sub-class (Boland and Hall 1994).

2.3.1.2 Symptoms

Traditionally, infection and symptom development by *S. sclerotiorum* has been associated with petal drop; however, infections can occur at any point during the host life cycle. In canola, if the plant has been previously wounded, symptoms can appear before flowering, or a

late-stage infection can be seen as lesions on pods (Abawi and Grogan 1979). Senescing petals are the most common plant part to transport germinated ascospores into healthy tissue in the canopy, but other organs, such as stamens, pollen, anthers, filaments and leaves, can also be colonized (Huang and Kokko 1992; Qandah and del Río Mendoza 2011; Stelfox et al. 1978; Young and Werner 2012).

Characteristic symptoms on canola begin as bleaching or development of water-soaked lesions under areas where infected plant parts have fallen and adhered (Abawi and Grogan 1979; Bolton et al. 2006; Jamaux et al. 1995; Willetts and Wong 1980). These areas tend to be on leaves, leaf axils, and the stems in the lower third of the plant, although any aboveground plant part can be infected (Bolton et al. 2006; Qandah and del Rio Mendoza 2012; Young and Werner 2012). Under favourable conditions, lesions can be fully developed within 48-72 h, but this can be expedited by other stress factors, and older lesions take on a shredded appearance (Ficke et al. 2018; Willetts and Wong 1980). As the disease continues to develop, the pathogen colonizes the pith, where sclerotia will form after several days (Bolton et al. 2006; Kamal et al. 2016; Willetts and Wong 1980). Other symptoms that appear after the stem has been infected include premature ripening, girdling, shredding and shattering of infected tissues, and lodging (Bolton et al. 2006; Kamal et al. 2016). If stem rot is severe and moist conditions occur, fluffy, white mycelium will begin to form on the outside surface of the plant, with sclerotia eventually developing (Bolton et al. 2006; Willetts and Wong 1980).

2.3.2 Disease Cycle

2.3.2.1 Sclerotia and Apothecia Formation

The sclerotia are critically important in the disease cycle, with *S. sclerotiorum* spending 90% of its life in this stage. Sclerotia formation occurs asexually at the end of the season, and are considered as primary survival and reproductive structures, overwintering in adverse conditions for several years (Adams and Ayers 1979; Derbyshire and Denton-Giles 2016; Willetts and Wong 1980). The sclerotia develop in three distinct phases: initiation, growth, and maturation (Townsend and Willetts 1954). Initiation includes the formation of sclerotial primordials on mycelial mats within the pith of the canola. Initiation is followed by the growth phase, where the sclerotia reach their maximum size. The last and final phase is maturation,

which includes pigmentation and dehydration (Willetts and Wong 1980). The fully developed hyphal aggregate is oval and can vary in size from 3-10 mm (Willetts and Wong 1980). The mature sclerotium consists of three regions. The first region, a black outer rind, aids in protection and longevity, consists of sealed hyphal tips (Bell and Wheeler 1986; Willetts and Wong 1980). The second region contains an almost indiscernible layer of interwoven hyphae, called the cortex (Willetts and Wong 1980). The largest and innermost region, the medulla, is hyaline, comprised of prosenchymatous cells, and consists mainly of carbohydrates (Townsend and Willetts 1954; Willetts and Wong 1980).

Sclerotia are long-lived in the soil, surviving anywhere from 4-10 years, although estimates in the field are hard to gather (Adams and Ayers 1979). Generally, longevity is affected by several factors, including temperature, soil moisture, soil microorganisms, and burial depth. Stable conditions have little to no effect on survival, but high temperatures (≥ 35˚C) and saturated soil for an extended period reduce the survival of sclerotia in soil (Adams and Ayers 1979; Ćosić et al. 2012; Grogan and Abawi 1975; Matheron and Porchas 2005).

Biological activity within the soil has the most detrimental effect on sclerotia (Adams and Ayers 1979; Willetts and Wong 1980). Over 30 species of microorganisms have an antagonistic or parasitic effect on sclerotia of *S. sclerotiorum*, with the most notable being *Coniothyrium minitans, Bacillus subtilis,* and species of *Trichoderma* (Adams and Ayers 1979; Kamal et al. 2016; Willetts and Wong 1980). The literature regarding the influence of burial depth on sclerotium viability is contradictory and may reflect crop management practices. Some researchers have reported a reduction in viability with increased burial depth (between 10 and 20 cm) (Kurle et al. 2001; Rakesh and Singh 2015), while others found a reduction in viability mainly within the top 5 cm of soil (Ćosić et al. 2012; Willetts and Wong 1980). In either case, there can be a reduction of viability because of increased wet and dry cycling and increased degradation due to microorganisms (Bolton et al. 2006; Ćosić et al. 2012; Kurle et al. 2001; Rakesh and Singh 2015; Willetts and Wong 1980). Sclerotia occur in clusters within a field, residing where they fell after the plants were harvested or decayed. However, sclerotia can be transported in irrigation runoff to other fields, in contaminated seed, or in contaminated soil carried on machinery (Abawi and Grogan 1979; Adams and Ayers 1979; Schwartz and Steadman 1978).

Sclerotia can germinate myceliogenically or carpogenically when exposed to optimal conditions (Le Tourneau 1979; Willetts and Wong 1980). Myceliogenic germination produces mycelium, which can only infect plants found within a very short distance of the sclerotium (Bardin and Huang 2001). While myceliogenic germination can occur in canola crops, it is not the primary means of infection. Carpogenic germination of sclerotia results in the formation of apothecia, the sexual fruiting bodies, which produce ascospores that can infect aboveground susceptible tissue up to several hundred metres away. Qandah and del Rio Mendoza (2012) reported that significant levels of the disease occur mostly within 25 m of the source, although plants as far as 40 m away can develop symptoms of infection (Bardin and Huang 2001; Ben-Yephet and Bitton 1985; Qandah and del Rio Mendoza 2012).

Initiation of stipes or carpophores on the sclerotium is termed carpogenic germination. The expansion of the tip of the stipe into a fully differentiated disc represents apothecium formation. Each stipe above the soil surface produces a single apothecium, which is a cup-shaped, concave disc up to 10 mm in diameter (Willetts and Wong 1980). Generally, under optimal lab conditions, stipes can appear within 25 days, with mature apothecia in 35 days (Wu and Subbarao 2008). Successful carpogenic germination depends on two key factors: temperature and moisture, although burial depth and the temperature at which the sclerotia formed are also contributing factors. Carpogenic germination of sclerotia requires continuous moisture for about 10 days, with fluctuations in moisture inhibiting germination (Abawi and Grogan 1979; Nepal and del Río Mendoza 2012). The optimum range is between 95%-100% soil saturation, with sclerotia imbibing 80% of their water content within 5 h of being buried in the soil (Nepal and del Río Mendoza, 2012). A sclerotium can produce stipes when it has taken in 70%-80% of its total water content, which can occur in soils kept as low as 25% water saturation. Carpogenic germination can occur well below field capacity (Nepal and del Río Mendoza 2012; Teo and Morrall 1985). Exposure to extreme conditions, such as prolonged dry periods or flooding, has a detrimental effect on carpogenic germination (Adams and Ayers 1979; Grogan and Abawi 1975; Morrall 1977; Wu and Subbarao 2008). Sclerotia exposed to extreme drought for more than 10 days stopped germination completely, although new stipes were produced after a 35-day rewetting period (Wu and Subbarao 2008).

Matheron and Porchas (2005) reported a significant reduction in sclerotial viability following a flooding event over an extended period. Regardless of soil type, no sclerotia

germinated when they were exposed to continuous soil moisture for 3-4 weeks at mean soil temperatures of 30-33˚C. At temperatures between 40˚C and 50˚C, no viable sclerotia were recovered after 1-2 weeks of exposure (Matheron and Porchas 2005). Ćosić et al. (2012) observed similar results when sclerotia were exposed to above average precipitation for a month under field conditions. In the top 5 cm of soil, only 50% of recovered sclerotia produced stipes after 27 days (Ćosić et al. 2012).

The ambient temperature required for carpogenic germination ranges between 5˚C - 25˚C, with a reduction in germination or abnormal stipe formation outside of this range (Clarkson et al. 2004; Hao et al. 2003). Exposure to 30˚C did not cause a significant reduction in already germinated sclerotia (Wu and Subbarao 2008), while Grogan (1979) noted that there was no stipe formation on sclerotia exposed to 30 ˚C for 21 days (Abawi and Grogan 1979). The range in temperature requirements has been attributed to the geographic origin and the ambient temperature during sclerotium formation (Bardin and Huang 2001). Researchers have found that sclerotia formed in cooler climates germinate more readily (and without a cool conditioning requirement) than sclerotia formed in warm climates, which have a cool conditioning requirement to germinate (Abawi and Grogan 1975; Hao et al. 2003; Huang and Kozub 1991; Uloth et al. 2015; Willetts and Wong 1980; Wu and Subbarao 2008).

The stipes produced from successful carpogenic germination are positively phototropic and will not differentiate further into an apothecium without exposure to light, although stipes alone can be formed in the dark (Honda and Yunoki 1977; Le Tourneau 1979; Willetts and Wong 1980; Wu and Subbarao 2008). This ensures that only sclerotia near the soil surface (top 5 cm) will form apothecia (Abawi and Grogan 1979; Willetts and Wong 1980). Other factors affecting apothecial formation are moisture and temperature. Apothecium formation can be inhibited entirely under slight moisture tension (Abawi and Grogan 1979). Prolonged dry periods can stop apothecial production, but it can resume after a return to optimal moisture conditions (Wu and Subbarao 2008). Stipes develop within the 5˚C to 25˚C range, but apothecia only form between 10˚C and 20˚C (Willetts and Wong 1980). Temperatures outside of this range can prevent apothecium formation in already germinated sclerotia (Willetts and Wong 1980).

In canola, suitable temperature and moisture conditions are essential for sclerotial germination and apothecium formation. The potential for a favourable microenvironment occurs

once the canopy closes and rainfall occurs, allowing for stable microenvironmental conditions in the upper soil layers and within the canopy itself, encouraging rapid growth and expansion of *S. sclerotiorum* (Turkington and Morrall 1993; Williams and Stelfox 1980). This favourable microenvironment is typically cool and moist, with the canopy providing shade, while helping to maintain high relative humidity and water potential in the top few centimetres of the soil (Bolton et al. 2006; Morrall and Dueck 1982; Turkington, Morrall, Rude 1991).

2.3.2.2 Ascospore Development and Release

As the apothecia form and mature, they differentiate into layers and have the appearance of a golf tee (Willetts and Wong 1980). Attached to the stipe is the base layer (hypothecium) and a top layer called the hymenium, containing rows of spore sacs (asci) bounded by paraphyses (Le Tourneau 1979; Willetts and Wong 1980). In each ascus, self-fertilization occurs, resulting in the formation of eight ascospores that are forcibly released into the air above the apothecia at a maximum rate of 1600 spores per h (Abawi and Grogan 1979; Bolton et al. 2006; Clarkson et al. 2003; Willetts and Wong 1980). In still air, the apothecium will mass discharge spores in short bursts, called 'puffing' (Willetts and Wong 1980). This forms a column of ascospores in the air above the apothecium, ensuring that the slightest air current can take up ascospores. In turbulent air and high relative humidity, ascospores are released continuously for 4-6 h (Clarkson et al. 2003; Qandah and del Río Mendoza 2011; Willetts and Wong 1980). Although ascospores can potentially travel several kilometres (Abawi and Grogan 1979; Williams and Stelfox 1979), Qandah and Del Rio Mendoza (2012) found that it was only within 25 m of the source where infection levels were economically significant (Qandah and del Rio Mendoza 2012). Previously, Ben-Yephet and Bitton (1985) noted that most (77%-90%) ascospores landed within 100 m from where they were released (Ben-Yephet and Bitton 1985). Under optimal conditions, each apothecium can produce and release 3×10^7 ascospores over a period of 9-20 days, but this varies depending on environmental conditions and the size and maturity of the apothecia (Abawi and Grogan 1979; Clarkson et al. 2003; Schwartz and Steadman 1978).

Ascospore release is highly dependent on environmental conditions, but especially changes in relative humidity. Spore release can be triggered by slight moisture tension, changes in relative humidity, temperature, and even windy conditions (Abawi and Grogan 1979; Bolton

et al. 2006). Temperature has a more indirect effect on spore release, which can occur over a wide range from 4˚C to 32˚C (Qandah and del Río Mendoza 2011). Instead, temperature changes were closely tied to relative humidity changes, which have a more significant influence on release (Qandah and del Río Mendoza 2011). Under low temperatures (5 – 10˚C), few ascospores were detected, but as temperatures increased, so did spore levels (Clarkson et al. 2003). Similarly, fewer spores were detected at low relative humidity (RH; 65%-75%) versus high RH (90%-95%) (Clarkson et al. 2003). Relative humidity and temperature may affect processes within the apothecium, thereby influencing spore release. Clarkson et al. (2003) concluded that apothecia die more quickly at higher temperatures, which increase the rate of spore maturity, allowing more spores to be ready for release over a shorter period. In contrast, fewer spores may be released over a longer period in cooler conditions, because the apothecia survive longer (Clarkson et al. 2003; Qandah and del Rio Mendoza 2012). Rain may also contribute to ascospore release by prolonging high relative humidity; however, rainfall may push airborne spores to the ground, or prevent release entirely by capturing spores in water droplets that have accumulated on top of the apothecia (Qandah and del Río Mendoza 2011; Turkington et al. 1991).

Hartill (1980) reported that light also impacts ascospore release. During a 3-year experiment performed with a Hirst-type spore trap, most *S. sclerotiorum* ascospores were captured over a 2-3 h period between either 11 am to 1 pm or 12 pm to 2 pm in tobacco crops in New Zealand (Hartill 1980), suggesting diurnal fluctuations in atmospheric ascospore levels (Turkington et al. 1991). However, since ascospore release is tied tightly to environmental conditions, other research has suggested that there are higher levels of ascospores during the day because of the changes in temperature and relative humidity, and that in some cases, spore release could occur at night if that was when the conditions of release were met (Qandah and del Río Mendoza 2011). Qandah and del Rio Mendoza (2011) found that during dry years, most spores were collected between 2 am - 7 am and that nighttime release lasted longer (10 am - 1 pm) when compared with daytime release during wet years (Qandah and del Río Mendoza 2011). Clarkson et al. (2003) found that spore release occurred continuously under consistent lab conditions, no matter the light regime. In contrast, Ben-Yephet and Bitton (1985) and Turkington et al. (1991) showed that ascospore loads and petal infestation levels, respectively were highest around mid-day, as reported by Hartill (1980).

2.3.2.3 Plant Infection

Ascospores can survive outside of the apothecium for about 2 weeks under favorable conditions (Caesar and Pearson 1983; Grogan and Abawi 1975). Outside of the apothecium, mortality is dependent on temperature, relative humidity (RH) and ultraviolet (UV) radiation, with only a 22% survival rate after 4 days of exposure on the tops of bean leaves in the field (Caesar and Pearson 1983). Researchers have found that high temperatures (≥ 25˚C) paired with RH > 35% caused ascospore mortality rates to increase, with mortality positively correlated with temperature and RH (Caesar and Pearson 1983; Clarkson et al. 2003). Caesar and Pearson (1983) demonstrated that UV light and therefore placement in the canopy had an effect on ascospore survival; 20% more viable ascospores occurred on shaded bean leaves lower in the canopy than on the top of the canopy (Caesar and Pearson 1983).

Ascospore germination can occur on the host surface, but for disease progression to occur, continuous free moisture and wounded, dying, or senescent tissue is required (Jamaux et al. 1995; Shahoveisi and del Rio Mendoza 2020). In canola, infection begins with the colonization of flower petals, although most flower parts (anthers, stamens, pollen) and leaves can be infected by ascospores (Almquist and Wallenhammar 2015; Ficke et al. 2018; Jamaux et al. 1995; Jiang 2001). The petals eventually senesce and fall into the canopy, landing on leaves and branches, which provide the perfect microclimate for disease progression (Turkington and Morrall 1993). The first symptoms appear under colonized petal tissues, where the pathogen has spread from infected petals into healthy tissue (Jamaux et al. 1995). High temperatures and dry periods can slow infection and further development of stem rot (Grogan and Abawi 1975; Shahoveisi and del Rio Mendoza 2020). The infected petals provide the nutrients that the spores require to germinate and enter healthy host tissue. The pathogen quickly spreads from the leaves, leaf axils and bases into the stem tissues including the pith, where sclerotia will eventually form asexually from the mycelium (Lumsden 1979).

2.4 Management

2.4.1 Chemical

The application of a fungicide during early to full bloom of canola (BBCH 61 – 65; (Canola Council of Canada 2020b) is the most effective form of control, since no fully resistant varieties are available. While various foliar fungicides are registered, proper timing and application are essential for good efficacy (Bradley et al. 2006; Kutcher and Wolf 2006; Turkington and Morrall 1993; Turkington et al. 2011). For fungicides to prevent ascospore-mediated plant infection effectively, they must be applied before symptoms begin to appear (Steadman 1979). Twelve fungicides are registered for the control or suppression of SSR of canola (Crop Protection Guide for Alberta 2022). These fungicides belong to groups 2 (dicarboximides), 3 (triazoles), 7 (carboxamides) and 11 (strobilurin), and are formulated as suspensions, suspension concentrates, wettable granules and emulsifiable concentrates (Alberta Agriculture and Forestry 2020). They are registered for one to two applications at 20% bloom and 50% bloom, and all reduce final disease pressure.

Given limited management options, farmers have turned to routine fungicide applications for SSR control; however, ascospore levels and the occurrence of favourable weather conditions can vary seasonally and over locations and years, causing many routine applications to be economically inefficient (Ficke et al. 2018; Turkington and Morrall 1993). A fungicide application is considered cost-effective when the amount of yield gain and associated returns exceed the cost of chemical and application, and is warranted when disease incidence >17% is forecasted (Del Río et al. 2007; Ficke et al. 2018)

Another significant problem with routine fungicide applications is the potential for shifts in pathogen resistance to the chemicals used. Gossen et al. (2001) reported the first fungicide resistant population of *S. sclerotiorum* in Canada, with isolates found that showed resistance to benomyl (Benlate). At that time, this product was used extensively for SSR control in alfalfa and canola (Gossen et al. 2001). More recently, isolates of *S. sclerotiorum* with resistance to fungicides in groups 2 (dicarboximides) and 12 (phenylpyrroles) have been reported from China (Duan et al. 2014; Zhou et al. 2014). The emergence of fungicide resistant populations of *S. sclerotiorum* highlights the need for integrated pest management strategies (IPM), better

forecasting measures, prudent fungicide application, rotation of different fungicide groups, and the use of products with multiple modes of action.

2.4.2 Cultural

Crop rotation with non-hosts can be an effective management strategy to reduce the amount of primary inoculum in the field. In fact, Twengström et al. (1998) found that the odds of high disease incidence increased by a factor of 13 if there had been high disease pressure in the previous crop. Nonetheless, there is evidence that while crop rotation does reduce disease pressure, it is an ineffective management strategy on its own. Morrall and Dueck (1982) found that the sclerotia still germinate to produce ascospores in cereal crops grown for three consecutive years after rapeseed, which can infect any susceptible weeds in the field or surrounding ditches, negating attempts to reduce the number of viable sclerotia in soil. Ascospores can also be carried to neighboring fields on wind or by bees on infected pollen grains, rendering rotation less effective (Morrall and Dueck 1982; Schwartz and Steadman 1978; Williams and Stelfox 1979).

Research on the effect of different tillage regimes (deep ploughing vs. minimum vs. no-till) on sclerotial viability is often contradictory, although there are two clear findings: tillage affects the distribution of sclerotia in the soil profile and parasitism via soil microbial activity reduces sclerotia viability. Some researchers have reported that deep ploughing in the fall reduces disease pressure the following growing season by burying the sclerotia, inhibiting carpogenic germination and apothecium production (Abawi and Grogan 1975; Schwartz and Steadman 1978). However, if a second tillage event occurs, it can redistribute viable sclerotia back to the soil surface (Kurle et al. 2001; Steadman 1979). Minimum tillage or zero-till operations result in clusters of sclerotia scattered throughout the field, with little to no redistribution in the soil profile (Kurle et al. 2001). Sclerotia in the top 5 cm of the soil are more likely to germinate and form apothecia, but are also at greater risk of degradation due to weather and soil moisture fluctuations, and increased parasitism due to higher organic matter in the soil (Abawi and Grogan 1975; Garza et al. 2002; Rakesh and Singh 2015). Sclerotia exposed to different tillage regimes, combined with different burial depths for various lengths of time, have

been reported to show variable viability and apothecial production (Ćosić et al. 2012; Garza et al. 2002; Rakesh and Singh 2015; Wu and Subbarao 2008).

Soil fertility is another important component of stem rot management. It is clear that nitrogen (N) and potassium (K) have both individual and combined effects on the incidence and severity of SSR in canola/rapeseed. Zhang et al. (2020) described the effects of sufficient K supplied with a moderate supply of N as "profound" on mitigating the negative effects of SSR on seed yield, although the mechanisms surrounding the effects are unclear. When compared with the sole application of N, the combination of N and K reduced SSR incidence by 9.9% – 24.4% and 17.4% – 37.9% in the first and second years of the study, respectively. Consequently, Zhang et al. (2020) suggested a combination of 180 kg N ha^{-1} and 120 – 180 kg K_2O ha^{-1} to alleviate some symptoms of SSR, including smaller lesion length, and an increase in host resistance and the number of pods per plant and seeds per pod.

Other methods of cultural control targeting sclerotial viability in the soil are flooding and burning. Flooding has limited practicality for Prairie canola production, but when the soil was flooded continuously for 2-3 weeks, the viability of sclerotia in the top 5 cm was significantly reduced (Ćosić et al. 2012; Matheron and Porchas 2005). One study found that fall burning in alfalfa was effective at reducing sclerotia in the top 2 cm of soil by 95%, with the remaining sclerotia degrading steadily over the following year (Gilbert 1991). Research that is more recent suggests, however, that fire would not burn uniformly in a field or create sufficiently hot temperatures to kill the sclerotia (Kutcher and Malhi 2010). Moreover, burning of crop residues would contribute to soil degradation, nutrient loss due to volatilization, and loss of snow trapping, leading to reduced soil moisture in the spring. Kutcher and Malhi (2010) concluded that there was generally no yield increase and no consistent effects on SSR levels in the following year if the previous crop stubble had been burnt.

Manipulation of crop phenology to create an unfavourable microenvironment for SSR development includes a later seeding date and altering the canopy density by using a lower seeding rate or wider row spacing. The efficacy of these tactics is variable, because the effects of crop manipulation may not be the same across years, fields or host varieties. This could result in yield loss due to a shorter growing period, reduced plant density and/or increased weed pressure, which would negate attempts to reduce disease as the weeds may become infected by *S.*

sclerotiorum (Ziesman 2016). Seeding later in the spring reduces the overlap between the infective stage of the stem rot pathogen and the susceptible stage of canola, potentially reducing the incidence of SSR (Morrall and Dueck 1982). Jurke and Fernando (2008) found that a higher seeding rate led to an increased potential for the crop to lodge, increasing plant-to-plant spread of *S. sclerotiorum* by mycelia, although seeding rate was not significantly correlated with disease incidence. Gugel and Morrall (1986), Turkington and Morrall (1993) and Turkington (1991) did not find strong relationships between seeding rate or stand counts and SSR levels. A higher seeding rate can also lead to a more conducive microenvironment for SSR development, given a higher relative humidity in dense canopies and longer periods of leaf wetness favorable for apothecium formation and ascospore germination (Jurke and Fernando 2008; Jurke 2003; Turkington and Morrall 1993). Kumar et al. (2015) found that a row spacing of 45 cm compared with 30 cm or 15 cm reduced stem rot incidence, but could also result in an increase in weed pressure due to less competition from the canola crop (Kumar et al. 2015). While a less favorable microclimate for SSR is ideal, Kutcher et al. (2013) reported that when row spacing was increased from 23 cm to 61 cm, there was a 26% decrease in plant density and an 11% reduction in yield.

When using cultural control methods to reduce the amount of *S. sclerotiorum* inoculum in the soil, it is crucial to employ an IPM strategy that includes weed control. Many broad leaf weeds found in Canada are hosts of the stem rot pathogen, including dandelion (*Taraxacum officionale)*, sow thistle (*Sonchus spp.*) and Canada thistle (*Cirsium arvense* (L.) Scop.) (Boland and Hall 1994). Vrandečić et al. (2003) found that velvetleaf (*Abutilon theophrasti* Medik.) and ragweed (*Ambrosia artemisiifolia* L.) in Croatia produced isolates of *S. sclerotiorum* that had the potential to be even more pathogenic on susceptible crops than the isolates collected from susceptible crop plants. Without an appropriate weed control strategy, most cultural methods to reduce SSR pressure could be ineffective, since the weeds would negate the effects of practices such as crop rotation or increased row spacing.

2.4.3 Biological

At least 30 species of insects, fungi and bacteria feed or parasitize on *S. sclerotiorum* (Adams and Ayers 1979). Biocontrol mechanisms include the parasitism of sclerotia,

mechanical obstruction, nutrient and space competition, antibiosis and the induction of host resistance to reduce the incidence of stem rot (Adams and Ayers 1979; Kamal et al. 2016; Smolińska and Kowalska 2018). Successful biocontrol requires an effective delivery system and survival of the biocontrol agent under field conditions (Sundh and Goettel 2013). However, due to regulatory challenges and a lack of success under field conditions, there are only a few biological control organisms approved for SSR management in Canada. A recent review of biological control agents by Smolińska and Kowalska (2018) concluded that while biocontrol methods are safer for the environment, their effectiveness is limited and they should be incorporated as part of an integrated management strategy that also includes chemical control, crop rotation and resistant cultivars (Smolińska and Kowalska 2018).

In Canada, two biological agents are currently registered and available for use for control of stem rot in canola: Contans WG (PCP# 29066, Bayer CropScience) and Serenade OPTI (PCP# 31666, Bayer CropScience) (Alberta Agriculture and Forestry 2020). The active ingredient in Contans WG is *Coniothyrium minitans*, a fungal mycoparasite applied to the soil at least 3 months before the field is to be planted (Sutton and Peng 1993), or used as a foliar biofungicide that can be applied directly to the crop during flowering (Smolińska and Kowalska 2018). *Coniothyrium minitans* parasitizes the sclerotia, thereby reducing the amount of primary inoculum (Huang et al. 2000). The active ingredient in Serenade OPTI is *Bacillus subtilis*, a bacterium that inhibits hyphal growth of *S. sclerotiorum*, and which can be applied as a seed coating or as a biofungicide during flowering (Hu et al. 2014).

The effectiveness of biocontrol agents registered in Canada is variable, with their efficacy highly dependent on field conditions, amount of sclerotia in the soil, tillage regimes and the crop planted. For example, a two-year study by Manitoba Agriculture (2010) showed that treatment with Contans WG (*C. minitans)* reduced levels of Sclerotinia white mould on carrots, while treatment with Serenade (*B. subtilis*) did not have a significant effect on disease (Manitoba Agriculture). In another study by Faechner et al. (2011), variable efficacy was reported for both Contans WG and Serenade, which was attributed to the timing, rate and method of incorporation of the biocontrol agents, as well as the soil conditions and the presence of inoculum blown in from neighboring fields (Faechner et al. 2011).

2.4.4 Host Plant Resistance

Resistance to *S. sclerotiorum* remains elusive, and no current host varieties offer complete protection from the pathogen. In previous studies, Young and Werner (2012) and Jiang (2001) tested apetalous rapeseed varieties to determine if a lack of canola petals was an effective avoidance mechanism to escape stem rot infection. Jiang (2001) found that while apetalous varieties developed some stem rot, it was less than either partially apetalous or fully petalled varieties. However, there was no significant difference in the severity of the infections between an early flowering, fully petalled variety and the apetalous varieties (Jiang 2001). Young and Werner (2012) confirmed the results from Jiang (2001), showing that fully petalled varieties developed more disease than apetalous varieties. They also found that infection of the apetalous varieties resulted from colonized, senescent stamens that adhered to the leaves (Young and Werner 2012). Kutcher et al. (2001) also found that the apetalous canola variety cv. Hylite 201 had lower stem rot levels versus two fully petalled canola varieties. However, the two petal canola varieties had higher yields even with higher sclerotinia, and this was likely due to cv. Hylite 201 being lower yielding and having a lower canopy density (T.K. Turkington personal communication). Kutcher et al. (2003) also found that between the two petalled varieties SSR levels were higher for the variety that was more lodging susceptible).

There has been limited success in finding canola germplasm with a high level of resistance (Young and Werner 2012). This is because resistance to *S. sclerotiorum* is polygenic and quantitative (Mei et al. 2020; Sharma et al. 2015). Researchers have identified strong partial resistance in relatives of *B. napus*, including *B. oleracea* and *B. carinata* (Mei et al. 2011). Recent advances in technology, including mapping of quantitative trait loci (Navabi et al. 2010), sequencing of the *Brassica napus* genome (Mei et al. 2013) and marker-assisted selection, have led researchers closer to developing a resistant variety of *B. napus*. Mei et al. (2020) successfully crossed a partially resistant *B. napus* cultivar 'Zhongshuang 9' with a highly resistant genotype of *B. incana*, a wild relative of *B. oleracea*, generating two highly resistant *B. napus* lines. Using marker-assisted selection, they found that the progeny plants expressed higher resistance than the parental lines only after pyramiding three quantitative trait loci (QTL) responsible for major resistance (Mei et al. 2020). Currently, there are only three registered canola varieties offering resistance or improved tolerance to stem rot of canola in Canada. However, all three require a fungicide application when conditions are conducive for SSR and

high disease pressure is forecasted (Canola Council of Canada 2020). Brett Young® offers two varieties on the 'DefendR' trait platform, ‘6074 RR' and ‘6076 CR', which have improved tolerance to stem rot (BrettYoung 2016). Pioneer® offers one variety, 'VR9561GS', rated as ‘resistant’ on the Protector trait platform (Pioneer 2021).

2.5 Forecasting and Risk Assessment Tools for Sclerotinia Stem Rot

Creating a reliable, fast, and accurate forecasting system for SSR is a global effort, as *S. sclerotiorum* occurs in all canola-growing regions and year-to-year disease severity is difficult to predict (Bolton et al. 2006). The implementation of forecasting tools can lead to increased confidence in SSR management decisions, such as applying fungicide only when it is cost-effective for the farmer. Forecasting tools can also reduce environmental impacts by eliminating unnecessary fungicide applications, reduce the risk of the pathogen developing resistance to fungicides, and provide better economic returns for farmers. While many forecasting systems have focused on only one or a few critical components of the disease triangle, multiple factors should be considered, including weather, inoculum pressure, petal infestation, crop density and field-specific data (Turkington and Morrall 1993; Twengström et al. 1998).

Currently, there are several different methods for forecasting SSR. These methods can be regional or field-specific, vary in complexity and labour intensity, and target various aspects of the disease cycle. Although there have been advances in relation to SSR forecasting, the nature of the disease makes it difficult to develop one method suitable for all growing regions. Attempts at forecasting SSR began in Canada in the 1980s with a checklist based on factors believed to affect disease incidence, in order to aid farmers in spray timing decisions (Thomas 1984). Since then, research to provide more efficient and accurate tools for informed SSR management decisions has continued, but many factors need to be considered for a successful forecasting tool (Bom and Boland 2000).

2.5.1 Prediction Methods Based on Petal Infestation and Airborne Inoculum

2.5.1.1 Airborne Inoculum Sampling Methods

Traditionally, air samplers used Sclerotinia selective media (SSM) or Vaseline coated slides to capture airborne inoculum, which would then be subjected to culturing or light

microscopy for pathogen identification (Rogers et al. 2009). However, the disadvantages with culturing and microscopy-based approaches, such as the three-day turnaround for results from culturing and the expertise required for correct fungal identification under light microscopy, have driven researchers to find more precise and efficient methods for capturing and identifying airborne *S. sclerotiorum* inoculum (Parker et al. 2014; West and Kimber 2015). These include innovative approaches such as combining air sampling with molecular diagnostic techniques such as qPCR and biosensing to allow for more efficient and accurate quantification of airborne inoculum (Almquist and Wallenhammar 2015; Freeman et al. 2002; Reich et al. 2016b; Rogers et al. 2009; Shoute et al. 2018; West and Kimber 2015). Incorporating air sampling equipment into forecasting methods can enable the early detection of an epidemic by providing information on the presence of ascospores before flowering begins (Freeman et al. 2002; Parker et al. 2014). This can allow farmers to optimize their management strategies without having to wait for flowers to be fully open before utilizing a petal test to aid in a management decision.

There are currently two types of spore trap air samplers used to monitor airborne inoculum in crops; active and passive spore traps (Mahaffee 2014; West and Kimber 2015). Passive spore traps have the simplest mechanisms that allow airborne inoculum to be funnelled through to a specialized filter that can trap spores, or be passively impacted onto an adhesive surface (West and Kimber 2015). Active spore traps are volumetric and use mechanical means, such as a rotating arm or vacuum, to collect airborne inoculum (West and Kimber 2015). An advantage of an active spore trap is that the number of particles per cubic meter of air can be calculated, since the spore traps can be set to run for a specific time and are independent of wind speed and direction (West and Kimber 2015). Other air samplers have been thoroughly reviewed by West and Kimber (2015).

Parker et al. (2014) compared three air sampling methods for the detection and quantification of *S. sclerotiorum* ascospores in carrots, including two active spore traps (Anderson air sampler and Burkard volumetric sampler) and a single passive trap (Blue Plate test). Parker et al. (2014) found that the most efficient was the Burkard volumetric sampler, which could be combined with qPCR to give results within 5 h of air sampling. In contrast, the Anderson air sampler consistently captured lower numbers of ascospores than the Blue-Plate test or the Burkard sampler, causing the authors to remove it from the study. A limitation of the Blue-Plate test, which passively deposits ascospores from bioaerosols onto a SSM, was the 3 day

incubation period required to confirm the presence of ascospores; it was also not quantitative (Parker et al. 2014).

The Burkard sampler is the most commonly used spore trap to collect aerosol samples of *S. sclerotiorum* in research studies. It can be used with various post-sampling techniques to quantify the number of ascospores in a sample, is easy to use, and can be left in the field for a week at a time (Almquist and Wallenhammar 2015; Parker et al. 2014; Reich et al. 2016a; Reich et al. 2016; West and Kimber 2015; Young et al. 2020). While air sampling can allow for early detection, most equipment still requires samples to be sent to a lab to be processed (Shoute et al. 2018). Multiple samples must also be collected up to and during the flowering period to determine disease risk accurately, while also monitoring environmental conditions (Turkington et al. 1991). The location of the air sampler and the time of deployment are also critical factors for determining SSR risk in a field (West and Kimber 2015).

2.5.1.2 Conventional Techniques

Petal infestation is the main path for pathogen ingress into a healthy host, and is a critical step in life cycle of *S. sclerotiorum* (Jamaux et al. 1995; Morrall and Dueck 1982; Willetts and Wong 1980). The level of petal infestation has shown to be positively correlated with final SSR disease pressure, is more accurate than weather-based algorithms or risk point systems, is field-specific and provides a quantitative estimate of infestation (Makowski et al. 2005; Turkington et al. 1991). Variability in the statistical relationship between petal infestation and disease level has been attributed to several factors, including environmental conditions leading up to and during the flowering period, time of petal collection, cropping variables, crop canopy density, crop stage and changes in inoculum pressure (Turkington et al. 1991; Turkington et al. 1991; Turkington and Morrall 1993).

Agar plate testing of colonized canola petals has been one of the traditional methods used to identify and predict stem rot in canola. Commercially developed by Morrall and Thomson (1991) at the University of Saskatchewan, Saskatoon, SK, agar plate testing includes collecting canola petals at early bloom (BBCH 61) onto potato dextrose agar (PDA) to identify fungal colonies visually after a 3-5 day incubation period to predict yield loss. Turkington et al. (1991) correctly predicted 73% of forecasts based on early bloom agar plates, with the greatest accuracy

in fields given a low disease risk. However, the authors noted that the accuracy of the forecasts would have improved if petal infestation had been reassessed throughout the bloom period, to account for changes in petal infestation due to weather fluctuations (Turkington et al. 1991). Currently, petal test kits based on the protocol developed by Morrall and Thompson (1991) are commercially available to Canadian growers (Discovery Seed Labs Ltd., Saskatoon SK). Petal testing detects pathogen inoculum produced internally or externally on the petal tissues, and is less labour-intensive than looking for apothecia that may not be in the same field (Gugel and Morrall 1986). However, there are some disadvantages associated with this technique. At least two tests are required between the first fully open flower and 50% bloom, which can be labour intensive as 160 petals from 4-10 different sites are recommended for the published plate assay (Morrall and Thomson 1991; Turkington et al. 1991) and 128 petals are required for a commercial version of the test (Discovery Seed Labs Ltd.). The incubation time needed for fungal growth is also a disadvantage, whereby the 5-7 day period can preclude timely fungicide use decisions. The period available for fungicide application is relatively short, and farmers must wait for the flowers to be fully open (BBCH 61) before conducting a petal test (McLaren et al. 2004). Finally, skill in the identification of the fungal colonies is required, as misidentification can lead to incorrect predictions, resulting in an unwarranted fungicide application (Rogers et al. 2009)

2.5.1.3 Molecular Techniques

Molecular based techniques have advanced tremendously over the last decade and are becoming the dominant method for detection and quantification of *S. sclerotiorum* in canola (Almquist and Wallenhammar 2015; Freeman et al. 2002; Parker et al. 2014; Reich et al. 2016; Rogers et al. 2009; Yin et al. 2009; Ziesman et al. 2016). This is because there are many advantages to using a molecular-based technique, including eliminating the incubation time required for culturing on media, a reduction in misidentification caused by human error or inexperience, and the ability to test for the presence of the pathogen in different substrates, like plant or soil or in collections of airborne spores (Almquist and Wallenhammar 2015). Some companies are molecular techniques (Discovery Seed Labs, Saskatoon, SK; Quantum Genetix,

Saskatoon, SK) to determine the concentration of *S. sclerotiorum* DNA in infected petals and the disease risk.

Quantitative PCR assays were developed for the detection and quantification of *S. sclerotiorum* DNA in air and plant tissue samples (Almquist and Wallenhammar 2015; Parker et al. 2014; Rogers et al. 2009; Yin et al. 2009; Ziesman et al. 2016). While they have the sensitivity and selectivity to detect as few as one ascospore per sample, extensive testing is required to ensure that the assays can distinguish between closely related *Sclerotinia* species and other similar fungi, like *Botrytis cinerea* (Ziesman 2016). Another consideration is the genetic diversity between *S. sclerotiorum* populations, requiring validation of new assays with regional collections of the pathogen (Kohli et al. 1992). Ziesman et al. (2016) developed a qPCR assay for the rapid detection and quantification of *S. sclerotiorum* DNA in canola petals from western Canada. The reported limit of detection was 8.0×10^{-4} ng of pathogen DNA, which was equivalent to 2.3 ascospores per petal (1 ascospore = 3.5×10^{-4} ng DNA (Rogers et al. 2009)). However, the measurement of *S. sclerotiorum* DNA in canola petals can reflect increases in pathogen biomass resulting from ascospore germination (hyphal development), so pathogen quantity should be expressed in nanograms of DNA. The assay of Ziesman et al. (2016) was highly specific for *S. sclerotiorum* and was able to differentiate between other species of *Sclerotinia*, as well as *B. cinerea*. To develop a reliable forecasting system based on this qPCR assay, however, the relationship between petal infestation and final SSR disease levels needs to be determined.

While qPCR for the detection and quantification of *S. sclerotiorum* DNA is a valuable tool, this method does have some drawbacks. Quantitative PCR analysis can be costly and requires specialized equipment, making its application most feasible for professional researchers with access to laboratory infrastructure (Shoute et al. 2018). Nonetheless, on the Canadian Prairies, several private sector seed testing labs have developed molecular-based expertise and offer a range of DNA-based tests for various pathogens including the causal agents of Fusarium head blight, clubroot, blackleg and Sclerotinia stem rot. As with agar plate testing of petal samples, some researchers have noted changes in petal infestation over the bloom period, suggesting that multiple petal samples must be evaluated to obtain an accurate assessment of inoculum levels (Almquist and Wallenhammar 2015; Turkington and Morrall 1993; Ziesman 2016).

2.5.2 Forecasts Based on Weather, Management Practices and Field Specific Data

2.5.2.1 Risk Point Tables

Risk point tables or 'checklists' are the oldest forecasting method for predicting *S. sclerotiorum* in western Canada. In these checklists, 'risk points' are awarded based on the presence/absence of factors shown to affect disease development, including factors related to environmental conditions, cropping history and previous disease pressure (Thomas 1984; Twengström et al. 1998). The accumulated points are then compared with a threshold value; if the points are greater than the threshold value, the recommendation is to apply a fungicide. The Canola Council of Canada recommends using a risk point table developed by Twengstrom et al. (1998) for spring-seeded rapeseed in Sweden. In this table, the factors considered include the number of years since the last host crop, SSR incidence in the last host crop, crop density, rainfall over the past two weeks, the current weather forecast and regional risk for apothecial development. If a score is > 40 points, a fungicide application is recommended (Canola Council of Canada 2020). Evaluations of this risk point table conducted in 1996-1997 indicated that a recommendation to spray was given in 75% of fields where it was required, and in 16% of fields where it was not (Twengström et al. 1998). Risk tables such as this are quick, field-specific, easy to access and use, and account for factors shown to have a significant effect on SSR development (Shoute et al. 2018). Nonetheless, checklists also have some shortcomings, including a lack of predictive power and an inability to account for ascospore spread (Derbyshire and Denton-Giles 2016; Turkington 1991).

While risk point tables provide farmers with a more informed idea of whether or not a fungicide application is warranted, they do not incorporate wind-dispersed ascospores into their predictions; windborne ascospores have been shown to contribute to SSR disease pressure, and hence their exclusion may influence the accuracy of a risk assessment (Turkington 1991; Twengström et al. 1998). Furthermore, Ficke et al. (2018) found that precipitation, particularly when measured at a weather station kilometers away from the field in question, is not a reliable predictor of stem rot infection in canola. In the same study, Ficke et al. (2018) suggested incorporating petal and leaf tests and soil moisture into a risk point table to estimate inoculum availability, as they these also influence stem rot incidence.

2.5.2.2 Forecast Maps and Risk Algorithms

Forecast maps for SSR are no longer produced for Canada, but they have been the basis for similar systems found in the United Kingdom and the United States. Weather-based forecast maps are used to monitor the risk of SSR development during the flowering period of canola (McLaren et al. 2004). Forecasts are generated up to 72 h in advance, giving farmers time to make a spray decision and an application, depending on the stage of their crop. The forecast maps used in Canada were described by McLaren et al. (2004), with three different maps generated twice a week from June until the end of July. The three maps included a map of the risk forecast, a map of growing degree-days, and a top-zone soil moisture map. A collaboration in 2001 between Manitoba, North Dakota and Minnesota further developed the risk maps to incorporate more factors influencing the risk of SSR on canola (McLaren et al. 2004). North Dakota State University (2009) has since updated its forecast system to include a 'Risk Calculator,' which allows a farmer to input cropping variables to create a more field-specific recommendation. A 'live' SSR forecast map that issues an alert when weather conditions are optimal for disease development during flowering (≥ 7˚C and ≥ 80 % RH for 23 consecutive hours) is also used in the United Kingdom (Agriculture and Horticulture Development Board 2021). While a weather-based forecasting system alone reduced unnecessary fungicide applications by 26% in the UK, it is considered a risk-averse system that can overestimate the occurrence of a high risk of sclerotinia (Young et al. 2020). Incorporating an inoculum-based forecast tool can turn a regional-based forecast into a field-based one and confirm the weather-based alert, as inoculum within the field is variable over the flowering period (Makowski et al. 2005; Turkington and Morrall 1993).

A crop loss-related model developed by Koch et al. (2007) in Germany gave recommendations based on climate, crop rotation and economy. While the model was 70% accurate in Germany, further investigations deemed the tool was not sufficiently accurate for use in other countries (Almquist and Wallenhammar 2015). However, Ficke et al. (2015) suggested that the use of petal and leaf assessments combined with in-crop microenvironment monitoring, measured in infection hours as demonstrated by Koch et al. (2007), could significantly improve prediction models.

Clarkson et al. (2014) developed a model for the progression of white mould in lettuce, based on an infection court concept. This model suggested there are micro-sites on leaf axils that provide conducive conditions for ascospore germination, even in dry conditions under the canopy. This model used mathematical relationships between weather factors and spore density during crucial stages of the disease cycle to predict the disease incidence within a population (Clarkson et al. 2014). Clarkson et al. (2014) pointed out that ascospore density is essential for disease progression; therefore, combining the simulation model with a method for determining ascospore density will be required to validate the model for field conditions. A logistical model to predict SSR incidence using only weather-based factors was developed by Sharma et al. (2015) for oilseed brassicas in India. Although the model produced accurate predictions (93%), it did not account for inoculum in the field (Sharma et al. 2015). A logistic model that was developed for predicting white mould in bean was able to accurately predict disease incidence using weather-based factors when incidence was high (>20%), but overestimated risk when it was low (Harikrishnan and Rio 2008).

2.6 Conclusion

More research is needed to improve SSR forecasting system. Ultimately, successful forecasting of this disease should utilize ascospore detection as a field-specific indicator of inoculum and incorporate environmental conditions into the forecasts; disease progression cannot occur if there are no ascospores present and/or if weather is not conducive. For farmers, such a system should be cheap, accessible, accurate, reliable, and easy to use. Insights into the relationships between environmental conditions, inoculum load over the flowering period, and final SSR incidence can help to develop forecasting systems that provide farmers with the information needed to make informed and timely spray decisions.

3 Influence of Environmental Conditions on *Sclerotinia sclerotiorum* Inoculum Pressure and Final Disease Levels

3.1 Introduction

Sclerotinia sclerotiorum, a necrotrophic, polyphagous fungus, causes the important canola (oilseed rape; *Brassica napus*) disease known as sclerotinia stem rot (SSR). In Canada, SSR is found across all canola-growing regions, but its incidence and severity are closely tied to environmental conditions, especially June and July precipitation. For example, 2016 was one of the wettest years on record, and all three Prairie Provinces had higher SSR disease incidence than in previous years; this was followed by a sharp decrease in incidence in 2017 due to record-breaking hot and dry weather (Anonymous 2021; Government of Canada 2017; Government of Canada 2018). The sporadic occurrence of the disease can be attributed to the fact that every stage of the *S. sclerotiorum* lifecycle has a specific set of environmental conditions required for progression into the next stage (Willetts and Wong 1980).

The persistent resting and primary reproductive structures (sclerotia) of the pathogen are found in the soil, overwintering for several years until conditions are favourable for germination (Abawi and Grogan 1979). While the temperature at which germination occurs can vary for sclerotia of isolates from different regions, the overall range is quite broad. Therefore, the main factor affecting germination and apothecium formation remains the same, i.e., soil moisture (Adams and Ayers 1979; Ćosić et al. 2012; Hao et al. 2003; Huang and Kozub 1991; Matheron and Porchas 2005; Nepal and del Río Mendoza 2012; Uloth et al. 2015; Willetts and Wong 1980; Wu and Subbarao 2008). Carpogenic germination of sclerotia, or production of stipes, requires continuous moisture, with the optimal range of soil saturation between 95-100%, although germination can occur well below field capacity (Nepal and del Río Mendoza 2012; Teo and Morrall 1985). Exposure to extreme conditions, such as extended dry periods or flooding, has a detrimental effect on both the sclerotia and apothecia, although apothecium formation can resume once conditions have returned to optimal after a dry period (Abawi and Grogan 1979; Adams and Ayers 1979; Ćosić et al. 2012; Matheron and Porchas 2005; Wu and Subbarao 2008). The ambient temperature required for carpogenic germination ranges between 5 – 25 ˚C, with reduced or abnormal stipe formation outside of this range (Clarkson et al. 2004; Hao et al. 2003). After the formation of stipes, differentiation into cup-shaped apothecia occurs when the stipes

are exposed to light, although this process can be inhibited entirely by slight moisture tension (Abawi and Grogan 1979; Honda and Yunoki 1977; Willetts and Wong 1980; Wu and Subbarao 2008). The ambient temperature required for the formation of apothecia is between 10 – 20 ˚C, with temperatures outside this range inhibiting already germinated sclerotia (Willetts and Wong 1980). Ascospores develop in the hymenium layer of the apothecia, with the potential of each apothecium to produce 3×10^7 ascospores over 9-20 days, depending on conditions, size, and maturity of the sclerotia (Clarkson et al. 2003; Le Tourneau 1979; Schwartz and Steadman 1978; Willetts and Wong 1980).

Spore release is closely associated with environmental conditions and depends on several factors, including moisture tension, relative humidity (RH) and wind (Abawi and Grogan 1979; Bolton et al. 2006). Temperature is indirectly related to ascospore release, which can occur between 4 – 32 ˚C. Instead, temperature influences RH, which has a direct influence on spore release. Fewer spores are detected at low temperatures (5-10 ˚C) and low RH (65% - 75%), with the number of spores released increasing as temperature and RH increased (Clarkson et al. 2003; Qandah and del Río Mendoza 2011). Temperature can also influence the longevity of an apothecium, with higher temperatures increasing the rate of ascospore maturity, allowing more spores to be released in a short period of time (Clarkson et al. 2003; Qandah and Del Rio Mendoza 2012). Rain can contribute to ascospore release by lengthening periods of relative humidity, but can also push airborne spores to the ground and trap spores in water droplets accumulated on top of the apothecia (Qandah and del Río Mendoza 2011; Turkington 1991). Outside of the apothecium, ascospore survival is dependent on temperature, RH and ultraviolet (UV) radiation (Caesar and Pearson 1983; Turkington 1991). High temperatures ($\leq$ 25°C), paired with a RH of $>$35%, caused spore mortality rates to increase, with mortality increasing further as RH and temperature increased (Caesar and Pearson 1983; Clarkson et al. 2003; Turkington 1991). UV radiation also impacts spore survivability, with Caesar and Pearson (1983) observing that 20% more ascospores survived on the lower leaves within a bean canopy versus leaves in the upper canopy, which was exposed more extensively to solar radiation (Caesar and Pearson 1983).

To initiate ascospore germination and infection, continuous free moisture is required. Wounded or senescent tissue in the form of canola petals is also needed, although other flower parts can also be colonized by *S. sclerotiorum* (Almquist and Wallenhammar 2015; Ficke et al.

2018; Jamaux et al. 1995; Jiang 2001; Shahoveisi and del Rio Mendoza 2020). When infested canola petals fall through the canopy and are caught on leaves, leaf axils and bases, they provide the nutrients required for the ascospores to germinate and for the pathogen to infect the plant (Jamaux et al. 1995). Clarkson et al. (2014) observed that under periods of high RH (70% - 100%), the first symptoms of SSR appeared within 5-7 days at 20˚C under laboratory conditions. Symptoms developed more slowly, and fewer plants became infected, when the RH was < 70% (8-12 days) (Clarkson et al. 2014), which was consistent with a report by Koch et al. (2007) indicating that 80% RH was the threshold at which stem infections occurred in oilseed rape.

While the significance of environmental conditions on the development of stem rot cannot be overlooked, the pathogen must also be present in a particular area, in sufficient quantities, for disease to occur. This is why monitoring only environmental conditions from a weather station kilometers away can over- or underestimate disease pressure (Ficke et al. 2018; Harikrishnan and Rio 2008; Koch et al. 2007; Sharma et al. 2015; Young et al. 2018). Conversely, monitoring only ascospore pressure or petal infestation, especially at one point in time over the flowering period, may also be misleading for assessing the risk of SSR, since petal infestation and weather conditions favourable for host infection can change over time (Clarkson et al. 2014; Makowski et al. 2005; Turkington and Morrall 1993; Turkington 1991; Ziesman 2016). A forecasting system would ideally implement all possible factors contributing to epidemics of SSR, but primarily environmental and field-specific factors, like inoculum pressure, to produce a reliable, accurate and efficient forecast easily accessible to farmers.

The main objective of this chapter was to explore (statistical) relationships between ambient and in-canopy temperature and RH and determine whether data collected from nearby weather stations was consistent with field-specific conditions. Specific objectives included (1) to determine if inoculum pressure and final disease incidence were related to RH and temperature conditions, (2) to assess the relationship between results from a passive spore trap and those of a quantitative volumetric trap and determine how measurements from both types of traps were related to weather conditions and final disease levels, and (3) to evaluate three different methods of petal testing utilizing different qPCR methodologies and compare them with weather conditions and final disease levels. The ultimate goal was to determine if spore traps alone could accurately predict SSR, or if petal tests were also required.

3.2 Materials and Methods

3.2.1 Field Selection and Site Locations

Commercial fields were selected for this study based on the following criteria: fields had to have had symptoms of *S. sclerotiorum* infection within the last 3 years if possible, no tall trees or shelterbelts across the north side of the field to allow prevailing winds to pass unencumbered, and an SSR-resistant variety could not be growing in the selected field during the year of the study. No fields were used consecutively over the two years of the study, but all were located within 50 km of Edmonton, AB. In 2019, four fields were selected on June 17th with assistance from Dr. T. K. Turkington (AAFC Lacombe) from field options provided by the staff at the Fort Saskatchewan Nutrien® retail location (https://www.nutrien.com/offices). All fields selected had a history of SSR and were in a two to three-year rotation with a cereal crop. These fields were sown to the canola cultivars 'L255PC' (Field 1 (F1)), 'Proven 581GC' (F2 and F4), and 'L255PC' (F3). In 2020, five fields were chosen on May 25th with the assistance of Dr. T. K. Turkington from several options that were provided by farmers, which were sown to the canola cultivars 'L241C' (F1, F2 and F3) and 'L234PC' (F4 and F5).

Experimental sites within all fields in 2019 and 2020 were selected based on access points, with preference given to the southeast corners of fields to catch prevailing winds. Each site was placed approximately 30 m from the field margin and included one Spornado spore sampler (20/20 Seed Labs Inc, Nisku, AB), one GRIPST rotorod sampler (Aerobiology Research Laboratories, Nepean, ON), two RH and temperature gauges, one tipping bucket rain gauge and one wind speed and direction sensor (anemometer) (Onset, Bourne, MA, USA). The fields F1 and F2 in 2019 received a single application of Proline (prothioconazole) (Bayer CropScience, Calgary, AB) on July 10th, while field F3 in 2019 did not receive a fungicide application. In 2019, field F4 received one application of Cotegra (BASF, Missisauga, ON) on July 15, with a fungicide-free check strip left where the spore traps were located. In 2019, fields F4 and F5 received one application each of Cotegra on July 6th and July 11th, respectively, with fungicide-free check strips included where the spore traps were located. Fields F1, F2 and F3 in 2020 received an application of Proline on July 12.

A companion fungicide timing trial was also included, and was carried out in 2019 at 10 sites across AAFC and Alberta Agriculture, Forestry and Rural Economic Development (AAFRED) stations in Alberta (Beaverlodge, Brooks, Edmonton, Lacombe, Lethbridge), Saskatchewan (Indian Head, Melfort, Outlook, Scott), Manitoba (Brandon) and Quebec (Normandin). At each of these sites, ascospore load assessments and measurements of weather variables (in-canopy and ambient RH and temperature, rainfall amounts) were carried out in check plots (no fungicide applied). Field locations were selected based on previous cropping history, with preference given to sites with a history of SSR in susceptible crops, with a rotation of 1-2 susceptible crops followed by the small plot study. Each field site contained one Spornado, one rotorod sampler, two RH and temperature gauges and one tipping bucket rain gauge. The setup of these was similar to that described above for the farmer fields in the Edmonton region. The companion trial had two seeding rates, low (60 seeds/m^2) and high (120 seeds/m^2) and was seeded to the hybrid canola 'L255PC' at all sites except Normandin, where 'L241C' was used. Unfortunately, COVID-19 restrictions resulted in cancellation of the fungicide trial in 2020.

Environmental data from the nearest commercially available public weather station was obtained from either Environment Canada (https://climate.weather.gc.ca/historical_data/search_historic_data_e.html) or Alberta Climate Information Services (ACIS, https://acis.alberta.ca/acis/weather-data-viewer.jsp). Publicly available weather stations ranged from 2 km (F3, 2020 to Oliver Environment Canada weather station) to 28 km (F2, 2020 to Radway Environment Canada weather station) away. The AAFC/AAFRED research locations had their own onsite weather stations recording data for the Environment Canada website, and therefore were generally within closer proximity to a publicly available weather station than commercial fields.

3.2.2 Weather Equipment

3.2.2.1 RH/Temperature

Two HOBO U23-002 relative humidity (RH) and temperature units were used to measure in-canopy and ambient RH and temperature (Onset Computer Corporation, https://www.onsetcomp.com/products/data-loggers/weather-stations/). The units were attached

to the same pipe (2-3 m length × 12.7 mm diam. metal electrical conduit or equivalent), with one unit placed 20 cm from ground level, and the second unit placed 154 cm from ground level. Pipes were within 1 m of the spore traps in an area where the crop canopy could fully grow to cover the lower unit. The in-canopy and ambient units were set to record every 15 min, with the average, minimum and maximum RH and temperature recorded daily. Measurements in 2019 were collected from June 28, 2019 to August 9, 2019. In 2020, the units began recording on June 22, 2020, and stopped on August 24, 2020. For all fields in both years, the RH temperature units were placed during the rosette – bolting stages of canola (GS 50-53) and removed after flowering had finished (GS 72).

For the companion fungicide trial, canopy and ambient RH and temperature units were placed on a similar singular pole in check plots with the high seeding rate. The set up was similar to that described for the commercial fields. Units were typically placed around the rosette growth stage (GS 50) and removed after disease assessments had been completed by AAFC/AAFRED staff before swathing or harvesting.

3.2.2.2 Wind Speed and Direction

A single HOBO Davis Wind Speed and Direction Smart Sensor (S-WCF-M003) was used in each field, placed on a tripod 2 m above ground level and within 3 m of the spore traps, with preference given to areas east, west, and south of the spore traps (Onset Computer Corporation, https://www.onsetcomp.com/products/data-loggers/weather-stations/). Each unit recorded direction, maximum wind, and maximum gust speed every 15 min. In 2019, data were recorded from July 8, 2019, to August 9, 2019. In 2020, data were recorded from July 13, 2020, to August 24, 2020. An anemometer was not used at any location for the companion fungicide test in 2019.

3.2.2.3 Precipitation

A single HOBO Bucket Rain Gauge (RG3-M) was used at each site and placed near field margins away from overhanging debris that would hinder rainfall, with tall grasses and weeds removed as necessary (Onset Computer Corporation,

https://www.onsetcomp.com/products/data-loggers/weather-stations/). Each rain gauge recorded daily rain events in increments of 0.2 mm. In 2019, rain gauges were placed in fields from June 28, 2019, to August 9, 2019, while in 2020, rain gauges were set out June 1, 2020, and removed on August 24, 2020.

For the companion fungicide-timing trials using similar procedures, units were typically placed in the field around the rosette growth stage (GS 50) and removed after disease assessments were completed by AAFC/AAFRED staff before swathing or harvesting. The only sites under irrigation were at Brooks, AB, and Outlook, SK.

3.2.3 Spore Traps

3.2.3.1 Spornado

For the commercial field and fungicide trial study, the passive Spornado spore trap was used. The Spornado is a funnel-type spore trap that uses a funnel with a tail fin mounted on a ball joint that swivels to catch prevailing winds. Spores that may be in the air are carried through the funnel trap and captured on a specialized membrane cassette (Figure 3.1. a and b). One spore trap was mounted on a pipe (2-3 m length × 12.7 mm diam. metal electrical conduit or equivalent) at 154 cm above ground level for each field. In 2019, the Spornado cassettes were changed every Monday and Thursday starting on June 28, 2019, until August 9, 2019 (GS 53 – 72). Cassettes were stored at room temperature in bags labelled with the location (Spornado number), crop stage, date and time of deployment and collection, and delivered to 20/20 Seed Labs Inc. (Nisku, Alberta, https://2020seedlabs.ca/) on Tuesdays. In 2020, the Spornado cassettes were replaced every 3-4 days (F1, F2 and F3 on Mondays and Thursdays, F4 and F5 on Tuesdays and Fridays, until July 20, at which point all fields had cassettes collected and replaced on Mondays and Thursdays) from July 6, 2020, until August 24, 2020 (GS 58 – 72).

Spornado traps were also mounted at approximately 150 cm above ground level at sites conducting the companion fungicide trial, with cassettes deployed on Tuesdays between 8:30 and 9:00 AM and collected on Thursdays between 3:30 and 4 PM. A second cassette was deployed on Friday mornings between 8:30 and 9:00 AM and collected from 3:30 to 4:00 PM the following Monday.

Testing for *S. sclerotiorum* DNA was conducted by 20/20 Seed Labs Inc., Nisku, AB, with a proprietary testing procedure. The results of the DNA testing were classified as follows: 1) not detected (coded as no to low risk); 2) trace level (*S. sclerotiorum* DNA found at less than the level of detection (LOD) and may not be replicable (coded as low risk)); and 3) detected (*S. sclerotiorum* DNA was greater than or equal to the LOD and (coded as moderate to high risk)) (E. McBain and Dr. T. K. Turkington, personal comm.).

3.2.3.2 Rotorod Sampler

A GRIPST-2009 Rotation Impact Sampler (rotorod sampler) from Aerobiology Research Laboratories, Nepean, ON, is a mechanically armed, timed sampling device that uses silicone-covered rods to collect airborne particles. A 12 V solar-powered battery spins the motor attached to the sampling head at approximately 2400 RPM. Airborne particles are impacted on the leading edge of the rod when they extend from the sampling head due to centrifugal force (Figures 3.2 a and b). The amount of particles collected can be analyzed and calculated based on rod size, rotation speed and length of exposure, and is a function of the volume of air sampled (AeroBiology Research Laboratories 2009)

The rotorod samplers were mounted on helical piles to hold two silicone-covered rods approximately 150 cm above ground level. The motor and timing device were solar-powered, with the motor set on a 10% duty cycle, spinning the rods for 1 min then resting for 9 min, from 8 AM – 8 PM, seven days a week. Rods were replaced every 3-4 days (generally on Mondays and Thursdays).

In 2019, deployment of the rotorod samplers began on July 8, 2019, and continued until August 9, 2019 (GS 58-72). In 2020, the rotorod samplers were deployed from July 6, 2020, until August 24, 2020 (GS 58 – 72). Collected rods were stored in 10 mL vials and labelled with the location, crop stage, date and time set out, and date and time collected. After collection, all rods were frozen until delivery to the Plant Health Lab (AAFRED, Edmonton, AB) for DNA extraction and qPCR analysis.

Helical piles were also used to mount the rotorod samplers at approximately 150 cm from ground level at the companion fungicide trial sites. The rotorods were placed at the trial sites

between the canola rosette stage and before flowering (GS 19 – 39), and removed after flowering ended (GS 79). The rotorod spore sampler ran on Mondays, Wednesdays, and Fridays from 9 AM – 4 PM, with the specific set out and collection times recorded. The solar-powered timer was set to a 10% duty cycle, seven days per week from 8 AM – 8 PM. Rods were stored in small PCR vials and stored at 2-3˚C before shipping to the Plant Health Lab (AAFRED, Edmonton, AB) for *S. sclerotiorum* DNA extraction and qPCR testing. One rod was tested upon receipt, while the second was stored as a backup for both the commercial field samples and most of the companion field trial samples (with the exception of rods collected from the AAFC/AAFRED sites in Alberta, where the second rod was used for unrelated research (T.K. Turkington, personal comm.)).

DNA extraction and subsequent quantitative PCR (qPCR) analysis followed Ziesman et al. (2016) with some modifications. Briefly, the rods were placed in 2 mL microcentrifuge tubes with approximately 300 mg of glass beads, 400 μL of AP1 buffer and 4 μL RNase A from an DNeasy Plant Mini Kit (Qiagen N.V., Hilden, Germany). The tubes with rods were placed horizontally in a cell disruptor (Mini-BeadBeater24, Biospec Products Inc.) and run for 3 min at 3000 rpm. After removal from the cell disruptor, the tubes were incubated for 10 min at 65˚C and inverted 2-3 times. After 10 min, 130 μL of P3 buffer (Qiagen) was added, mixed, and then incubated on ice for 5 min. The lysate was then centrifuged for 5 min at 14,000 RPM. Samples were placed in a QIAcube (Qiagen) liquid-handling platform and subjected to qPCR based on Ziesman et al. (2016) using *S. sclerotiorum*-specific primers and a hydrolysis probe.

3.2.4 Petal Tests

3.2.4.1 Commercial Petal Test 1

Two commercial petal test kits were compared. Commercial Petal Test 1 (CPT1) was obtained from 'Commercial Testing Lab 1' and used according to the supplier instructions, which included the collection petals from fully opened flowers from eight different sites around the spore trapping equipment within the fungicide-free test strip. Each package provided eight strips of eight tubes with caps that required two fully opened, intact petals per tube. Forecepts were used to pull petals from the plant; the tweezers were disinfected with 'Spray Nine' (Permatex, Inc., Ohio, USA) between individual petals and plants. Due to sampling issues in

2019, no tests were conducted with 'Commercial Testing Lab 1' that year. In 2020, the testing kits were used at approximately 10% flower (GS 61), 20% flower (GS 62), 30% flower (GS 63) and 50% flower (GS 65). Petal test kit vials were then stored at 3-4 ˚C and shipped as soon as possible to 'Commercial Testing Lab 1' for their proprietary DNA testing. Their results were returned as a percentage of the canola petals infested with *S. sclerotiorum*, where: 0 – 45% = Low Risk; 45 – 90% = Moderate Risk; and 90 – 100% = High Risk. From there, a chart provided by the supplier indicated the percent probability of diseased plants in the crop (0 – 20%, 20 – 40%, and >40% respectively), and the probable percent yield loss (0 – 10%, 10 – 20% and >20%, respectively).

Petal samples collected from untreated check plots from the AAFC/AAFRED fungicide trial sites followed the instructions for CPT1. Each strip of eight tubes included a label with the plot number, site, date, and year. Petal test kit vials were then stored at 3-4 ˚C and shipped as soon as possible to the 'Commercial Testing Lab 1' as above. The companion fungicide trial sites utilized two tests per site in 2019, with one used at around full bloom (BBCH 65) and the second approximately one week later.

3.2.4.2 Commercial Petal Test 2

Commercial Petal Test 2 (CPT2) was obtained from 'Commercial Testing Lab 2' and used following supplier instructions, which included sampling eight plants at five different sites within the field surrounding the spore trap equipment in the fungicide-free check strip. Each kit contained five small plastic bags containing eight tubes for petal samples in which three petals from the same plant were collected. Due to sampling issues in 2019, no petal tests were conducted with CPT2 that year. In 2020, one petal test kit was used per single field site between 20% flower (BBCH 62) and 50% flower (BBCH 65). Three petals were selected from the top, middle and bottom of the flowering sections of the plants, as per the provided CPT2 protocol. The kits were stored between 3-4˚C until delivery to 'Commercial Testing Lab 2', where the petals were analyzed using their proprietary PCR methodology to determine the number of petals that tested positive for *S. sclerotiorum*. The results from the lab included the percentage of positive petal tests (indicating amplification of *S. sclerotiorum* DNA), where: 0 – 20% positive tests indicated 'Low Risk'; 20 – 40% indicated 'Moderate Risk'; and ≥ 40 % indicated high risk.

Based on the CTP2 instructions, an overall positive percentage >40% would correspond to an SSR incidence ≥15%, equating to a yield loss of ≥7.5%; this would justify a fungicide application, according to Canola Council of Canada recommendations (https://www.canolacouncil.org/canola-encyclopedia/diseases/sclerotinia-stem-rot/).

The CPT2 was not used for samples collected from the AAFC/AAFRED companion fungicide trial sites in 2019, and given COVID-19 restrictions, CPT2 was only used for the commercial field component of the project in 2020.

3.2.4.3 Petal Testing at the Plant Health Lab, AAFRED

A qPCR-based petal test was performed at the Plant Health Lab (AAFRED, Edmonton, AB) to determine the amount of *S. sclerotiorum* DNA per petal. Fifteen fully open and intact flowers were collected from canola plants immediately surrounding the spore traps in the fungicide-free test strip by using tweezers to break the flower off at the peduncle. The tweezers were disinfected with 'Spray Nine' between flowers and plants. Flowers were stored in Petri dishes or small plastic bags and given a label corresponding to the location, crop stage, date, and time of collection. Flowers were frozen until delivery to the lab. Flowers were collected at each site twice per week from the area around every spore trap, July 3, 2020, until August 24, 2020 (when flowering had ended). Pathogen quantification by qPCR was performed following Ziesman et al. (2016) for samples from the AAFC/AAFRED companion fungicide trials and commercial field sites in 2019 and 2020, respectively. Forty petals were collected from the commercial field sites at each sampling date, while for the companion fungicide trial, 10 petals were collected from each of four untreated check reps for one seeding rate, and processed according to Ziesman et al. (2016).

3.2.5 Disease Incidence and Severity

Sclerotinia stem rot ratings were recorded at each field site around the time that swathing would normally occur (BBCH 83). Ratings were made on a 0-5 scale (Kutcher and Wolf 2006), where: 0, no symptoms; 1, only pods are infected with *S. sclerotiorum*; 2, lesions found on the main stem or secondary branches in the upper canopy, impacting up to 25% of seed formation; 3,

lesions found in the upper canopy on numerous branches or on the main stem, impacting up to 50% of seed formation; 4, lesions in the upper canopy on numerous branches or on the main stem, impacting up to 75% of seed formation; and 5, a main stem lesion low enough to impact seed formation for the entire plant.

One hundred plants in the area immediately surrounding each spore-trapping site were rated, with 25 plants assessed in each compass direction from the traps in fungicide-free test strips. An additional 400 plants were rated within the field at four separate sites (100 plants per site, 25 plants in each compass direction of the selected spot) to mimic plants rated in the grid field. The additional four sites were selected from areas where fungicide may have been applied. In the companion fungicide trial sites, 25 plants at each of four randomly selected spots per plot (100 plants total per plot) were rated. Assessments were from the fungicide-untreated plots.

3.2.6 Statistical Analysis

For AAFC/AAFRED producer fields in 2019 and 2020 and the 2019 companion AAFC/AAFRED trial sites, relationships between environmental conditions, atmospheric ascospore levels, petal infestation and disease incidence were analyzed with RStudio program v. 1.3.1093 (Integrated Development for R. RStudio, PBC, Boston, MA). Data were separated into individual years and variables for statistical analysis, but were combined to determine linear relationships. All tests for assumptions included assumptions for normality (Kolmogorov – Smirnov test or Shapiro test) and homogeneity of variance (F Test or Levene Test). Assumptions were tested to compare weather data between ambient and canopy RH and temperature. If the assumptions were met, a paired two-sample T-test was employed to determine if there was a significant difference. If violated, a Mann – Whitney – Wilcoxon signed-rank test (non-parametric test) was used. To determine if ambient weather conditions were significantly different from weather conditions recorded at the nearest weather station, assumptions were tested, and if they were met, a two-sided paired T-Test was used. A Mann – Whitney – Wilcoxon signed-rank test (non-parametric test) was used if assumptions were not met. The Mann – Whitney – Wilcoxon signed-rank test was also used to determine significant differences in weather conditions between years.

Rotorod samples between fields in both years were compared using a Kruskal – Wallis test (non-parametric one-way ANOVA) after testing for assumptions, while a Mann – Whitney – Wilcoxon non-parametric test was used to determine if there was a significant difference between the amount of DNA captured between years. After the Kruskal – Wallis test was used and if differences were significant, medians were re-ordered and then compared using a Dunn Test of Multiple Comparisons, which determined differences in medians among groups. Rotorod samples at AAFC/AAFRED locations were not tested for significant differences among fields because quantifiable inoculum was detected at only one location (Beaverlodge, AB).

Results from petal tests conducted at the Plant Health Lab (AAFRED, Edmonton, AB) were compared with a Kruskal – Wallis test (non-parametric one-way ANOVA) after testing assumptions to determine significant differences between the amount of DNA per petal between fields in 2020. A one-way ANOVA was used for both CPT1 and CPT2 when all assumptions for the test had been met.

To determine significant differences between disease incidence and severity, and if the distribution of SSR was similar across fields and years, a Chi-Squared test on a contingency table was used.

Multiple linear regression was used first to determine the relationship between environmental factors on spore detection methods (Spornado risk level, SSR DNA on rotorods and petal infestation levels) and secondly, on airborne inoculum, and petal infestation levels coupled with environmental variables on final disease incidence and severity. To begin the regression analysis, all independent variables, including weather variables, spore traps and Plant Health Lab petal tests, were separated into early-mid flower (first half of the flowering period (BBCH 60 – 65)), mid-late flower (second half of the flowering period (BBCH 66-69)) and total average over the flowering period (BBCH 60-69) with some exceptions. Wind and gust speed were not included in models for determining environmental effects on airborne ascospore levels or petal infestation levels, as the equipment often malfunctioned in the field, and data were only obtained at the commercial field locations. No models were generated for environmental effects on CPT1 and CPT2 because of the limited sampling size for both petal test kits. They were then assembled into a model format for each category, with each spore trap and Plant Health Lab petal test evaluated separately and together. All linear models were tested for multicollinearity

before proceeding to stepwise progression. If variance inflation factors (VIF) for each independent variable were <10, with ≥10 considered severe (García et al. 2015), then all independent variables were included in stepwise selection, with the final suggested model having the lowest Akaike's Information Criterion (AIC). If an independent variable exhibited a VIF value greater than 10, this was an indication that multicollinearity was occurring, which can influence the values of the coefficients and the p-value; however, it does not affect the goodness of fit (Kutner et al. 2004). A separate model was generated in which the independent variables were removed to determine the changes that would occur in the final model if the variable were removed from the equation. If no changes occurred, then the variable with a high VIF value was retained in the model. If the excluded variable influenced the p-value or coefficients, further investigation between variables was conducted, via a correlation chart to determine correlations between final variables. In most cases, the equation with the removed variable was kept because of a low sample size and multicollinearity could cause the coefficients to become sensitive to small changes (Frost 2022).

Interactions were also tested between each period (early – mid flower, mid-late flower, total flowering average), and none was found to be significant. The model with the lowest AIC values after all directions of the stepwise regression had been tested (backward, forward, and both) indicated which variables were statistically significant to the dependent variable. The residuals of the final model were tested for normality (formal tests, residual vs. fitted plots, and Q-Q plots). The models were tested at the 5% significance level, with the null hypothesis (that there is no relationship between the dependent variable and independent variable) rejected if $P > 0.05$. If assumptions of the residuals were violated, outliers were investigated and removed, if necessary. To compare the models with different amounts of independent variables, the adjusted R^2 was used.

3.3 Results

3.3.1 Disease Incidence

Stem rot disease incidence (DI) in 2019 was high for commercial fields sampled in the Edmonton region, with the average DI between 56% - 74% (Table 3.1). Statistical analysis indicated that the DI and severity between different commercial fields in 2019 had similar

distributions. Fields F1 and F4 had the highest average DI (77% and 66%, respectively) and the highest average severity ratings (1.5 for both) in 2019 (Table 3.1). Fields F1, F2 and F4 had the highest number of individual severity ratings of '5' (9.8%, 7.6% and 10.8% respectively, data not shown). F4 also had the highest number of upper canopy infections of all fields in 2019 (i.e., ratings of '1' and '2') (data not shown).

Compared with 2019, DI was not as high in 2020, with average DI between 22% and 39% (Table 3.1). Statistical analysis showed that the severity distribution between fields in 2020 was similar (data not shown). Fields F3 and F5 had the highest average severity ratings at 0.7 and 0.9, respectively (Table 3.1); F3 and F5 also had the highest number of severity ratings of '5' (7.8% and 7.4%, respectively, data not shown). Disease incidence and severity between 2019 and 2020 were significantly different (Table 3.1).

At the AAFC/AAFRED sites, only two locations, Beaverlodge, AB, Outlook, SK, had sufficient disease incidence in the check plots, with limited to no disease at the other trial locations. At Beaverlodge, DI ranged from 7.4% to 35.6%, with average severity ratings between 0.2 and 1.0 (Table 3.2). Outlook had much lower DI, with the maximum in the check treatments only reaching 2.5%. Disease severity was also low, with the highest severity being 0.1 in the untreated checks (Table 3.2).

3.3.2 Precipitation

In 2019, a rainfall event occurred between 57% - 77% of the time during the flowering period and on 71%-84% of days during the pre-flowering period, up to 3 weeks before the first open flower (Table 3.3). The maximum amount of rainfall captured in a single day was 39.4 mm on July 17th, 2019, in F1 (Figure A.1). F3 had the highest percentage of days where there was a rain event over 0 mm (81.8% during early flower and 72.7% during late flowering). However, the highest percentage of days where there was a rain event totalling over 2.5 mm and 5 mm, respectively, during the flowering period occurred in F2 (36.8%) and F1 (22.7%) (Tables 3.3, 3.4). The total amount of rain during the flowering period in 2019 ranged from 116 mm in F3 to 176 mm in F1 (data not shown), with the most precipitation occurring in the early flowering period. Overall, the early flowering period had a higher percentage of days with rain over 0 mm,

and the late flowering period had a higher percentage of days with rain amounts over 2.5 mm and 5 mm (Tables 3.3, 3.4; Figures A.1, A.2, A.3, A.4).

The amount of rain collected during the flowering period in 2020 was significantly less than in 2019 (Figures A.5, A.6, A.7, A.8, A.9). The highest amount of rain captured in a single day was 28.8 mm on July 1, 2020, in F2, which would have occurred just before flowering began (Figure A.6). Both F3 and F5 had the same percentage of days (78.3%) during the flowering period where there was a rain event above 0 mm, but F3 had the highest percentage of days where the total rainfall was above 2.5 mm and 5 mm (Tables 3.3, 3.4). F3 also had a higher percentage of days in both categories (2.5 mm and 5 mm) in the early flowering period compared with the late flowering period. The total amount of rain collected during flowering was between 34 mm in F2 to 90 mm in F3 (data not shown), with the most precipitation occurring during the early flowering period for each field.

Of all the AAFC/AAFRED sites across Canada, the highest accumulated daily rainfall occurred in Scott, SK, on July 14th, 2019, with a total amount of 46.2 mm (Figure A.10). The highest percentage of days during the flowering period where the rain amounts totalled over 2.5 mm (51.2% of days) and 5 mm (41.5% of days) occurred in Brooks (Tables 3.5, 3.6). The irrigation regime in Outlook, SK, added 10 mm of precipitation in May 2019, 25 mm in June (12.5 mm each on June 7th and June 14th), 40 mm in July (12.5 mm each on July 5th and July 10th, 15 mm on July 24th) and 40 mm in August (25 mm on Aug. 1, and 15 mm on Aug. 6) (Figure A.11). Irrigation at Brooks, AB occurred more frequently, beginning on May 24th, 2019, and ending July 26th, 2019, in increments of either 7.6 mm or 15.2 mm, approximated based on the speed of CDCS lateral irrigation. In May, irrigation added approximately 60.8 mm of precipitation, 83.6 mm in June and 98.8 mm in July (Figure A.12). However, Beaverlodge, AB, had the highest percentage of days during the flowering period with rain events above 0 mm (Table 3.5, Figure A.13). Lethbridge, AB, consistently had the lowest amount of rain over the flowering period (Figure A.14). The tipping rain gauge at Lacombe, AB, malfunctioned in 2019; therefore, rain data was taken only from the Environment Canada weather station (https://climate.weather.gc.ca).

When the amount of rain recorded in a field was compared against rain collected at the nearest weather station, it was not significantly different during 2019 (Tables A.1, A.2, A.3 and

A.4). This suggested that rain collected at the nearest publicly available Environment Canada weather station or ACIS station would be an accurate representation of the rain collected in each field, at least under the conditions experienced in 2019. However, there was some variation across years and sites; in 2020, the amount of rain at 89% of the sites was not significantly different from the nearest publicly available weather station, while in 2019, rainfall at 67% of the AAFC/AAFRED locations was not significantly different. The AAFC/AAFRED sites at Brooks, AB, and Outlook, SK, were both under irrigation, and in Brooks, the amount of precipitation was significantly different from the nearest weather station. In Outlook, however, it was not significantly different (Tables A.5, A.6). The amount of rain collected per year was significantly different, with rain occurring more frequently and in higher amounts in 2019 than in 2020.

3.3.3 Relative Humidity

In 2019, the daily average canopy RH was greater than the daily average ambient RH (Table 3.7; Figures A.15, A.16, A.17, A.18). The range in differences between the daily average canopy RH and daily average ambient RH was large, from 4.5% to 28.8% RH. The total percentage of days with the daily average canopy RH over 80% was 100% in all fields (Table 3.8). Fields 2, 3 and 4 in 2019 had 100% of days during the flowering period where the daily average canopy RH was over 90% (Table 3.8). In F1, only 81.8% of total days during the flowering period had daily average canopy RH >90%, and there were more days in the late flowering period (90.9%) with RH >90% than the early flowering period (72.7%, Table 3.8). The mean daily canopy RH during early and late flowering in 2019 was similar in early and late flower, generally within 4% of each other; however, the late flowering period had slightly higher RH values, except in F4, where the RH was 0.1% higher in the early flowering period (Table 3.10). The mean canopy RH values did not fall below 93% during the entirety of the flowering period in 2019. The ambient mean RH was also high, with means in all fields during pre-flower, early flower, and late flower >80% RH (Table 3.11). In 2019, the ambient RH reached 80% RH on 60-80% of days in all flowering periods (pre-flowering, early and late), and was >90% RH on less than 23% of days (Table 3.9)

In 2020, ambient and canopy relative humidity was more variable, with the lowest daily average RH reaching 64% on July 13th in F3 (Figure A.19). The average minimum canopy RH

did not drop below 60% after canopy closure in any field (Figure A.20, A.21, A.19, A.22, A.23), and was generally equal to or higher than the ambient average RH. The difference between daily average canopy RH and daily average ambient RH was as high as 34% in F5 (Figure A.23). The only field that recorded daily average canopy RH >80% on all days during the flowering period was F5 (Table 3.8). Other fields in 2020 had an average daily canopy RH >80% between 56.3% - 88.9% of days during the flowering period (Table 3.8). In F1 and F2, there were more days during the late flowering period where the daily average canopy RH was >80% (100% and 68.8%, respectively), but the opposite was true for F3 (Table 3.8). Similarly, a high percentage of days where the daily average canopy RH was > 90% during the flowering period (78.3%) also occurred in F5; however, there were more days in the late flowering period that had an RH >90% under the canopy in F5 (81.8%, Table 3.8). The highest mean canopy and ambient RH in 2020 was also detected in F5 (92.8% in the canopy and ambient RH of 80.4%; Table 3.7). Fields 1, 2 and 3 had a higher percentage of days where the RH was >90% during the early flowering period. The percentage of days with ambient RH >90% was low, at 16.7% of days in the early flowering period for all fields in 2020 (Table 3.11). Field 2 and F3 had no days during the late flowering period where the average ambient RH was >90% (Table 3.11). Mean canopy RH during flowering was slightly lower in 2020 vs. 2019, with average canopy RH between 81.0% and 92.8% in 2020 and between 94.6% and 98.6% in 2019 (Table 3.7). The mean ambient RH was also lower in 2020, with the range between 72.8% - 80.4% in 2020 and between 82.7% - 84.3% in 2019 (Table 3.7). The same trend was observed for the mean RH in the pre-flowering period, with 2019 (81.4% - 82.1%) higher than in 2020 (74.1% - 77.0%) (Table 3.11).

The AAFC/AAFRED sites across western Canada, Beaverlodge, AB, Lacombe, AB, and Outlook, SK, all had more than 90% of days during the flowering period where the daily average canopy RH was >80% (Table 3.12; note that the Brooks canopy RH was unavailable due to sensor malfunction). These same sites were the only ones to experience 50% of days during the flowering period where the daily average canopy RH was >90% (Table 3.12). Lacombe was the only site to experience >80% RH every day during the early flowering period, but this decreased to 95% of days during late flowering (Table 3.12). Normandin, QC, had only 26.7% of days during the early flowering period where RH >80%; however, this increased to 92.9% of days during late flowering (Table 3.12). The percentage of days where the ambient RH reached >80% and >90% during the flowering period was lower than canopy values at the same sites, but

Lacombe had the highest percent of days >80% (68.3%, Table 3.13). Beaverlodge had the highest percentage of days with RH >90% during flowering, at 17%; however, most of those days occurred during the late flowering period (Table 3.13). Outlook, SK, showed a different trend when comparing the percentage of days when the daily average ambient RH and canopy RH reached >80% during the flowering period, with only 2.9% of days reaching >80% RH above the canopy (ambient, Table 3.13) and 94.3% under the canopy (Table 3.12), likely because of the irrigation regime.

The mean canopy RH in Beaverlodge was similar to the commercial field sites in 2019, with percentages that rarely dropped below 80% (Figure A.24) and 91.1% of days during the flowering period above 80% (Table 3.14). At the AAFC/AAFRED sites in Lacombe, AB, Outlook, SK, Scott, SK, and Melfort, SK, average daily canopy RH frequently was >80% but did not reach 100% (Figures A.25, A.26, A.27, A.28). The other AAFC/AAFRED sites had lower RH values, with daily averages generally <80%, although maximum RH values approached 100% (A.29, A.30, A.31, A.32, A.33).

Overall, when the average daily canopy RH was tested against the average daily ambient RH, all commercial fields and AAFC/AAFRED sites were significantly different, with canopy RH generally higher than ambient RH (Tables A.1 – A.19). When the daily ambient RH was tested against the nearest publicly available Environment Canada weather station, 78% of the commercial field sites were significantly different from the nearest weather station, while 90% of the AAFC/AAFRED sites were significantly different (Tables A.1 – A. 19). This suggests that the ambient RH at the nearest weather station may not accurately represent the ambient RH at the field level.

3.3.4 Temperature

In 2019, the maximum ambient temperature recorded was 28.5˚C on July 22 in F1 and F4, a day that fell outside of the flowering period (data not shown) (Figures A.34, A.35). All fields in 2019 had multiple days when the canopy minimum and maximum temperatures were above the respective ambient temperatures (Figures A.34, A.36, A.37, A.35), but were generally within 3˚C of each other. In F3, the daily mean canopy temperature during the flowering period was higher than the mean ambient temperature, and among all fields in 2019, F3 had the highest

daily mean canopy temperature and lowest mean ambient temperature (Table 3.7). In F1, F2 and F4, the mean daily ambient temperature was higher than the canopy temperature during the flowering period (Table 3.7). In all fields in 2019, the mean canopy temperature was slightly higher in the late flowering period than in the early flowering period (Table 3.10). The same was true for the mean ambient temperature; however, the lowest mean temperatures for all fields were in the pre-flowering period, and the highest mean ambient temperatures were in the late flowering period (Table 3.11).

The maximum temperature in 2020 was 31.4˚C on August 18 in F2, after flowering was completed (Figure A.38). Field 1, F2, F3, and F4 had days when the maximum canopy temperatures exceeded the maximum ambient temperatures (Figures A.39, A.38, A.40, A.41). The same fields also reached daily maximum canopy temperatures >25˚C more often compared with the daily maximum ambient temperature (Figures A.39, A.38, A.40, A.41). Mean canopy temperatures were more variable in 2020, with the highest mean temperature observed during late flowering in F1 (16.1˚C), F2 (18.3˚C) and F3 (17.1˚C). Field 1 had a consistently lower mean canopy temperature during pre-flower and early flower, at 15.7˚C, but F2 had the lowest mean canopy temperature at 16.6˚C during pre-flower, and F3 saw its lowest mean canopy temperatures during early flower at 15.6˚C (Table 3.10). In F4 and F5, the highest mean temperature was recorded during the pre-flower period (15.5˚C and 16.0˚C, respectively), although the mean canopy temperature remained consistent during the flowering period in F4 (15.4˚C), while in F5, the lowest mean canopy temperature (14.9˚C) occurred during early flowering (Table 3.10; Figures A.41, A.42). The mean ambient temperatures in each field were highest during the late flowering period and lowest during pre-flower in F1, F2, and F4 (Table 3.11). Field 3 had the highest mean ambient temperatures during late flower (16.9˚C), but the lowest during early flower at 15.6˚C (Table 3.11). The trend for mean ambient temperature in F5 was the same as for mean canopy temperature, where the highest temperature occurred during the pre-flower period (16.0˚C), and the lowest occurred in early flower (14.9˚C, Table 3.11). Among the five fields in 2020, F2 had the highest canopy and ambient temperatures during the flowering period, while F5 had the lowest (Table 3.7).

At the AAFC/AAFRED trial sites in 2019, the location that had the lowest number of days where the maximum ambient temperature reached 25˚C was Beaverlodge, AB, with only 6% of days between June 25th, 2019, and August 31st, 2019, equal to or above 25˚C (data not

shown, Figure A.43). In Beaverlodge, there was only one day when the maximum canopy temperature in the canopy reached 23.8˚C, whereas in Lethbridge, AB, consistently higher maximum canopy temperatures were observed; these were also above the maximum ambient temperatures (25˚C and 35˚C) during the flowering period (Figures A.43, A.44). Lethbridge also had the highest maximum ambient and maximum canopy temperatures recorded among all of the AAFC/AAFRED sites during the flowering period, at 34.3˚C and 36.1˚C, respectively, on August 2, 2019 (Figure A.44). Indian Head, SK, also consistently experienced higher maximum canopy temperatures than the corresponding maximum ambient temperatures during the flowering period (Figure A.45). The temperature trends at Beaverlodge, Lacombe, Melfort and Scott were very similar, with temperatures generally <25˚C, and average temperatures remaining between 10 and 20˚C for most of the summer (Tables 3.14, 3.15, 3.16, Figures A.43, A.46, A.47, A.48). The Brooks, Indian Head, Outlook, Brandon and Normandin sites had average temperatures between 15 and 25˚C, with some maxima >30˚C (Tables 3.14, 3.15, 3.16, Figures A.49, A.45, A.50, A.51, A.52). Generally, the difference between the canopy and ambient temperature was about 5˚C or less, with the canopy temperature lower than the ambient temperature.

Most (78%) of the commercial fields in 2019 and 2020 showed significantly different daily average ambient vs. canopy temperatures, with the former generally higher than the latter (Tables A.1 – A.9). Among the nine AAFC/AAFRED sites where canopy temperature was recorded, 89% had significantly different daily average canopy vs. ambient temperatures, with canopy temperatures generally higher than ambient temperatures, although this varied depending on location (Tables A.10 – A.19). The daily average ambient temperature in 55% of the commercial field sites was significantly different from the nearest publicly available Environment Canada weather station, with canopy temperatures generally greater than ambient temperatures (Tables A.1 – A.9). In contrast, the daily average ambient temperature in 80% of the AAFC/AAFRED locations was similar to the nearest publicly available Environment Canada weather station (Tables A.10 – A.19).

3.3.5 Wind Speed and Direction

The daily average wind speed in 2019 was about 5 km/h, while gust speeds stayed below 10 km/h during the flowering period (Table 3.7, 3.11, Figures A.53, A.54, A.55, A.56). There was one occasion when the daily average gust speed peaked between 27 km/h and 30 km/h on July 25th, 2019, in F1, F2 and F4 (Figures A.53, A.54, A.56), with the data not available for F3. The prevailing wind direction in 2019 was 186˚ in a southerly direction (data not shown).

The 2020 growing season was slightly more volatile in terms of wind, with average daily wind and gust speeds between 5 and 15 km/h (Tables 3.7, 3.11); however, F2 and F4 did not capture any data before July 13th and July 17th, respectively. Field 1 and F5 were gustier, with daily gust speeds averaging over 15 km/h between 31% - 40% of the time on days when data were recorded (Figures A.57, A.58), while all other fields in 2020 had less than 8% of days recorded when daily average gust speed reached over 15 km/h (Figures A.59, A.60, A.61). There was one significantly windy week in F1 and F5 over the flowering period, when the gust speed was over 18 km/h for four days from July 9th to July 16th in F1 and from July 8th to July 15th in F5 (Figures A.57, A.58). The prevailing wind direction in 2020 was 212˚, south-southwest on the compass rose.

The wind speed measured with the Onset equipment in the commercial fields was significantly different at eight of nine sites (Tables A.1 – A.9). These differences may have reflected various factors, including tall shelterbelts surrounding some fields and rolling topography (Bolton et al. 2006).

3.3.6 Airborne Inoculum over the Flowering Period

Variable airborne inoculum levels were detected with both spore-trapping methods, i.e., the Spornado and Rotorod samplers. In 2019, the most consistent Spornado results over the flowering period were found in F2 and F4, with most of the results rated as a ‘3’, indicating a “moderate” risk level (Table 3.17, Figures A.62 and A.63). The highest airborne inoculum levels recorded over the flowering period in 2019 with the Spornado were found in F4, which were rated as a ‘3’ (“moderate” risk level), with a standard deviation of zero (Figure A.8a, Table 3.17). The results from F1 and F3 over the flowering period were more variable, with flushes of

ascospores detected in both fields throughout the growing season (Table 3.17; Figures A.64, A.65)

The minimum rating observed in F1, F3 and F5 based on the Spornado sampling throughout flowering in 2020 was '2' ("trace" levels; Table 3.1, Figures A.66, A.67, A.68). In contrast, a rating of '3' ("moderate" risk) was detected in F4 during flowering, with a reduction in airborne inoculum level after July 20th, 2020, which coincided with the end of flowering (Figure A.69). Flushes of ascospores later in the growing season were detected in all of the fields, generally after July 20th, 2020 (Figures A.66, A.70, A.67, A.69 A.68). Ascospore pressure at early and late flowering was consistent in most fields, with standard deviations ≤ 1 (Table 3.17). Field 4 had an average rating of 3.0, with a standard deviation of zero during early flowering, indicating that every Spornado cassette was rated as a '3' during this critical period for disease development (Table 3.17).

Spornado ratings at the AAFC/AAFRED fungicide trial sites were variable. At Beaverlodge, AB, ratings were mostly returned as "detected" throughout the flowering period, with three returned as "trace limits detected", once in the middle of flowering (July 23rd, 2019 – July 25th, 2019) and the rest at the end of flowering (Figure A. 71). The results from Melfort, SK, were similar, with most cassettes returned as "detected" until the end of the flowering period; two cassettes in a row were returned as "trace levels detected" (Figure A. 72). Ratings in Brooks, AB, were more variable, with all three levels ("detected", "trace levels detected" and "not detected") identified multiple times over the flowering period (Figure A.73). Similar results were found for Lacombe, AB, Indian Head, SK, Outlook, SK, Scott, SK, and Brandon, MB (Figures A.74, A.75, A.76, A.77 and A.78), suggesting that multiple flushes of ascospores occurred at each of these locations. At Lethbridge, AB, most Spornado ratings were returned as "not detected" except for a single cassette on July 19th, 2019, which came back as "trace levels detected" (Figure A. 79). Similarly, in Normandin, QC, most cassettes were returned as "not detected" during the flowering period, except for one cassette corresponding to July 23rd-July 25th, 2019, which was returned as "trace levels detected" (Figure A.80).

The highest amount of *S. sclerotiorum* DNA found in the rotorod samples in 2019 was 2.1×10^{-3} ng DNA/m^3/h, captured in F4 over July 15th - July 18th (Figure A. 81). This amount was about 1.5-fold greater than what was captured in any other field during the 2019 growing

season (Table 3.17; Figures A.83, A.84, A.85). Nonetheless, all fields showed an increase in ascospore pressure from July 15th to July 18th, 2019, corresponding to about 30% flower (BBCH 63). Ascospore levels before July 15th – July 18th were comparatively lower; however, flushes occurred throughout the growing season. The lowest amount of *S. sclerotiorum* DNA was detected in F2 during the early flowering period, but pathogen DNA increased during late flowering, with samples from this field ending up having the second-highest amount (1.5×10^{-4} ng DNA/m^3/h) in 2019 (Table 3.17). The lowest amount of DNA during the late flowering period was detected in F1, with only one big flush of ascospores over July 15th – July 18th; this field also had the lowest amount of DNA on rotorods during the entire flowering period (Figure A. 83).

In 2020, the rotorod samplers caught much fewer airborne ascospores than in 2019. In fields F2 and F3, there was only one period in each field (July 13th – July 16th (early flower) and July 20th – July 23rd (late flower), respectively) when quantifiable amounts of *S. sclerotiorum* DNA were found (Table 3.17, Figures A. 86, A. 87). Field 1 had the most ascospores caught during early flowering, with inoculum pressure decreasing after July 9th, 2020, although smaller flushes occurred until the spore traps were removed from the field (Figure A. 88). The amount of pathogen DNA captured on the rotorods in F4 increased over the flowering period, peaking during the July 20th – July 23rd period at 1.24×10^{-4} ng DNA/m^3 air/h; this was also the maximum amount of DNA captured in 2020 in any of the rotorod samplers (Figure A. 89). In F5, moderate amounts of *S. sclerotiorum* DNA were detected at early flowering, but this increased during the late flowering period (Table 3.17; Figure A.90). The highest average amount of pathogen DNA over the entire flowering period was collected in F5 (3.8×10^{-5} ngDNA/m^3/h Table 3.1)

At the AAFC/AAFRED fungicide trial sites in Brooks, AB, Lacombe, AB, Lethbridge, AB, Indian Head, SK, Scott, SK, Normandin, QC, and Brandon MB, all rotorod samples tested negative (Table 3.2). At Melfort, SK, and Outlook, SK, one and two samples, respectively, tested weakly positive, although the exact amounts of *S. sclerotiorum* DNA were not determined; all other samples at these two sites were negative (Table 3.2). In Beaverlodge, AB, the amount of pathogen DNA in the rotorod samples peaked at 3.9×10^{-3} ng DNA/m^3/h from July 23rd – July 27th, 2019, with DNA also detected in late June (Figure A. 91). The mean amount of DNA detected was consistent throughout early and late flowering at Beaverlodge (Table 3.2).

For the rotorod samples collected in 2019, a non-parametric one-way ANOVA showed that the amount of *S. sclerotiorum* DNA detected was significantly different across fields (Table 3.1). Post-hoc analysis indicated that there was only a significant difference between F1 and F4 ($P < 0.05$). Fields in 2020 were also significantly different from each other; however, post-hoc analysis showed that F2 and F3 were significantly different from F4 and F5, while F1 was not significantly different from any field (Table 3.1, data not shown).

3.3.7 Petal Infestation over the Flowering Period

The results of the petal tests based on qPCR analysis performed by the Plant Heath Lab (AAFRED, Edmonton, AB) from samples collected in 2020 were variable. The maximum amount of *S. sclerotiorum* DNA per petal reached 2.2×10^{-2} ng DNA/petal in F3 (Table 3.1), approximately seven times higher than the second-highest amount in F5 (3.1×10^{-3} ng DNA/petal, Table 3.1). In F3, F4, and F5, higher petal infestation levels were detected in late flower, while in F1 and F2, the fields with the lowest petal infestation, higher levels were observed in early flower (Table 3.1, 3.18; Figures A.92, A.93, A.94, A.95, A.96). When subjected to a Kruskal-Wallis non-parametric test, the population means of the petal infestation for each field were not significantly different at $P < 0.05$, suggesting similar levels of petal infestation across all fields in 2020.

Tests were also conducted by the Plant Health Lab to determine the amount of *S. sclerotiorum* DNA on petals from the AAFC/AAFRED fungicide trial sites in 2019. All Normandin, QC, petal samples returned a negative result, while no samples were available for Scott, SK or Lethbridge, AB. At Lacombe, AB, Indian Head SK, and Brandon, MB, there were three sampling days or fewer where a small amount of DNA was detected (Figures A.97, A.98, A.99, A.100). During early flowering, petal samples collected in Brooks, AB, showed a small amount of the pathogen DNA (Figure A. 101). On July 31, 7.5×10^{-3} ng DNA/petal was detected in a low seeding rate treatment (60 seeds/ m^2), and 3.2×10^{-3} ng DNA/petal was detected in a high seeding rate treatment (120 seeds/m^2), which were the highest amounts of *S. sclerotiorum* DNA found among all AAFC/AAFRED sites in 2019 (Table 3.19). Brooks had the highest amounts of *S. sclerotiorum* DNA during the flowering period, while Beaverlodge, AB, had the highest amount of mean DNA during the early flower period (Table 3.19). The second

highest amount of pathogen DNA during flowering was detected in Beaverlodge, AB, with the peak occurring on July 25, 2019, near mid-flower (Figure A.102). At a high seeding rate (120 seeds/m^2), the highest amount of DNA in Melfort occurred on August 7, 2019, near the end of flowering, while the peak at the lower seeding rate (60 seeds/ m^2) occurred during early-mid flowering on July 17th, 2019 (Figure A. 103).

In 2020, petal infestation levels as determined via CPT1 were low (<45%) over the whole flowering period in F2, with a maximum of 36.3% detected on July 16th, 2020 (Table 3.1). In contrast, the highest petal infestation was observed in F1, where an infestation level of 100% recorded on July 13th, 2020. Moderate levels of petal infestation were observed in F3 (55%), F4 (53%) and F5 (58%) between July 10th and 16th, 2020. One-way ANOVA indicated that the mean infestation levels determined via CPT1 in each field were not significantly different when tested at $P < 0.05$ (data not shown). When linear regression was used to determine if weather variables influenced the level of petal infestation, no significant correlations were found (data not shown). Commercial Petal Test 1 results for fungicide trial sites in 2019 showed that Beaverlodge, AB, had the highest average percent petal infestation, followed by Melfort, SK (52% and 34.5% respectively, Table 3.2). However, Beaverlodge would have been the only site with a "moderate" amount of disease, resulting in a predicted yield loss of 10-20%, according to instructions sent with the CPT1. All other AAFC/AAFRED sites would have been designated as low risk because of the lower petal infestation levels observed at these sites.

Using CPT2, the greatest petal infestation level (82.5%) was detected in F4, and all fields with the exception of F5 had petal infestations >40% (Table 3.1). Given that only one CPT2 was used at each site, the data were not tested to determine significant differences.

3.3.8 Influence of Environmental Conditions on Petal Infestation and Airborne Inoculum

The influence of in-field environmental conditions on petal infestation and airborne inoculum pressure was investigated using multiple linear regression. Since there was a limited number of petal samples from the commercial fields in 2019 and 2020, and sampling could not proceed at the AAFC/AARED fungicide trials in 2020, all sampling years and locations were included in the creation of the models, with some exceptions. All variables, with the exception of wind, CPT1 and CPT2, were averaged over early-mid flowering, mid-late flowering and the

total flowering period for each field. Wind and gust speed were not included in models for determining environmental effects on airborne ascospore levels or petal infestation levels, as the equipment often malfunctioned in the field, and data were only obtained at the commercial field locations. No models were generated for environmental effects on CPT1 and CPT2 because of the limited sampling size for both petal test kits. In 2019, Scott, SK, and Outlook, SK, were removed from the models to predict the impact of weather variables on petal infestation levels, as these sites had no petal samples undergo testing for the presence of pathogen DNA at the Plant Health Lab (AAFRED, Edmonton, AB).

For determining the Spornado risk level during the early flowering period, VIF values were <10, so the model proceeded to stepwise regression using all weather variables. After stepwise regression, the model with the lowest AIC values included only early average canopy RH as a predictor for the early Spornado risk level. However, the model was not statistically significant ($p = 0.1925$), with a low coefficient of determination ($R^2 = 0.12$ and adjusted $R^2 = 0.05$) (Table 3.20). When a model was created for determining the mid-late flower average Spornado risk level, the VIF function indicated that there was multicollinearity between the mid-late flower average canopy temperature and the mid-late average ambient RH, and all temperature and RH variables were tested for interaction. The interaction terms were not significant ($p > 0.5$), so the variable with the higher VIF value was removed (mid-late average ambient RH) and proceeded with the regression analysis after the VIF values indicated no further multicollinearity. Following stepwise regression, there were no environmental variables that were statistically significant for the prediction of the late average Spornado rating (Table 3.20). When outliers were investigated, the residuals vs. fitted plots indicated that Indian Head, SK, and Normandin, QC, were outliers. When Indian Head was removed, the model with the lowest AIC was still one without any environmental variables included in the final model. The same was true when Indian head was put back into the equation and Normandin removed. However, when both outliers were removed (and mid-late average ambient RH because of a high VIF value), the model with the lowest AIC included mid-late canopy RH and mid-late average rain, although the model was not significant ($p = 0.1218$) (Table 3.20). If the mid-late average ambient RH was included in the model, the final model was the same as when it was excluded (Table 3.20).

The original model for the averaged Spornado rating during the total flowering period indicated multicollinearity. The variable with the highest VIF value was removed (average

canopy temperature). The final model indicated that average ambient temperature was the only variable for determining total average Spornado ratings during the flowering period, although the model itself was not significant ($p = 0.1326$). If the average canopy temperature was included in the model, the final model suggested that only the average canopy temperature was significant for predicting the average Spornado risk level during the flowering period, but the model was still not significant and did not change the coefficient of determination or adjusted R^2 ($p = 0.1266$) (Table 3.20).

The model created for determining the amount of airborne ascospores (*S. sclerotiorum* DNA) collected in the rotorod samplers during early-mid flowering showed no multicollinearity when all weather variables were included in the model (early-mid average canopy RH and temperature, ambient RH and temperature and average rain). The final model suggested after stepwise regression indicated that early-mid canopy temperature, early-mid canopy and ambient RH influenced the level of pathogen DNA on the rotorods. The final model suggested was not statistically significant ($p = 0.4945$) with low coefficients of determination ($R^2 = 0.17$ and adjusted $R^2 = -0.03$) (Table 3.20). The late-mid flowering model showed multicollinearity. The weather variable with the highest VIF value was removed (mid-late flower average ambient RH), and the final model suggested from stepwise regression indicated that only mid-late canopy temperature had a significant relationship with late average DNA amounts on the rotorod. The model was also statistically significant ($p = 0.03216$) and accounted for 29% (R^2) of the variation for *S. sclerotiorum* DNA captured on the rotorods during the mid-late flowering period (adjusted $R^2 = 0.24$) (Table 3.20). If mid-late average ambient RH was retained in the model, the same final model was suggested at the end of stepwise regression for predicting the mid-late average rotorod DNA. The model for total average DNA captured on rotorods showed multicollinearity so the variable with the highest VIF (average ambient temperature) was removed. After stepwise regression, the suggested model included the remaining weather variables (average canopy RH, average canopy temperature, average ambient RH and average rain), and was statistically significant ($p = 0.01208$). This suggested model showed that average canopy RH explained 66% (R^2) of the variation found for the total amount of *S. sclerotiorum* DNA on the rotorods during flowering, while 54% (Adjusted R^2) could be explained by the other independent variables in the model. If the average ambient temperature remained in the model, the same final model was suggested by stepwise regression.

Environmental variables during early-mid flowering on petal infestation levels showed no multicollinearity and these were kept in the model. The resulting suggested model after stepwise regression was not statistically significant ($p = 0.1106$) and only indicated that early average ambient temperature was significant for predicting early average petal infestation levels with a small coefficient of determination ($R^2 = 0.24$ and adjusted $R^2 = 0.16$). The model created for the mid-late average amount of petal infestation level showed multicollinearity when all weather mid-late variables were included (mid-late average canopy temperatures), but the model with the lowest AIC values indicated that no weather variables were statistically significant for predicting the amount of petal infestation for the mid-late flowering period. Outliers were investigated, and when the outlier (F3 in 2020) was removed from the model, it was not significant ($p = 0.121$). Three weather variables were included in the final model (mid-late flowering average canopy and ambient RH and late ambient temperature), with higher coefficients of determination ($R^2 = 0.54$ and adjusted $R^2 = 0.35$) and a lower AIC value (data not shown) than if no weather variables were included (Table 3.20). If mid-late average canopy temperatures were included in the model, the final suggested model changed to indicate late canopy temperature as statistically significant for the mid-late average petal infestation. It also changed the *p*-value ($p = 0.01445$). In this case, due to a relatively small sample size, multicollinearity would make the equation sensitive to minor changes, and so it should not be ignored for this equation.

The model to determine environmental conditions averaged over the total flowering period on the average amount of petal infestation level showed multicollinearity when all the variables were included. The variable with the highest VIF value was removed from the model (average canopy temperature). After stepwise regression, the final model with the lowest AIC values suggested that no weather variables were significant. Outliers were investigated, and from residual vs. fitted plots, they showed that F3 in 2020 was the outlier (data not shown). When this outlier was removed, the resulting model became significant ($p = 0.04049$) and included total average canopy RH, average ambient temperature, and total rain. The AIC value for the model with the outlier removed was lower than the one suggesting that no weather variables influenced the total average petal infestation levels, and the coefficient of determination was increased ($R^2 = 0.67$ and adjusted $R^2 = 0.53$) (Table 3.20). If average canopy temperature was retained in the model, the final suggested model was the same.

Ultimately, many of the models suggested for predicting ascospore/*S. sclerotiorum* DNA levels (Spornado, rotorod and petal infestation levels) were not statistically significant at $P < 0.05$. Only three were significant: mid-late average environmental conditions for predicting DNA levels on rotorods; total average environmental conditions for predicting DNA levels on the rotorods; and total average environmental conditions for predicting the petal infestation level (Table 3.20). Of the three that were statistically significant, the total average environmental conditions for predicting the DNA amounts on the rotorods accounted for the highest amount of variation (adjusted $R^2 = 0.54$), although the equation for the total average petal infestation levels was very close (adjusted $R^2 = 0.53$) (Table 3.20).

3.3.9 Influence of Inoculum Load and Weather on Final Disease Incidence and Severity

Following a similar procedure for exploring the influence of in-field environmental conditions on ascospore detection methods, DI and severity were investigated using multiple linear regression. The CPT1 and CPT2 were not included in the models because of the limited sampling size for both petal test kits. The fungicide trial sites at Scott, SK, and Outlook, SK, in 2019 were removed from models aiming to predict the level of petal infestation and weather effects, and their combined influence on DI and severity, as no petal testing was conducted for these sites at the Plant Health Lab (AAFRED, Edmonton, AB).

Before the regressions were performed, correlations were determined using a correlation chart and the linear models tested for multicollinearity using the VIF function. Interactions between every period (early-mid flower, mid-late flower, total flowering average) were also tested, and none was found to be significant. A stepwise regression procedure was then used to reduce the models' independent variables to those that had significant relationships to the dependent variable, in this case DI or severity.

Each spore detection method (Spornado, rotorod sampler and Plant Health Lab petal samples) was used singularly, but in combination with in-field environmental variables (average canopy RH and temperature, average ambient RH and temperature and average rain), to determine if one spore detection method had better predictive power for DI and severity than others. Other models were created and tested to determine if a certain period could better predict DI and severity using the same sets of variables above (early-mid, mid-late and total average).

Inclusion of all ascospore/*S. sclerotiorum* DNA detection methods together at each time with all environmental variables was also investigated.

3.3.9.1 Significant Regression Analysis to Determine the Relationship between the Spornado and Environmental Variables on DI and Severity

The model created for determining DI using only the early average Spornado as the ascospore/pathogen detection method plus the early-mid flower averaged in-field environmental variables showed no multicollinearity. Stepwise progression reduced the model to show that only early canopy RH was significant to DI, with the model itself significant with a $p = 0.003$ ($R^2 = 0.48$ and adjusted $R^2 = 0.44$) (Table 3.21). The model using only the mid-late average Spornado rating, and mid-late average environmental variables, showed multicollinearity, with mid-late ambient RH having the highest VIF value (data not shown). After removing mid-late average ambient RH, the remaining variables had VIF values <10 and stepwise progression proceeded. The final suggested model indicated that mid-late canopy RH, mid-late average Spornado ratings, mid-late average rain and mid-late ambient temperature all were significant to the final DI. The model was also statistically significant ($p = 0.001776$, $R^2 = 0.77$ and adjusted $R^2 = 0.68$) (Table 3.21). If mid-late ambient RH was retained, the model with the lowest AIC included mid-late canopy temperature, mid-late ambient temperature and mid-late canopy RH. In the original model, the two variables with the highest VIF were mid-late canopy temperature and mid-late ambient RH. In this case, the multicollinearity could not be ignored, as the two variables are highly correlated, as indicated by a correlation chart (data not shown), and a change in one of the two variables can cause a shift in the other.

The model created for the total average Spornado ratings and total average weather variables showed multicollinearity. Average ambient temperature had the highest VIF value (data not shown), so it was removed from the model; however, when it was re-introduced into the model for testing, the final model did not change. The only variable remaining after stepwise regression in the statistically significant model ($p = 0.002051$) was average canopy RH, which accounted for 50% of the variation found in DI (adjusted $R^2 = 0.47$) (Table 3.21).

A second set of models was generated for using only early-mid Spornado ratings and early to mid-average environmental variables for predicting the average level of severity. In the

first model, there was no multicollinearity within the variables, so the model proceeded to stepwise regression. The final model was statistically significant ($p = 0.008139$, $R^2 = 0.40$ and adjusted $R^2 = 0.36$) (Table 3.22). The model that included the mid-late average Spornado rating and mid-late average environmental variables showed multicollinearity in mid-late ambient RH, so this parameter was removed from the model. The resulting model showed no further multicollinearity and was subjected to stepwise regression. The final suggested model was statistically significant ($p = 0.01048$), and included mid-late canopy RH, mid-late average Spornado ratings and mid-late average rain ($R^2 = 0.60$ and adjusted $R^2 = 0.49$) (Table 3.22). When mid-late ambient RH was added back into the model to test the multicollinearity, the final suggested model showed both mid-late canopy temperature and mid-late ambient RH, which have a high amount of correlation (data not shown), and therefore multicollinearity cannot be ignored. A model generated using the total average of each environmental variable and the total average Spornado ratings during the flowering period showed multicollinearity in the average ambient temperature. After the variable was removed, the rest of the independent variables had VIF values <10, and so proceeded through stepwise regression. The final suggested model included only average ambient RH as statistically significant in determining average severity when only the Spornado was included in the model, and the average rotorod DNA and petal infestation levels were excluded. The model was also significant ($p = 0.01161$, $R^2 = 0.38$ and adjusted $R^2 = 0.33$) (Table 3.22). When the effect of multicollinearity was tested by adding excluded variables to the original equation, the final model was unchanged.

3.3.9.2 Regression Analysis to Determine the Relationship between the Rotorod Sampler and Environmental Variables on DI and Severity

A model using early environmental variables and only early averaged rotorod DNA showed no multicollinearity, so all variables (early average rotorod DNA, early average canopy RH and temperature, early average ambient RH and temperature and early average rain) were retained. After stepwise regression, the final model including early rotorod results could account for 52% of the variation in DI, with 45% of variation attributed to the remaining variable, and was statistically significant ($p = 0.008137$) (Table 3.21).

The model using late variables and the rotorod for spore detection showed multicollinearity, and the mid-late average canopy temperature was removed from the model. After testing the VIF values, mid-late ambient RH also showed multicollinearity after mid-late canopy temperature had been removed, so late ambient RH was also removed. Before stepwise progression proceeded, mid-late canopy temperature was added back to the model to determine if the VIF value would be lower if the late ambient RH was removed, but it still had a high VIF value, so both variables were removed from the model before proceeding to stepwise progression. This left mid-late average DNA on rotorods, mid-late ambient temperature, mid-late average canopy RH and mid-late average rain in the model before stepwise regression. The variables left in after the stepwise regression were mid-late average canopy RH and late average rain (Table 3.21). The model was also statistically significant ($p = 0.003081$, $R^2 = 0.59$ and adjusted $R^2 = 0.53$) (Table 3.21). When the regression was run with both removed values reintroduced to the original model, the resulting final model included mid-late canopy RH, mid-late ambient RH (the previously removed variable) and mid-late average rain. However, mid-late canopy RH and mid-late ambient RH were highly correlated, so the model excluding mid-late average ambient RH and mid-late canopy temperature was retained. The model using total average weather variables and total average rotorod DNA showed multicollinearity when the model was first created, which resulted in the removal of average ambient temperature. The final model suggested was statistically significant ($p = 0.002359$) and included total average rotorod DNA and average ambient RH were significant for predicting DI ($R^2 = 0.61$ and adjusted $R^2 = 0.55$) (Table 3.21). When average ambient temperature was added back into the model to test if multicollinearity could be ignored, there were no changes in the final model suggested.

The same variables (early-mid average DNA on rotorods, early-to-mid average environmental variables (canopy RH and temperature, ambient RH and temperature and rain)) were used for predicting the level of stem rot severity. There was no multicollinearity in the original model, so it proceeded to stepwise regression. The final suggested model included early-mid canopy RH and early-mid average rotorod DNA. This model was also statistically significant ($p = 0.01057$, $R^2 = 0.50$ and adjusted $R^2 = 0.43$) (Table 3.22). The original model that included mid-late average rotorod DNA amounts and environmental independent variables showed multicollinearity in mid-late average ambient RH, so this was removed from the model. The resulting model also showed multicollinearity in mid-late canopy temperature. To ensure

that there was multicollinearity in both independent variables, mid-late ambient temperature was inserted back into the model and re-tested, but still showed multicollinearity, so both independent variables were removed. After stepwise regression, the final model was statistically significant ($p = 0.007203$) and suggested that mid-late canopy RH accounted for 53% of the variation in average disease severity, while mid-late average DNA amount on the rotorods accounted for 46% (Table 3.22). When the regression was run with both removed values reintroduced to the original model, the resulting final model included all the original independent variables; however, mid-late canopy RH and mid-late ambient RH were highly correlated, so multicollinearity cannot be ignored. The model created for using the total average environmental variables during the flowering period coupled with the total average DNA amounts captured on the rotorods showed multicollinearity in the average ambient temperature, so the average ambient temperature as removed from the model. The final model indicated that the average DNA amount collected on the rotorods and average ambient RH were statistically significant for predicting severity ($p = 0.0016$, $R^2 = 0.63$ and adjusted $R^2 = 0.57$) (Table 3.22). When multicollinearity was tested by adding in the excluded variable into the original model and run through stepwise regression, the final suggested model was unchanged.

3.3.9.3 Regression Analysis to Determine the Relationship between AAFRED Petal Samples and Environmental Variables on DI and Severity

A model created that used early environmental variables and only early averaged petal test DNA showed no multicollinearity when all variables were included. After stepwise regression, the model showed that early ambient temperature, early petal test DNA amounts, early canopy temperature and early canopy RH were statistically significant in determining the final level of DI. The model was statistically significant ($p = 0.004156$), with a coefficient of determination of 86% (adjusted $R^2 = 0.78$) (Table 3.21). Mid-late variables with the petal test as the spore detection method were very similar for the model for mid-late average rotorod results on DI, with the VIF high for both late canopy temperature and late ambient RH. After stepwise regression, the final suggested model included late average petal infestation and late canopy RH. The model, however, was not statistically significant ($p = 0.1147$, $R^2 = 0.38$ and adjusted $R^2 = 0.25$) (Table 3.21). If the excluded variable had been included in the model, then they both

would have ended up in the final model, but because both were correlated strongly with mid-late canopy RH, the multicollinearity cannot be ignored. The model generated for the total average petal infestation levels and environmental conditions over the flowering period on the level of stem rot incidence showed multicollinearity, so the independent variable (average canopy temperature) with the highest VIF value was removed. After the model went through stepwise regression, the final suggested model was statistically significant ($p = 0.003617$) and included all environmental variables included in the original model after testing for multicollinearity, but not total average petal infestation levels ($R^2 = 0.80$ and adjusted $R^2 = 0.72$) (Table 3.21). When the original model that included average canopy temperature was tested, the final model was unchanged.

Early-mid average environmental variables and early-mid average petal infestation were also used to predict the level of severity. No multicollinearity was found in the original regression model, so stepwise progression proceeded. The final model indicated that the combination of early-mid average canopy temperature, early-mid ambient RH and temperature, and early-mid average petal infestation level was statistically significant to the average severity rating ($p = 0.003348$, $R^2 = 0.87$ and adjusted $R^2 = 0.79$) (Table 3.22). The equation modelling the mid-late petal infestation levels and mid-to-late environmental independent variables on average severity showed multicollinearity in the mid-late canopy temperature variable. After it was removed from the model, no other variables showed multicollinearity. The final model (mid-late PT, canopy RH, ambient RH, ambient temperature and rainfall) suggested was statistically significant ($p = 0.005054$, $R^2 = 0.91$ and adjusted $R^2 = 0.83$) (Table 3.22). When the excluded variable was introduced back into the original model to determine if multicollinearity could be ignored, the final model included every variable from the original equation. Multicollinearity can influence which variables are significant to average disease severity, so it cannot be ignored for this equation. The model created for predicting severity using the average level of petal infestation during the flowering period coupled with environmental variables averaged over the flowering period showed multicollinearity in average canopy temperature, so it was removed from the model. The remaining independent variables showed no further multicollinearity. The final model suggested after stepwise regression indicated that average ambient RH and temperature, as well as the level of petal infestation, could be used to predict the severity ($p = 0.001795$, $R^2 = 0.83$ and adjusted $R^2 = 0.77$) (Table 3.22). When multicollinearity

was tested by adding in the excluded variable (average canopy temperature), the coefficients had changed. In this case, multicollinearity cannot be ignored because it can influence which variables are significant to the average severity.

3.3.9.4 Regression Analysis Using Petal Infestation Levels, Spornado Risk Levels and Rotorod *S. sclerotiorum* DNA Amounts Coupled with Environmental Variables on DI and Severity

Using all available early average spore detection methods and variables, the original model showed multicollinearity, with early average ambient temperature having the highest VIF value. Once early average ambient temperature was removed, all remaining VIF values were <10, so the model proceeded through stepwise regression. The final suggested model indicated that the *S. sclerotiorum* DNA amount as determined by the Plant Health Lab (AAFERD, Edmonton, AB) from early-mid flower collected petals, the early-mid flower Spornado ratings and early-mid average rain were statistically significant to determining final DI, and the model itself was also statistically significant ($p = 0.024$, $R^2 = 0.68$, adjusted $R^2 = 0.55$) (Table 3.21). If early-mid average ambient temperature was retained in the model, the final suggested model after stepwise regression included all seven independent variables for the early-mid flowering period (canopy temperature and RH, ambient temperature and RH, average rain, average petal infestation level, average Spornado rating and average DNA amounts of rotorods). The final model summary for this equation was statistically significant ($p = 0.01345$) and had a strong relationship with DI (data not shown); however, due to a small sample size, the multicollinearity found in this model could cause a problem with predicting final DI, so it cannot be ignored. The model that included all variables (all ascospore/DNA detection methods plus all weather variables) averaged over the mid-late flowering period showed multicollinearity in the mid-late average ambient RH and mid-late canopy temperature. The final suggested model included late average canopy RH, late average petal infestation and late average Spornado ratings. However, the model was not significant ($p = 0.09729$, $R^2 = 0.53$, adjusted $R^2 = 0.35$) (Table 3.21). When both variables were kept, the final model suggested inclusion of both of the excluded variables. The multicollinearity could not be ignored for this model, as mid-late ambient RH was highly correlated with both mid-late average canopy temperature and mid-late canopy RH. Mid-late

average canopy temperature still had high VIF values after mid-late ambient RH was removed, so it too cannot be ignored. The model created for all variables averaged over the total flowering period showed multicollinearity in average ambient temperature and average ambient relative humidity in the following model. The remaining model included average canopy RH, average canopy temperature, average rain, average DNA on the rotorods, average Spornado rating and average petal infestation levels (total average over the flowering period) before stepwise regression. After stepwise regression, the remaining variables in the model included average canopy temperature, average canopy RH, average rain and average rotorod DNA. This model was statistically significant ($p = 0.02242$, $R^2 = 0.77$, adjusted $R^2 = 0.63$) (Table 3.21). When the original model, which included all variables, was tested, the coefficients had changed; however, the multicollinearity cannot be ignored because it can influence which coefficients are significant to the dependent variable.

All early-mid average environmental variables and ascospore/DNA detection methods were used to investigate how they influenced the level of severity. With all independent variables included, the original model showed multicollinearity in early ambient temperature, so it was removed from the regression equation. The remaining variables were all under the acceptable VIF values. After stepwise regression, the resulting final model showed that early-to-mid average rain, petal infestation level, Spornado risk level and amount of *S. sclerotiorum* DNA on the rotorods as significant to the average severity levels; however, the model was not significant ($p = 0.07011$, $R^2 = 0.67$, adjusted $R^2 = 0.48$) (Table 3.22). When the original model that included all variables was tested to determine if multicollinearity could be ignored, the resulting model was different from the model proposed when early ambient temperature was not included. In this case, multicollinearity cannot be ignored because it can affect which coefficients are significant to the average severity. The model that included all spore detection methods and environmental variables during the mid-late flowering period showed multicollinearity in the mid-late canopy temperature and late average ambient RH, so both were removed from the model. The final model suggested that all remaining independent variables could be used to predict the average stem rot severity and was significant ($p = 0.04836$, $R^2 = 0.79$, adjusted $R^2 = 0.61$) (Table 3.22). When the variables were added back to the model to test the effects of multicollinearity, this changed the suggested coefficients that were included. Mid-

late canopy temperature and mid-late ambient RH were highly correlated; therefore, multicollinearity cannot be ignored for this model.

The model created for the prediction of the average severity that included all independent variables (all averaged over the total flowering period) showed multicollinearity with average ambient temperature and average ambient RH. After stepwise progression, the final model included the remaining environmental variables, the total average DNA found on rotorods during the flowering period and the petal infestation level, and was statistically significant ($p = 0.02441$, $R^2 = 0.84$, adjusted $R^2 = 0.70$) (Table 3.22). Multicollinearity was tested by running the original equation through stepwise regression as if there was no multicollinearity. The resulting model included both previously excluded variables, which had a high amount of correlation (data not shown), and therefore the multicollinearity cannot be ignored.

3.4 Discussion

Various tools have been developed to forecast Sclerotinia stem rot in Canada and internationally. Many of these tools target key aspects of the disease cycle, which influence the levels of disease in the field, such as weather conditions. However, exact disease levels are difficult to predict and weather stations may be many kilometers away from a particular field. Some systems have had great success with prediction models using only weather-based variables. For example, a logistical model for quantitatively predicting stem rot levels in oilseed Brassicas in India based on weather-based variables could accurately predict final disease incidence with 93% accuracy, although the authors indicated that accounting for in-field inoculum could further improve this accuracy (Sharma et al. 2015). Other forecasting methods have been developed, but are considered 'risk averse', and can better predict fields with low amounts of forecasted disease levels than fields at higher risk (Turkington et al. 1991a; Young et al. 2020). These same researchers have suggested that some form of measuring inoculum density in the field would be beneficial, as the best indication of no risk is an absence of inoculum. Accurate predictions of stem rot are essential, as the surest method of effective disease control is a fungicide applied during flowering. Such a fungicide targets the germinating ascospores of *S. sclerotiorum* on the canola petals and/or progression of the pathogen from petals into plant tissues, and should be applied prior to the appearance of symptoms. Therefore, being

able to detect ascospore levels before and during the critical phases of flowering (generally up to 50% flower) can aid farmers in making cost-effective spray decisions. In western Canada, there are only a few such methods for ascospore detection, offered by private sector seed test laboratories, which provided DNA-based sampling services through proprietary protocols.

Weather data collected from all fields in this study was used to explore the relationship between ambient weather variables versus in-canopy weather variables and ambient versus recorded weather conditions at the nearest Environment Canada weather station (or ACIS station). The relationships between variables varied between years and locations; the only relationship that remained consistent between years and across all field locations was that the in-canopy RH was significantly different from the ambient RH. In-canopy temperature was also generally significantly different from ambient temperature, as only 16% of all fields, including AAFC/AAFRED sites, had similar in-canopy and ambient temperatures. From these observations, we can conclude that ambient RH and temperature may not provide an accurate representation of the in-canopy conditions (Tables A.1-A.19). Based on the data collected in this study, canopy RH was generally about 6% above ambient RH. Thus, if in-canopy monitoring is not possible due to cost or practicality, then one may be able to use ambient RH, with the understanding that canopy RH will likely be 3 to 13 % above ambient RH. When the ambient RH measured in-field was compared with the nearest weather station, it was generally significantly different at most of the commercial field sites and AAFC/AAFRED locations. In contrast, ambient in-field temperatures tended to be closer to the weather station measurements (not significantly different in nearly half of the commercial fields). At the AAFC/AAFRED sites, the in-field ambient temperature was not significantly different from the public weather station data at 80% of the locations. This was likely because public weather stations were often located on government research stations associated with the AAFC/AAFRED sites; in contrast, public weather stations were between 2 km and 28 km away from the commercial field sites. The greatest extent of agreement between in-field weather data and data recorded at the nearest weather station occurred for rainfall, the values of which were similar for most commercial fields and AAFC/AAFRD sites. Wind speeds recorded at the commercial field sites were generally significantly different from the public weather station data, likely because this parameter may be affected by more factors at the field level, such as topography and nearby vegetation (Tables A.1-A.19) (Ruel et al. 1998).

Conditions favouring Sclerotinia stem rot development have been described by Bolton et al. (2006), Morrall and Dueck (1982) and Turkington et al. (1991b), and include moderate temperatures and frequent rainfall resulting in higher RH values and leaf wetness. Such favourable conditions were observed in 2019 due to frequent rain events, which resulted in higher RH and the collection of free moisture in leaf axils and bases, which likely contributed to the moderate to severe levels of stem rot recorded. While moisture is a key driving factor related to stem rot development, excessive rainfall can negatively affect disease by limiting ascospore dispersal. Water droplets can form on the cup-like portion of the apothecia, preventing their discharge into the air, with rain also potentially washing the ascospores from the air and/or from canola petals (Kamal et al. 2016; Qandah and del Río Mendoza 2011; Turkington et al. 1991b). The weather conditions at most of the AAFC/AAFRED sites in 2019 likely limited the risk and development of Sclerotinia stem rot. Most sites experienced dry conditions in June and to a certain extent in July, which reduced treatment effects. The exception was Beaverlodge, AB, which received a moderate amount of rainfall and had weather conditions consistent with commercial fields in the Edmonton region in 2019 and 2020.

In terms of the relationship between ascospore detection methods and weather variables (CPT1 and CPT2 were not tested because of a low sample size), the weather variables could be used to predict *S. sclerotiorum* DNA levels on the rotorods and Plant Health Lab-tested petal samples. However, the models were significant only with averages for each variable obtained from the mid-late flowering period and over the total flowering period. No models were significant for predicting the Spornado risk level or for any spore detection methods during early-mid flowering. The strongest relationships for both the rotorod and Plant Health Lab-tested petal samples occurred with averages from the total flowering period (adjusted $R^2 = 0.54$ and 0.53, respectively). The lack of strength in the models could indicate that additional factors need to be included, such as soil moisture, which other researchers have found to increase the strength of their models for predicting DI (Bom and Boland 2000; Sharma et al. 2015). Soil moisture is an indirect measure of moisture availability under the canopy, and can give insights into the environmental conditions present in a field for sclerotial germination, ascospore production and disease development (Bom and Boland 2000). The incorporation of soil moisture may be advantageous for predicting DI where environmental conditions seem unfavorable, but petal infestation is high (Bom and Boland 2000). While weather, especially moisture, is a

critical factor in the stem rot cycle, another key factor is the population of sclerotia in the soil. Thus, relying exclusively on weather to predict spore load is problematic, as there may be scenarios where the weather is predicting increased ascospore loads, but due to no to low sclerotial populations, the pathogen is simply not present in sufficient quantities to respond to these favourable weather conditions. This was illustrated by the results from the AAFC/AAFRED fungicide trial sites in Brooks and Lacombe, AB, where weather conditions were somewhat favourable for stem rot, but limited to no inoculum was detected via spore sampling or petal testing. No relationship between early-mid weather variables and inoculum levels is significant because that is the time when fungicide decisions are made, and infections that occur during the same period are more likely to develop on the main stem of canola and become more established (Gugel 1986).

The relationship between final disease incidence, ascospore detection method and weather variables during early-mid flower, mid-late flower and the total flowering period was variable. The strongest relationship was detected between final stem rot incidence, Plant Health Lab-tested petal samples and weather variables averaged over the early-mid flowering period (R^2 = 0.86, Table 3.21), which is the most critical time for making fungicide decisions. When trying to predict stem rot severity, the strongest relationship was found between average severity, petal samples and weather variables averaged over the mid-late flowering period (Table 3.22). Regression analysis in this study did not take into account year-to-year variation, or variation between sites, due to a small sampling size. Other research has supported that petal infestation levels were highly correlated with final disease incidence (Ficke et al. 2018; Rogers et al. 2009; Turkington et al. 1991a). Ziesman (2016) found the strongest relationship between final DI and petal samples at full bloom (R^2 = 0.919, p = 0.002). For the other two spore detection methods, the strongest relationships with DI were found in the mid-late flowering period and the total flowering average for the Spornado and rotorod samplers, respectively. The lack of significant relationships during early-mid flower for the Spornado and rotorod samplers could be attributed to peaks in the quantity of *S. sclerotiorum* DNA detected after the early-mid flowering period, which likely reflected environmental conditions that would have influenced sclerotial germination, apothecial development and ascospore production and release. Qandah and del Río Mendoza (2011) found that peaks in *S. sclerotiorum* ascospore levels occurred after the daily

average canopy RH was >85% for seven days. Other researchers have indicated that apothecia did not appear in the field before the bud stage or early flower (Morrall and Dueck 1982).

During mid-late bloom, leaf abscission begins on the canola plant, reducing the potential for infection sites (Gugel 1986; Turkington and Morrall 1993). The loss of leaves on the plant also creates a less favorable environment for infection; however, prolonged favorable conditions for canola growth (e.g., cool, moist weather) can delay leaf abscission, and therefore increase the period for potential main stem or branch infections. Rude (1989) found that a late application of fungicide reduced disease incidence (compared with a check) by >80% at four of five locations, with the greatest control at locations with the highest inoculum during full bloom. However, they also found that plants infected at late bloom did not experience a significant yield reduction (8% yield loss per plant), unlike plants infected at early bloom. Young et al. (2020) also found that when a weather-based alert was issued during late (or very early) flowering, and a fungicide was applied, it provided "significant disease control", indicating that enough yield damage could still occur during late flowering to justify a fungicide application. Other researchers, however, reported that fungicide applications at full flower (BBCH 65) made no significant difference, with the application having a 4% increase in effectiveness, but having no effect on yield (Spitzer et al. 2017).

Variability in stem rot incidence and severity, even when the canopy microenvironment had less desirable conditions (F1 vs. F4 in 2019, F1 vs. F5 in 2020), indicates that predicting the exact value of DI, even with known inoculum pressure, can be very challenging. Thus, instead of exact values, a minimum threshold level of inoculum pressure could be determined with set levels of optimal weather conditions, whereby there is risk of stem rot that warrants a fungicide application. From the data in this study, it appears that at least 1.0×10^{-4} ng *S. sclerotiorum* DNA/m^3 air/h or 1.0×10^{-4} ng *S. sclerotiorum*/petal was required during early flowering with optimal weather conditions (primarily RH >80%) for moderate to severe infections to occur. Less than 1.0×10^{-4} ng DNA/m^3 air/h or per petal resulted in a lower DI with a lower average severity rating (Tables 3.1, 3.17, 3.2). This was illustrated when comparing *S. sclerotiorum* DNA amounts in samples from the commercial fields and Beaverlodge, AB, in 2019 (all of which were $>1.0 \times 10^{-4}$ ng DNA/m^3 air/h), with the 2020 commercial fields and most of the fungicide trial sites in 2019 (generally $<1.0 \times 10^{-4}$ ngDNA/m^3 air/h or $<1.0 \times 10^{-4}$ ng/petal). Another factor that may have contributed to the variable DI observed in F1, F2 and F3 in 2020

was the infection of many plants in these fields by *Plasmodiophora brassicae* (the cause of clubroot disease), which can affect leaves (potential infection sites) and petals, canopy density and result in premature ripening (Hwang et al. 2012).

Other researchers have found that there is a level of inoculum above which lesions and DI do not increase further, even with the addition of more inoculum. Such levels have been reported on lettuce (87 ascospores/cm^2), pinto beans (2.0×10^2 ascospores/flower), and oilseed rape (5.0×10^2 ascospores/petal or 1.5×10^2 ascospores m^{-3}) (Clarkson et al. 2014; Harikrishnan and Rio 2008; Heran et al. 1999; McCartney et al. 1999). This suggests that thresholds can be determined as to the number of ascospores required (as measured via DNA-based testing), per either petal or airborne spore concentrations, to cause an epidemic that warrants a fungicide application. Rogers et al. (2009) reported negligible levels of stem rot on oilseed rape when a maximum of 4 ascospores/m^3/day were detected at the start of flowering in Rothamsted, UK. They also found an "unusually high for the region" incidence of stem rot when the number of airborne ascospores peaked at 12 m^3/day during the flowering period. Young et al. (2020) also found that sites with a low DI had <3% petal infestation.

Comparison of two different spore trap methods, one passive and one volumetric, as well as the Plant Heath Lab petal results, highlighted two key findings. First, a single sample during early flowering does not provide an accurate representation of the overall airborne inoculum, and flushes of ascospores can occur throughout the flowering period. This is consistent with the observations of Qandah and del Río Mendoza (2011, 2012), Turkington and Morrall (1993), Turkington et al. (1991a) and Young et al. (2020). The rotorod sampler better monitored flushes of ascospores that occurred during the flowering period, detecting the fluctuations in *S. sclerotiorum* DNA associated with these flushes; in contrast, the Spornado did not catch the smaller flushes of ascospores. Second, while a definitive amount of ascospore pressure can be helpful for forecasting Sclerotinia stem rot, it does not guarantee the presence or absence of lesions, nor the severity of disease, if only ascospore pressure is being monitored. Overall and on its own, the Spornado did not accurately reflect final disease levels, and could potentially indicate false positives in relation to inoculum levels that suggested the need for fungicide. Ultimately, there was a higher amount of airborne inoculum captured by the rotorod samplers in 2019; however, this was not reflected in the results obtained with the Spornado. In general, the use of inoculum load assessments will be more beneficial if coupled with a forecasting system,

as is done in the UK, where ambient weather is monitored for risk during the flowering period, and an alert issued if conditions are met (Young et al. 2018). Moreover, monitoring aerial spore load and necessary weather conditions (RH >80%) prior to and during flowering may help to guide fungicide timing, whether at or just prior to early bloom or later as the crop progresses into full bloom. For example, favourable weather and increased inoculum loads prior to and during the start of flowering may indicate the need for fungicide application earlier in the bloom period. If these favourable conditions persist into full bloom then a second application of fungicide may be warranted depending on yield potential and commodity price. Conversely, if weather and inoculum loads are not favourable prior to and during early flowering, but then become more favourable as the crop progresses towards full bloom, a full bloom application of fungicide may be more effective.

The different petal tests evaluated in 2020 gave variable results. Nonetheless, CPT1 seemed to be the best correlated with final stem rot levels and petal tests conducted by the Plant Health Lab, especially in fields with low amounts of inoculum pressure and DI. At the AAFC/AAFRED sites, CPT1 (but not CPT2) was assessed in 2019, with similar correlations found between CPT1 and petal tests conducted by the Plant Health Lab. Potential reasons for discrepancies between the rotorod and Plant Health Lab inoculum assessments and those for the Spornado and CPT1 and CPT2 may reflect inherent differences in the protocols, although the specificity of the molecular tests is likely also a factor. Ziesman (2016) and Ziesman et al. (2016) found specificity issues for tests reported in the literature, with some cross-reacting with fungi other than *S. sclerotiorum*. Previous research has already shown that the level of petal infestation varies over time (Almquist and Wallenhammar 2015; Turkington and Morrall 1993; Turkington et al. 1991a; Turkington 1991; Ziesman et al. 2016), which was confirmed in this study. Nonetheless, including petal tests or spore trapping in a Sclerotinia stem rot forecasting system, at least once during the critical flowering stages (BBCH 61-65), may be more reflective of accumulated risk conditions, helping to synthesize host, pathogen and environmental responses in relation to disease risk.

Overall, the results of this study highlight the need for an integrated forecasting system to generate the most accurate stem rot predictions. As others have found (Turkington 1991; Young et al. 2020), the most precise stem rot forecasts occurred when there was no risk of disease due to a lack of inoculum, as determined by petal infestation levels or airborne ascospores, coupled with

unfavourable weather conditions. Much like what was proposed by Bom and Boland (2000), a stepwise sampling technique can be employed if conditions are favorable and there has been some indication that airborne ascospore pressure is present. For example, if there is a significant history of Sclerotinia stem rot in a particular field, or spore traps give a positive result (a Spornado result of at least "detected", or a rotorod sampler result of $\geq 1.0 \times 10^{-4}$ ng DNA/m^3 air/h), then a petal test could be used to confirm if inoculum is sufficiently high to result in a DI >10-20%. Specific fungicide application decisions could then be made depending on commodity price and overall yield potential). The current results suggest that stem rot risk and the potential need for fungicide are elevated at an infestation level of $\geq 1.0 \times 10^{-4}$ ng DNA/petal at flowering, a high average canopy RH (>80% on at least 85% of days), at least 50% of days with rain > 0 mm during the flowering period, and a low canopy temperature (between 13˚C and 18˚C). This was best demonstrated in all commercial fields in 2019 and the AAFC/AAFRED sites at Beaverlodge, AB, Brooks, AB, Lacombe, AB, and Outlook SK, where all four sites had similar conditions. Most other AAFC/AAFRED sites (Lethbridge, AB, Indian Head, AB, Melfort, SK, Scott, SK, Brandon, MB and Normandin, QC) did not experience levels of petal infestation likely required for an outbreak (i.e., 1.0×10^{-4} ng DNA/petal), while they also had generally unfavourable environmental conditions that were not conducive for disease progression.

The advent of DNA-based testing technologies, improved pathogen identification and rapid results can greatly enhance our ability to assess *S. sclerotiorum* inoculum loads and stem rot risk in canola in a timely fashion (Ziesman 2016; Ziesman et al. 2016). Nonetheless, while existing tests for the occurrence and severity of stem rot can provide an indication of risk, further refinements in testing procedures may improve forecasts. Overall at a particular point in time, inoculum load assessments synthesize the favourability of the weather (for inoculum production) and the potential for the stem rot pathogen to be present in sufficient quantities. This latter aspect is in relation to sclerotial load in the field and adjacent fields and the extent of sclerotial germination and apothecia production. Ultimately, measures of pathogen (ascospore) inoculum level, while important, need to be considered in light of environmental conditions, as well as field history (e.g., presence of sclerotia in the soil). An integrated forecasting system, which takes into account all components of the disease triangle, will be most effective for predicting Sclerotinia stem rot of canola as well as other diseases.

3.5 Tables

Table 3.1 Average, minimum and maximum Sclerotinia stem rot disease incidence, disease severity ratings, Spornado ratings, ngDNA contained on rotorod samples and petal infestation levels reported by AAFRED Plant Health Lab, Commercial Petal Test 1 and Commercial Petal Test 2 in 2019 and 2020 in commercial fields near Edmonton, AB.

Year	Field	Descriptive Statistics	DI[a]	Disease Severity[a]	Spornado Rating[b]	Rotorod DNA (ngDNA/m^3/h)[c]	Petal Infestation Level (ngDNA/petal)[d]	Commercial Petal Test 1 (PPI)[e]	Commercial Petal Test 2 (PPI)[f]
2019	F1	Average	77.0	1.5	2.0	1.1E-04	N/A	N/A	N/A
		Min	70.0	1.1	1.0	2.3E-05			
		Max	93.0	1.9	3.0	4.6E-03			
	F2	Average	64.4	1.1	2.6	1.5E-04	N/A	N/A	N/A
		Min	56.0	0.8	1.0	1.3E-05			
		Max	73.0	1.6	3.0	3.6E-04			
	F3	Average	56.0	0.6	2.3	1.2E-04	N/A	N/A	N/A
		Min	51.0	0.6	1.0	1.5E-05			
		Max	64.0	0.7	3.0	2.2E-04			
	F4	Average	66.0	1.5	3.0	8.7E-04	N/A	N/A	N/A
		Min	53.0	0.7	3.0	2.6E-04			
		Max	81.0	2.0	3.0	2.1E-03			
2020	F1	Average	37.2	0.7	2.4	1.4E-05	2.1E-04	65.0	70
		Min	23.0	0.4	2.0	0.0	0.0	23.0	-
		Max	50.0	1.0	3.0	5.5E-05	1.0E-03	100.0	-
	F2	Average	21.6	0.4	2.3	7.9E-06	1.1E-04	12.9	45
		Min	17.0	0.3	1.0	0.0	4.2E-05	2.4	-
		Max	28.0	0.4	3.0	6.3E-05	3.2E-04	36.3	-
	F3	Average	24.8	0.7	2.2	2.2E-06	2.8E-03	26.0	72.5
		Min	13.0	0.2	2.0	0.0	0.0	4.0	
		Max	28.0	1.0	3.0	1.6E-05	2.2E-02	55.0	
	F4	Average	27.2	0.6	3.0	1.9E-05	3.5E-04	30.6	82.5
		Min	20.0	0.3	3.0	0.0	0.0	15.2	
		Max	34.0	0.8	3.0	1.2E-04	1.3E-03	53.2	
	F5	Average	39.4	0.9	2.2	3.8E-05	8.5E-04	37.2	37.5
		Min	10.0	0.2	2.0	0.0	3.3E-05	14.3	
		Max	59.0	1.9	3.0	8.4E-05	3.1E-03	58.5	

[a] DI is defined as the percentage of plants with SSR symptoms developed by Kutcher and Wolf (2006). Ratings were taken around BBCH 83 at different sites throughout the same field, with 100 plants rated at each site. Average disease severity ratings are based on Kutcher and Wolfe (2006) Sclerotinia Stem Rot Rating scale of 0 – 5, taken at BBCH 83-85, when 30-50% of seeds were black. 0 = no symptoms and 5 = lesions on the main stem with the potential to affect seed formation. The minimum and maximum severity were found by taking the average severity from each site within the rated field and ranking them.

[b] Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA), and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Inc., Nisku, Alberta, Canada. Spornado cassettes were changed twice weekly for about five weeks in 2019 and 10 weeks in 2020.

[c] Rotorod ng *S. sclerotiorum* DNA, measured in ngDNA/m^3/hr, was determined using the protocol described in chapter 3.2.5.2. Rotorods were exchanged twice per week for about five weeks in 2019 and 10 weeks in 2020.

[d] Petal infestation level represents the amount of *S. sclerotiorum* DNA (ng) per canola petal as determined by qPCR analysis designed by Ziesman (2016) and Ziesman et al. (2016) and performed by AAFRED Plant Health Lab.

[e] PPI is the percent petal infestation reported by commercial petal tests 1 (company 1), using petals selected from canola plants near spore traps and subjected to their proprietary PCR test for *S. sclerotiorum* DNA.

[f] PPI is the percent petal infestation reported by commercial petal test 2 (company 2), using petals selected from canola plants near the spore trapping site, which were then subjected to their proprietary PCR test for *S. sclerotiorum* DNA.

Table 3.2 Average percentage petal infestion (PPI), Sclerotinia stem rot disease incidence and disease severity from 10 AAFC sites across Canada. Average Spornado ratings, rotorod and petal infestation levels of *Sclerotinia* sclerotiorum DNA observed during the flowering period from the same 10 AAFC sites, 2019.

Location			DI[a] (%)		Disease Severity[a]		Spornado Rating[c]		Rotorod DNA (ngDNA/m^3/h)[d]		Petal Infestation Level (ngDNA/petal)[e]	
			Seeding Rate (Low = 60 seeds/m^2, High = 120 seeds/m^2)									
	Average PPI[b] (%)	Descriptive Statistics	High[f]	Low[f]	High	Low	High	Low	High	Low	High	Low
Beaverlodge, AB	52.0	Average	33.5	31.3	1.0	0.9	2.7	2.7	1.4E-03	1.4E-03	1.5E-04	1.2E-04
		Min	24.0	17.0	0.8	0.6	2.0	2.0	4.4E-04	4.4E-04	8.8E-05	4.4E-05
		Max	42.0	40.0	1.2	1.2	3.0	3.0	3.9E-03	3.9E-03	2.3E-04	1.9E-04
Brooks, AB	2.95	Average	LD	LD	LD	LD	2.2	2.2	NEG	NEG	8.3E-04	1.9E-03
		Min					1	1			2.0E-05	2.0E-05
		Max					3	3			3.2E-03	7.5E-03
Lacombe, AB	17.5	Average	LD	LD	LD	LD	2.0	2.0	NEG[g]	NEG[g]	2.0E-05	2.6E-05
		Min					1	1			1.3E-05	2.1E-05
		Max					3	3			2.6E-05	3.0E-05
Lethbridge, AB	1.25	Average	LD	LD	LD	LD	1.1	1.1	NEG	NEG	N/A	N/A
		Min					1	1				
		Max					2	2				
Indian Head, SK	2.45	Average	LD	LD	LD	LD	2.6	26	NEG	NEG	5.5E-05	NEG
		Min					1	1			4.1E-05	
		Max					3	3			6.9E-05	
Melfort, SK	34.5	Average	LD	LD	LD	LD	2.8	2.8	POS[h]	POS[h]	6.5E-05	6.7E-05
		Min					2	2			4.2E-05	1.8E-05
		Max					3	3			1.0E-04	1.2E-04
Outlook, SK	18.0	Average	2.8	2.3	0.14	0.098	1.9	1.9	POS	POS	2.2E-05*	2.8E-05
		Min	0	1	0	0.05	1	1			-	2.2E-05
		Max	6	3	0.3	0.15	3	3			-	3.4E-05
Scott, SK	4.15	Average	LD	LD	LD	LD	2.3	N/A	NEG	NEG	N/A	N/A
		Min					1					
		Max					3					
Brandon, MB	0.8	Average	LD	LD	LD	LD	1.8	1.9	NEG	NEG	NEG	1.6E-05
		Min					1	1				1.5E-05
		Max					3	3				1.6E-05
Normandin QC	0.4	Average	LD	LD	LD	LD	1.1	1.1	NEG	NEG	NEG	NEG
		Min					1	1				
		Max					2	2				

[a] DI defined as percentage of plants with SSR severity symptoms developed by Kutcher and Wolfe (2006). Ratings were taken at BBCH 83. If no disease was found in check plots or the first replication, then no further disease ratings were taken and designated as "LD" (Low Disease). Average disease severity ratings are based off Kutcher and Wolfe (2006) Sclerotinia Stem Rot Rating scale of 0 – 5, taken at BBCH 83-85, when 30-50% of seeds were black. 0 = no symptoms and 5 = lesions on the main stem with the potential to affect seed formation.
[b] PPI is the average percent petal infestation reported by commercial petal test 1. Petal samples for commercial petal test 1 were taken twice during the flowering period, at approximately the third and fourth weeks after the yellow bud stage (BBCH 59)
[c] Spornado ratings were categorized by Dr. T. K. Turkington and Eleanor McBain as 1 = no risk (none detected), 2 = low risk (trace levels detected) and 3 = moderate risk (limit of detection). A fungicide application was recommended if a "Limit of detection" result was returned. Spornado cassettes were set out on Tuesdays and Fridays between 8:30 and 9 am and collected the following Thursday and Monday between 3:30 and 4pm, respectively.
[d] Rotorod ngDNA, measured as ngDNA/m^3/hr, was determined using the protocol described in chapter 3.2.5.2. Rotorods were exchanged 3 times per week on Mondays, Wednesdays, and Fridays from 9 am – 4 pm.
[e] Petal infestation level represents the amount of *S. sclerotiorum* DNA (ng) per canola petal as determined by qPCR analysis designed by Ziesman (2016) and performed by AAFRED Plant Health Lab.
[f] High and low represents the flowering periods for the high seeding rate (HSR) and low seeding rate (LSR), which was 120 seeds/m^2 and 60 seeds/m^2 respectively.
[g] NEG denotes that all samples tested returned negative results.
[h] POS represents sites that had rotorod samples test positive for *Sclerotinia sclerotiorum* DNA, however no discernable amount was detected.
* Only one petal sample in the LSR at the Outlook, SK site tested positive for DNA.

Table 3.3 The average percentage of days during the early and late flowering period with any rain event >0 mm, and ≥ 2.5 mm for four commercial fields in 2019 and five commercial fields in 2020.

Year	Location	Percent of days with rain above 0				Percent of days with rain equal to or above 2.5 mm			
		Pre-Flower[a]	Early Flower[b]	Late Flower[c]	Total Flower[d]	Pre-Flower	Early Flower	Late Flower	Total Flower
2019	F1	71.4	54.0	54.0	63.6	42.9	36.4	36.4	36.4
	F2	81.0	60.0	55.6	57.9	42.9	20.0	55.6	36.8
	F3	81.0	81.8	72.7	77.3	42.9	27.3	45.5	36.4
	F4	71.4	60.0	55.6	57.9	42.9	10.0	22.2	15.8
2020	F1	66.7	61.5	58.3	60.0	28.6	30.8	33.3	32.0
	F2	71.4	56.3	12.5	34.4	33.3	25.0	12.5	18.8
	F3	66.7	83.3	72.7	78.3	28.6	66.7	27.3	47.8
	F4	57.1	66.7	44.4	55.6	33.3	33.3	11.1	22.0
	F5	42.9	91.7	58.3	78.3	23.8	54.5	25.0	39.1

[a] The pre-flowering includes the three weeks previous to the first open flower (BBCH 60). 2019 pre-flowering data was obtained from the nearest publicly available Environment Canada weather station at https://climate.weather.gc.ca or https://acis.alberta.ca/acis/township-data-viewer.jsp until in-field monitoring equipment was placed on July 9th, 2019.
[b] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[c] Late flowering included the second half of the flowering period (BBCH 66-69).
[d] Total flowering included the entire flowering period, from BBCH 60 – 69.

Table 3.4 Average percentage of days during the early and late flowering period with any rain event ≥5mm at four commercial fields in 2019 and five commercial fields in 2020. Based on in-field monitoring equipment and Environment Canada weather stations.

Year	Location	Percentage of days with rain equal to or above 5mm			
		Pre-Flower[a]	Early Flower[b]	Late Flower[c]	Total Flower[d]
2019	F1	28.6	18.2	27.3	22.7
	F2	28.6	20.0	22.2	21.1
	F3	28.6	9.1	27.3	18.2
	F4	28.6	10.0	11.1	10.5
2020	F1	19.0	30.8	25.0	28.0
	F2	28.6	18.8	6.3	12.5
	F3	14.3	33.3	18.2	30.4
	F4	19.0	33.3	11.1	22.2
	F5	14.3	36.4	16.7	26.1

[a] The pre-flowering includes the three weeks previous to the first open flower (BBCH 60). 2019 pre-flowering data was obtained from the nearest publicly available Environment Canada weather station at https://climate.weather.gc.ca or https://acis.alberta.ca/acis/township-data-viewer.jsp until in-field monitoring equipment was placed on July 9th, 2019.
[b] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[c] Late flowering included the second half of the flowering period (BBCH 66-69).
[d] Total flowering included the entire flowering period, from BBCH 60 – 69.

Table 3.5 Average percentage of days during pre-flower, early flower and late flower that had a rain event, and a rain event ≥ 2.5 mm at 10 AAFC research sites in Alberta, Saskatchewan, Manitoba and Quebec in 2019.

	Average percentage of days with rain above 0				Average percentage of days with rain equal to or above 2.5 mm			
Location, Province	Pre-Flower[a]	Early Flower[b]	Late Flower[c]	Total Flower[d]	Pre-Flower	Early Flower	Late Flower	Total Flower
Beaverlodge, AB	57.1	57.1	65.0	61.0	28.6	28.6	35.0	31.7
Brooks, AB	71.4	73.3	80.0	56.1	57.1	66.7	73.3	51.2
Lacombe, AB *	42.9	45.0	50.0	46.3	28.6	25.0	15.0	46.3
Lethbridge, AB	42.9	26.7	6.7	16.7	9.5	6.7	0.0	3.3
Indian Head, SK	42.9	57.9	22.2	40.5	9.5	31.6	16.7	24.3
Melfort, SK	57.1	40.9	50.0	43.2	38.1	27.3	18.2	20.5
Outlook, SK	71.4	61.1	41.2	51.4	42.9	33.3	23.5	28.6
Scott, SK	42.9	66.7	35.3	51.4	23.8	50.0	5.9	28.6
Brandon, MB	47.9	50.0	25.0	37.5	28.6	33.3	16.7	25.0
Normandin, QC	38.1	46.7	50.0	48.3	23.8	13.3	42.9	27.6

[a] The pre-flowering includes the three weeks previous to the first open flower (BBCH 60).
[b] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[c] Late flowering included the second half of the flowering period (BBCH 66-69).
[d] Total flowering included the entire flowering period, from BBCH 60 – 69.
* Lacombe, AB rain averages were obtained from https://climate.weather.gc.ca/

Table 3.6 Average percentage of days during pre-flower, early flower and late flower that had a rain ≥ 5 mm at 10 AAFC research sites in Alberta, Saskatchewan, Manitoba and Quebec in 2019.

	Average percentage of days with rain above 5 mm			
Location, province	Pre-Flower[a]	Early Flower[b]	Late Flower[c]	Total Flower[d]
Beaverlodge, AB	23.8	19.0	20	19.5
Brooks, AB	38.1	53.3	60.0	41.5
Lacombe, AB *	23.8	20.0	5.0	12.2
Lethbridge, AB	9.5	6.7	0.0	3.3
Indian Head, SK	4.8	21.1	16.7	18.9
Melfort, SK	19.0	27.3	13.6	18.2
Outlook, SK	38.1	22.2	17.6	20.0
Scott, SK	23.8	44.4	0.0	28.6
Brandon, MB	23.8	33.3	16.7	25.0
Normandin, QC	14.3	13.3	35.7	24.1

[a] The pre-flowering includes the three weeks previous to the first open flower (BBCH 60).
[b] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[c] Late flowering included the second half of the flowering period (BBCH 66-69).
[d] Total flowering included the entire flowering period, from BBCH 60 – 69.
* Lacombe, AB rain averages were obtained from https://climate.weather.gc.ca/

Table 3.7 Mean, average daily minimum and daily maximum for relative humidity, temperature, wind, and wind gusts during the flowering period for four fields in 2019 and five fields in 2020, near Edmonton, AB, based on in-field monitoring equipment and Environment Canada weather stations.

				RH (%)[c]			Temperature (˚C)[c]			Wind (km/h)[e]		Gust (km/h)[e]
Year	Closest Weather Station (WS)	Field	Descriptive Statistics	Canopy	Ambient	WS[b]	Canopy	Ambient	WS[b]	Ambient	WS[b]	Ambient
2019	Oliver	F1	Mean	94.6	83.0	80.8	15.8	16.5	17.8	3.7	7.2	7.3
			Min	84.3	70.3	59.3	13.3	13.1	14.8	1.4	4.3	3.4
			Max	99.8	92.5	92.5	15.7	20.6	20.8	10.7	18.1	19.2
	Namao	F2	Mean	96.4	84.3	79.7	15.5	16.3	16.5	5.1	9.7	10.6
			Min	91.5	75.0	63.1	13.4	13.2	12.8	2.4	6.6	6.5
			Max	99.9	94.7	92.7	18.4	19.9	20.8	15.2	16.7	28.3
	Namao	F3	Mean	98.6	83.7	80.1	16.1	15.3	16.4	4.6	9.7	4.3
			Min	95.7	73.1	63.1	12.8	13.0	12.8	2.5	6.6	0.0
			Max	100	93.5	92.7	19.8	18.2	20.8	7.5	16.7	14.7
	Oliver	F4	Mean	97.8	82.7	80.0	15.9	16.7	17.6	4.9	7.1	10.7
			Min	92.9	73.8	59.3	13.5	13.2	14.8	1.6	4.3	5.2
			Max	100	93.1	92.5	19.0	20.2	20.8	13.5	18.1	27.1
2020	Radway	F1	Mean	86.4	77.6	77.5	15.9	16.7	16.7	N/A*	7.6	N/A
			Min	70.5	61.8	63.9	13.3	14.0	14.8		3.2	
			Max	96.9	91.4	93.3	18.5	10.6	19.9		17.3	
	Radway	F2	Mean	81.0	72.8	73.3	17.6	18.1	17.8	8.7	8.4	9.7
			Min	64.8	59.9	56.7	12.7	13.3	13.9	3.3	3.2	4.5
			Max	94.7	90.0	91.7	22.0	22.9	21.8	22.1	17.3	22.1
	Oliver	F3	Mean	81.2	77.1	77.7	16.6	16.6	16.5	4.5	7.7	8.6
			Min	64.3	60.1	57.3	14.0	14.1	13.9	1.1	3.3	3.4
			Max	95.1	92.8	94.3	19.4	19.7	19.2	11.8	16.8	20.0
	Legal, AB	F4	Mean	86.0	79.7	83.1	15.4	15.7	15.9	N/A*	13.2	9.5
			Min	73.6	65.8	72.1	12.8	13.3	13.2		6.3	5.6
			Max	96.9	94.4	97.0	17.6	18.5	18.6		24.5	13.2
	Legal, AB	F5	Mean	92.8	80.4	84.7	15.2	15.2	15.9	7.7	12.5	14.3
			Min	83.0	65.3	72.1	12.4	12.4	12.9	3.4	6.3	7.4
			Max	99.1	94.7	97.1	17.6	18.6	18.6	17.3	24.5	30.5

[a] Flowering period is defined as the first open flower on the main raceme (BBCH 60) to when most of the petals have senesced and fallen into the canopy (BBCH 69) (Canola Council of Canada 2020).
[b] WS = Weather station data obtained from the nearest publicly available Environment Canada weather station. Data was obtained from https://climate.weather.gc.ca/historical_data/search_historic_data_e.html.
[c] RH and Temperature were monitored within the canopy, 20 cm from the soil surface, and above the canopy (ambient) at 150cm above the soil surface during the flowering period. Both were measured with two HOBO U23-002 units mounted on the same pole.
[d] Precipitation was measured with a HOBO Bucket Rain Gauge (RG3-M) with the cumulative amount recorded for each day and averaged during the flowering period.
e Wind and gust speed were measured with a HOBO Davis Wind Speed and Direction Smart Sensor mounted 2 meters above the soil surface.
*Wind data unavailable or not represented during the flowering period.

Table 3.8 Average percentage of days with relative humidity ≥ 80% and 90% under the canopy during pre-flowering, early and late flowering for four commercial fields in 2019 and five in 2020, near Edmonton, AB based on in-field monitoring equipment.

Year	Location	Average percentage of days with RH equal to or above 80%				Average percentage of days with RH equal to or above 90%			
		Pre-Flower[a]	Early Flower[b]	Late Flower[c]	Total Flower[d]	Pre-Flower	Early Flower	Late Flower	Total Flower
2019	F1	N/A	100	100	100	N/A	72.7	90.9	81.8
	F2	N/A	100	100	100	N/A	100	100	100
	F3	N/A	100	100	100	N/A	100	100	100
	F4	N/A	100	100	100	N/A	100	100	100
2020	F1	52.4	76.9	100	88.0	9.5	15.4	6.3	36.0
	F2	52.4	56.3	68.8	62.5	14.3	12.5	6.3	9.4
	F3	38.1	66.7	45.1	56.5	4.8	25.0	9.1	17.4
	F4	57.1	88.9	88.9	88.9	14.3	22.2	33.3	27.8
	F5	47.6	100	100	100	4.8	75.0	81.8	78.3

[a] The pre-flowering includes the three weeks previous to the first open flower (BBCH 60).
[b] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[c] Late flowering included the second half of the flowering period (BBCH 66-69).
[d] Total flowering included the entire flowering period, from BBCH 60 – 69.

Table 3.9 Average percentage of days with relative humidity ≥ 80% and 90% above the canopy (pre-flowering, early and late flowering) for four commercial fields in 2019 and five in 2020, near Edmonton, AB based on in-field monitoring equipment.

Year	Location	Percentage of days with RH ≥ 80%				Percentage of days with RH ≥ 90%			
		Pre-Flower[a]	Early Flower[b]	Late Flower[c]	Total Flower[d]	Pre-Flower	Early Flower	Late Flower	Total Flower
2019	F1	66.7	72.7	72.7	72.7	9.5	18.2	18.2	18.2
	F2	71.4	80	66.7	73.7	14.3	10	33.3	21.1
	F3	71.4	81.8	63.6	72.7	14.3	18.2	18.2	22.7
	F4	66.7	60.0	66.7	63.2	9.5	10.0	22.2	15.8
2020	F1	47.6	30.8	58.2	44.0	9.5	7.7	0.9	8.0
	F2	42.9	25.0	6.3	15.6	14.3	6.3	0.0	3.1
	F3	33.3	41.7	9.1	26.1	4.8	16.7	9.1	13.0
	F4	52.4	55.6	33.3	44.4	14.3	11.1	0.0	5.6
	F5	47.6	58.3	54.5	56.5	9.5	16.7	9.1	13.0

[a] The pre-flowering includes the three weeks previous to the first open flower (BBCH 60). 2019 pre-flowering data was obtained from the nearest publicly available Environment Canada weather station at https://climate.weather.gc.ca or https://acis.alberta.ca/acis/township-data-viewer.jsp until in-field monitoring equipment was placed on July 9th, 2019.
[b] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[c] Late flowering included the second half of the flowering period (BBCH 66-69).
[d] Total flowering included the entire flowering period, from BBCH 60 – 69.

Table 3.10 Mean and standard deviation of relative humidity and temperature under the canopy for the pre-flowering, early and late flowering for four sites in 2019 and five sites in 2020 near Edmonton, AB based on in-field monitoring equipment.

		Relative Humidity (%)[a]						Temperature (˚C)[a]					
		Pre-Flower[b]		Early Flower[c]		Late Flower[d]		Pre-Flower		Early Flower		Late Flower	
Year	Field	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2019	F1	N/A	N/A	93.0	4.9	96.1	3.2	N/A	N/A	15.5	1.7	16.2	1.7
	F2	N/A	N/A	95.7	1.7	97.1	2.8	N/A	N/A	15.4	1.4	15.7	1.7
	F3	N/A	N/A	97.9	1.2	99.2	0.7	N/A	N/A	15.6	1.9	16.7	1.9
	F4	N/A	N/A	97.8	1.3	97.7	2.3	N/A	N/A	15.7	1.4	16.2	1.8
2020	F1	78.0	10.0	82.9	6.7	90.1	4.8	15.7	2.6	15.7	1.3	16.1	1.6
	F2	78.9	11.2	81.2	7.8	80.8	5.9	16.6	2.3	16.9	1.2	18.3	3.2
	F3	75.6	10.0	84.7	6.9	76.3	5.6	16.2	2.8	15.6	1.1	17.1	1.4
	F4	77.9	11.9	86.5	6.6	85.6	5.6	15.5	2.5	15.4	1.2	15.4	1.1
	F5	74.4	12.4	92.1	4.5	93.6	4.2	16.0	2.9	14.9	1.4	15.6	1.3

[a] RH and Temperature were monitored within the canopy, 20 cm from the soil surface during the flowering period. Both were measured with two HOBO U23-002 units mounted on the same pole.
[b] The pre-flowering includes the three weeks previous to the first open flower (BBCH 60).
[c] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[d] Late flowering included the second half of the flowering period (BBCH 66-69).

Table 3.11 Mean and standard deviation of relative humidity and temperature above the canopy (ambient) for the pre-flowering, early and late flowering four sites in 2019 and five sites in 2020 near Edmonton, AB based on in-field monitoring equipment.

		Relative Humidity (%)[a]						Temperature (˚C)[a]						Wind (km/h)[b]					
		Pre-Flower[c]		Early Flower[d]		Late Flower[c]		Pre-Flower		Early Flower		Late Flower		Pre-Flower		Early Flower		Late Flower	
Year	Field	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2019	F1	81.4	8.9	82.7	6.4	83.3	6.7	13.8	2.1	15.8	1.9	17.2	2.0	7.3	2.6	3.0	0.8	4.5	2.5
	F2	82.1	9.4	84.0	4.6	84.6	7.0	13.6	2.2	15.8	1.7	16.8	2.3	11.4	3.4	3.8	0.8	6.5	3.8
	F3	82.1	9.4	83.8	5.5	83.7	6.9	13.6	2.2	15.0	1.5	15.6	1.4	11.4	3.4	5.3	1.6	9.7	4.2
	F4	81.4	8.9	81.8	5.3	83.7	6.7	13.8	2.1	16.3	1.9	17.1	2.3	7.3	2.6	3.7	1.0	6.2	3.3
2020	F1	76.5	10.5	75.6	8.2	79.7	6.9	15.8	2.8	16.2	1.3	17.2	1.0	7.3	3.0	9.1	4.1	5.8	2.8
	F2	76.2	11.1	75.3	7.5	70.4	5.7	16.2	2.2	17.0	1.3	19.1	3.3	6.4	1.9	9.4	5.7	7.8	3.0
	F3	74.1	11.0	80.4	8.6	72.2	6.4	16.0	2.8	15.6	1.2	16.9	1.4	6.3	1.8	4.9	1.9	7.5	3.0
	F4	77.0	12.2	81.2	7.8	78.2	5.8	15.5	2.6	15.6	1.4	15.8	1.1	9.1	2.5	10.8	3.1	12.4	6.7
	F5	74.1	13.4	81.4	8.1	79.4	6.6	16.0	2.9	14.9	1.4	15.6	1.3	9.2	2.7	11.6	3.2	8.3	4.6

[a] RH and Temperature were monitored above the canopy (ambient) at 150 cm above the soil surface during the flowering period. Both were measured with two HOBO U23-002 units mounted on the same pole.
[b] Wind speed was measured with a HOBO Davis Wind Speed and Direction Smart Sensor mounted two meters above the soil surface.
[c] The pre-flowering period includes the three weeks previous to the first open flower (BBCH 60). 2019 pre-flowering data was obtained from the nearest publicly available Environment Canada weather station at https://climate.weather.gc.ca or https://acis.alberta.ca/acis/township-data-viewer.jsp until in-field monitoring equipment was placed on July 9th, 2019.
[d] Early-mid flowering was the first half of the flowering period (BBCH 60 – 65).
[e] Late flowering included the second half of the flowering period (BBCH 66-69).

Table 3.12 Average percentage of days during pre-flower, early and late flower before where the canopy relative humidity was ≥ 80% and 90% at 10 AAFC sites in Alberta, Saskatchewan, Manitoba and Quebec in 2019.

	Average percentage of days with RH ≥ 80%				Average percentage of days with RH ≥ 90%			
Location, Province	Pre-Flower[a]	Early Flower[b]	Late Flower[c]	Total Flower[d]	Pre-Flower	Early Flower	Late Flower	Total Flower
Beaverlodge, AB	52.4	95.2	95.0	95.1	9.5	47.6	75	61.0
Brooks, AB	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Lacombe, AB	57.1	100	95.0	95.1	19.0	70.0	35.0	51.2
Lethbridge, AB	14.3	6.7	0.0	3.3	4.8	6.7	0.0	3.3
Indian Head, SK	23.8	84.2	16.7	51.4	19.0	26.3	5.6	16.2
Melfort, SK	28.6	72.7	86.4	79.5	0.0	36.4	22.7	29.5
Outlook, SK	46.7	94.4	88.2	94.3	23.8	61.1	52.9	60.0
Scott, SK	38.1	66.7	58.8	62.9	4.8	16.7	58.8	8.6
Brandon, MB	38.1	41.7	0.0	20.8	4.8	8.3	0.0	4.2
Normandin, QC	19.0	26.7	92.9	58.6	0.0	6.7	28.6	17.2

[a] The pre-flowering includes the three weeks previous to the first open flower (BBCH 60).
[b] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[c] Late flowering included the second half of the flowering period (BBCH 66-69).
[d] Total flowering included the entire flowering period, from BBCH 60 – 69.

Table 3.13 Average percentage of days during pre-flower, early and late flower before where the ambient relative humidity was equal to or above 80% and 90% at 10 AAFC sites in Alberta, Saskatchewan, Manitoba, and Quebec in 2019.

	Average percentage of days with RH ≥ 80%				Average percentage of days with RH ≥ 90%			
Location, Province	Pre-Flower[a]	Early Flower[b]	Late Flower[c]	Total Flower[d]	Pre-Flower	Early Flower	Late Flower	Total Flower
Beaverlodge, AB	28.6	23.8	70.0	46.3	4.8	9.5	25	17.1
Brooks, AB	9.5	6.7	0.0	2.4	0.0	0.0	0.0	0.0
Lacombe, AB	47.6	70.0	70.0	68.3	4.8	10.0	15.0	12.2
Lethbridge, AB	14.3	6.7	0.0	3.3	0.0	0.0	0.0	0.0
Indian Head, SK	23.8	25.0	11.1	18.9	14.3	0.00	5.6	2.7
Melfort, SK	28.6	40.9	36.4	38.6	0.0	9.1	0.0	4.5
Outlook, SK	28.6	22.2	0.0	2.9	4.8	0.0	0.0	0.0
Scott, SK	47.6	100	82.4	97.1	4.8	38.9	17.6	28.6
Brandon, MB	4.8	8.3	0.0	0.0	4.8	0.0	0.0	0.0
Normandin, QC	14.3	13.3	78.6	44.8	0.0	0.0	7.1	3.4

[a] The pre-flowering includes the three weeks previous to the first open flower (BBCH 60).
[b] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[c] Late flowering included the second half of the flowering period (BBCH 66-69).
[d] Total flowering included the entire flowering period, from BBCH 60 – 69.

Table 3.14 Average, minimum, maximum, and median relative humidity (RH), temperature, precipitation, and wind during the canola flowering period in high and low seeding treatments at 10 AAFC fungicide trial sites located in Alberta, Saskatchewan, Manitoba, and Quebec in 2019.

		Environmental Conditions					
		Relative Humidity (%)[a]			Temperature (˚C)[a]		
Location, Province	Descriptive Statistics	Canopy	Ambient	Closest Weather Station (WS)[b]	Canopy	Ambient	Closest Weather Station (WS)[b]
Beaverlodge, AB	Average	91.1	79.4	77.3	13.7	14.5	14.0
	Min	77.2	60.4	57.8	7.9	7.7	7.6
	Max	99.8	95.6	94.8	18.0	19.7	19.2
Brooks, AB	Average	N/A	65.9	61.1	N/A	19.1	19.2
	Min		50.5	42.1		12.7	13.0
	Max		85.9	84.3		23.7	24.8
Lacombe, AB	Average	89.8	83.0	78.7	14.9	15.5	15.3
	Min	76.8	64.9	57.5	9.6	9.9	10.5
	Max	99.1	93.9	89.5	19.4	21.3	21.1
Lethbridge, AB	Average	59.7	53.8	56.8	20.1	20.1	20.0
	Min	41.8	35.1	36.8	12.4	11.8	11.8
	Max	98.7	89.3	94.1	24.3	25.0	24.8
Indian Head, SK	Average	78.2	73.7	71.4	18.7	18.4	17.9
	Min	59.5	60.6	57.3	12.2	12.2	12.3
	Max	94.3	90.4	86.4	24.7	24.8	24.6
Melfort, SK	Average	85.7	79.6	74.6	16.9	17.1	16.9
	Min	73.3	70.9	65.1	11.9	12.0	12.3
	Max	96.2	91.7	87.1	21.6	22.5	22.5
Outlook, SK	Average	89.4	77.0	71.4	17.0	18.2	18.0
	Min	77.5	66.7	61.7	12.8	13.1	13.1
	Max	97.8	93.3	89.3	20.8	22.5	22.4
Scott, SK	Average	82.4	88.1	73.3	16.0	16.5	16.6
	Min	74.9	79.6	64.9	11.8	11.9	12.1
	Max	96.7	98.1	90.0	21.1	22.4	22.9
Brandon, MB	Average	75.0	70.4	65.4	19.7	19.9	19.9
	Min	64.3	61.5	53.9	15.5	15.7	15.6
	Max	94.7	85.1	81.5	23.9	24.2	24.3
Normandin, QC	Average	81.3	76.5	76.5	17.9	18.0	17.8
	Min	56.6	52.8	60.4	12.8	12.9	12.7
	Max	93.9	92.4	91.8	25.0	25.1	23.0

[a] RH and Temperature were monitored both within the canopy, 20 cm from the soil surface and above the canopy (ambient) at 150cm above the soil surface during the flowering period. Both were measured with two HOBO U23-002 units mounted on the same pole.
[b] WS = Weather station data obtained from the nearest publicly available Environment Canada weather station. Data was obtained from https://climate.weather.gc.ca/historical_data/search_historic_data_e.html .

Table 3.15 Mean and standard deviation of relative humidity and temperature during pre-flower, early and late flower under the canola canopy at 10 AAFC research sites in Alberta, Saskatchewan, Manitoba, and Quebec in 2019.

	Relative Humidity (%)[a]						Temperature (˚C)[a]					
	Pre-Flower[b]		Early Flower[c]		Late Flower[d]		Pre-Flower		Early Flower		Late Flower	
Location, Province	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Beaverlodge, AB	79.2	10.2	89.9	5.2	92.4	5.3	13.3	1.8	14.6	1.8	12.7	2.3
Brooks, AB	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Lacombe, AB	81.3	9.6	91.2	4.9	88.4	5.5	13.1	2.3	15.0	2.2	14.8	1.7
Lethbridge, AB	68.4	13.3	63.2	13.7	56.2	5.6	15.4	2.5	19.3	3.1	20.9	1.5
Indian Head, SK	74.5	12.0	85.4	5.8	70.7	9.1	17.0	2.8	18.7	2.2	18.7	3.5
Melfort, SK	74.5	11.0	86.2	7.8	85.3	5.0	16.3	2.8	17.5	2.2	16.3	2.8
Outlook, SK	77.2	13.6	89.8	5.9	89.0	6.0	14.9	2.1	16.8	1.9	17.3	2.4
Scott, SK	77.1	7.9	83.8	6.2	80.9	3.4	15.3	2.3	16.3	2.3	15.8	2.7
Brandon, MB	72.8	10.4	79.6	7.6	70.2	4.6	19.9	2.0	19.3	1.8	19.9	2.8
Normandin, QC	73.3	9.2	75.3	9.4	87.7	4.2	19.3	2.2	19.1	3.3	16.6	2.2

[a] RH and Temperature were monitored both within the canopy, 20 cm from the soil surface and above the canopy (ambient) at 150 cm above the soil surface during the flowering period. Both were measured with two HOBO U23-002 units mounted on the same pole.
[b]The pre-flowering includes the three weeks before the first open flower (BBCH 60).
[c] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[d] Late flowering included the second half of the flowering period (BBCH 66-69).

Table 3.16 Mean and standard deviation of relative humidity and temperature during the pre-flowering period (approximately 3 weeks before first open flower) above the canola canopy (ambient) at 10 AAFC research sites in Alberta, Saskatchewan, Manitoba and Quebec in 2019.

	Relative Humidity (%)[a]						Temperature (˚C)[a]					
	Pre-Flower[b]		Early Flower[c]		Late Flower[d]		Pre-Flower		Early Flower		Late Flower	
Location, Province	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Beaverlodge, AB	76.4	9.5	75.1	9.5	83.8	7.4	13.2	1.9	13.3	2.7	13.3	2.7
Brooks, AB	66.7	11.0	70.4	7.5	61.3	5.9	15.7	3.2	17.8	2.5	20.4	1.6
Lacombe, AB	78.2	8.6	82.4	6.7	83.6	6.5	13.2	2.2	15.7	2.5	15.3	1.9
Lethbridge, AB	66.7	12.6	56.9	13.2	50.7	10.4	15.0	2.5	19.3	3.3	20.9	1.8
Indian Head, SK	72.9	12.4	77.4	5.7	69.8	8.3	16.9	2.7	18.8	2.2	18.1	3.7
Melfort, SK	73.4	11.3	80.3	6.8	78.9	4.5	16.0	2.6	17.6	2.3	16.6	3.1
Outlook, SK	72.0	12.8	75.3	5.1	78.9	5.7	15.0	2.3	18.0	2.3	18.3	2.8
Scott, SK	79.1	8.6	89.2	5.3	86.9	3.0	15.0	2.3	16.7	2.6	16.3	3.0
Brandon, MB	69.0	7.9	73.8	5.4	66.8	4.0	20.0	20.2	19.6	1.9	20.1	2.9
Normandin, QC	70.0	9.7	71.6	9.6	81.7	6.4	19.4	2.4	19.1	3.3	16.8	2.4

[a] RH and Temperature were monitored both within the canopy, 20 cm from the soil surface and above the canopy (ambient) at 150 cm above the soil surface during the flowering period. Both were measured with two HOBO U23-002 units mounted on the same pole.
[b]The pre-flowering includes the three weeks before the first open flower (BBCH 60).
[c] Early flowering was the first half of the flowering period (BBCH 60 – 65).

[d] Late flowering included the second half of the flowering period (BBCH 66-69).

Table 3.17 Mean and standard deviation of Spornado and rotorod ratings for early and late flowering periods for detection of *S. sclerotiorum* ascospores in four commercial fields in 2019 and five in 2020.

Year	Location	Spornado Ratings [a]						Rotorod DNA (ngDNA/m^3/h)[b]					
		Early Flower [c]		Late Flower [d]		Total[e]		Early Flowering		Late Flowering		Total	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2019	F1	2.0	0.9	2.0	1.0	2.0	1.0	1.9E-04	2.4E-04	3.3E-05	1.5E-05	1.1E-04	1.7E-04
	F2	2.3	1.2	3.0	0.0	2.6	0.9	2.1E-04	1.6E-04	4.9E-05	5.1E-05	1.5E-04	1.4E-04
	F3	2.0	1.0	2.3	1.2	2.2	1.0	1.7E-04	4.7E-05	7.6E-05	8.9E-05	1.2E-04	8.2E-05
	F4	3.0	0.0	3.0	0.0	3.0	0.0	1.1E-03	9.5E-04	5.3E-04	3.7E-04	8.7E-04	7.6E-04
2020	F1	2.5	0.6	2.3	0.6	2.4	0.5	2.1E-05	2.9E-05	7.7E-06	9.6E-06	1.4E-05	2.0E-05
	F2	2.3	1.0	2.3	1.0	2.3	0.9	1.6E-05	3.1E-05	0.0	0.0	7.9E-06	2.2E-06
	F3	2.0	0.0	2.3	0.6	2.2	0.4	0.0	0.0	3.9E-06	7.8E-06	2.2E-06	5.9E-06
	F4	3.0	0.0	3.0	0.0	3.0	0.0	8.1E-06	7.1E-06	3.6E-05	2.2E-05	1.9E-05	1.9E-05
	F5	2.0	0.0	2.3	0.6	2.2	0.4	1.4E-05	1.3E-05	6.1E-05	3.4E-05	3.4E-05	3.0E-05

[a] Spornados ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA), and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada). Spornado cassettes were changed twice weekly for about five weeks in 2019 and 10 weeks in 2020.
[b] Rotorod ngDNA was determined using the protocol described in chapter 3.2.5.2. Rotorods were exchanged three times per week on Mondays, Wednesdays and Fridays from 9 am – 4 pm.
[c] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[d] Late flowering included the second half of the flowering period (BBCH 66-69).
[e] Total flowering included the entire flowering period, from BBCH 60 – 69.

Table 3.18 Mean and standard deviation for petal infestation levels based on Ziesman et al. (2016) during early and late flowering for four commercial fields in 2019 and five in 2020.

Year	Location	Petal Infestation Levels [a]					
		Early Flower [b]		Late Flower [c]		Total [d]	
		Mean	SD	Mean	SD	Mean	SD
2019	F1	N/A	N/A	N/A	N/A	N/A	N/A
	F2	N/A	N/A	N/A	N/A	N/A	N/A
	F3	N/A	N/A	N/A	N/A	N/A	N/A
	F4	N/A	N/A	N/A	N/A	N/A	N/A
2020	F1	3.6E-04	6.0E-04	1.1E-04	1.2E-04	2.1E-04	3.8E-04
	F2	1.4E-04	1.6E-04	7.3E-06	1.0E-05	1.1E-04	1.4E-04
	F3	1.3E-05	1.1E-02	5.6E-03	1.1E-02	2.8E-03	8.0E-03
	F4	3.7E-04	1.1E-04	5.7E-04	6.6E-04	3.5E-04	4.6E-04
	F5	1.4E-04	1.5E-04	1.4E-03	1.6E-03	8.5E-04	3.3E-05

[a] Petal infestation level represents the amount of *S. sclerotiorum* DNA (ng) per canola petal as determined by qPCR analysis designed by Ziesman (2016) and Ziesman et al. (2016) and performed by AAFRED Plant Health Lab.
[b] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[c] Late flowering included the second half of the flowering period (BBCH 66-69).
[d] Total flowering included the entire flowering period, from BBCH 60 – 69.

Table 3.19 Mean and standard deviation for petal infestation levels at 10 AAFC sites in Alberta, Saskatchewan, Manitoba and Quebec during pre-flowering, early flowering, and late flowering.

	Petal Infestation Level (ngDNA/petal)[b]											
	Early Flower[c]				Late Flower[d]				Total Flowering			
	Seeding Rate (Low = 60 seeds/m^2, high = 120 seeds/m^2)											
	High		Low		High		Low		High		Low	
Location, Province	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Beaverlodge, AB	2.2E-04	1.9E-05	1.3E-04	7.1E-05	1.1E-04	1.6E-05	1.1E-04	6.0E-05	1.5E-04	6.3E-05	1.2E-04	5.7E-05
Brooks, AB	3.1E-05	1.5E-05	3.9E-05	2.7E-05	1.6E-03	2.2E-03	3.8E-03	5.3E-03	8.3E-04	1.6E-03	1.92E-03	3.7E-03
Lacombe, AB	2.0E-05	9.2E-06	2.6E-05	6.4E-06	NEG	NEG	NEG	NEG	2.0E-05	9.2E-06	2.6E-05	6.4E-06
Lethbridge, AB	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Indian Head, SK	4.1E-05	N/A	NEG	NEG	6.9E-05	1.9E-05	NEG	NEG	5.5E-05	NEG	2.0E-05	NEG
Melfort, SK	5.3E-05	1.4E-05	6.9E-05	7.3E-05	1.0E-04	N/A	6.4E-05	1.6E-05	6.5E-05	2.7E-05	6.7E-05	4.3E-05
Outlook, SK	NEG	NEG	2.2E-05	N/A	2.8E-05	8.6E-06	NEG	NEG	2.8E-05	8.6E-06	2.2E-05	N/A
Scott, SK	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Brandon, MB	NEG	NEG	1.6E-05	6.8E-07	NEG	NEG	NEG	NEG	NEG	NEG	1.6E-05	6.8E-07
Normandin QC	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG

[a] Petal infestation level represents the amount of *S. sclerotiorum* DNA (ng) per canola petal as determined by qPCR analysis designed by Ziesman (2016) and performed by AAFRED Plant Health Lab.
[b] The pre-flowering includes the three weeks previous to the first open flower (BBCH 60).
[c] Early flowering was the first half of the flowering period (BBCH 60 – 65).
[d] Late flowering included the second half of the flowering period (BBCH 66-69).
[e] NEG denotes that all samples tested returned negative results.
[f] POS represents sites that had rotorod samples test positive for *Sclerotinia sclerotiorum* DNA, however a discernable amount was not detected.

Table 3.20 Significant regression models between environmental variables and spore detection methods (Spornado risk level, DNA amount of *Sclerotinia sclerotiorum* captured on rotorods, and petal infestation levels) at early-mid flower, mid-late flower and total flowering period, and the disease incidence (DI) of Sclerotinia stem rot in fields located near Edmonton, AB in 2019 and 2020, and AAFC/AAFRED CDCN trials across western and central Canada.

Spore Detection Method	Flowering Period	Suggested model[a]	Coefficient of Determination (R^2)	Adjusted R^2	Model Significance (p-value)
Spornado	Early – Mid	$EarlySR^b = (-0.02) + (0.03)\ ERHCan^q + \varepsilon^z$	0.12	0.055	p-value: 0.1925
	Mid – Late	$LateSR^c * = (0.68) + (0.02)\ LRHCan^r + (-0.01)\ LRain^x + \varepsilon^z$	0.32	0.19	p-value: 0.1218
	Total Average	$avSR^d = (4.45) + (-0.13)\ avRHAmb^v + \varepsilon^z$	0.16	0.01	p-value: 0.1266
Rotorod	Early – Mid	$EarlyRR^e = 2.40 + (-0.09)\ EtempCan^k + (0.01)\ ERHAmb^t + (0.01)\ ERHCan^q + \varepsilon^z$	0.17	-0.031	p-value: 0.4945
	Mid – Late	$LateRR^f = (1.09E\text{-}03\ ) + (-6.09E\text{-}05\ )\ LtempCan^l + \varepsilon^z$	0.29	0.24	p-value: 0.03216
	Total Average	$avRR^g = (3.87E\text{-}03) + (5.59E\text{-}05)\ avRHCan^s + (-1.45E04)\ avTempCan^m + (-8.98e\text{-}05)\ avTempAmb^p + (8.04E\text{-}06)\ avRain^y + \varepsilon^z$	0.66	0.54	p-value: 0.01208
AAFRED Petal Test	Early – Mid	$EarlyPT^h = 6.71E\text{-}04\ + (-3.33E\text{-}05)\ EtempAmb^n + \varepsilon^z$	0.24	0.16	p-value: 0.1106
	Mid – Late	$LatePT^i * = (7.26E\text{-}03) + (7.63E\text{-}05)\ LRHCan^r + (-1.34E\text{-}04)\ LRHAmb^u + (-1.85E\text{-}04)\ LtempAmb^o + \varepsilon^z$	0.54	0.35	p-value: 0.121
	Total Average	$avPT^j * = (6.63E\text{-}03) + (-3.89E\text{-}05)\ avRHCan^s + (-1.72E\text{-}04)\ avTempAmb^p + (-2.14E\text{-}06)\ avRain^y + \varepsilon^z$	0.67	0.53	p-value: 0.04049

[a] The suggested model was the final model with the lowest AIC after stepwise progression. Models were also tested for multicollinearity, and if independent variables had a VIF value over 10, the variable was removed from the original model before proceeding through stepwise progression, which reduced independent variables to ones statistically significant to the dependent variable.
[b] Average Spornado risk level during the early-mid flowering period (BBCH 60-65).
[c] Average Spornado risk level during the mid-late flowering period (BBCH 66-69).
[d] Average Spornado risk level during the total flowering period (BBCH 60-69).
[e] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA/m^3 air/hour on rotorods during the early - mid flowering period (BBCH 60-65)
[f] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA/m^3 air/hour on rotorods during the mid-late flowering period (BBCH 65-69)
[g] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA/m^3 air/hour on rotorods during the total flowering period (BBCH 60-69)
[h] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA per petal during the early - mid flowering period (BBCH 60-65)
[i] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNAper petal during the mid-late flowering period (BBCH 65-69)
[j] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA per petal during the total flowering period (BBCH 60-69)
[k] Average canopy temperature during early-mid flowering period (BBCH 60-65) measured in ˚C.
[l] Average canopy temperature during the mid-late flowering period (BBCH 66-69) measured in ˚C.

[m] Average canopy temperature during the total flowering period (BBCH 60-69), measured in ˚C.
[n] Average ambient temperature during the early -mid flowering period (BBCH 60-65) measured in ˚C.
[o] Average ambient temperature during the late flowering period (BBCH 66-69) measured in ˚C.
[p] Average ambient temperature during the total flowering period (BBCH 60-69), measured in ˚C.
[q] Average canopy relative humidity during the early -mid flowering period (BBCH 60-65) measured in ˚%.
[r] Average canopy temperature during the late flowering period (BBCH 66-69) measured in ˚%.
[s] Average canopy relative humidity, measured in %, calculated from the total flowering period (BBCH 60-69).
[t] Average ambient relative humidity during the early -mid y flowering period (BBCH 60-65) measured in ˚%.
[u] Average ambient temperature during the late flowering period (BBCH 66-69) measured in ˚%.
[v] Average ambient relative humidity, measured in %, calculated from the total flowering period (BBCH 60-69).
[w] Average daily rainfall during the early -mid flowering period (BBCH 60-65), measured in mm, collected from a HOBO rain gauge.
[x] Average daily rainfall during the late flowering period (BBCH 66-69), measured in mm , collected from a HOBO rain gauge.
[y] Average daily rainfall, measured in mm, collected over the flowering period (BBCH 60-69).
ε^z Residual error term.
*Outliers were investigated and removed from the model.

Table 3.21 Significant regression models for the relationship between Spornado risk levels, amount of *Sclerotinia sclerotiorum* DNA captured on rotorods and petal infestation levels per canola petal, as determined by qPCR analysis at early-mid flower, mid – late flower and total flowering period, and the disease incidence (DI) of Sclerotinia stem rot in fields located near Edmonton, AB in 2019 and 2020, and AAFC/AAFRED CDCN trials across western and central Canada.

Spore Detection Method	Flowering Period	Suggested model [a]	Coefficient of Determination (R^2)	Adjusted R^2	Model Significance (p-value)
Spornado	Early – Mid	DI [b] = (-222.54) + (2.85) $ERHCan^r + \varepsilon^{ab}$	0.48	0.44	p-value: 0.003009
	Mid – Late	DI = (-325.58) + (2.34) $LRHCan^s$ + (20.42) $LateSR^d$ + (0.47) $LRain^y$ + (4.77) $LtempAmb^p + \varepsilon^{ab}$	0.77	0.68	p-value: 0.001776
	Total Average	DI = (-217.30) + (2.82) $avRHCan^t + \varepsilon^{ab}$	0.50	0.47	p-value: 0.002051
Rotorod	Early – Mid	DI = (-314.56) + (20721.08) $EarlyRR^f$ + (4.29) $ERHAmb^u + \varepsilon^{ab}$	0.52	0.45	p-value: 0.008137
	Mid – Late	DI = (-169.84) + (2.06) $LRHCan^s$ + (0.38) $LRain^y + \varepsilon^{ab}$	0.59	0.53	p-value: 0.003081
	Total Average	DI = (-234.64) + (20600.52) $avRR^h$ + (3.26) $avRHAmb^w + \varepsilon^{ab}$	0.61	0.55	p-value: 0.002359
AAFRED petal samples	Early – Mid	DI = (148.17) + (-9.64) $EtempAmb^o$ + (56300.19) $EarlyPT^i$ + (5.09) $EtempCan^l$ + (-0.74) $ERHCan^r + \varepsilon^{ab}$	0.86	0.78	p-value: 0.004156
	Mid – Late	DI = (-80.45) + (1.10) $LRHCan^s$ + (4460.02)$LatePT^j + \varepsilon^{ab}$	0.38	0.25	p-value: 0.1147
	Total Average	DI = (718.06) + (-5.17) $avRHAmb^w$ + (-16.84) $avTempAmb^q$ + (-0.15) $avRain^z + \varepsilon^{ab}$	0.80	0.72	p-value: 0.003617
All spore detection methods	Early – Mid	DI^a = (1.1E+01) + (1.1E+05) $EarlyPT^i$ + (-1.4E+01) $EarlySR^c$ + (4.2E-01) $ERain^x + \varepsilon^{ab}$	0.68	0.55	p-value 0.024
	Mid – Late	DI = (-108.57) + (1.13) $LRHCan^s$ + (4101.22) $LatePT^j$ + (11.58)lateSR $+ \varepsilon^{ab}$	0.53	0.35	p-value: 0.09729
	Total Average	DI = (480.80) + (-11.23) $avTempCan^n$ + (-3.03) $avRHCan^t$ + (-0.22) $avRain^z$ + (17331.75) $avRR^h + \varepsilon^{ab}$	0.77	0.63	p-value: 0.02242

[a] The suggested model was the final model with the lowest AIC after stepwise progression. Models were also tested for multicollinearity, and if independent variables had a VIF value over 10, the variable was removed from the original model before proceeding through stepwise progression, which reduced independent variables to ones statistically significant to the dependent variable.
[b] DI is defined as percentage of plants with SSR symptoms developed by Kutcher and Wolf (2006). Ratings were taken around BBCH 83 at 5 different sites throughout the same field, with 100 plants rated at each site.
[c] Average Spornado risk level during the early -mid flowering period (BBCH 60-65).
[d] Average Spornado risk level during the mid-late flowering period (BBCH 66-69).
[e] Average Spornado risk level during the total flowering period (BBCH 60-69).
[f] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA/m^3 air/hour on rotorods during the early - mid flowering period (BBCH 60-65)
[g] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA/m^3 air/hour on rotorods during the mid-late flowering period (BBCH 65-69)

[h] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA/m^3 air/hour on rotorods during the total flowering period (BBCH 60-69)
[i] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA per petal during the early - mid flowering period (BBCH 60-65)
[j] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNAper petal during the mid-late flowering period (BBCH 65-69)
[k] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA per petal during the total flowering period (BBCH 60-69)
[l] Average canopy temperature during the early -mid flowering period (BBCH 60-65) measured in ˚C.
[m] Average canopy temperature during the mid-late flowering period (BBCH 66-69) measured in ˚C.
[n] Average canopy temperature during the total flowering period (BBCH 60-69), measured in ˚C.
[o] Average ambient temperature during the early -mid flowering period (BBCH 60-65) measured in ˚C.
[p] Average ambient temperature during the late flowering period (BBCH 66-69) measured in ˚C.
[q] Average ambient temperature during the total flowering period (BBCH 60-69), measured in ˚C.
[r] Average canopy relative humidity during the early -mid flowering period (BBCH 60-65) measured in °%.
[s] Average canopy temperature during the late flowering period (BBCH 66-69) measured in ˚%.
[t] Average canopy relative humidity, measured in %, calculated from the total flowering period (BBCH 60-69).
[u] Average ambient relative humidity during the early -mid y flowering period (BBCH 60-65) measured in ˚%.
[v] Average ambient temperature during the late flowering period (BBCH 66-69) measured in ˚%.
[w] Average ambient relative humidity, measured in %, calculated from the total flowering period (BBCH 60-69).
[x] Average daily rainfall during the early -mid flowering period (BBCH 60-65), measured in mm, collected from a HOBO rain gauge.
[y] Average daily rainfall during the late flowering period (BBCH 66-69), measured in mm, collected from a HOBO rain gauge.
[z] Average daily rainfall, measured in mm, collected over the flowering period (BBCH 60-69).
ε^{ab} Residual error term.

Table 3.22 Significant regression models for the relationship between Spornado risk levels, amount of *Sclerotinia sclerotiorum* DNA captured on rotorods and petal infestation levels per canola petal, as determined by qPCR analysis at early-mid flower, mid – late flower and total flowering period, and the average severity (avSev) of Sclerotinia stem rot in fields located near Edmonton, AB in 2019 and 2020, and AAFC/AAFRED CDCN trials across western and central Canada.

Spore Detection Method	Flowering Period	Suggested model [a]	Coefficient of Determination (R^2)	Adjusted R^2	Model Significance (p-value)
Spornado	Early – Mid	avSev [b] = (-4.03) + (0.05) ERHCan^{r} + ε^{ab}	0.40	0.36	p-value: 0.008139
	Mid – Late	avSev = (-3.62) + (0.03) LRHCan^{s} + (0.38) LateSR^{d} + (0.01) LRain^{y} + ε^{ab}	0.60	0.49	p-value: 0.01048
	Total Average	avSev = (-5.32) + (0.08) avRHAmb^{w} + ε^{ab}	0.38	0.33	p-value = 0.01161
RR	Early – Mid	avSev = (-3.19) + (0.04) ERHCan^{r} + (373.21) EarlyRR^{f} + ε^{ab}	0.50	0.43	p-value: 0.01057
	Mid – Late	avSev = (-2.13) + (0.03) LRHCan^{s} + (1028.00) LateRR^{g} + ε^{ab}	0.53	0.46	p-value: 0.007203
	Total Average	avSev = (-3.36) + (577.59) avRR^{h} + (0.05) avRHAmb^{w} + ε^{ab}	0.63	0.57	p-value: 0.0016
AAFRED petal samples	Early – Mid	avSev = (4.47) + (-0.27) EtempAmb^{o} + (0.14) EtempCan^{l} + (788.23) EarlyPT^{i} + (-0.03) ERHAmb^{u} + ε^{ab}	0.87	0.79	p-value: 0.003348
	Mid – Late	avSev = (7.07) + (65.20) LatePT^{j} + (0.09) LRHCan^{s} + (-0.14) LRHAmb^{v} + (-0.21) LtempAmb^{p} + (0.01) LRain^{y} + ε^{ab}	0.91	0.83	p-value: 0.005054
	Total Average	avSev = (15.56) + (-0.38) avTempAmb^{q} + (-0.11) avRHAmb^{w} + (107.68) avPT^{k} + ε^{ab}	0.83	0.77	p-value: 0.001795
All spore detection methods	Early – Mid	avSev = (0.31) + (0.01) ERain^{x} + (2224.48) EarlyPT^{i} + (266.76) EarlyRR^{f} + (-0.37) EarlySR^{c} + ε^{ab}	0.67	0.48	p-value: 0.07011
	Mid – Late	avSev = (-5.65) + (160.50) LatePT^{j} + (0.27) LateSR^{d} + (1433.98) LateRR^{g} + (0.04) LRHCan^{s} + (0.12) LtempAmb^{p} + ε^{ab}	0.79	0.61	p-value: 0.04836
	Total Average	avSev = (9.09) + (533.28) avRR^{h} + (-0.06) avRHCan^{t} + (-0.22) avTempCan^{n} + (-0.004) avRain^{z} + (124.41) avPT^{k} + ε^{ab}	0.84	0.70	p-value: 0.02441

[a] The suggested model was the final model with the lowest AIC after stepwise progression. Models were also tested for multicollinearity, and if independent variables had a VIF value over 10, the variable was removed from the original model before proceeding through stepwise progression, which reduced independent variables to ones statistically significant to the dependent variable.

[b] Average disease severity ratings are based off of Kutcher and Wolfe (2006) Sclerotinia Stem Rot Rating scale of 0 – 5, taken at BBCH 83-85, when 30-50% of seeds were black. 0 = no symptoms and 5 = lesions on the main stem with the potential to affect seed formation. The minimum and maximums for severity were found by taking the average severity from each site within the rated field and ranking them.
[c] Average Spornado risk level during the early -mid flowering period (BBCH 60-65).
[d] Average Spornado risk level during the mid-late flowering period (BBCH 66-69).
[e] Average Spornado risk level during the total flowering period (BBCH 60-69).
[f] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA/m^3 air/hour on rotorods during the early - mid flowering period (BBCH 60-65)
[g] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA/m^3 air/hour on rotorods during the mid-late flowering period (BBCH 65-69)
[h] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA/m^3 air/hour on rotorods during the total flowering period (BBCH 60-69)
[i] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA per petal during the early - mid flowering period (BBCH 60-65)
[j] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA per petal during the mid-late flowering period (BBCH 65-69)
[k] Average *Sclerotinia sclerotiorum* DNA amounts, measured in ngDNA per petal during the total flowering period (BBCH 60-69)
[l] Average canopy temperature during the early -mid flowering period (BBCH 60-65) measured in ˚C.
[m] Average canopy temperature during the mid-late flowering period (BBCH 66-69) measured in ˚C.
[n] Average canopy temperature during the total flowering period (BBCH 60-69), measured in ˚C.
[o] Average ambient temperature during the early -mid flowering period (BBCH 60-65) measured in ˚C.
[p] Average ambient temperature during the late flowering period (BBCH 66-69) measured in ˚C.
[q] Average ambient temperature during the total flowering period (BBCH 60-69), measured in ˚C.
[r] Average canopy relative humidity during the early -mid flowering period (BBCH 60-65) measured in ˚%.
[s] Average canopy temperature during the late flowering period (BBCH 66-69) measured in ˚%.
[t] Average canopy relative humidity, measured in %, calculated from the total flowering period (BBCH 60-69).
[u] Average ambient relative humidity during the early -mid y flowering period (BBCH 60-65) measured in ˚%.
[v] Average ambient temperature during the late flowering period (BBCH 66-69) measured in ˚%.
[w] Average ambient relative humidity, measured in %, calculated from the total flowering period (BBCH 60-69).
[x] Average daily rainfall during the early -mid flowering period (BBCH 60-65), measured in mm, collected from a HOBO rain gauge.
[y] Average daily rainfall during the late flowering period (BBCH 66-69), measured in mm , collected from a HOBO rain gauge.
[z] Average daily rainfall, measured in mm, collected over the flowering period (BBCH 60-69).
ε^{ab} Residual error term.

3.6 Figures

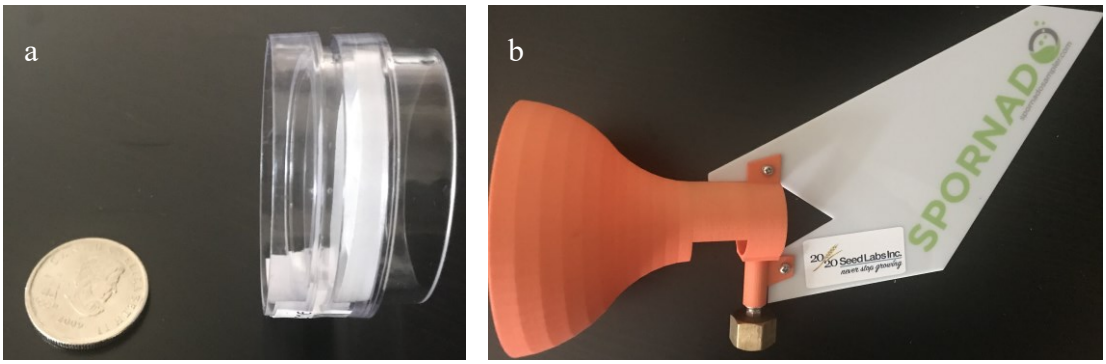

Figure 3.1. Spornado passive spore trap used to monitor *Sclerotinia sclerotiorum* ascospore levels in this study. a) Spornado cassette and b) Spornado. Cassettes were switched every 3-4 days from just before flowering until flowering had completed.

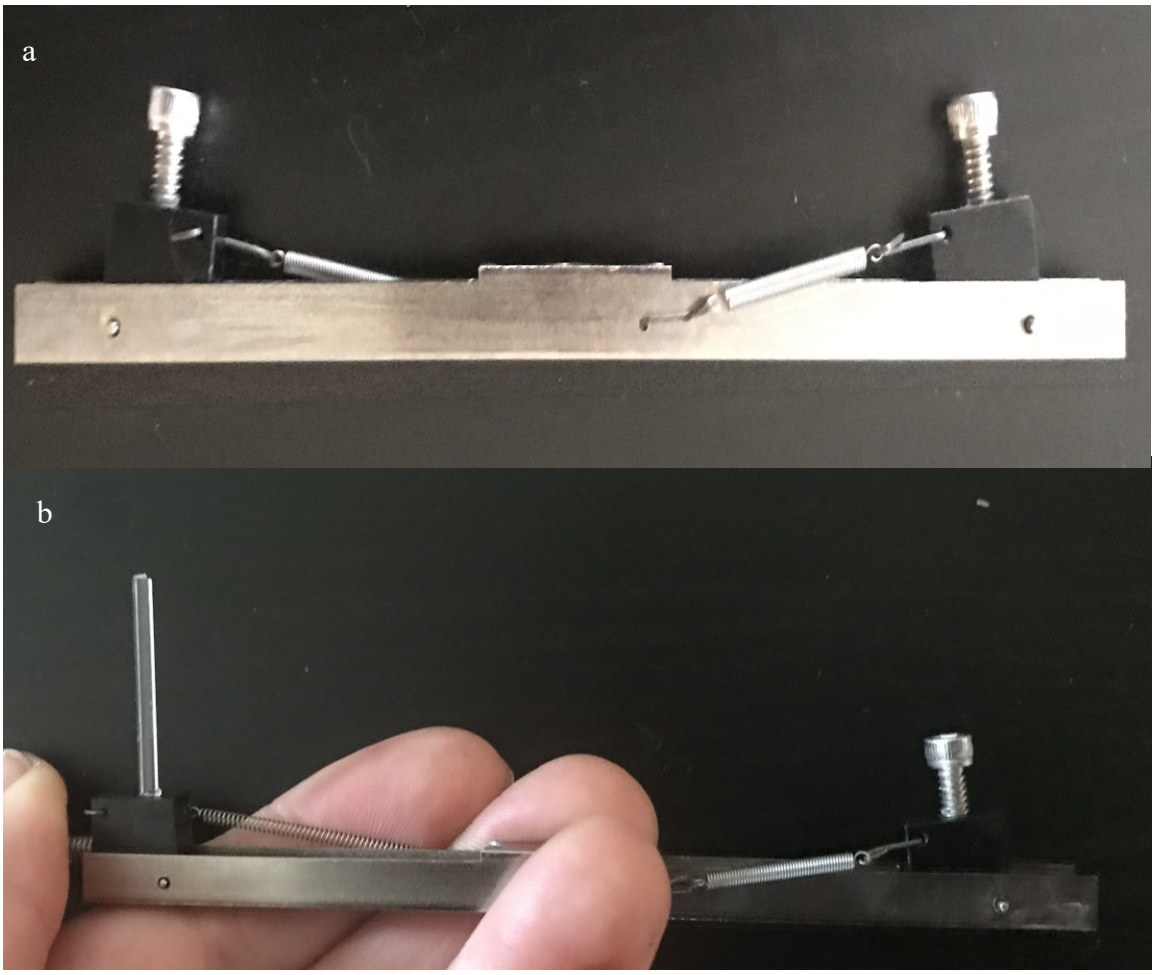

Figure 3.2 GRIPST-2009 Rotation Impaction Sampler (rotorod) used to monitor *Sclerotinia sclerotiorum* ascospore levels in this study. Close up of a) sampling head at rest with rods retracted and b) sampling head with a single silicone covered rod exposed.

4 Monitoring of In-Field *Sclerotinia sclerotiorum* Inoculum over the Flowering Period

4.1 Introduction

Sclerotinia stem rot, caused by the fungal pathogen *Sclerotinia sclerotiorum* (Lib.) de Bary, is an economically significant disease of canola (*Brassica napus* L.) in Canada and occurs in most canola growing regions worldwide (Adams and Ayers 1979; Purdy 1979; Rothmann and McLaren 2018; Sharma et al. 2016; Willetts and Wong 1980). Stem rot infections are typically white to greyish white in appearance and can occur on infected stems, branches, and pods. When the disease is severe, stem rot interferes with water and nutrient transport within the plant, while infected tissues are very brittle and easily shred and shatter. Severe infections can cause premature ripening and lodging, both of which directly affect yield via fewer and smaller seeds, poor seed set, pod shattering and grain contaminated with sclerotia (Adams and Ayers 1979; Ficke et al. 2018; Kamal et al. 2016; Morrall and Dueck 1982). Other impacts of stem rot include production losses due to an underestimates of disease risk or improperly timed or applied fungicide, resulting in yield loss as well as negative net returns in relation to the cost of control measures (Kamal et al. 2016).

Sclerotia, the long-lived resting structures of *S. sclerotiorum*, can remain in the soil for several years. Under optimal conditions, the sclerotia germinate either myceliogenically, via direct production of mycelia, or carpogenically, involving the formation of sexual apothecia (Le Tourneau 1979; Willetts and Wong 1980). While myceliogenic germination can result in the infection of nearby canola roots, the most important inoculum in relation to canopy infection (ascospores) results from carpogenic germination. In this process, the sclerotia produce apothecia and ultimately windblown ascospores that can infect canola petals (Le Tourneau 1979; Willetts and Wong 1980). Infection can occur as far away as 40 m, although most severe infections occur within 25 m of the source (Bardin and Huang 2001; Qandah and Del Rio Mendoza 2012). Canola infection begins with the colonization of the petals, which naturally senesce and fall from the plant to be caught on leaf axils and bases (Jamaux et al. 1995). Nutrients from the infected petal support further disease progression into the healthy tissue of the leaves or stems, with the canopy providing shade and a stable microenvironment crucial for disease development (Jamaux et al. 1995; Lumsden 1979; Turkington and Morrall 1993). The first symptoms of stem rot appear under adhered colonized petals, as bleaching or water-soaked

lesions on leaves, leaf axils and bases, with the pathogen progressing into stem tissues and eventually colonizing the pith, where new sclerotia will form (Abawi and Grogan 1979; Bolton et al. 2006; Jamaux et al. 1995; Willetts and Wong 1980). When the stem is infected, yield-reducing symptoms occur as stem girdling, premature ripening, lodging and pod shatter (Bolton et al. 2006; Ficke et al. 2018; Willetts and Wong 1980).

Stem rot management can be challenging, due to a lack of host resistance, a wide host range, persistent sclerotia in the soil, windborne dispersal of ascospores and variability in disease levels between years, regions and fields (Morrall and Dueck 1982; Turkington 1991; Willetts and Wong 1980; Ziesman 2016). The efficacy of many control strategies, including cultural practices such as tillage and extended rotations, is limited, creating uncertainty when it comes to effective risk management of stem rot (Abawi and Grogan 1975; Ćosić et al. 2012; Garza et al. 2002; Rakesh and Singh ; Schwartz and Steadman 1978; Wu and Subbarao 2008). As a result, reliance on fungicides has increased, as it is the most effective form of control (Rogers et al. 2009). Routine fungicide application, however, increases the chances of the pathogen developing resistance to commonly used chemicals (Gossen et al. (2001), while having potential adverse environmental effects. Fungicide use can also be cost-ineffective due to the need to apply the product before stem rot symptoms are visible, the variable nature of the disease, and changes in inoculum and weather conditions over the flowering period (Rogers et al. 2009; Turkington and Morrall 1993; Ziesman 2016).

Attempts to provide producers with tools to aid in their decision-making have resulted in the development of various stem rot forecasting methods. In Canada, the first forecasting method, and the basis for the risk point table recommended by the Canola Council of Canada today, was a checklist developed by Thomas (1984). This checklist used a summation of point values based on answers to questions related to pathogen characteristics, cropping variables and environmental conditions to generate a predicted risk level for stem rot in a field. The checklist has since been modified to include parts of a Swedish risk assessment table, developed by Twengström et al. (1998), as well as other stem rot related factors, to create a more suitable tool for Canadian canola producers (Canola Council of Canada 2021). Several other forecasting systems utilize various tools to provide regional or field-specific forecasts of varying complexity, with the choice of system often dependent on available resources (Parker et al. 2014). These include the collection of airborne inoculum or petal tests, coupled with microscopy and fungal

culture or molecular techniques such as quantitative PCR (qPCR) (Almquist and Wallenhammar 2015; Freeman et al. 2002; Morrall and Thomson 1991; Parker et al. 2014; Reich et al. 2016; Rogers et al. 2009; Shoute et al. 2018; Turkington 1991; West and Kimber 2015; Yin et al. 2009; Ziesman et al. 2016). Methods that do not directly account for inoculum load, like the risk point table, include weather-based forecasts (McLaren et al. 2004; University 2009) and risk algorithms (Clarkson et al. 2014; Harikrishnan and Rio 2008; Koch et al. 2007; Sharma et al. 2015).

Given the variable nature of stem rot of canola, a single prediction system may not be suitable for all growing regions. Moreover, no matter the model or method used, there is consensus that the accuracy of predictions improves with the addition of spore detection methodologies at the field level, as this is the best predictor of risk (Almquist and Wallenhammar 2015; Clarkson et al. 2014; Parker et al. 2014; Turkington 1991; Young et al. 2018). Previously, researchers have shown that petal infestation and airborne inoculum levels fluctuate over the flowering period (Almquist and Wallenhammar 2015; Turkington and Morrall 1993; Young et al. 2020; Ziesman 2016). As more airborne inoculum detection tools become available, new questions are emerging, such as how many spore traps are required to obtain an accurate overview of ascospore levels in a field. Although information related to the number of plant and petal samples is available (Turkington et al. 1988), less in known regarding more recent spore trapping techniques. The main objective of this chapter is to provide insight into the placement of spore traps, including the Spornado (2020 Seed Labs, Inc. 2021) and rotorod samplers, and determine how many would be required to provide an accurate prediction of stem rot when coupled with in-field and in-canopy monitoring of weather conditions. A secondary objective was to compare protocols for assessment of aerial spore loads.

4.2 Materials and Methods

4.2.1 Field Selection and Site Location

One commercial field per year was designated as a "Grid field" in 2019 and 2020, and were selected based on the following criteria: field had to have a history of stem rot within the last 3 years, there should not be any tall trees or shelterbelts across the north side of the field, to allow prevailing winds to pass unencumbered, and a stem rot-resistant canola cultivar was not

being grown during the study. Preference for the grid fields was given to half sections (approximately 130 ha or 320 acres). No fields were included consecutively over the two years of the study, but both were located within 50 km from Edmonton, AB. In 2019, the grid field was selected on June 17th from field options provided by the Fort Saskatchewan Nutrien® retail location (https://www.nutrien.com/offices). All fields selected (one per year of study) had a history of Sclerotinia stem rot and were in a short rotation with cereal crops. In 2020, the grid field was chosen on May 25th from several options suggested by farmers. In 2019, one application of Proline (Bayer Crop Science) was applied on July 10th over the entire field covering all within field sampling locations. In 2020, one application of fungicide (Cotegra, BASF) was applied to on July 6th, 2020, with all field-sampling sites sprayed except for a fungicide-free strip close to the southeast sampling location.

Spore trapping sites were set up in an X pattern (Figure 4.1) and were designated according to the compass directions: NE (Northeast), NW (Northwest), SE (Southeast), SW (Southwest), and C (Center). The SE location housed the weather monitoring equipment (Chapter 3) in both years. At this location in both years, in-canopy and ambient relative humidity (RH)/temperature sensors, a tipping bucket rain gauge, and a wind speed and direction sensor were set up according as described in Chapter 3. In 2019, monitoring occurred throughout the growing period, while in 2020, monitoring at all other sites occurred over the early-mid bloom period.

4.2.2 Spore Traps

4.2.2.1 Spornado

One Spornado trap (2020 Seed Labs 2021) was mounted on a pipe 154 cm above ground level at each site. The Spornado is a passive spore trap that relies on a fin mounted on the back of a funnel-like unit, which directs the trap into the direction of the wind (Chapter 3). Spores that are in the air move through the funnel trap and are captured on a specialized membrane cassette. Each grid field contained five sites in an X pattern in a half-section field. In 2019, the cassettes for the Spornado were changed every Monday and Thursday starting June 28, 2019 until August 9, 2019 (GS 53 – 72). In 2020, the Spornado cassettes were replaced every 3-4 days (Tuesdays and Fridays), beginning from when the spore traps were set out on July 6, 2020,

until 50% flowering (BBCH 65), which was reached July 16th. The SE location was considered a single site, with sampling continuing until August 7th, 2020, while samples at the other four sites ceased.

The Spornado cassettes were stored at room temperature in bags labelled with the location, crop stage, date and time of deployment and collection, then delivered to 20/20 Seed Labs Inc. (Nisku, AB) for testing for the presence of *S. sclerotiorum* DNA by a proprietary technology. The results were provided by 20/20 Seed Labs Inc. as follows: 1) not detected (coded as no to low risk); 2) limit of detection (coded as low risk); and 3) detected (coded as moderate to high risk).

4.2.2.2 Rotorod Samplers

GRIPS Rotation Impact Samplers (rotorods; Aerobiology Research Laboratories, Ottawa, ON) were mounted on helical piles to hold two silicone-covered rods approximately 150 cm above ground level. The motor and timing device were solar-powered, with the motor set to be on a 10% duty cycle, spinning the rods for 1 min then resting for 9 min, from 8 AM – 8 PM, seven days a week. Both rods were replaced every 3-4 days (generally on Mondays and Thursdays or Tuesdays and Fridays). For the grid fields, five rotation impact samplers were placed in an X pattern of sites within 2 m of the Spornado spore samplers.

In 2019, deployment of the rotorod samplers began on July 8 and continued until August 9 (GS 58-72). In 2020, the rotorod samplers were deployed from July 6th until July 16th (50% flower, BBCH 65). After July 16th, 2020, the SE location remained a "single-site" with regular rod replacement until the end of flowering on August 7th (GS 58 – 72). Collected rods were stored in 1.7 mL microcentrifuge tubes labeled with the location, crop stage, date and time set out, and the date and time collected. After collection, all rods were stored frozen until delivery to the Plant Health Lab, Alberta Agriculture, Forestry and Rural Economic Development (AAFRED), Edmonton, AB, for DNA extraction and qPCR analysis following Ziesman et al. (2016).

4.2.3 Petals Tests

4.2.3.1 Commercial Petal Test Kits

In 2020, two commercially available DNA-based petal test kits were also used to monitor *S. sclerotiorum* (due to sampling issues in 2019, no petal tests were used in that year). Details regarding the first kit (Commercial Petal Test 1 (CPT1) are provided in Chapter 3, but samples were collected in a grid pattern as described above. The CPT1 kits were used at approximately 10% flower (GS 61), 20% flower (GS 62), 30% flower (GS 63) and 50% flower (GS 65) at each of the five locations within the Grid field. Samples were then stored at 3-4˚C in vials provided with the kits and shipped as soon as possible to the 'Commercial Testing Lab 1' for their proprietary DNA testing. Results were returned as a percentage of the canola petals infested with *S. sclerotiorum*, where: 0 – 45% = Low Risk; 45 – 90% = Moderate Risk; and 90 – 100% = High Risk. A chart provided by the supplier indicated the potential percent probability of diseased plants in the crop (0 – 20%, 20 – 40%, and >40% respectively), and the probable percent yield loss (0 – 10%, 10 – 20% and >20% respectively).

A second kit, Commercial Petal Test 2 (CPT2; Chapter 3), was also used in 2020. One kit was deployed at each of the five spore trapping sites in the field, with the crop sampled at 30% flower (BBCH 63). Each kit included five small plastic bags containing eight tubes for petal samples, in which three petals from the same plant were collected. Petals were selected with tweezers from the top, middle and bottom of the flowering sections of the plants. Vials with petals were stored at 3-4˚C until delivery to the lab that supplied CPT2, where the petals were analyzed with a proprietary PCR protocol to determine the number of petals that tested positive for *S. sclerotiorum*. The results were reported as: 0 – 20% = 'Low Risk'; 20 – 40% ='Moderate Risk'; and ≥ 40% = high risk. If the overall positive percent of petal tests was >40%, the CPT2 manual suggested that this would correspond to a stem rot incidence of ≥15%, equating to a yield loss of ≥7.5%; this level of disease which would justify a fungicide application according to Canola Council of Canada recommendations (https://www.canolacouncil.org/canola-encyclopedia/diseases/sclerotinia-stem-rot/).

4.2.3.2 qPCR Petal Test

A qPCR-based petal test was performed at the Plant Health Lab (AAFRED, Edmonton, AB), to determine the amount of *S. sclerotiorum* DNA per petal. Fifteen fully open and intact flowers were collected from plants immediately surrounding the spore traps by using tweezers to break the flower off at the peduncle. The tweezers were disinfected with 'Spray Nine' (Permatex, Inc., Ohio, USA) between flowers and plants. Flower collections were made at the NE, NW, C, and SW points of each field twice per week (when fields were visited to maintain the spore traps) from July 3rd, 2020, to July 16th, 2020 (until 50% flower). At the SE location, petal collections were made from July 6th until August 7th, 2020. Flowers were stored in Petri dishes or small plastic bags and labeled with the location, crop stage, date and time of collection; they were stored frozen until delivery to the Plant Health Lab where they were subjected to PCR analysis following Ziesman et al. (2016).

4.2.4 Disease Incidence and Severity

Similar to the stem rot disease ratings taken for individual fields (Chapter 3), disease ratings for the Grid fields were taken at each of the five sampling sites around the time at which swathing would normally occur (BBCH 83). Stem rot incidence and severity were assessed on a 0-5 scale following Kutcher and Wolf (2006), where: 0, no symptoms; 1, only pods infected; 2, lesions found on the main stem or secondary branches in the upper canopy, impacting up to 25% of seed formation; 3, lesions found in the upper canopy on numerous branches or on the main stem, impacting up to 50% of seed formation; 4, lesions found in the upper canopy on numerous branches or on the main stem, impacting up to 75% of seed formation; and 5, a main stem lesion low enough to impact seed formation on the entire plant. One hundred plants around each of the five spore-trapping sites were rated, with 25 plants evaluated in each compass direction from the traps.

4.2.5 Statistical Analysis

The 2019 and 2020 Grid field data were separated into individual years and variables for statistical analysis. All analyses included tests for assumptions for normality (Kolmogorov –

Smirnov test or Shapiro test) and homogeneity of variance (F Test or Levene Test). To determine significant differences between sites within a field, tests included the Mann – Whitney – Wilcoxon paired non-parametric t-test and Kruskal – Wallis one-way ANOVA and Chi-Squared test on a contingency table.

To determine significant differences between rotorod samples, Spornado cassettes and petal tests from within the same fields in 2019 and 2020, a Kruskal – Wallis test (non-parametric one-way ANOVA on ranks) was used after testing for assumptions. A Dunn Test of Multiple Comparisons was carried out if the test was significant. A Chi-squared test on a contingency table was used to determine if the distribution of stem rot disease incidence and severity were similar across the same field.

4.3 Results

4.3.1 Monitoring of Airborne Inoculum

The Spornado results were variable within the same field on the same days in 2019 and 2020, but all five locations were generally within one category of each other (Figure A.104). In both years, consistent "trace" to "moderate" levels (risk levels of 2 and 3, respectively) of ascospores were detected in the Grid fields over the flowering period (Figure A.104). In 2019 and 2020, the SW corner consistently showed "trace levels detected" (category 2, low risk) during the flowering period, except for one time period in both years (July 18th – July 16th, 2019, and July 6th – 10th, 2020), where a "detected" (category 3, moderate risk) result was returned (Figure A.104, A.105). In 2019, ascospore levels for the SE, NE and NW corners were defined as "detected" (category 3, moderate risk) based on spore trapping with the Spornado. Similarly, in 2020, ascospores were consistently "detected" (category 3, moderate risk) in the SE and NE corners of the Grid field until after July 20, 2020 (Figure A.105). All other locations varied between risk levels of 2-3 during the flowering period.

In 2019, the rotorod results indicated flushes of ascospores at the start of flowering, mid-flowering and after flowering had ended. No location or time showed consistent trends during the flowering period, although the NE location tended to have the largest amounts of *S. sclerotiorum* DNA caught from July 15th, 2019, until monitoring ceased (Figure A.106). The greatest amount of DNA (4.3×10^{-4} ng DNA/m^3 air/h) was detected at the NE location on July

26th – July 29th, 2019 (Figure A. 106, Table 4.1). In 2020, the C location generally returned higher amounts of DNA on most of the rod samples during early flowering (between 10% - 30% flower, BBCH 61 – 63). Nonetheless, the highest quantity of *S. sclerotiorum* DNA in 2020 (3.4×10^{-5} ng DNA/m^3 air/h) was found at the NW location during the period of July 14th – July 17th, 2020 (Table 4.1, Figure A.107).

While the above trends were observed, when both the Spornado and rotorod results for locations were subjected to a non-parametric Kruskal-Wallis H test, the results for the five locations within a field were not significantly different from each other (Table 4.2).

4.3.2 Evaluation of Petal Infestation Levels

During the flowering period (BBCH 61-69), the results obtained with CPT1 indicated anywhere from 9% (low) to 53% (moderate) petal infestation, with variability in sampling date and location (Table 4.1). The SE, SW, and NE locations showed a peak in petal infestation on July 10th, 2020. At the C location, petal infestation as determined with the CPT1 increased over time, with the highest amount detected on the last day of testing on July 14th, 2020 (33.9% (low)) (Table 4.1). ANOVA on ranks indicated no significant differences in population means at $P > 0.05$ for CPT1 among the five locations within the same field (Table 4.2).

The results with CPT2 indicated that the NE and C sites had the highest level of petal infestation (85%) on July 10th, 2020, although only one test per site was conducted. The percent petal infestation ranged from 68% to 85% across the five locations over the same field and taken from the same area as CPT1. As with CPT1, one-way ANOVA on ranks showed that the population means among the five locations were not significantly different at $P > 0.05$ within the same field (Table 4.2).

The overall trend with the petal results by the Plant Health Lab (AARED, Edmonton, AB) indicated a spike in petal infestation on July 10th, 2020, with a smaller second flush on July 17th, 2020 (Figure A.108). When subjected to the Kruskal-Wallis non-parametric test, the results showed that the population means were not significantly different between tests at the five locations within the same field (Table 4.2). Although locations were not significant, the

numerically greatest amount of *S. sclerotiorum* DNA per petal (1.3×10^{-2} ng DNA) was found at the SE location on July 17th, 2020, at BBCH 67 near the end of the flowering period (Table 4.1).

4.3.3 Stem Rot Incidence and Severity

Disease incidence in 2019 ranged from 56% to 73%, depending on location within the field. This was in the moderate to high range and would have warranted fungicide application (Table 4.1). Stem rot severity ranged from 0.8-1.5, which in general would be considered low to moderate (Table 4.1). In 2020, disease incidence ranged from 20% to 34%, depending on the location within the field, but all locations were in the moderate range suggesting that fungicide would have been warranted (Table 4.1). Stem rot severity in 2020 ranged from 0.3-0.8, which would be considered low (Table 4.1). The Chi-square test for independence indicated that stem rot severity were dependent on location and were not evenly distributed across the field in 2019 or 2020 (2019 severity: $\chi^2 = 22.7$, $p < 0.001$; 2020 severity: $\chi^2 = 25.2$, $p = 0.003$). Disease incidence in 2019 also showed a site dependence, indicating that stem rot incidence was not uniform across the field ($\chi^2 = 22.7$, $p < 0.001$). In contrast, in 2020, the disease incidence was similar at each location ($\chi^2 = 1.4$, $p = 0.2$).

4.4 Discussion

In Canada, several private sector seed testing laboratories have developed a range of molecular-based techniques for detection of specific pathogens, including the causal agents of Fusarium head blight in cereals, clubroot and blackleg in canola, as well as Sclerotinia stem rot of canola. In the case of stem rot, these DNA-based tests can be used in conjunction with other risk assessment tools, such as a Sclerotinia checklist and weather monitoring, to aid in making fungicide spray decisions. The Sclerotinia checklist is based on the risk point tables of Thomas (1984) and Twengström et al. (1998), and consists of questions related to factors that influence stem rot, like crop history, environmental conditions and the previous disease history of a field. Depending on the answers to these questions, risk point values are assigned and then totalled; if the total is above a critical threshold, fungicide application is recommended. The Sclerotinia checklist on its own, however, does not account for *S. sclerotiorum* ascospore levels during

flowering, which has been cited as one of the shortcomings of the checklist (Derbyshire and Denton-Giles 2016; Turkington 1991).

The only airborne spore trap commercially available in Canada for the detection of *S. sclerotiorum* ascospores is called the Spornado; and consists of a funnel ending in a fin mounted on a ball joint ensuring the trap is always facing prevailing winds (Van der Heyden et al. 2021; West and Kimber 2015). The passive Spornado trap uses a specialized filter cassette assembly to collect airborne inoculum, which is then subjected to proprietary testing for *S. sclerotiorum* DNA. Although this passive trap is relatively inexpensive and can be used for large-scale deployment, it is also non-quantitative. However, 2020 Seed Labs Inc. provides results based on Spornado samples that are classified into three groups: "Detected" (*S. sclerotiorum* DNA detected at replicable levels, greater than or equal to the limit of detection), "Trace Levels Detected" (pathogen detected at less than the level of detection and may not be replicable) and "Not Detected." The reported limit of detection is about 40 genomes, which is equivalent to 4.5 ascospores (2020 Seed Labs, Inc., personal communication). In the current study, this spore trap was compared with rotation impact samplers (rotorods) that can provide a quantitative sample; the rotorods are employed for research purposes but at present are not available for commercial use (Chapter 3). Five spore traps were deployed per field to balance reasonable coverage of the field with the cost and practicality of using multiple spore traps. This number is also similar to that used by Turkington et al. (1988), who found that a minimum of 4-5 sampling sites within a field were needed to provide accurate determination of petal infestation levels.

The Grid fields experienced within-field variability and year-to-year variability for *S. sclerotiorum* DNA found in all petal tests and air samplers. There was also variability found in final stem rot incidence and severity within fields and between years. The differences between years highlight the importance of the environmental factors in stem rot development; these factors influence not only ascospore germination and disease progression, but also sclerotial germination, rate of maturity for apothecia, ascospore release and petal adherence (Abawi and Grogan 1979; Almquist and Wallenhammar 2015; Schwartz and Steadman 1978; Wu and Subbarao 2008). In addition, crop history, management and crop canopy and yield potential may also contribute to year-to-year variability in disease (Jurke and Fernando 2008; Kutcher et al. 2005; Turkington and Morrall 1993; Twengström et al. 1998; Yin-shui et al. 2013; Zhang et al.

2020). The variability observed could reflect differences in the microclimates, crop canopy density or inoculum gradients.

The observation of changes in petal infestation in the currently study are consistent with previous research by Almquist and Wallenhammar (2015), Turkington et al. (1991a), Turkington and Morrall (1993) and Ziesman (2016). We can only speculate on the reason for the differences in percent petal infestation between petal tests, since only the protocol used by the Plant Health Lab is publicly available. It is possible that the tests used in the CPT1 and CPT2 have some cross-reactivity with *B. cinerea* or other *Sclerotinia* species. Some variability within petal tests could also be attributed to the age of the petals, as petals can remain in the flower for up to six days (Turkington et al. 1991b). However, statistical analysis showed that the means of the same petal tests were not significantly different between the five sites within each field. Overall, qPCR-based methods have increased the sensitivity of detection and can quantify flushes of increased petal infestation occurring in a field, which in turn can be related to a possible minimum level of petal infestation required for a high final incidence of stem rot.

Airborne inoculum, as measured by the Spornado or rotorod samplers, varied across the flowering period in both years, consistent with previous research in which researchers observed multiple flushes of ascospores during flowering. The placement of the spore traps is important, as only large amounts of spores can be captured when apothecia are near the traps, with the gradient decreasing further away from the source (Ben-Yephet and Bitton 1985; Hartill 1980). Qandah and Del Rio Mendoza (2012) reported high stem rot incidence within 25 m of the inoculum source, although symptoms of disease were detected up to 40 m away. In the current study, significance testing showed that in both years, results from the rotorod and Spornado samplers were not significantly different between the five sites within each field. Thus, a single spore trap could be located anywhere in a field; however, ease of sampling also needs to be considered for trap placement, as farmers cannot be expected to switch out samples for multiple spore traps in one or more fields where the traps are not easily accessible. In this experiment, the traps were placed in half-section (~130 ha) fields, while smaller 'quarter' section (~65 ha) fields may be expected to be even more uniform. However, more research is needed to confirm this.

Overall, the current study suggests that only one spore sampler may be required to provide a reasonable estimation of aerial ascospore levels and the potential risk of stem rot of

canola. Concerns regarding variability of results are inherently less of a concern for petal testing, given that petal samples are typically collected throughout the field. For spore traps such as the Spornado, a single trap is likely sufficient, while also limiting cost, although locating the trap in the centre of a field may provide better exposure to inoculum from all areas of the field. Alternatively, if field site access and time are concerns, one could look at locating the trap in a downwind area of the field, depending on prevailing wind directions. In this study, there was variability in stem rot severity among locations within the fields in both years, while differences in disease incidence were only significant in 2019. It is important to note that factors other than inoculum load, including variable environmental conditions in the canopy, crop canopy density, lodging, etc., may affect final stem rot levels for different locations within a field. Nonetheless, disease levels were broadly similar across each field in 2019 and 2020, and the canola crops would have likely benefited from fungicide application.

4.5 Tables

Table 4.1 Overall stem rot incidence and severity, average, minimum and maximum Spornado ratings, ngDNA contained on rotorod samples and petal infestation levels reported by the Alberta Plant Health Lab, Commercial Petal Test 1 and Commercial Petal Test 2 at five sites within a single commercial field near Edmonton, AB, per year in 2019 and 2020.

Year	Field	Site	DI[a]	Disease Severity[a]	Descriptive Statistics	Spornado Rating[b]	Rotorod DNA (ngDNA)[c]	Petal Infestation Level (ngDNA)[d]	CPT 1 (%)[e]	CPT 2 (%)[f]
2019	F2	Southeast	73	1.6	Average	2.6	1.5E-04	N/A	N/A	N/A
					Min	1	1.3E-05			
					Max	3	3.6E-04			
		Centre	56	0.8	Average	2.4	1.6E-04	N/A	N/A	N/A
					Min	1	1.8E-05			
					Max	3	2.6E-04			
		Southwest	61	1.4	Average	2.0	8.7E-05	N/A	N/A	N/A
					Min	1	1.1E-05			
					Max	3	2.6E-04			
		Northwest	65	0.8	Average	2.6	6.1E-05	N/A	N/A	N/A
					Min	1	1.2E-05			
					Max	3	1.5E-04			
		Northeast	67	0.9	Average	2.8	1.8E-04	N/A	N/A	N/A
					Min	2	3.8E-05			
					Max	3	4.0E-04			
2020	F4	Southeast	21	0.5	Average	2.9	1.1E-05	4.9E-04	30.6	82.5
					Min	2	0.0	0.0	15.2	-
					Max	3	1.2E-04	1.3E-03	53.2	-
		Centre	34	0.7	Average	2.7	3.4 E-05	6.8E-05	29.4	85
					Min	2	2.9E-05	4.4E-05	26.0	-
					Max	3	3.8 E-05	1.1E-04	33.9	-
		Southwest	29	0.6	Average	2.3	1.23E-05	1.4E-04	25.5	75.0
					Min	2	0	6.6E-05	9.9	-
					Max	3	2.3E-05	2.8E-04	42.7	-
		Northwest	20	0.3	Average	2.7	2.4E-05	1.5E-04	12.6	67.5
					Min	2	0	4.2E-05	9.0	-
					Max	3	5.7E-05	3.2E-04	18.0	-
		Northeast	32	0.8	Average	3	2.3E-05	4.7E-05	25.9	85.0
					Min	3	1.5E-05	0.0	15.2	-
					Max	3	3.4E-05	1.1E-04	37.5	-

[a] DI, disease incidence, is defined as the percentage of plants with stem rot symptoms, based on a scale developed by Kutcher and Wolfe (2006). Ratings were taken around BBCH 83 at each of the five different sites throughout the same field, with 100 plants rated at each site.

Disease severity is rated on a scale of 0 – 5 (taken at BBCH 83-85, when 30-50% of seeds are black), where: 0 = no symptoms and 5 = lesions on the main stem with the potential to affect seed formation.

[b] Spornado ratings were categorized as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (trace levels detected) and 3 = moderate risk (limit of detection).

[c] Rotorod ngDNA represents ng *S. sclerotiorum* DNA, determined using the protocol Chapter 3.

[d] Petal infestation level represents the amount of *S. sclerotiorum* DNA (ng) per canola petal as determined by qPCR analysis following and Ziesman et al. (2016) as determined by the AAFRED Plant Health Lab.

[e] PPI, percent petal infestation, was as determined with Commercial Petal Test 1 (CPT1), using petals selected from canola plants near spore traps and subjected to a proprietary PCR test.

[f] PPI, percent petal infestation, was as determined with Commercial Petal Test 2 (CPT2), using petals selected from canola plants near the spore trapping site, which were then subjected to a proprietary PCR test.

Table 4.2 One-way ANOVA on ranks significance tests to determine if variables are significantly different at each of five sites within the same field near Edmonton, AB, in each of 2019 and 2020.

Year	Variable	Df	H-value	p-value
2019	Spornado [a]	4	4.5	0.3
	Rotorod [b]	4	9.2	0.06
	CPT1 [c]	N/A	N/A	N/A
	CPT2 [d]	N/A	N/A	N/A
	Petal Infestation Level [e]	N/A	N/A	N/A
2020	Spornado [a]	4	2.6	0.6
	Rotorod [b]	4	3.1	0.5
	CPT1 [c]	4	4.9	0.2
	CPT2 [d]	4	4	0.4
	Petal Infestation Level [e]	4	2.1	0.6

[a] Spornado ratings were categorized as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (trace levels detected) and 3 = moderate risk (limit of detection).
[b] Rotorod ngDNA represents ng *S. sclerotiorum* DNA, determined using the protocol Chapter 3.
[c] PPI, percent petal infestation, was as determined with Commercial Petal Test 1 (CPT1), using petals selected from canola plants near spore traps and subjected to a proprietary PCR test.
[d] PPI, percent petal infestation, was as determined with Commercial Petal Test 2 (CPT2), using petals selected from canola plants near the spore trapping site, which were then subjected to a proprietary PCR test.
[e] Petal infestation level represents the amount of *S. sclerotiorum* DNA (ng) per canola petal as determined by qPCR analysis following and Ziesman et al. (2016) as determined by the AAFRED Plant Health Lab.

4.6 Figures

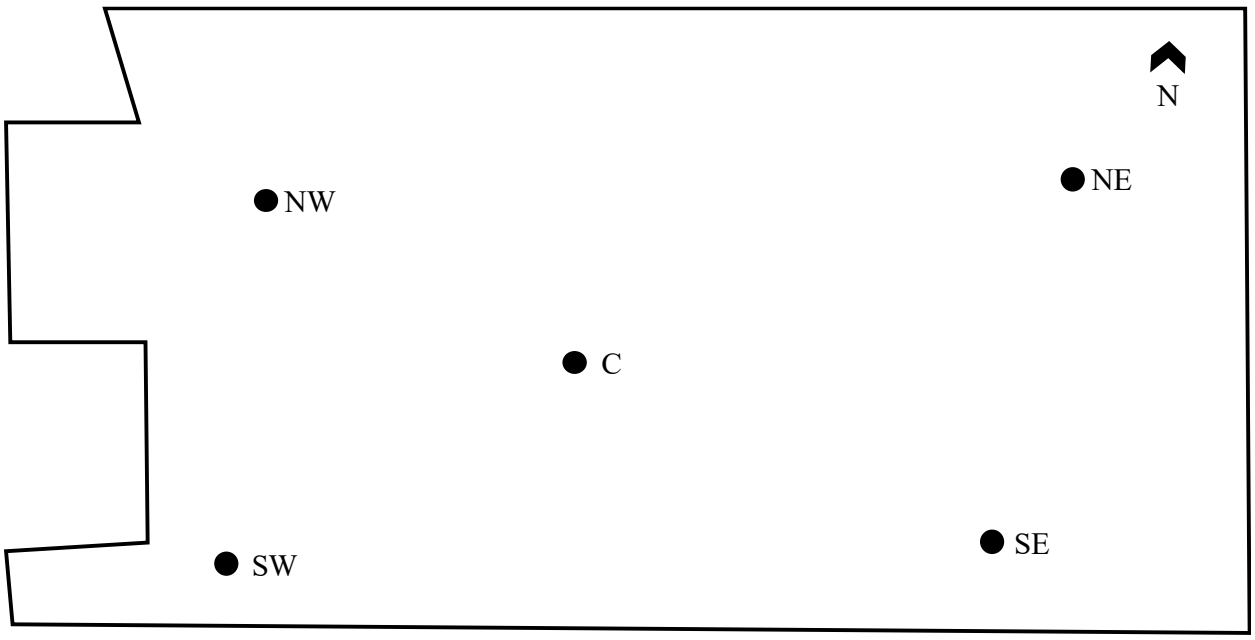

Figure 4.1 An example of a Grid field setup, with a rotorod sampler and Spornado located at each of five sites (circles) within the field. Separate canola petal tests were conducted at each site during the flowering period. "NW" = Northwest, "SW" = Southwest, "C" = Center, "NE" = Northeast, "SE" = Southeast. Weather monitoring equipment was placed in the SE corner.

5 General Discussion

Sclerotinia stem rot of canola remains a challenging disease to forecast and manage, given the wide host range, persistent resting structures and windborne ascospores of the causal agent, *Sclerotinia sclerotiorum*. Routine applications of fungicide are the primary method of control, but these can be cost-ineffective, have detrimental effects on the environment and select for populations of *S. sclerotiorum* with resistance to fungicides (Gossen et al. 2001). To improve the benefit and efficacy of fungicide application, risk assessment tools should include a field-specific form of inoculum detection, since the presence of inoculum, together with weather conditions, are the best indicators for low to no risk areas (Turkington 1991; Young et al. 2018). There are only a few commercially available methods for *S. sclerotiorum* ascospore detection in Canada, including a passive spore trap and petal test kits from private sector seed testing laboratories. The objectives of this thesis were to: 1) develop a better understanding of how environmental conditions influence *S. sclerotiorum* inoculum load and risk over the flowering period, and how these affect final stem rot incidence and severity; and 2) investigate the use of spore traps and DNA-based petal testing to assess airborne inoculum as a means of forecasting disease risk, and determine if a quantitative measure of risk is required for accurate predictions. These objectives were accomplished by: 1) comparing in-field weather conditions with the nearest publicly available Environment Canada weather station; 2) assessing commercially available tools to determine quantitative measures of petal infestation or airborne ascospore levels; 3) testing the relationship between ambient and in-canopy environmental conditions on all methods of spore detection; 4) evaluating the relationship between final disease levels and a combination of inoculum load and environmental conditions during flowering; and 4) monitoring inoculum load over the flowering period with multiple spore samplers and petal tests, to determine the recommended number and location of spore traps and tests required to provide an accurate estimate of inoculum pressure.

Understanding the influence of weather conditions on the disease cycle is key to understanding the variable nature of diseases including stem rot. Although most steps of the disease cycle occur under a broad range of conditions, if an element is missing, such as sufficient moisture early enough in the growing season, the subsequent steps can be affected, as can the time when infections will occur (Ficke et al. 2018). The impact of weather on each stage of the stem rot disease cycle has been researched extensively (Caesar and Pearson 1983; Clarkson et al.

2003; Clarkson et al. 2004; Ficke et al. 2018; Hao et al. 2003; Huang and Kozub 1991; Matheron and Porchas 2005; Nepal and del Río Mendoza 2012; Shahoveisi and del Rio Mendoza 2020; Teo and Morrall 1985; Turkington et al. 1991b; Wu and Subbarao 2008; Young et al. 2004). Comparisons made in this thesis (Chapter 3) suggest that in-field measures of influential weather conditions, such as daily average ambient RH and daily average ambient temperature, were generally significantly different (P =0.05) from corresponding conditions recorded at the nearest publicly available weather station. The only exception was total daily rainfall, which was generally similar to the amounts recorded at the closest weather stations. While most of the weather variables recorded in-field differed from the nearest public weather station, it has been traditionally thought that it is not realistic for a farmer to have weather-monitoring equipment present in all fields. However, over the past 20 years several companies offer weather station station set ups (RH, temperature and rainfall) and monitoring services for farmers and crop consultants (Decisive Farming 2020; Metos Canada 2021). Researchers have also found success using regional weather forecasts to create stem rot prediction models or alert systems. For example, Sharma et al. (2015) predicted stem rot incidence with an accuracy of 93% based on local weather forecasts, while Young et al. (2018) effectively reduced unnecessary sprays by 26% with weather-based alerts alone. Nonetheless, both research groups concluded that an in-field measurement of inoculum levels improves forecast accuracy.

Modern technology has allowed researchers to develop qualitative and quantitative PCR assays that can rapidly detect and measure *S. sclerotiorum* DNA in air and plant tissue samples (Almquist and Wallenhammar 2015; Parker et al. 2014; Rogers et al. 2009; Yin et al. 2009; Ziesman et al. 2016). The assay of Ziesman et al. (2016), which can be used to detect *S. sclerotiorum* DNA in plant or airborne samples, has undergone extensive testing to ensure no cross-reactivity with closely related species, such as *Botrytis cinerea* or other *Sclerotinia* species. It has also been tested for specificity to *S. sclerotiorum* populations from western Canada, as there is a large amount of genetic diversity between regional populations (Kohli et al. 1992). This assay has a reported limit of detection of 8.0×10^{-4} ng DNA, which is equivalent to 2.3 ascospores per petal (1 ascospore = 3.5×10^{-4} ng DNA; Rogers et al. 2009). This method was compared with two commercially available petal test kits (Canola Petal Test 1 (CPT1) and Canola Petal Test 2 (CPT2)) available in Canada that use proprietary DNA testing to give a percentage petal infestation, which correlates to predicted disease levels and yield loss. Petal

tests have been reported to be laborious, complex, costly, and samples must be sent to a lab that may be out of province (Shoute et al. 2018). Some of these concerns, however, may not be valid for the Canadian canola industry, given the ample lab capacity and expertise currently available across the Prairies, and the availability of accurate, low-cost tests that trained staff can conduct quickly. The cost of commercial petal testing or spore trapping can range from just under $100 CAD to $200-400 CAD, depending on the lab and testing process. Evaluation of petal samples has the advantage that it is based on monitoring inoculum that has already landed on, and potentially infected, host tissues (i.e., the petals). In contrast, monitoring of airborne ascospores quantifies inoculum that may or may not encounter the host.

Based on our testing, a minimum of 1.0×10^{-4} ng *S. sclerotiorum* DNA (per petal or m^{-3} $hour^{-1}$), which is equivalent to 0.3 ascospores (1 ascospore = 3.5×10^{-4} ng DNA; Rogers et al. 2009), appears to be needed during flowering especially early stages for epidemic levels of disease. Conducive weather conditions are also required, with Young et al. (2020) determining that 23 consecutive hours with ambient RH >80%, and ambient temperatures >7˚C, provided ideal conditions for disease development to occur in the UK; this was also supported by a model developed by Koch et al. (2007) for use in Germany. Rogers et al. (2009) reported that peak inoculum flushes of 12 ascospores m^{-3} day^{-1} (approximately 0.5 ascospores m^{-3} $hour^{-1}$) were needed during flowering for "higher than usual" levels of disease incidence (5.5% in untreated check plots), which is somewhat more than what was found in this thesis. However, Rogers et al. (2009) also noted that lower spore concentrations over a long period could cause severe epidemics, consistent with our results. Other researchers have also identified a maximum level of inoculum, beyond which additional ascospores will not increase disease further. Such maximum levels were reported for *S. sclerotiorum* on lettuce (87 spores cm^{-2}), pinto beans (200 spores per flower), and oilseed rape (80 ascospores per petal or 150 ascospores m^{-3}) (Clarkson et al. 2014; Harikrishnan and Rio 2008; Heran et al. 1999; McCartney et al. 1999). This suggests that thresholds can be established as to the number of ascospores required, either per petal or per volume of air, to cause an epidemic that warrants a fungicide application.

Results with the commercial petal test kits were variable and depended on proprietary testing at the respective labs. While there were not enough samples from either commercial petal test kit for early or late bloom to be evaluated separately, petal infestation levels determined following Ziesman et al. (2016) showed a stronger relationship to disease incidence than either

airborne spore trap. Test results with the CPT1, which predicted most fields in 2020 to have <20% disease incidence (0 – 45% percent petal infestation = low – no risk), were more consistent with the results from petal samples subjected to the protocol of Ziesman et al. (2016), which also indicated low amounts of petal infestation. The results with CPT2 showed greater variability, with a higher percent petal infestation found than with CPT1, making the former the more risk-averse of the commercial petal tests. Tests conducted with CPT2 predicted that most fields in 2020 would be above the 15% disease incidence threshold that the Canola Council of Canada indicates would benefit from an application of fungicide (i.e., >40% percent petal infestation). While the cost associated with the purchase of CPT2 limited the number of samples obtained, the results were consistent with what other researchers have found with respect to fluctuations in petal infestation over the flowering period. Namely, a prediction based on a single petal sample may not accurately reflect the final disease incidence (Almquist and Wallenhammar 2015; Parker et al. 2014; Rogers et al. 2009; Turkington et al. 1991a; Young et al. 2018; Ziesman 2016).

As studies on petal testing progresses, conflicting results are sometimes generated. Some researchers have observed variability in the strength of the relationship between petal infestation and disease incidence, depending on the growth stage of canola and environmental conditions over the flowering period (Ficke et al. 2018; Turkington et al. 1991a; Ziesman 2016), while others have found the relationship to be non-significant (Almquist and Wallenhammar 2015; Bom and Boland 2000). Variables such as sample size, canopy density, fluctuating inoculum levels, rainfall, light penetration, leaf area index and crop height may all have an impact on this relationship, making accurate predictions regarding final disease incidence or severity difficult (Turkington and Morrall 1993; Turkington et al. 1988; Turkington et al. 1991b). Petal tests, as opposed to airborne inoculum, are based on a later stage of the disease cycle (the pathogen has already encountered the host) and thus may provide a more accurate picture of potential disease progression. Overall at a particular point in time, petal infestation assessments are a closer stage to infected plants in the stem rot disease cycle. Petal infestation assessments as well as aerial spore load assessments synthesize the favourability of the weather (for inoculum production) and the potential for the stem rot pathogen to be present in sufficient quantities. This latter aspect is in relation to sclerotial load in the field and adjacent fields and the extent of sclerotial germination and apothecia production. In general, higher amounts of disease are found with

higher petal infestation and higher airborne inoculum levels. Our results of significance testing to determine the strength of the relationship between weather conditions and petal infestation levels were variable, with the only statistically significant model having included variables averaged over the total flowering period. Weather variables accounted for 67% of the variation in petal infestation. Across the three spore detection methods tested (the rotorod sampler, the Spornado and the analysis conducted by the Plant Health Lab), the strongest relationship was found when only petal samples and weather variables were used for predicting disease incidence (accounting for 86% of the variation in this parameter). During early to mid-flowering, when fungicide decisions are usually made, the Spornado and rotorod samplers accounted for only 48% and 52% of the variation in disease incidence, respectively. Petal samples also had the strongest relationship (coupled with weather variables) for predicting average stem rot severity. When using airborne ascospore-based detection methods, the inoculum averages over the mid-late flowering period showed the strongest relationship with disease severity ($R^2 = 0.91$), although the early-mid flowering period also showed a strong relationship ($R^2 = 0.87$).

Monitoring of airborne ascospore levels can indicate the potential for an epidemic level of disease even before flowering begins, allowing farmers to monitor weather conditions to determine whether these will also favour disease development (Freeman et al. 2002; Parker et al. 2014). Both types of airborne spore traps detected ascospore flushes, but the rotorod sampler better identified smaller peaks. While the Spornado was less sensitive to changes in inoculum levels and gave relatively broad categories for detection levels, it was able to detect fields at no risk of stem rot accurately. Significance testing between weather variables and airborne spore traps indicated that the only significant relationship was between weather variables and rotorod samples averaged over the total flowering period (Table 3.20), with weather variables accounting for 66% of the variation in the model. No models were significant for the Spornado or during early-mid flower or mid-late flower for either spore trap. This may indicate that other factors, such as soil moisture, were missing from the model. Soil moisture is an indirect measure of the total moisture available in the crop canopy. The absence of statistically significant models during the early-mid flowering period is important because that is when spray decisions are usually made. Other research has found that including soil moisture into an equation for predicting disease incidence improved model strength (Bom and Boland 2000; Sharma et al. 2015). The regression modeling in this thesis suggests that relying only on weather to predict

ascospore levels may be insufficient, because while weather conditions may predict increased ascospore loads, if there are no sclerotia present in the soil or in neighbouring fields, there may not be enough ascospores produced to cause enough disease to warrant a fungicide application.

The use of inoculum load measures in a stem rot forecasting system may be improved if combined with an assessment of weather conditions. For example, in ongoing experiments in the UK, a weather alert is issued if specific ambient conditions are met for disease progression (Young et al. 2018). A similar system could also be employed to predict if weather conditions between the rosette stage and early flowering (BBCH 20-61) are conducive for sclerotial germination, apothecial development, and ascospore development and release. Qandah and del Rio (2011) found that ascospore peaks occurred after several days of RH >85% under the canopy. However, such a system relies on the assumption that there are sufficient sclerotia present to benefit from the favorable weather, which often may not be the case. Therefore, field history should be considered, since it can indicate if sclerotia are likely to be present in the field or adjacent fields, while an airborne inoculum detection method can account for internally and externally produced inoculum (Bom and Boland 2000). Conversely, using only inoculum detection methods when the environmental conditions are not conducive for disease progression may also cause a farmer to apply a fungicide unnecessarily. For example, Turkington (1991) and Turkington et al. (1993) found late maturing fields with petal infestation levels >50%, which reflected previous rainfall events that promoted sclerotial germination and ascospore production. However, prevailing conditions at the petal infestation stage included daily highs of >30°C with little to no rainfall, and these unfavorable conditions for stem rot persisted for the subsequent 2-3 weeks. Therefore, although petal infestation levels were high, the non-conducive environmental conditions at flowering resulted in lower disease levels than predicted based on the petal tests. In contrast, earlier maturing fields had elevated inoculum loads and favourable weather conditions that promoted increased disease.

In terms of this study, the spore traps and the rotorod sampler, in particular, appeared to be more useful for providing an overall picture of inoculum load because they could be used before flowering starts (and while the first flowers are opening), indicating early ascospore levels. The spore traps are less labour-intensive compared with petal sampling, and in the case of the Spornado, are fairly inexpensive. A less labour-intensive sampling can be particularly advantageous, especially when inoculum needs to be monitored throughout the spray window.

Moreover, the detection of petal infestations may not always be an accurate measure of disease development; Almquist and Wallenhammar (2015) and Ficke et al. (2018) found significant stem rot incidence (up to 21%) when all petal tests had been negative, suggesting that infection had occurred through senescing leaves. Nevertheless, some measure of inoculum within a field, whether of airborne spores or petal infection, can aid in predicting stem rot, and the best indicator of no risk is either the absence of inoculum or weather conditions that are not conducive for disease (Clarkson et al. 2014; Parker et al. 2014; Rogers et al. 2009; Turkington 1991; Young et al. 2020). A more extensive sampling regime may provide more insights as to the differences in sampling methods.

In Chapter 4, significance testing indicated that petal samples taken from five different locations within a field did not show different population means, suggesting that one sample per field may be sufficient for monitoring purposes. However, these results should be regarded with caution, as sample sizes were small and petal samples were collected only in one year of the study for the Grid field. This was also true for the Spornado and rotorod samples, where population means were not significantly different at five different sites within a field. In 2019, a year with high petal infestation and airborne ascospore levels, as well as highly conducive weather for disease progression, stem rot incidence and severity were not uniform across fields. In contrast, in 2020, when there was generally lower petal infestation and airborne ascospore levels and less conducive weather, disease incidence was distributed uniformly (although severity was not). The differences observed between 2019 and 2020 highlight the effect of different environmental conditions on ascospore germination and disease progression, as well as sclerotial germination, rate of maturity for apothecia, ascospore release and petal adherence (Abawi and Grogan 1979; Almquist and Wallenhammar 2015; Schwartz and Steadman 1978; Wu and Subbarao 2008).

Generating ascospore and petal infestation thresholds may be necessary for improved stem rot forecasts. In this regard, quantitative measurements of ascospores are required, as threshold values for passive traps cannot be standardized because of the inconsistent air volume sampled (Parker et al. 2014). Other research has suggested that oilseed rape, lettuce and pinto beans can experience a maximum spore concentration over which lesions and disease incidence do not significantly increase with the addition of more spores (Clarkson et al. 2014; Harikrishnan and Rio 2008; Heran et al. 1999). Rogers et al. (2009) found negligible levels of stem rot incidence

on oilseed rape when there was a maximum of 4 ascospores m^{-3} day^{-1} detected at the start of flowering in Rothamsted, UK. Young et al. (2018) also found that a petal test showing >10% petal infestation indicated a risk for stem rot in oilseed rape in the UK, while approximately 200 spores/m^3 were "generally thought to indicate an infection risk." In this study, a minimum concentration of 1.0×10^{-4} ng DNA per canola petal or per cubic meter of air per hour during early flowering resulted in disease incidence levels >15%, at which fungicide is recommended by the Canola Council of Canada (https://www.canolacouncil.org/canola-encyclopedia/diseases/sclerotinia-stem-rot/#forecasting-and-risk-assessment).

Commercially available tools for predicting Sclerotinia stem rot need to be affordable, accurate, easy to use and accessible to farmers and agronomists. These tools should also be risk-averse, as underestimating disease risk and the potential yield loss from a missed fungicide application can be more costly than an unnecessary fungicide application (Parker et al. 2014). In Chapter 4, we found that population means for petal infestation and airborne ascospore levels were not significantly different among sampling sites, suggesting that only one trap may be needed per field. As mentioned previously, petal testing addresses potential variability across the field as samples can be collected from sites throughout the field. However, more testing is required, especially in 160 acre (64.7 ha) 'quarter-sections', which are more common than 'half-sections' (320 acres/129.5 ha), although smaller fields would be expected to be more uniform versus larger fields. In addition, others have suggested that ascospore release may not be synchronized with flowering and that it may occur before or after the late bud stage (BBCH 55). This would affect the timing of infection in relation to crop growth stage (Almquist and Wallenhammar 2015; Ficke et al. 2018; Gugel 1986; Qandah and del Río Mendoza 2011; Young et al. 2018). This can in turn affect the timing of fungicide application in relation to the early to mid-flowering periods. Given the wide range of variables required for disease progression, an integrated approach to forecasting should be employed that includes monitoring ambient weather conditions and an inoculum detection method to determine optimal timing for a fungicide application. Most fungicides in Canada are registered for application between 20%-50% flower (BBCH 62 – 65).

Based on the results of the current project, producers should monitor inoculum levels prior to and during the flowering period, along with in-field ambient and/or canopy RH levels. As reported by Young et al. (2018), we also found that a RH >80% was associated with increased

stem rot incidence, while a RH <80% was associated with lower disease incidence that would typically not require a fungicide application. Measurements of RH could be coupled with inoculum assessments based on spore trapping or petal testing, wherein 1.0×10^{-4} ng DNA per canola petal or per cubic meter of air per hour during early flowering would be expected to result in a disease incidence >15%, at which fungicide is recommended. Moreover, monitoring aerial spore load and necessary weather conditions (RH >80%) prior to and during flowering may help to guide fungicide timing, whether at or just prior to early bloom or later as the crop progresses into full bloom. For example, favourable weather and increased inoculum loads prior to and during the start of flowering may indicate the need for fungicide application earlier in the bloom period. If these favourable conditions persist into full bloom then a second application of fungicide may be warranted depending on yield potential and commodity price. Conversely, if weather and inoculum loads are not favourable prior to and during early flowering, but then become more favourable as the crop progresses towards full bloom, a full bloom application of fungicide may be more effective.

Of the inoculum load tests that were evaluated in the current study, the rotorod and Alberta Plant Heath Lab assessments tended to be more closely related to stem rot levels, while also accounting for the impact of weather, especially RH. The other tests were not as consistent, although they would still be useful for identifying fields at low risk where fungicide is not needed. Lower petal infestation and/or 'non-detection' to 'limit of detection' with the Spornado during the flowering period, coupled with in-field RH levels consistently <80%, would indicate a low risk and limited need for fungicide. Further refinements in terms of the testing procedures used and information provided may also help with these tests. The commercial supplier of the Spornado is looking at including additional information related to the 'detected' category, such as levels of *S. sclerotinia* DNA that correspond to 'no/low risk' to 'moderate to high risk' (R. Melenka, 20/20 Seed Labs Inc., personal communication). For both the petal tests and Spornado results, assessments of risk will be more accurate when combined with an evaluation of weather conditions.

Based on the results of Chapter 4, only one spore sampler may be needed per field for a reasonable estimate of airborne ascospore levels, which can limit costs. The location of such a spore trap could either be central, providing better exposure to airborne inoculum from all areas of the field, or in a downwind area of the field, depending on the prevailing wind direction. Petal

samples are usually collected throughout the field, so variability in results is less of a concern. The levels of Sclerotinia stem rot were similar across each field in 2019 and 2020, with differences in disease only significant in 2019. However, there was variability in stem rot severity between locations within the Grid fields. In both years, disease exceeded the 15% incidence required for a fungicide application; however, fertility levels, variable in-canopy environmental conditions, crop canopy density, and lodging, along with other variables, may also affect final disease in a field. As such, multiple risk assessment tools are warranted, including some form of measuring inoculum levels and RH, to provide the most robust predictions for stem rot in canola.

Advances in DNA-based technologies, refined pathogen identification and a rapid turnover for results can greatly improve our ability to predict *S. sclerotiorum* inoculum loads and stem rot risk in canola (Ziesman 2016; Ziesman et al. 2016). Further refinements in testing procedures to indicate risk and severity of stem rot may improve current forecasting models. Ultimately, measures of ascospore inoculum levels, while important, need to be considered together with environmental conditions and field history. An integrated forecasting system, which takes into account all components of the disease triangle, will be most effective for predicting Sclerotinia stem rot of canola as well as other diseases.

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Appendix

Table A. 1 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables from the nearest weather station (WS) for Field 1, 2019, near Edmonton, AB.

Location, Year	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables Tested	t value (Z or V value)[a]	p-value
F1 2019	Canopy Relative Humidity (RH)	93.7	4.9	0.85	Canopy vs. Ambient RH	t = 17.21	p <0.0001
	Ambient Relative Humidity	83.4	5.9	1.02	Ambient vs. WS RH	t = 3.47	p = 0.0015
	Weather station (WS) RH	81.4	7.2	1.3	-	-	-
	Canopy Temperature	16.1	1.6	0.28	Canopy vs. Ambient Temperature	t = -6.9	p <0.0001
	Ambient Temperature	16.7	1.8	0.32	Ambient vs. WS Temperature	t = -3.18	p = 0.003829
	Weather Station (WS) Temperature	17.4	1.6	0.28	-	-	-
	Daily Rain	5.2	11.1	1.9	Rain vs. WS Rain	Z = 0.269	p = 0.788
	Weather Station (WS) Rain	4.2	7.1	1.2	-	-	-
	Wind	3.6	1.7	0.30	Wind vs. Weather Station Wind	V = 0	p <0.0001
	Weather Station Wind	6.9	2.8	0.48	-	-	-

[a] The significance test used was based on assumptions tested. A paired t-test (t-value) was used if the normality assumption was met. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 2 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables from the nearest weather station (WS) for Field 2, 2019, near Edmonton, AB.

Location, Year	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables Tested	t value (Z or V value)[a]	p-value
F2 2019	Canopy Relative Humidity (RH)	95.5	3.4	0.59	Canopy vs. Ambient RH	t = 13.89	p <0.0001
	Ambient Relative Humidity	95.5	5.1	0.89	Ambient vs. WS RH	t = 7.27	p <0.0001
	Weather station (WS) RH	80.4	6.9	1.2			
	Canopy Temperature	15.6	1.4	0.24	Canopy vs. Ambient Temperature	V = 10	p <0.0001
	Ambient Temperature	16.3	1.7	0.31	Ambient vs WS Temperature	V = 171	p = 0.05
	Weather Station (WS) Temperature	16.5	2.1	0.36			
	Daily Rain	5.2	9.6	1.7	Rain vs WS Rain	Z = -0.595	p = 0.5517
	Weather Station (WS) Rain	4.0	6.3	1.1			
	Wind	4.9	2.5	0.43	Wind vs WS Wind	V = 0	p <0.0001
	Weather Station Wind	9.7	2.8	0.49			

[a] The significance test used was based on assumptions tested. A paired t-test (t-value) was used if the normality assumption was met. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 3 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables from the nearest weather station (WS) for Field 3, 2019, near Edmonton, AB.

Location, Year	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables tested	t value (Z or V value)[a]	p-value
F3 2019	Canopy Relative Humidity (RH)	97.9	2.9	0.05	Canopy vs. Ambient RH	V = 561	p <0.0001
	Ambient Relative Humidity	85.5	5.5	0.96	Ambient vs. WS RH	t = 7.13	p <0.0001
	Weather station (WS) RH	80.4	6.9	1.21			
	Canopy Temperature	16.2	1.8	0.31	Canopy vs. Ambient Temperature	Z = 1.686	p = 0.0917
	Ambient Temperature	15.5	1.4	0.25	Ambient vs. WS Temperature	t = -5.145	p <0.0001
	Weather Station (WS) Temperature	16.5	2.1	0.36			
	Daily Rain	3.5	7.1	1.2	Rain vs. WS Rain	Z = -0.175	p = 0.8611
	Weather Station (WS) Rain	4.2	6.5	1.1			
	Wind	3.0	2.7	0.46	Wind vs. WS Wind	Z = -6.421	p <0.0001
	Weather Station Wind	6.9	2.9	0.89			

[a] The significance test used was based on assumptions tested. A paired t-test (t-value) was used if the normality assumption was met. If the assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 4 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables from the nearest weather station (WS) for Field 4, 2019, near Edmonton, AB.

Location, Year	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables Tested	t value (Z or V value)[a]	p-value
F4 2019	Canopy Relative Humidity (RH)	95.6	4.3	0.74	Canopy vs. Ambient RH	V = 560	p <0.0001
	Ambient Relative Humidity	84.0	5.2	0.91	Ambient vs. WS RH	t = 4.08	p = 0.0003
	Weather station (WS) RH	81.4	7.2	1.26			
	Canopy Temperature	16.1	1.4	0.25	Canopy vs. Ambient Temperature	t = -6.756	p <0.0001
	Ambient Temperature	16.7	1.8	0.31	Ambient vs. WS Temperature	t = -3.02	p = 0.006
	Weather Station (WS) Temperature	17.4	1.6	0.28			
	Daily Rain	4.7	9.4	1.9	Rain vs. WS Rain	Z=0.215	p = 0.8297
	Weather Station (WS) Rain	4.2	7.1	1.2			
	Wind	4.5	2.6	0.45	Wind vs. WS Wind	V = 0	p <0.0001
	Weather Station Wind	6.9	2.8	0.48			

[a] The significance test used was based on assumptions tested. If the normality A-value) was used. A paired t-test (t-value) was used if the normality assumption was met. If the assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 5 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables located from the nearest weather station (WS) for Brooks, AB, 2019.

Location, Province	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables tested	t value (Z or V value)[a]	p-value
Brooks, AB	Canopy Relative Humidity (RH)	N/A	N/A	N/A	Canopy vs. Ambient RH	N/A	N/A
	Ambient Relative Humidity	67.9	9.5	1.1	Ambient vs. WS RH	t = 7.5	p < 0.0001
	Weather station (WS) RH	64.3	10.6	1.3			
	Canopy Temperature	N/A	N/A	N/A	Canopy vs. Ambient Temperature	N/A	N/A
	Ambient Temperature	17.3	2.8	0.3	Ambient vs. WS Temperature	t = -0.78	p = 0.44
	Weather Station Temperature	17.4	2.9	0.3			
	Daily Rain	4.0	4.6	0.5	Rain vs. WS Rain	Z = 4.9	p < 0.0001
	Weather Station Rain	1.3	3.6	0.4			

[a] The significance test used was based on assumptions tested. If the normality assumption was met, a paired t-test (t-value) was used. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 6 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables located from the nearest weather station (WS) for Outlook, SK, 2019.

Location, Province	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables tested	t value (Z or V value)[a]	p-value
Outlook, SK	Canopy Relative Humidity (RH)	87.2	7.3	0.9	Canopy vs. Ambient RH	t = 17.1	p < 0.0001
	Ambient Relative Humidity	76.2	7.2	0.9	Ambient vs. WS RH	t = 20.8	p < 0.0001
	Weather station (WS) RH	70.0	7.4	0.9	-	-	-
	Canopy Temperature	16.7	2.4	0.3	Canopy vs. Ambient Temperature	t = .13.1	p < 0.0001
	Ambient Temperature	16.9	2.8	0.3	Ambient vs. WS Temperature	t = 1.14	p = 0.26
	Weather Station (WS) Temperature	3.0	2.8	0.3	-	-	-
	Daily Rain	1.7	5.4	0.6	Rain vs. WS Rain	Z = 1.25	p = 0.21
	Weather Station (WS) Rain	1.7	4.5	0.5	-	-	-

[a] The significance test used was based on assumptions tested. If the normality assumption was met, a paired t-test (t-value) was used. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 7 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables from the nearest weather station (WS) for Field 1 in 2020, near Edmonton, AB.

Location, Year	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables Tested	t value (Z or V value)[a]	p-value
F1 2020	Canopy Relative Humidity (RH)	85.0	7.7	1.1	Canopy vs. Ambient RH	t = 16.381	p <0.0001
	Ambient Relative Humidity	76.5	8.3	1.2	Ambient vs. WS RH	t = 0.6331	p = 0.5296
	Weather station (WS) RH	76.0	9.4	1.3	-	-	-
	Canopy Temperature	16.6	2.3	0.32	Canopy vs. Ambient Temperature	t = -8.026	p <0.0001
	Ambient Temperature	17.3	2.5	0.36	Ambient vs. WS Temperature	t = 0.4282	p = 0.6704
	Weather Station (WS) Temperature	17.2	2.2	0.31	-	-	-
	Daily Rain	3.4	6.3	0.90	Rain vs. WS Rain	Z = -0.1226	p = 9.025
	Weather Station (WS) Rain	3.7	6.8	0.96	-	-	-
	Wind	7.6	3.9	0.56	Wind vs. WS Wind	Z = -0.9604	p = 0.3369
	Weather Station Wind	7.7	3.1	0.44	-	-	-

[a] The significance test used was based on assumptions tested. A paired t-test (t-value) was used if the normality assumption was met. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 8 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables from the nearest weather station (WS) for Field 2, 2020, near Edmonton, AB.

Location, Year	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables Tested	t value (Z or V value)[a]	p-value
F2 2020	Canopy Relative Humidity (RH)	81.6	8.0	1.0	Canopy vs. Ambient RH	t = 17.044	p <0.0001
	Ambient Relative Humidity	74.6	8.3	1.0	Ambient vs. WS RH	t = -1.789	p = 0.07846
	Weather station (WS) RH	75.6	8.8	1.1			
	Canopy Temperature	16.9	2.5	0.31	Canopy vs. Ambient Temperature	t = -3.6474	p = 0.0005
	Ambient Temperature	17.2	2.7	0.33	Ambient vs. WS Temperature	t = 2.204	p = 0.0314
	Weather Station (WS) Temperature	16.8	2.4	0.30			
	Daily Rain	2.8	5.8	0.72	Rain vs. WS Rain	Z = -0.7521	p = 0.452
	Weather Station (WS) Rain	3.3	6.2	0.78			
	Wind	7.8	4.2	0.52	Wind vs. WS Wind	V = 282	p = 0.0204
	Weather Station Wind	7.5	2.9	0.36			

[a] The significance test used was based on assumptions tested. A paired t-test (t-value) was used if the normality assumption was met. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 9 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables from the nearest weather station (WS) for Field 3, 2020, near Edmonton, AB.

Location, Year	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables Tested	t value (Z or V value)[a]	p-value
F3 2020	Canopy Relative Humidity (RH)	78.8	8.5	1.3	Canopy vs. Ambient RH	t = 10.802	p <0.0001
	Ambient Relative Humidity	75.3	9.5	1.4	Ambient vs. WS RH	t = -2.2976	p = 0.03295
	Weather station (WS) RH	76.5	9.5	1.4	-	-	-
	Canopy Temperature	17.8	2.8	0.42	Canopy vs. Ambient Temperature	Z = 0.2381	p = 0.8118
	Ambient Temperature	17.6	2.7	0.41	Ambient vs. WS Temperature	Z = 0.2219	p = 0.8244
	Weather Station (WS) Temperature	17.4	2.5	0.37	-	-	-
	Daily Rain	3.4	5.67	0.84	Rain vs. WS Rain	Z = -0.1525	p = 0.8788
	Weather Station (WS) Rain	3.4	5.69	0.85	-	-	-
	Wind	4.2	2.3	0.34	Wind vs. WS Wind	Z = -4.723	p <0.0001
	Weather Station Wind	7.1	2.7	0.40	-	-	-

[a] The significance test used was based on assumptions tested. A paired t-test (t-value) was used if the normality assumption was met. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 10 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables from the nearest weather station (WS) for Field 4, 2020, near Edmonton, AB.

Location, Year	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables Tested	t value (Z or V value)[a]	p-value
F4 2020	Canopy Relative Humidity (RH)	83.6	8.3	1.1	Canopy vs. Ambient RH	V = 1429	p <0.0001
	Ambient Relative Humidity	78.4	8.4	1.2	Ambient vs. WS RH	t = -4.1197	p = 0.00013
	Weather station (WS) RH	80.7	9.5	1.3	-	-	-
	Canopy Temperature	16.1	2.6	0.35	Canopy vs. Ambient Temperature	t = -4.4789	p <0.0001
	Ambient Temperature	16.4	2.7	0.37	Ambient vs. WS Temperature	t = -2.4661	p = 0.017
	Weather Station (WS) Temperature	16.5	2.6	0.35	-	-	-
	Daily Rain	3.2	6.9	0.95	Rain vs. WS Rain	Z = -0.06497	p = 0.9482
	Weather Station (WS) Rain	2.9	5.3	0.73	-	-	-
	Wind	7.2	2.7	0.37	Wind vs. WS Wind	t = -13.493	p <0.0001
	Weather Station Wind	12.7	4.6	0.64	-	-	-

[a] The significance test used was based on assumptions tested. A paired t-test (t-value) was used if the normality assumption was met. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 11 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables from the nearest weather station (WS) for Field 5, 2020, near Edmonton, AB.

Location, Year	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables Tested	t value (Z or V value)[a]	p-value
F5 2020	Canopy Relative Humidity (RH)	87.8	9.6	1.3	Canopy vs. Ambient RH	V = 1188	p <0.0001
	Ambient Relative Humidity	77.4	9.2	1.3	Ambient vs. WS RH	t = -5.868	p <0.0001
	Weather station (WS) RH	80.7	9.5	1.3	-	-	-
	Canopy Temperature	16.2	2.6	0.36	Canopy vs. Ambient Temperature	t = -5.657	p <0.0001
	Ambient Temperature	16.2	2.6	0.36	Ambient vs. WS Temperature	t = -1.254	p = 0.2155
	Weather Station (WS) Temperature	16.5	2.6	0.35	-	-	-
	Daily Rain	2.7	5.09	0.70	Rain vs. WS Rain	Z = 0.2376	p = 0.8122
	Weather Station (WS) Rain	2.9	5.33	0.73	-	-	-
	Wind	7.7	3.2	0.43	Wind vs. WS Wind	t = -14.963	p <0.0001
	Weather Station Wind	12.7	4.6	0.64	-	-	-

[a] The significance test used was based on assumptions tested. A paired t-test (t-value) was used if the normality assumption was met. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 12 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables located from the nearest weather station (WS) for Beaverlodge, AB, 2019.

Location, Province	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables tested	t value (Z or V value)[a]	p-value
Beaverlodge, AB	Canopy Relative Humidity (RH)	87.0	7.4	0.9	Canopy vs. Ambient RH	t = 13.3	p < 0.0001
	Ambient Relative Humidity	78.1	8.5	1.0	Ambient vs. WS RH	t = 9.1	p < 0.0001
	Weather station (WS) RH	76.3	8.7	1.0	-	-	-
	Canopy Temperature	13.5	2.0	0.2	Canopy vs. Ambient Temperature	t = -8.0	p < 0.0001
	Ambient Temperature	14.1	2.3	0.3	Ambient vs. WS Temperature	t = 8.3	p < 0.95
	Weather Station (WS) Temperature	13.6	2.3	0.3	-	-	-
	Daily Rain	2.7	6.3	0.7	Rain vs. WS Rain	V = 1113	p < 0.0001
	Weather Station (WS) Rain	2.4	5.5	0.7	-	-	-

[a] The significance test used was based on assumptions tested. If the normality assumption was met, a paired t-test (t-value) was used. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 13 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables located from the nearest weather station (WS) for Lacombe, AB, 2019.

Location, Province	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables tested	t value (Z or V value)[a]	p-value
Lacombe, AB	Canopy Relative Humidity (RH)	86.4	6.9	0.8	Canopy vs. Ambient RH	t = 14.8	p < 0.0001
	Ambient Relative Humidity	81.0	6.8	0.8	Ambient vs. WS RH	t = 15.4	p < 0.0001
	Weather station (WS) RH	77.1	7.5	0.9	-	-	-
	Canopy Temperature	14.0	2.1	0.2	Canopy vs. Ambient Temperature	t = -9.4	p < 0.0001
	Ambient Temperature	14.5	2.3	0.3	Ambient vs. WS Temperature	t = 1.5	p = 0.12
	Weather Station Temperature	14.4	2.3	0.3	-	-	-
	Daily Rain *	N/A	N/A	N/A	Rain vs. WS Rain	N/A	N/A
	Weather Station Rain	2.9	6.1	0.7	-	-	-

[a] The significance test used was based on assumptions tested. If the normality assumption was met, a paired t-test (t-value) was used. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.
*The HOBO tipping bucket rain gauge malfunctioned, therefore rain data from Environment Canada was used (https://climate.weather.gc.ca/)

Table A. 14 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables located from the nearest weather station (WS) for Lethbridge, AB, 2019.

Location, Province	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables tested	t value (Z or V value)[a]	p-value
Lethbridge, AB	Canopy Relative Humidity (RH)	65.8	13.7	1.6	Canopy vs. Ambient RH	t = 15.4	p < 0.0001
	Ambient Relative Humidity	60.5	14.3	1.7	Ambient vs. WS RH	t = 14.8	p < 0.0001
	Weather station (WS) RH	63.9	14.1	1.7	-	-	-
	Canopy Temperature	18.3	3.0	0.4	Canopy vs. Ambient Temperature	t = 15.4	p < 0.0001
	Ambient Temperature	18.2	3.2	0.4	Ambient vs. WS Temperature	t = 14.8	p < 0.0001
	Weather Station (WS) Temperature	17.9	3.2	0.4	-	-	-
	Daily Rain	1.3	4.8	0.6	Rain vs. WS Rain	Z= -0.003	p = 0.998
	Weather Station Rain	1.0	3.8	0.5	-	-	-

[a] The significance test used was based on assumptions tested. If the normality assumption was met, a paired t-test (t-value) was used. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 15 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables located from the nearest weather station (WS) for Indian Head, SK, 2019.

Location, Province	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables tested	t value (Z or V value)[a]	p-value
Indian Head, SK.	Canopy Relative Humidity (RH)	77.6	9.7	1.1	Canopy vs. Ambient RH	t = 8.5	p < 0.0001
	Ambient Relative Humidity	74.1	8.2	1.0	Ambient vs. WS RH	t = 7.3	p < 0.0001
	Weather station (WS) RH	71.7	7.4	0.9	-	-	-
	Canopy Temperature	17.5	3.0	0.4	Canopy vs. Ambient Temperature	t = 4.1	p < 0.0001
	Ambient Temperature	17.3	3.0	0.4	Ambient vs. WS Temperature	t = 8.4	p < 0.0001
	Weather Station (WS) Temperature	16.7	3.0	0.4	-	-	-
	Daily Rain	2.5	5.6	0.7	Rain vs. WS Rain	Z = 0.53	p = 0.59
	Weather Station (WS) Rain	2.2	4.9	0.6	-	-	-

[a] The significance test used was based on assumptions tested. If the normality assumption was met, a paired t-test (t-value) was used. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 16 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables located from the nearest weather station (WS) for Melfort, SK, 2019.

Location, Province	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables tested	t value (Z or V value)[a]	p-value
Melfort, SK	Canopy Relative Humidity (RH)	84.2	7.2	0.9	Canopy vs. Ambient RH	V = 2016	p < 0.0001
	Ambient Relative Humidity	79.5	6.4	0.8	Ambient vs. WS RH	V = 2019	p < 0.0001
	Weather station (WS) RH	74.7	6.1	0.7	-	-	-
	Canopy Temperature	16.3	8.9	0.3	Canopy vs. Ambient Temperature	t = -1.4	p = 0.167
	Ambient Temperature	16.3	3.0	0.4	Ambient vs. WS Temperature	t = 1.88	p = 0.065
	Weather Station (WS) Temperature	16.0	2.8	0.3	-	-	-
	Daily Rain	2.4	4.9	0.6	Rain vs. WS Rain	Z = 0.28	p = 0.78
	Weather Station (WS) Rain	2.0	4.2	0.5	-	-	-

[a] The significance test used was based on assumptions tested. If the normality assumption was met, a paired t-test (t-value) was used. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 17 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables located from the nearest weather station (WS) for Scott, SK, 2019.

Location, Province	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables tested	t value (Z or V value)[a]	p-value
Scott, SK	Canopy Relative Humidity (RH)	80.1	6.2	0.7	Canopy vs. Ambient RH	V = 8.00	p < 0.0001
	Ambient Relative Humidity	84.5	6.6	0.8	Ambient vs. WS RH	V = 2346	p < 0.0001
	Weather station (WS) RH	71.3	6.5	0.8	-	-	-
	Canopy Temperature	15.2	2.6	0.3	Canopy vs. Ambient Temperature	t = -4.85	p < 0.0001
	Ambient Temperature	15.4	2.8	0.3	Ambient vs. WS Temperature	t = -1.65	p = 0.1031
	Weather Station (WS) Temperature	15.5	2.7	0.3	-	-	-
	Daily Rain	2.5	6.8	0.8	Rain vs. WS Rain	Z = -0.51	p = 0.61
	Weather Station (WS) Rain	2.3	5.3	0.6	-	-	-

[a] The significance test used was based on assumptions tested. If the normality assumption was met, a paired t-test (t-value) was used. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 18 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables located from the nearest weather station (WS) for Brandon, MB, 2019.

Location, Province	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables tested	t value (Z or V value)[a]	p-value
Brandon, MB	Canopy Relative Humidity (RH)	76.5	8.6	1.0	Canopy vs. Ambient RH	t = 11.1	p < 0.0001
	Ambient Relative Humidity	72.2	7.7	0.9	Ambient vs. WS RH	t = 13.9	p < 0.0001
	Weather station (WS) RH	68.0	7.7	0.9	-	-	-
	Canopy Temperature	18.5	2.7	0.3	Canopy vs. Ambient Temperature	t = -4.94	p < 0.0001
	Ambient Temperature	18.7	2.9	0.3	Ambient vs. WS Temperature	t = 0.69	p = 0.492
	Weather Station (WS) Temperature	18.3	3.1	0.4	-	-	-
	Daily Rain	3.9	6.6	0.8	Rain vs. WS Rain	Z = 2.17	p = 0.030
	Weather Station (WS) Rain	1.9	4.3	0.5	-	-	-

[a] The significance test used was based on assumptions tested. If the normality assumption was met, a paired t-test (t-value) was used. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

Table A. 19 Significance tests to determine the relationship between the weather variables within the canopy compared to the same variables above the crop canopy (ambient). Ambient weather variables are also compared with the same variables located from the nearest weather station (WS) for Normandin, QC, 2019.

Location, Province	Variables	Mean	Standard Deviation	Standard Error of the Mean	Variables tested	t value (Z or V value)[a]	p-value
Normandin, QC	Canopy Relative Humidity (RH)	78.7	11.4	1.3	Canopy vs. Ambient RH	V = 2346.0	p < 0.0001
	Ambient Relative Humidity	774.5	11.1	1.3	Ambient vs. WS RH	t = -0.457	p = 0.650
	Weather station (WS) RH	74.7	10.3	1.2	-	-	-
	Canopy Temperature	17.9	2.7	0.3	Canopy vs. Ambient Temperature	t = -3.20	p = 0.002
	Ambient Temperature	18.0	2.7	0.3	Ambient vs. WS Temperature	t = 0.478	p = 0.6332
	Weather Station (WS) Temperature	17.8	2.6	0.3	-	-	-
	Daily Rain	2.6	4.6	0.6	Rain vs. WS Rain	Z = 0.649	p = 0.517
	Weather Station (WS) Rain	2.2	4.3	0.5	-	-	-

[a] The significance test used was based on assumptions tested. If the normality assumption was met, a paired t-test (t-value) was used. If the normality assumption was violated, a Mann-Whitney (Wilcoxon Signed Rank Test (V – value) or Asymptotic (Z – value)) non-parametric paired test was used.

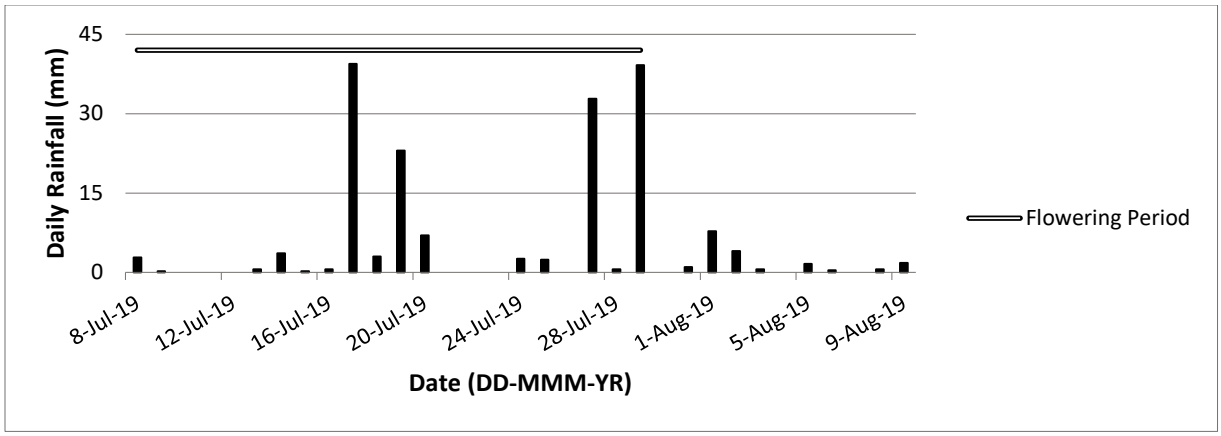

Figure A. 1 Daily rainfall in field 1 (F1) near Oliver, AB, 2019. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M).

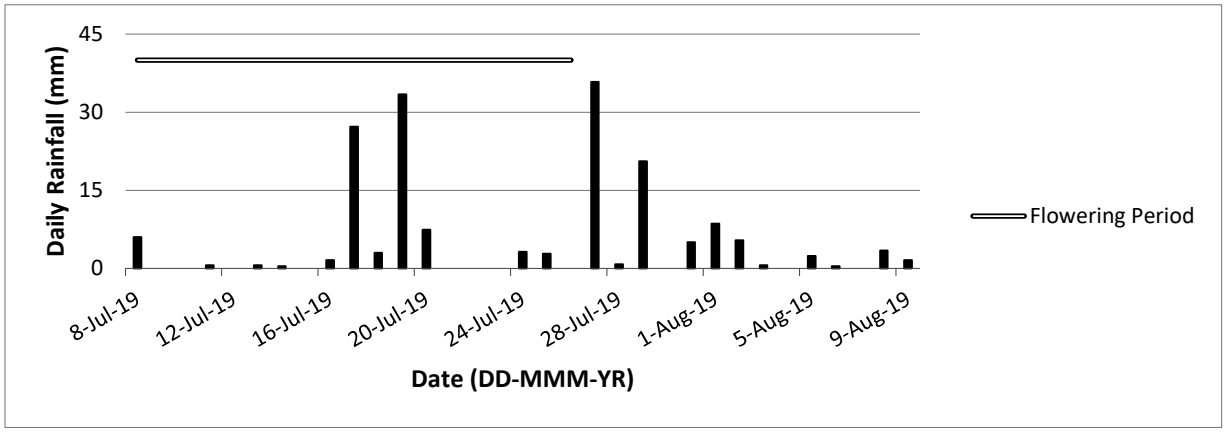

Figure A. 2 Daily rainfall in field 2 (F2), near Namao, AB, 2019. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M).

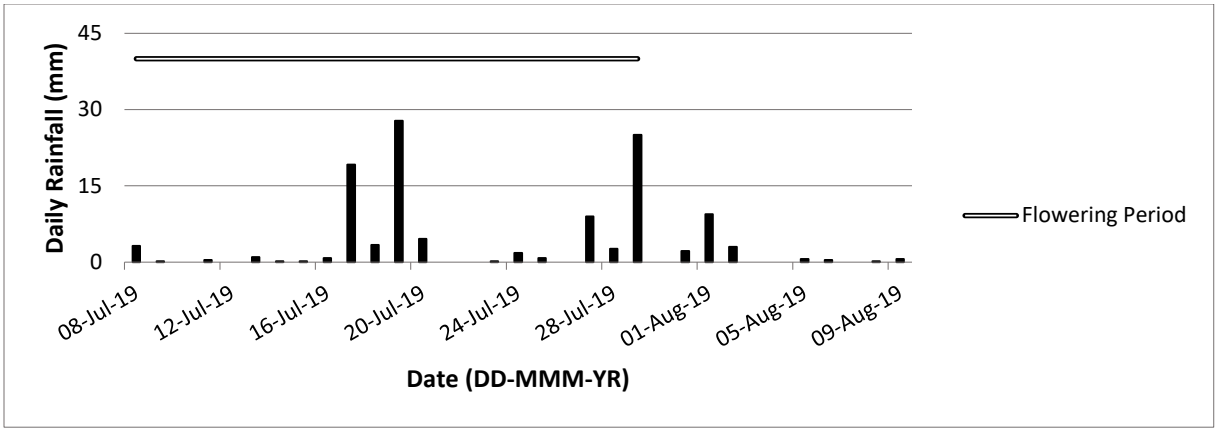

Figure A. 3 Daily rainfall in field 3 (F3), near Namao, AB, 2019. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M).

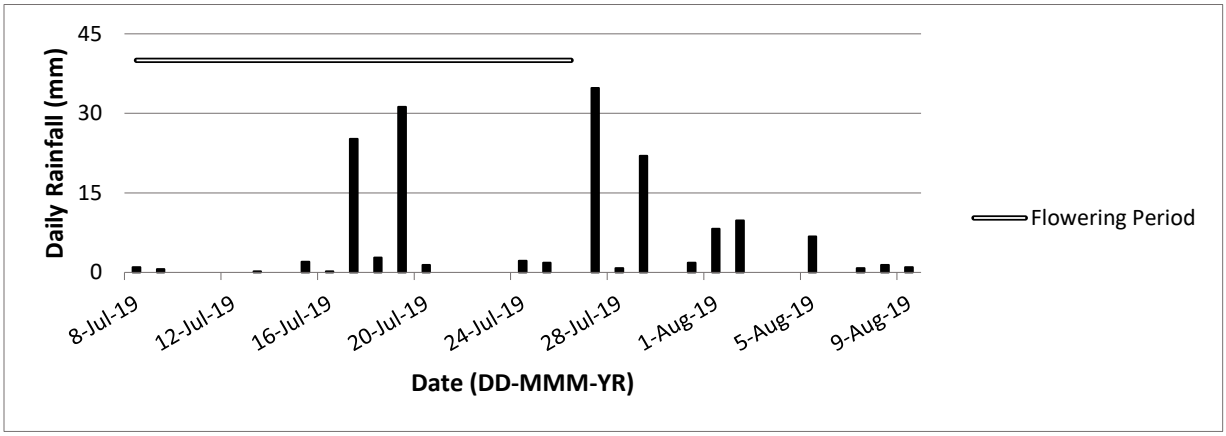

Figure A. 4 Daily rainfall in field 4 (F4), near Oliver, AB, 2019. Rain was measured with a HOBO Bucket Rain Gauge (RG3 – M).

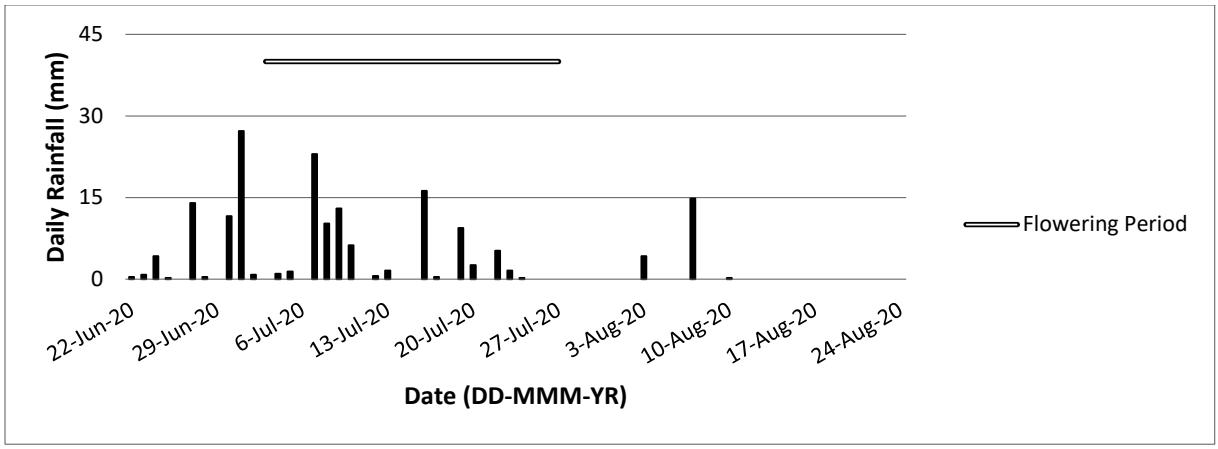

Figure A. 5 Daily rainfall in field 1 (F1), near Radway, AB, 2020. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M).

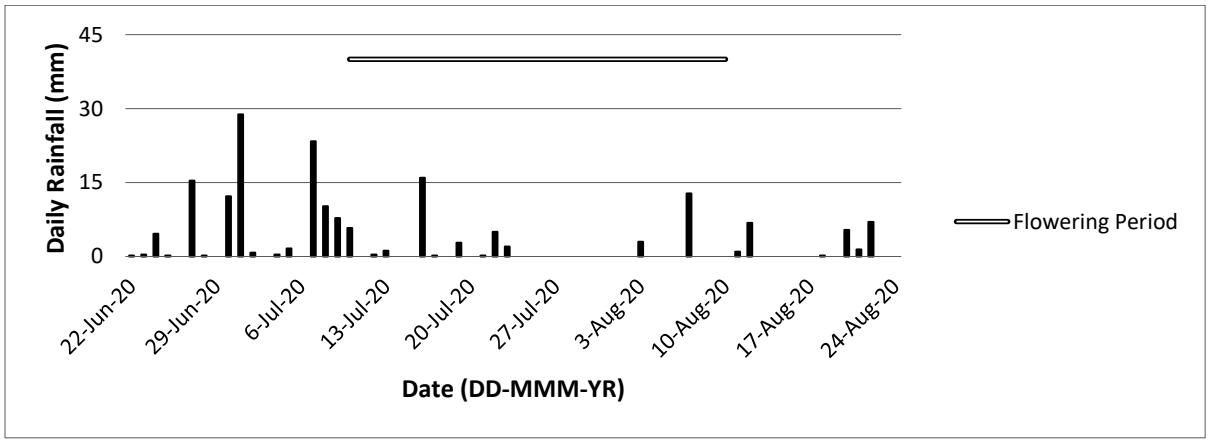

Figure A. 6 Daily rainfall in field 2 (F2), near Radway, AB, 2020. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M).

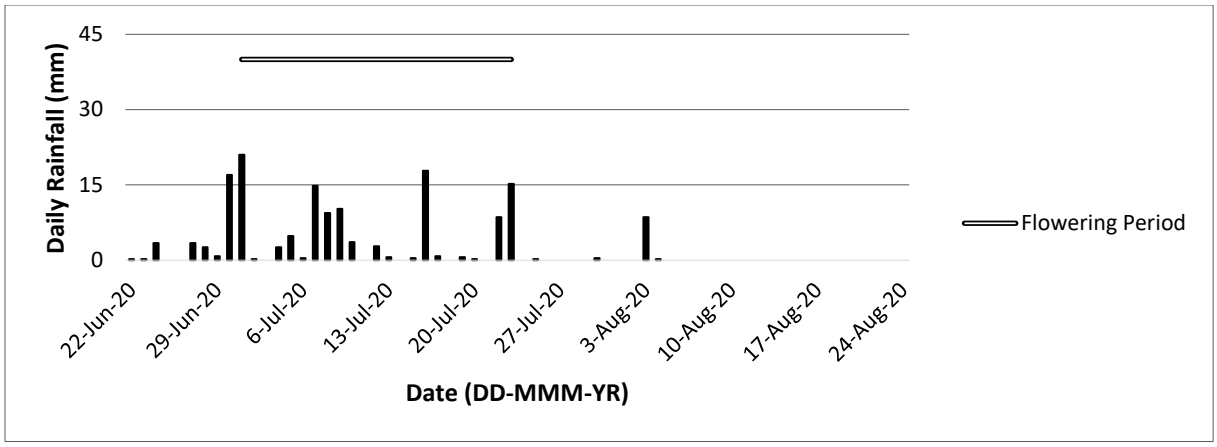

Figure A. 7 Daily rainfall in field 3 (F3), near Oliver, AB, 2020. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M).

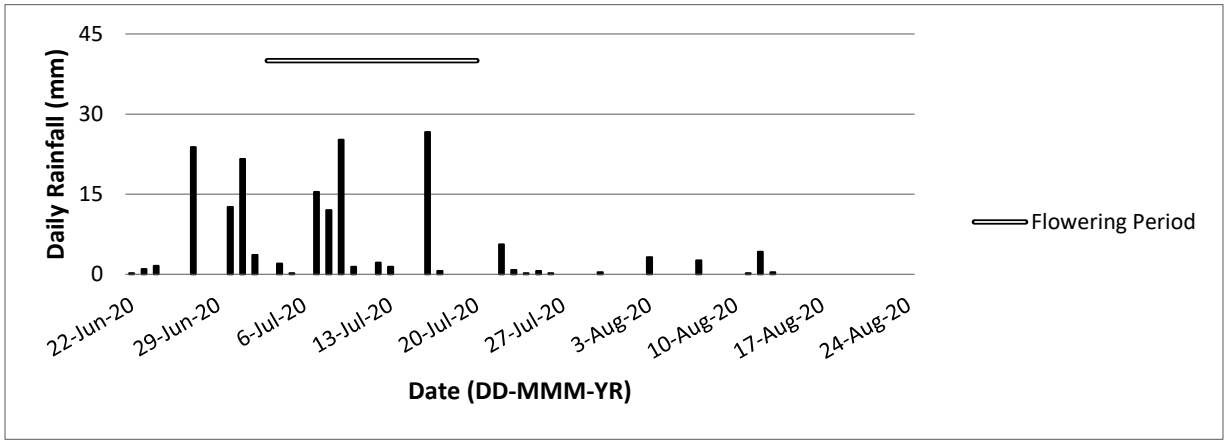

Figure A. 8 Daily rainfall in field 4 (F4), near Legal, AB, 2020. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M).

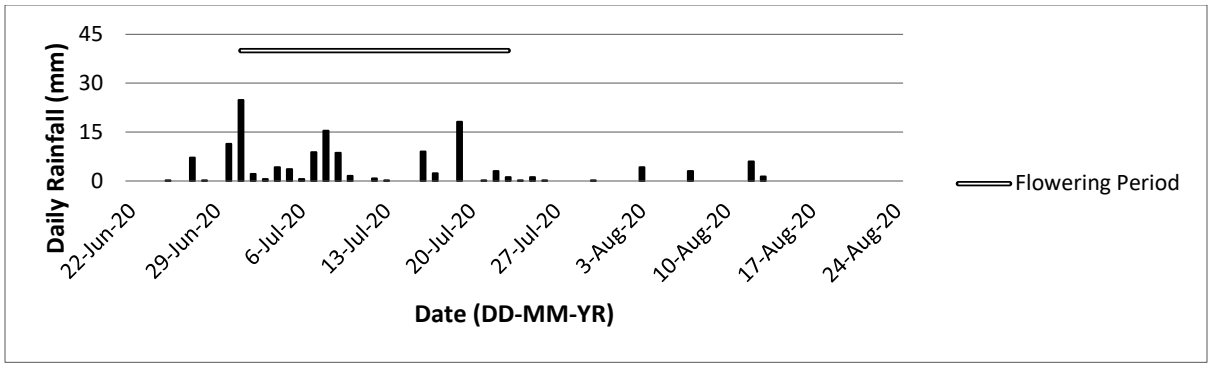

Figure A. 9 Daily rainfall in field 5 (F5), near Legal, AB, 2020. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M).

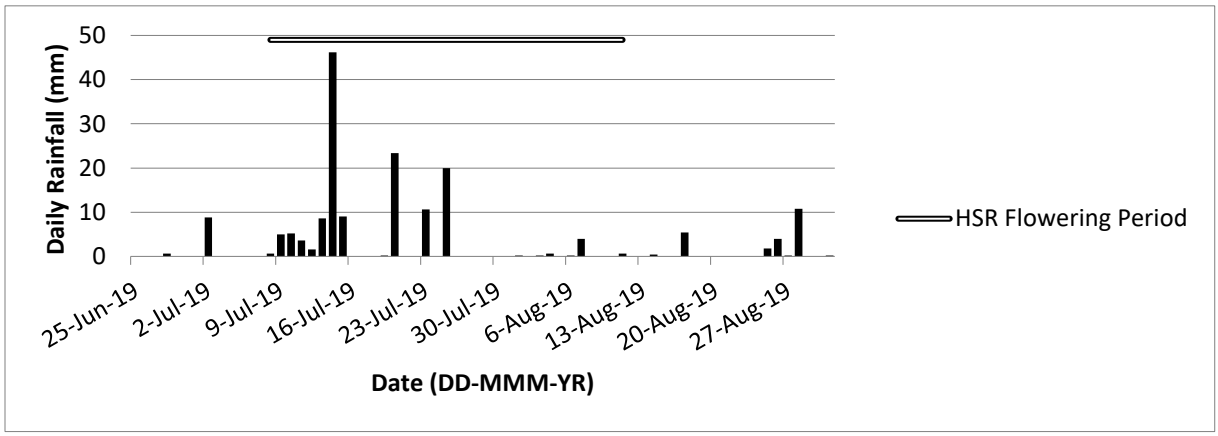

Figure A. 10 Daily rainfall near Scott, AB, 2019. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate), although no flowering dates were recorded the lower seeding rate for Scott.

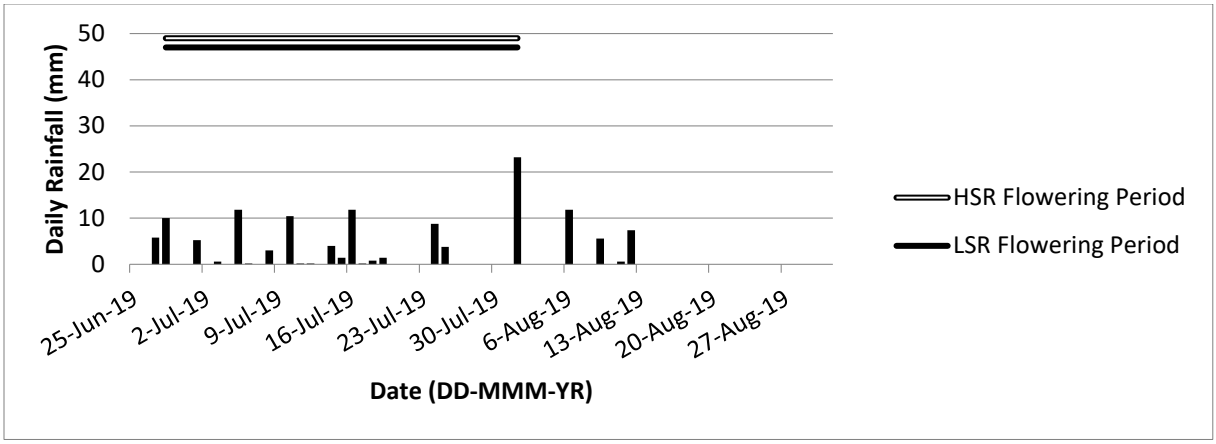

Figure A. 11 Daily rainfall near Outlook, SK, 2019. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

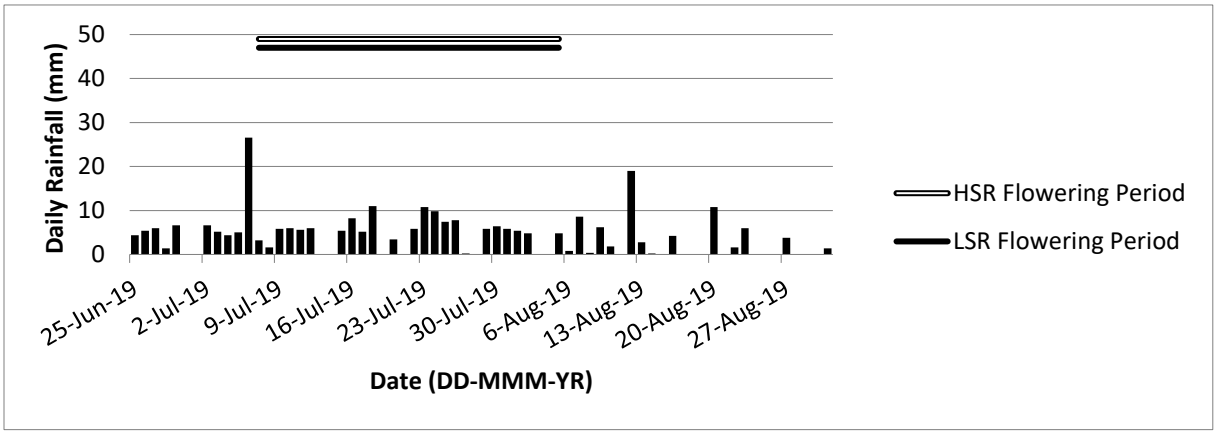

Figure A. 12 Daily rainfall near Brooks, AB, 2019. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

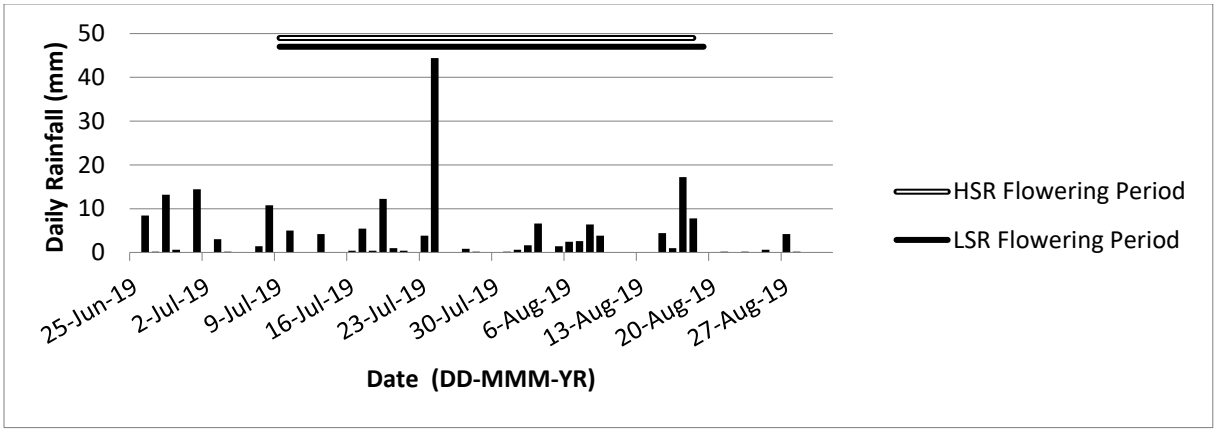

Figure A. 13 Daily rainfall near Beaverlodge, AB, 2019. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

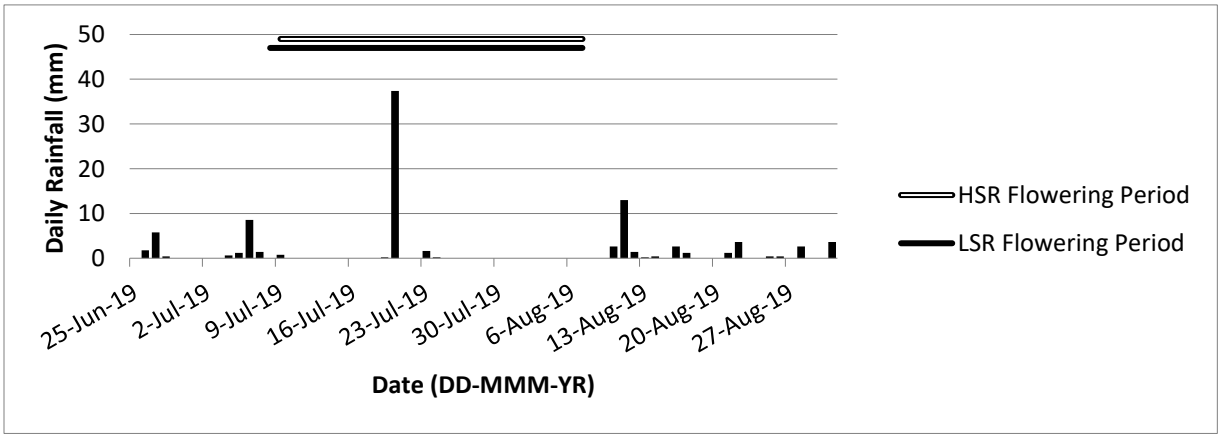

Figure A. 14 Daily rainfall near Lethbridge, AB, 2019. Rain (mm) was measured with a HOBO Bucket Rain Gauge (RG3 – M). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

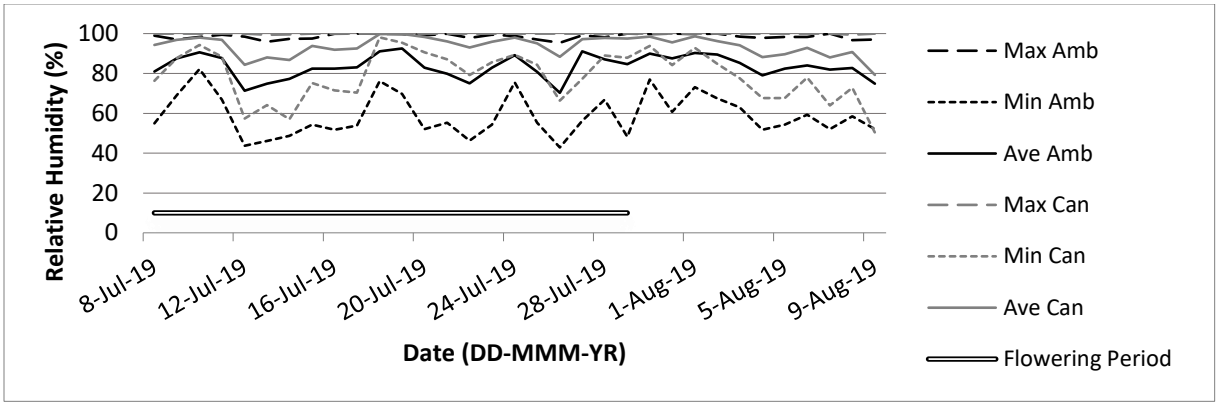

Figure A. 15 Relative humidity (RH) in field 1 (F1) near Oliver, AB, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

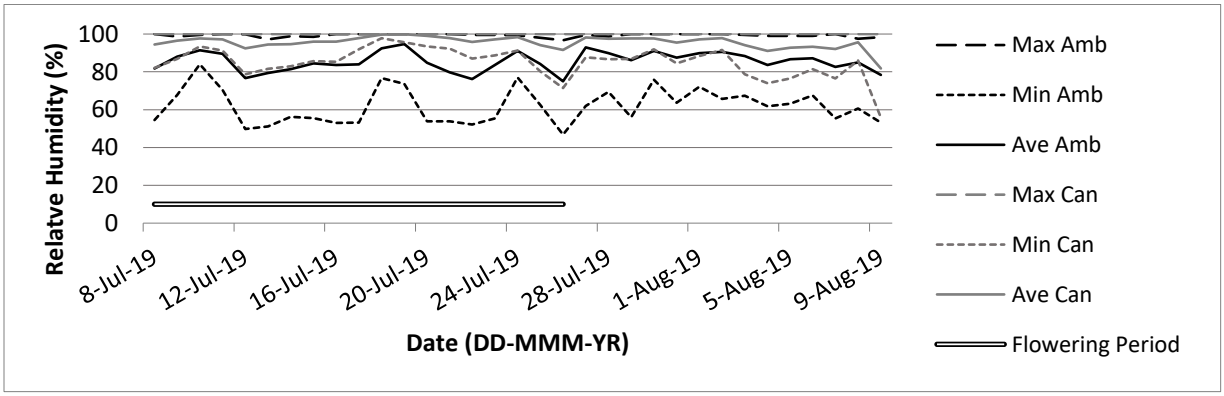

Figure A. 16 Relative humidity (RH) in field 2 (F2), near Namao, AB, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

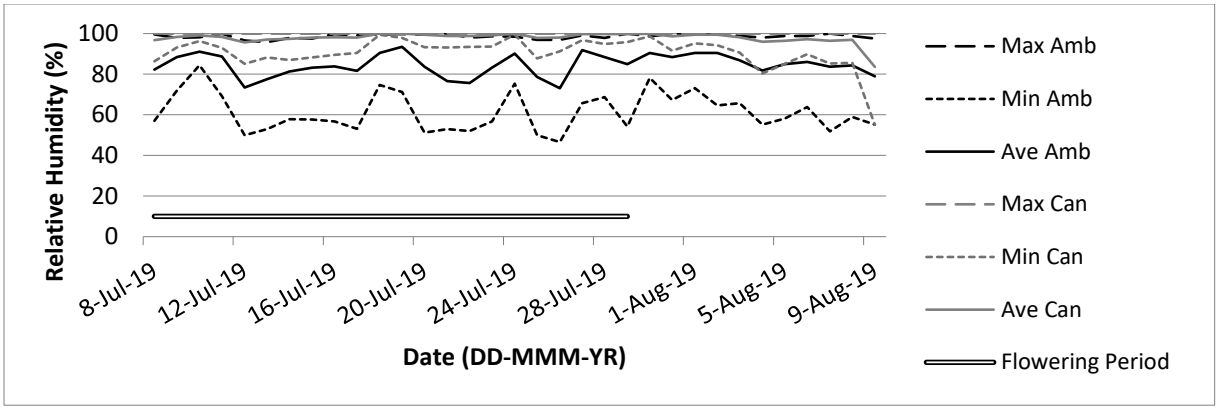

Figure A. 17 Relative humidity (RH) in field 3 (F3), near Namao, AB, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

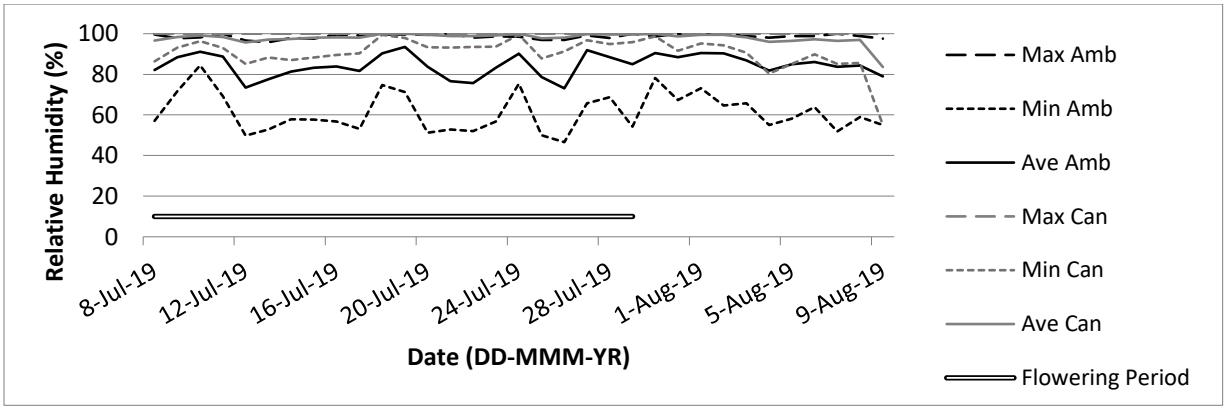

Figure A. 18 Relative humidity (RH) in field 4 (F4), near Oliver, AB, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

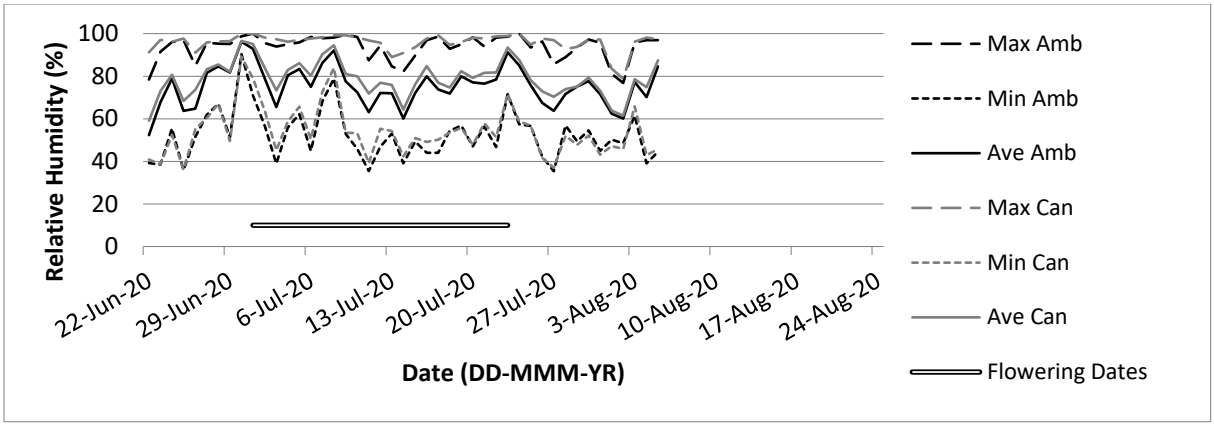

Figure A. 19 Relative humidity (RH) in field 3 (F3), near Oliver, AB, 2020. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

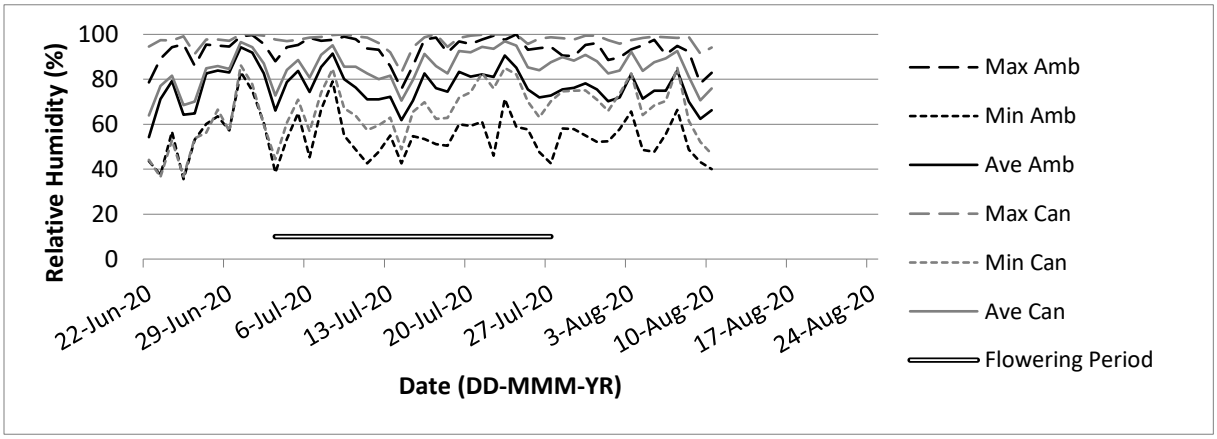

Figure A. 20 Relative humidity (RH) in field 1 (F1), near Radway, AB, 2020. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

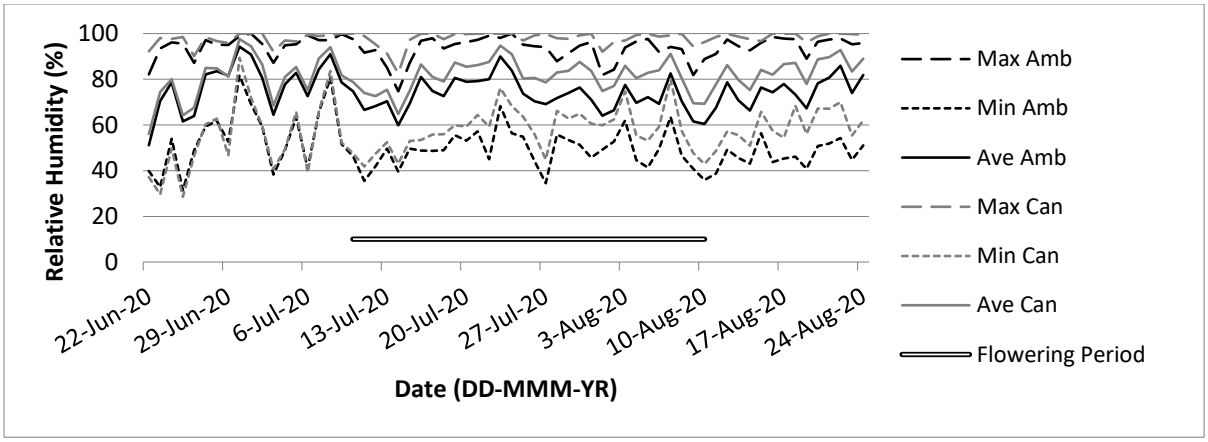

Figure A. 21 Relative humidity (RH) in field 2 (F2), near Radway, AB, 2020. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

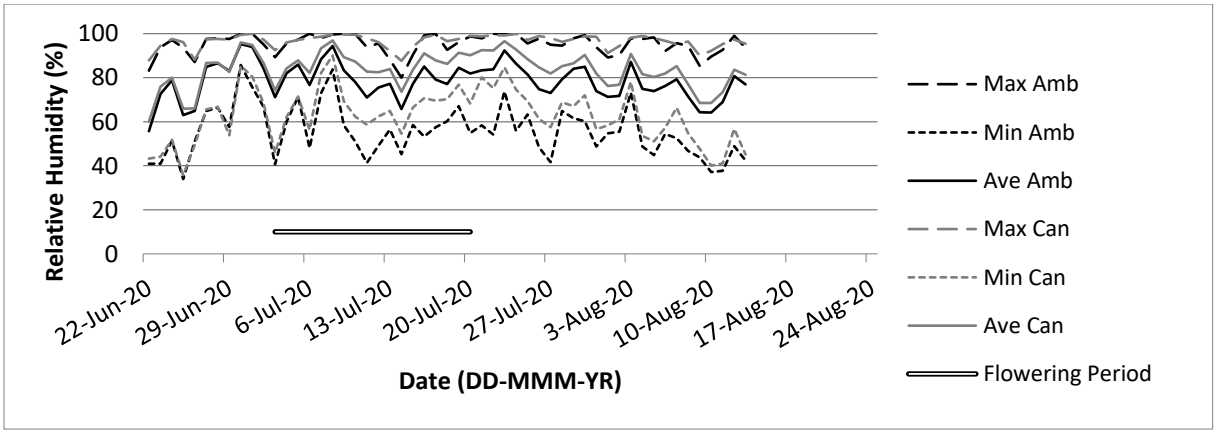

Figure A. 22 Relative humidity (RH) in field 4 (F4), near Legal, AB, 2020. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

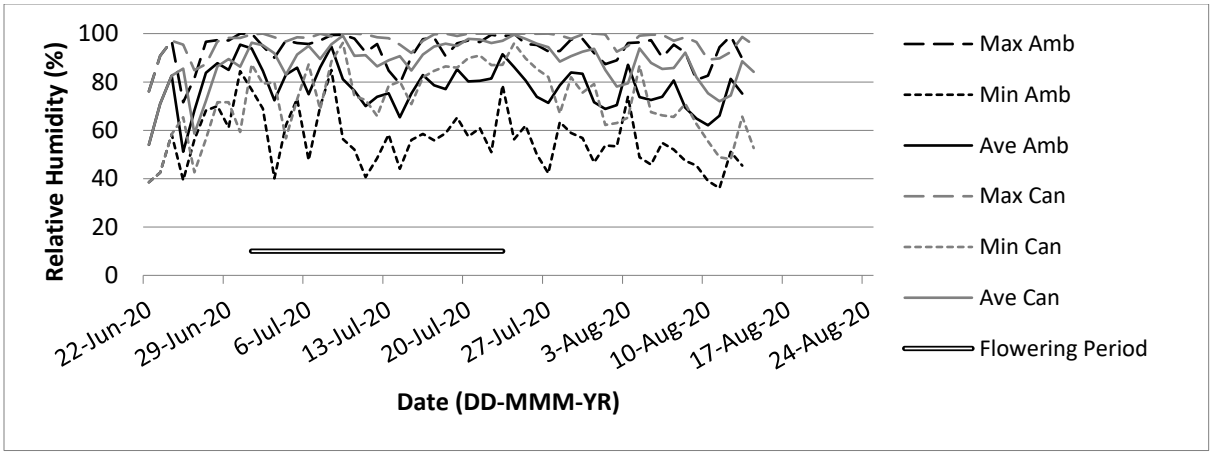

Figure A. 23 Relative humidity (RH) in field 5 (F5), near Legal, AB, 2020. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

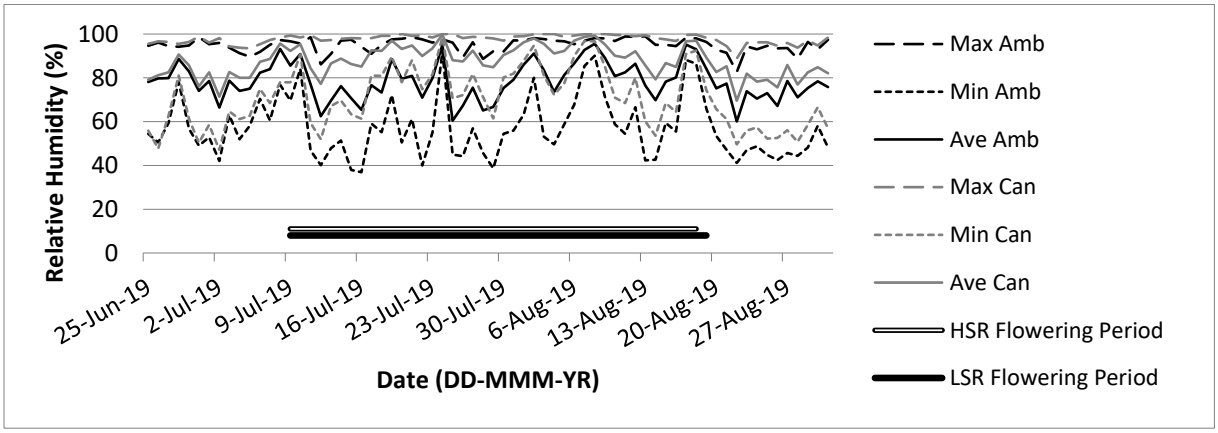

Figure A. 24 Relative humidity (RH) near Beaverlodge, AB, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

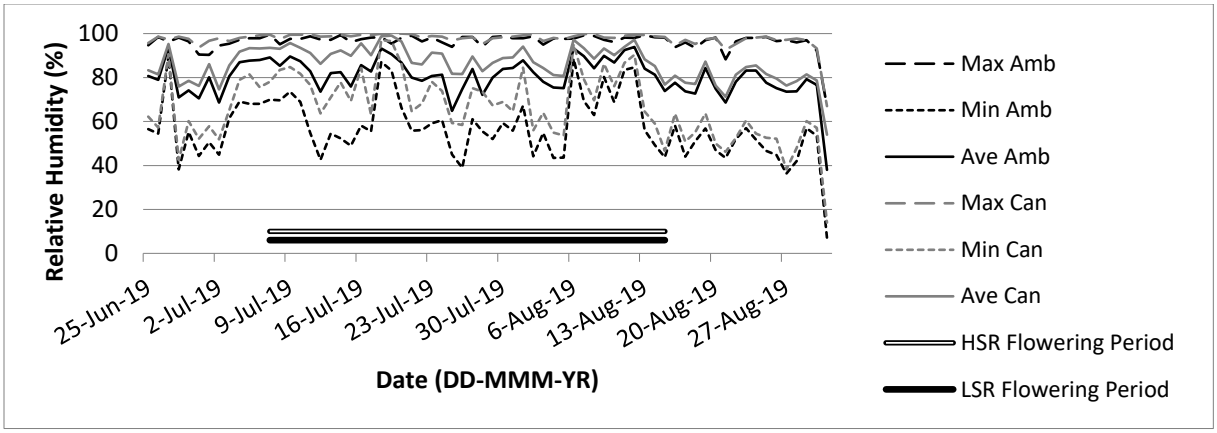

Figure A. 25 Relative humidity (RH) near Lacombe, AB, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

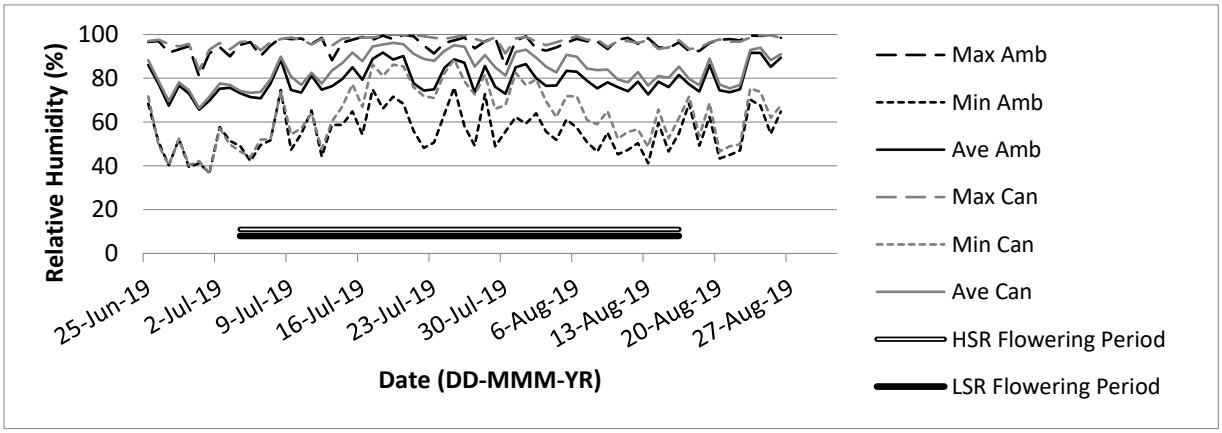

Figure A. 26 Relative humidity (RH) near Melfort, SK, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

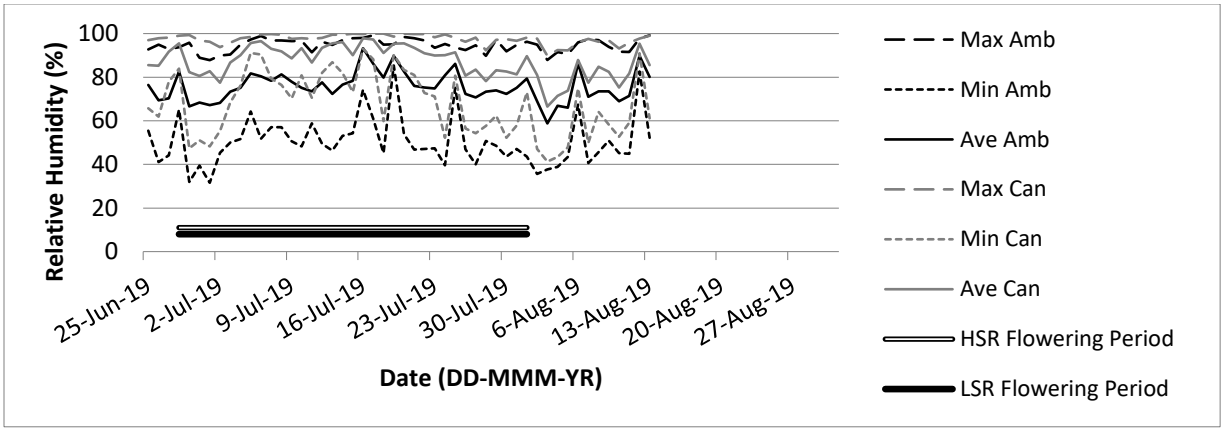

Figure A. 27 Relative humidity (RH) near Outlook, SK, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

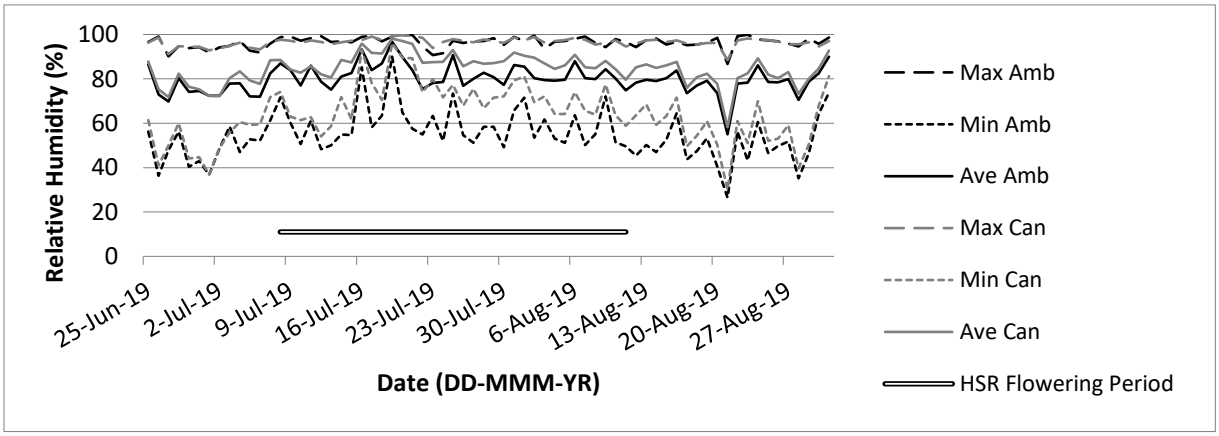

Figure A. 28 Relative humidity (RH) near Scott, AB, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate), although no flowering dates were recorded the lower seeding rate for Scott, AB.

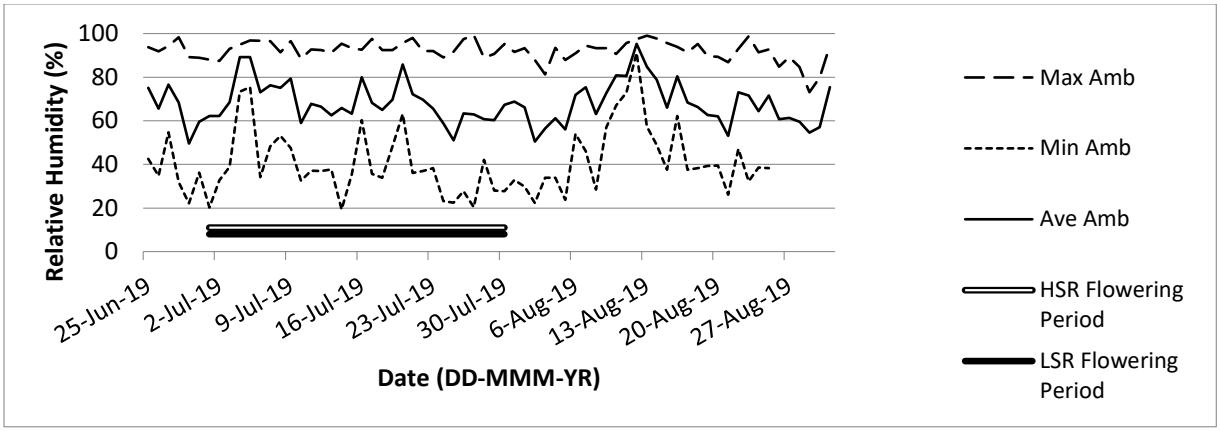

Figure A. 29 Relative humidity (RH) near Brooks, AB, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured above the crop canopy (Amb) with a HOBO U23-002 Temperature and Relative Humidity unit. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

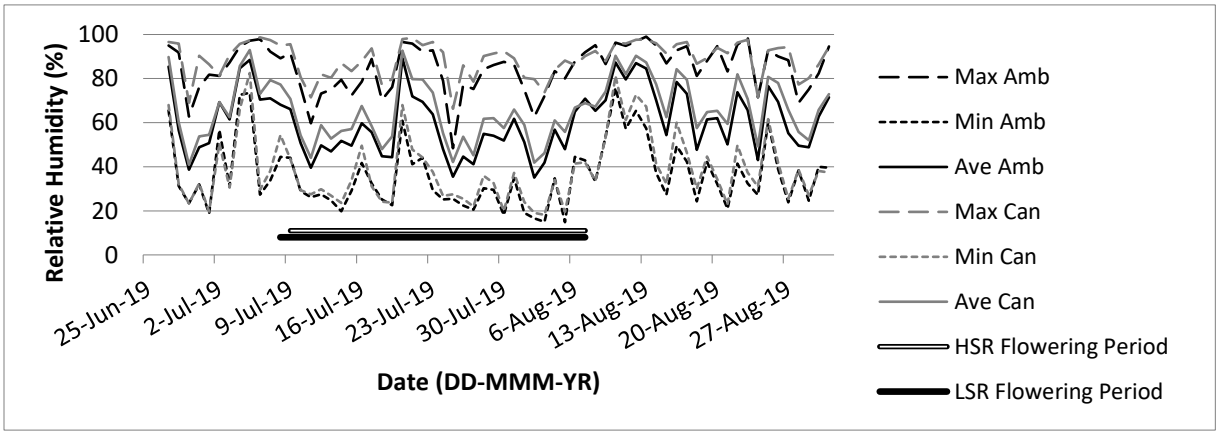

Figure A. 30 Relative humidity (RH) near Lethbridge, AB, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

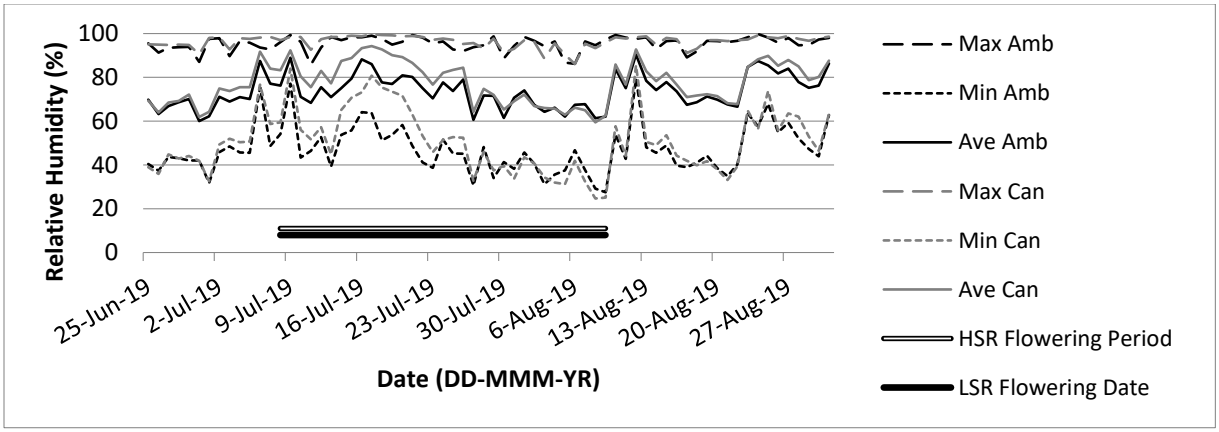

Figure A. 31 Relative humidity (RH) near Indian Head, SK, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

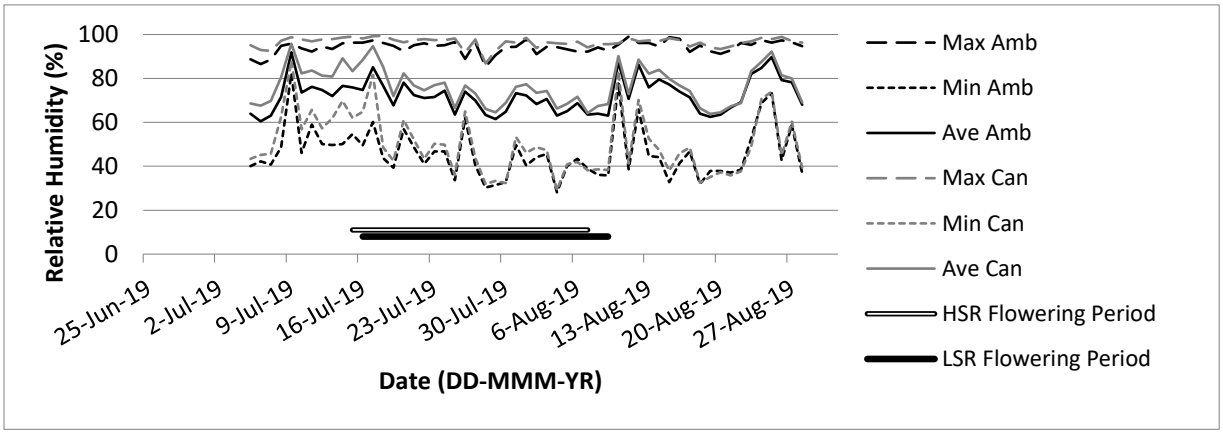

Figure A. 32 Relative humidity (RH) near Brandon, MB, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

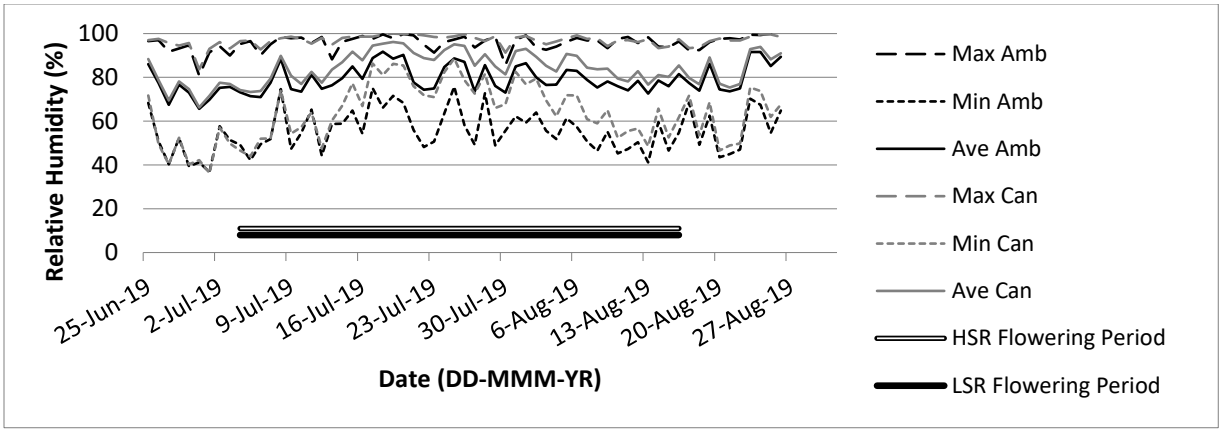

Figure A. 33 Relative humidity (RH) near Normandin, QC, 2019. The average (Ave) daily RH (%), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

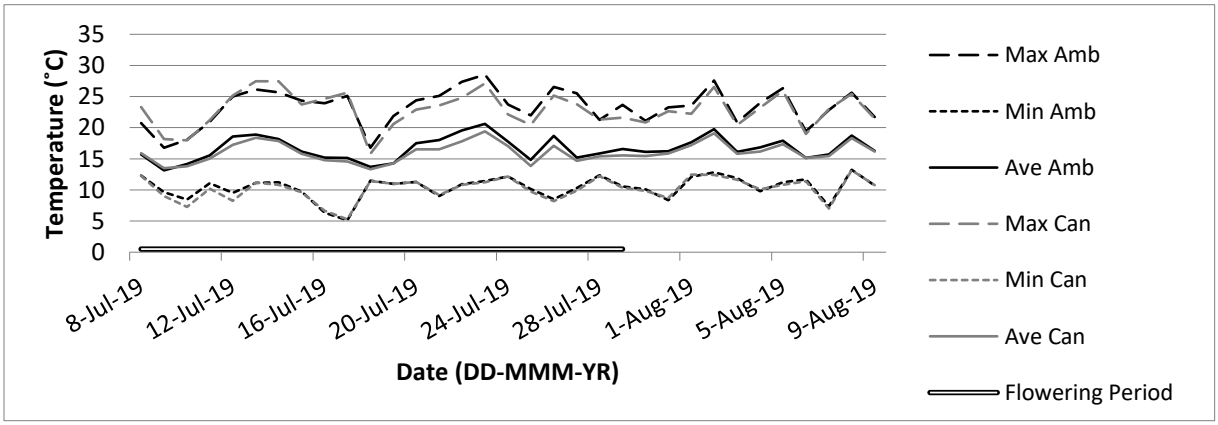

Figure A. 34 Temperature in field 1 (F1) near Oliver, AB, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

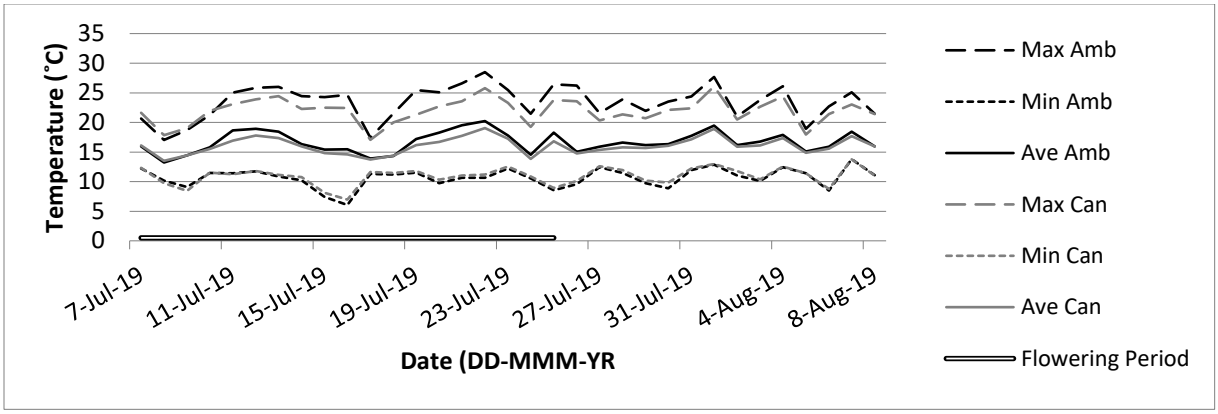

Figure A. 35 Temperature in field 4 (F4), near Oliver, AB, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

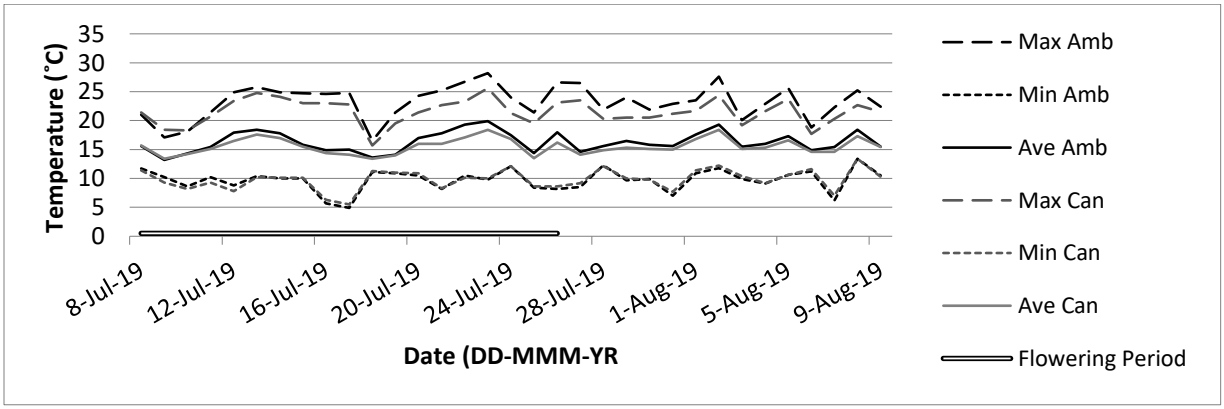

Figure A. 36 Temperature in field 2 (F2), near Namao, AB, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

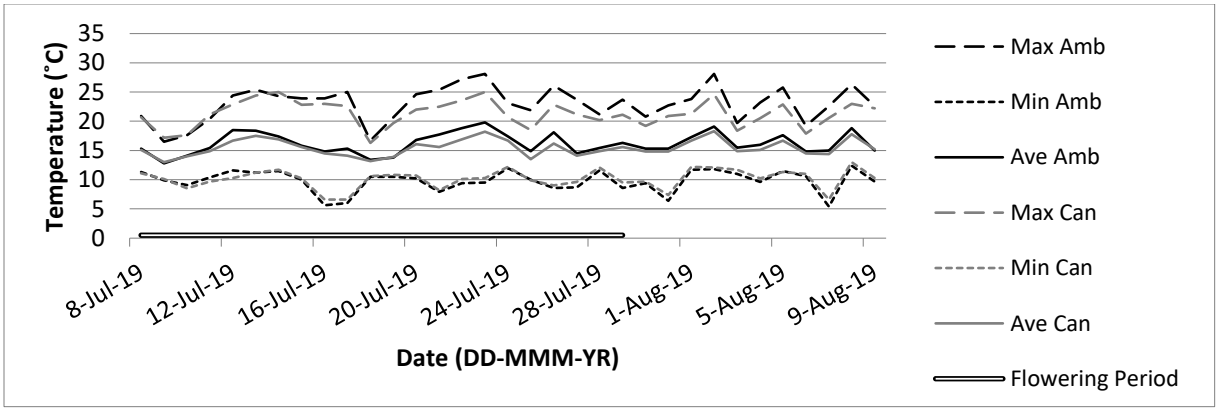

Figure A. 37 Temperature (a), relative humidity (b), rain (c), and wind (d) in field 3 (F3), near Namao, AB, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

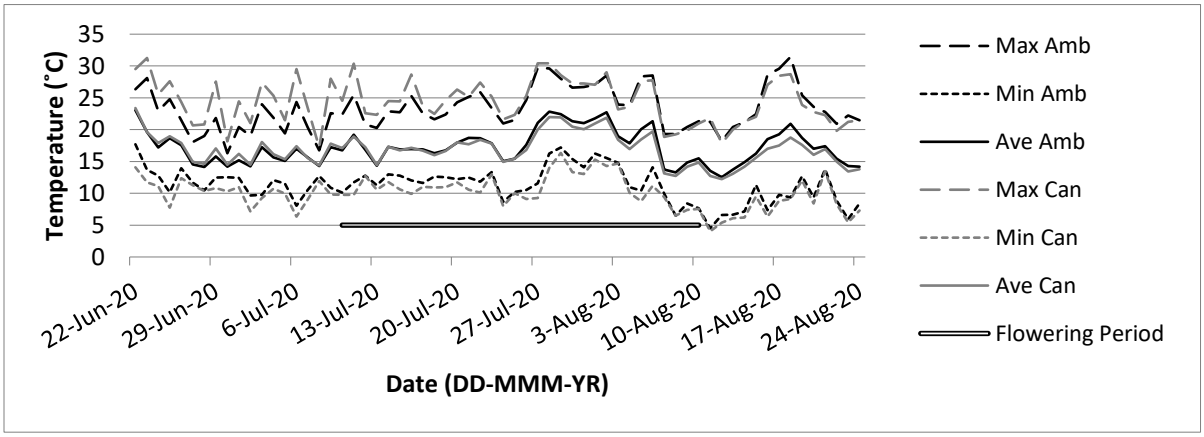

Figure A. 38 Temperature in field 2 (F2), near Radway, AB, 2020. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

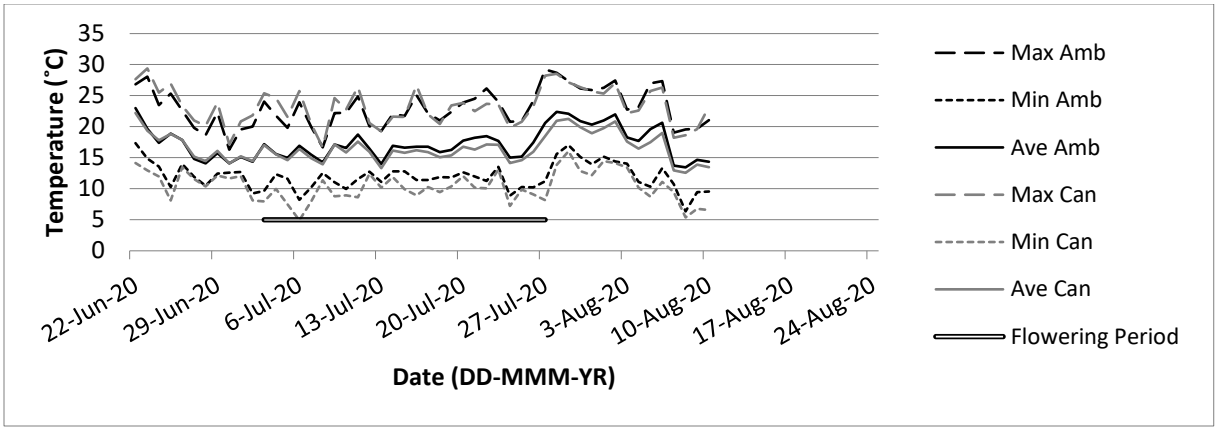

Figure A. 39 Temperature in field 1 (F1), near Radway, AB, 2020. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

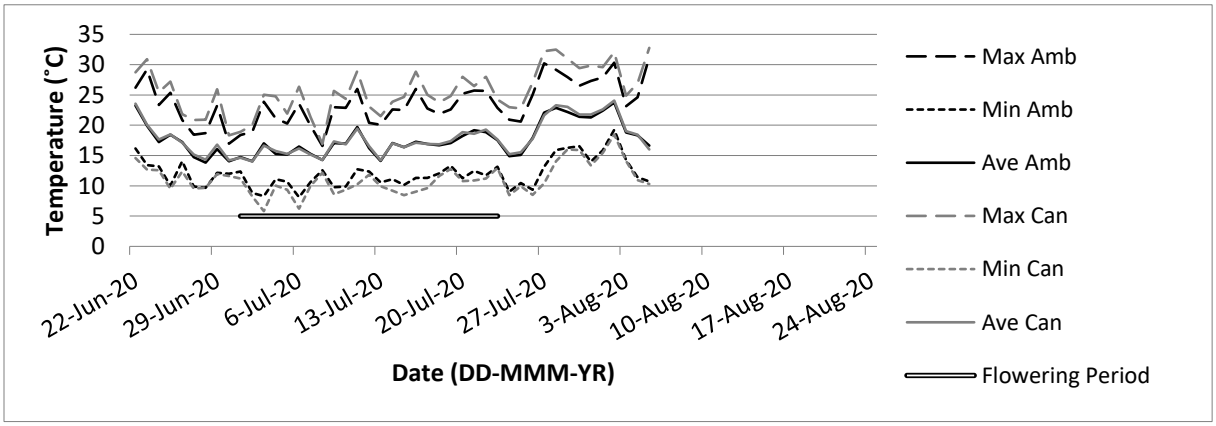

Figure A. 40 Temperature in field 3 (F3), near Oliver, AB, 2020. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

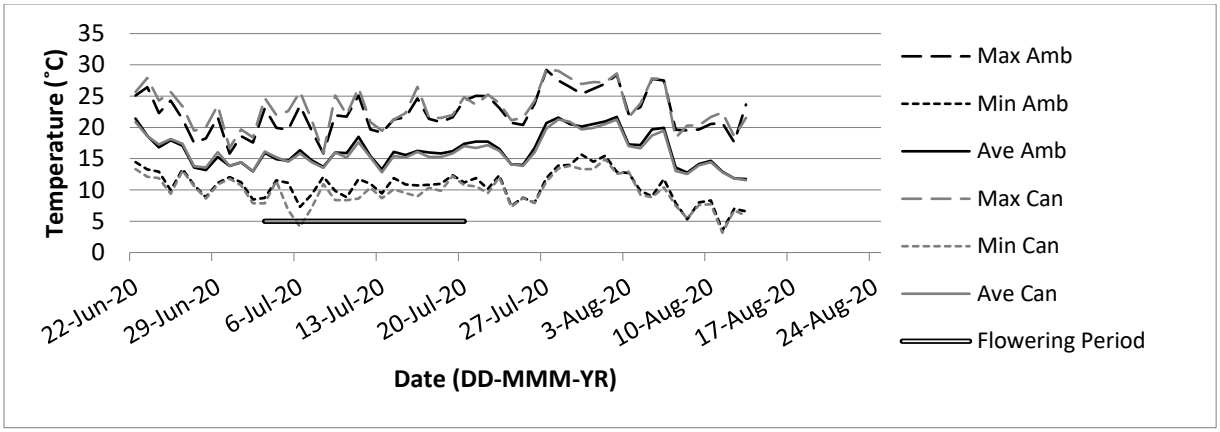

Figure A. 41 Temperature in field 4 (F4), near Legal, AB, 2020. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

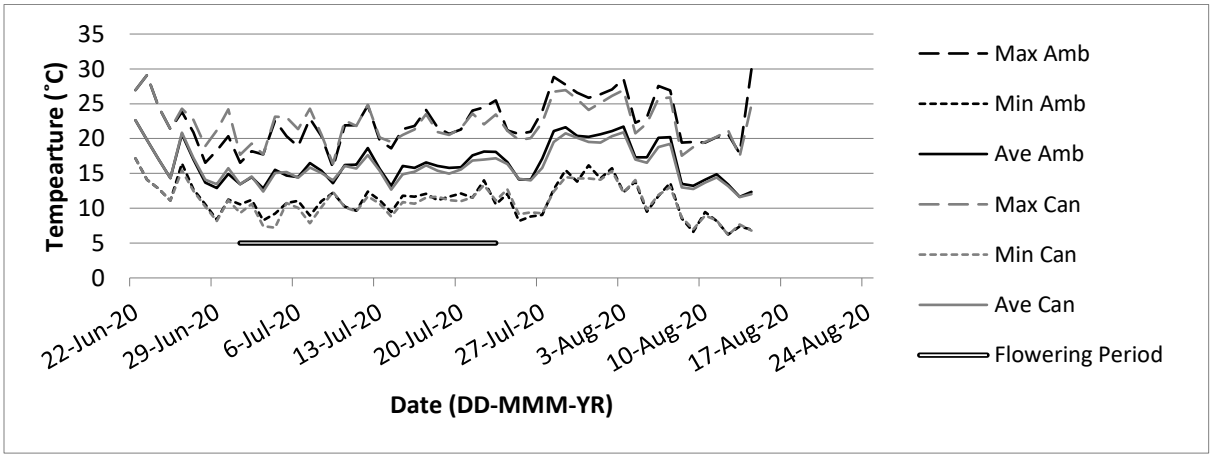

Figure A. 42 Temperature in field 5 (F5), near Legal, AB, 2020. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units.

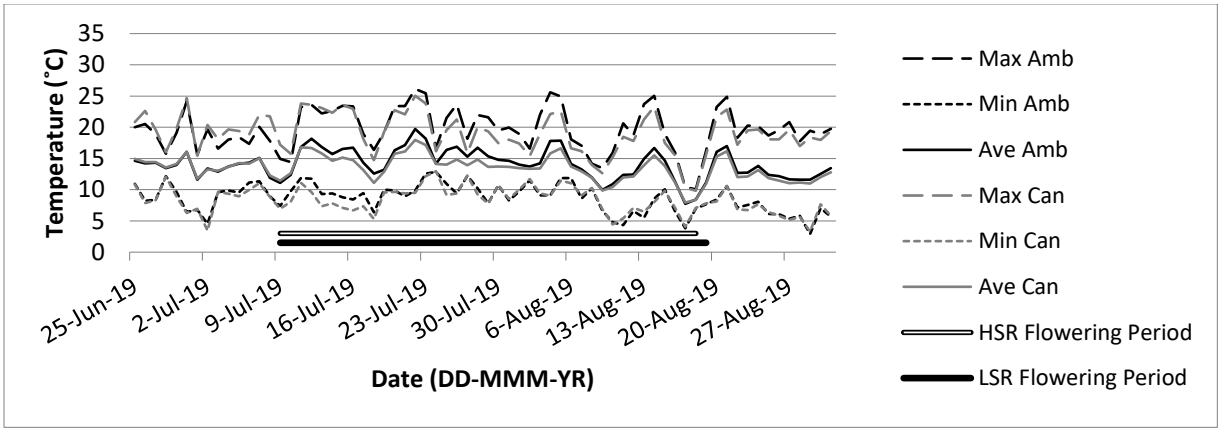

Figure A. 43 Temperature near Beaverlodge, AB, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

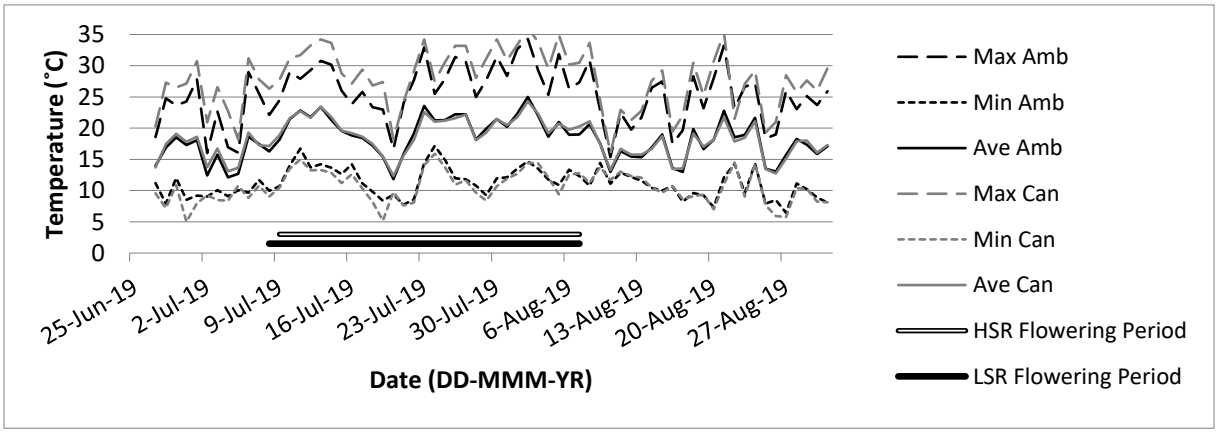

Figure A. 44 Temperature near Lethbridge, AB, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

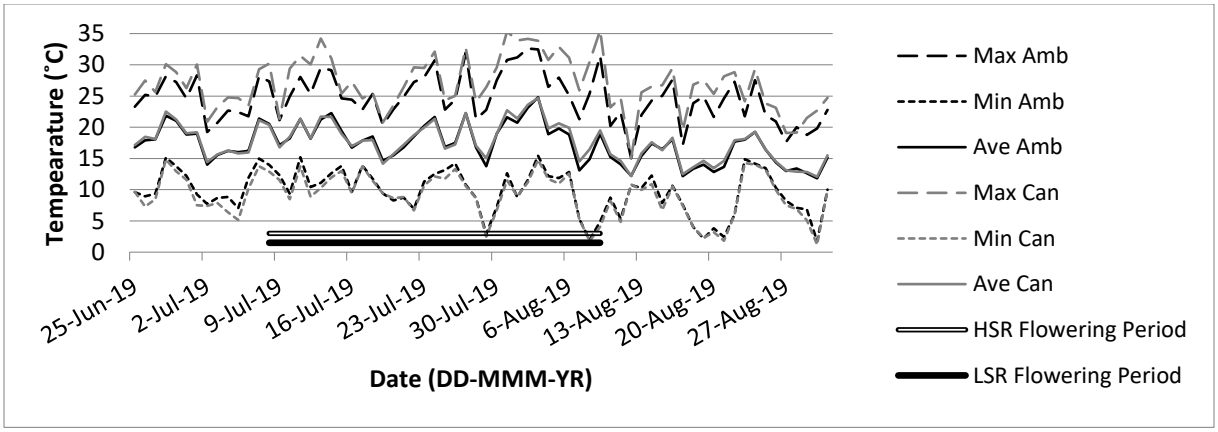

Figure A. 45 Temperature near Indian Head, SK, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

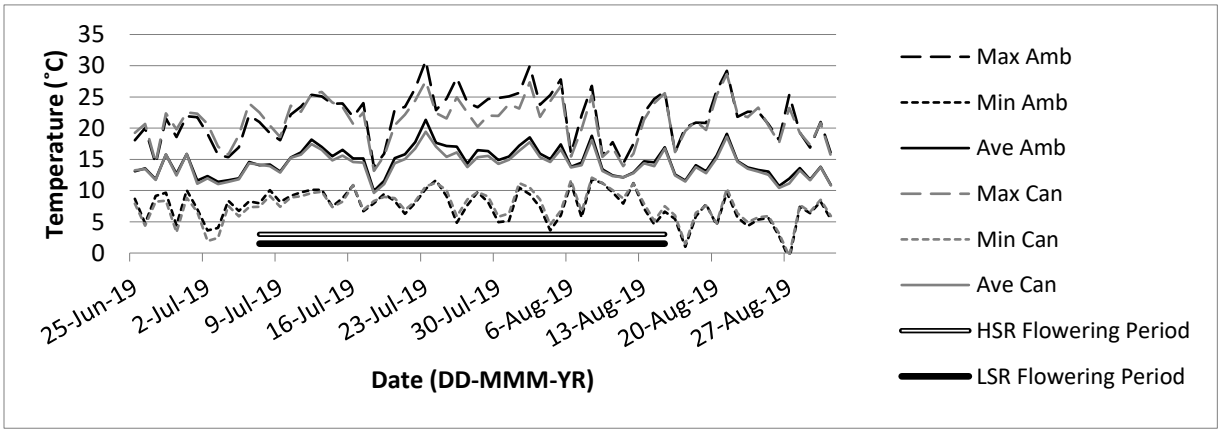

Figure A. 46 Temperature near Lacombe, AB, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

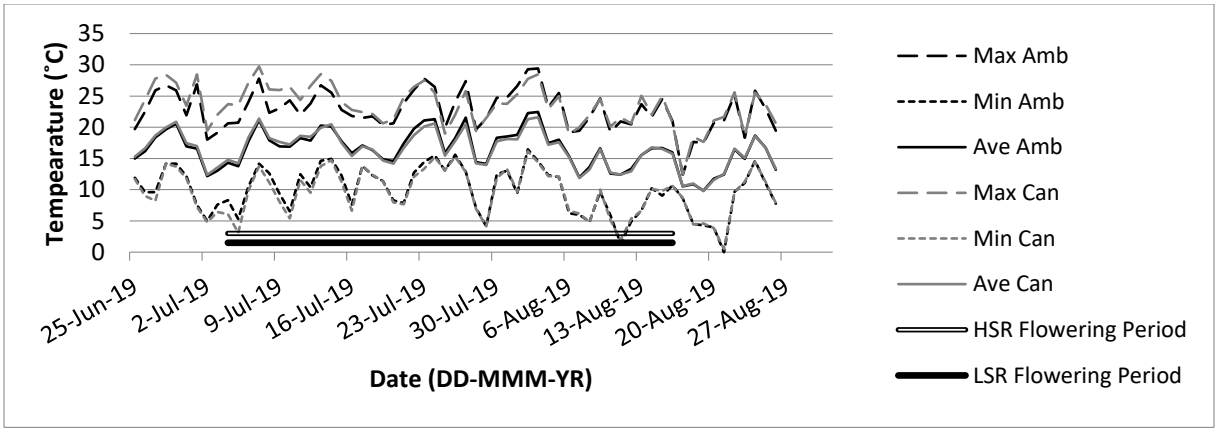

Figure A. 47 Temperature near Melfort, SK, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

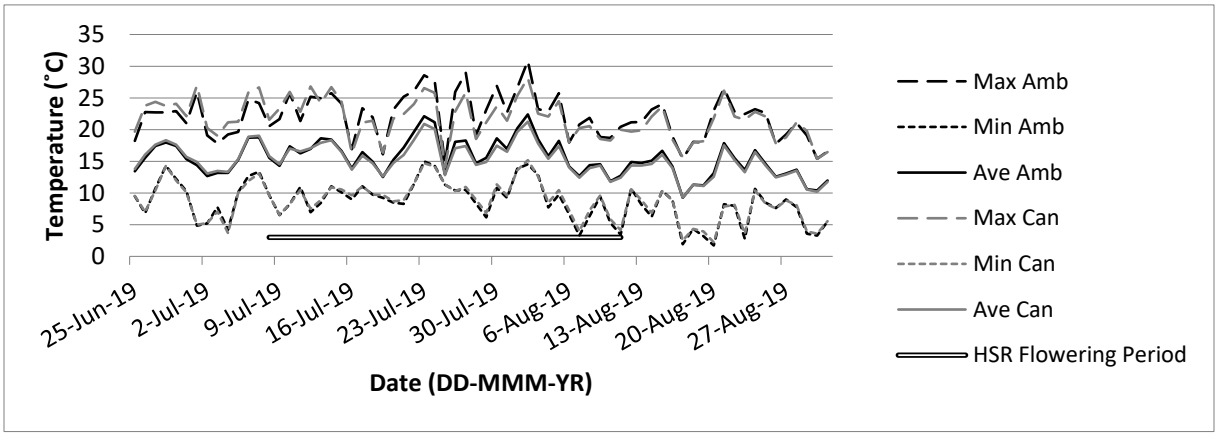

Figure A. 48 Temperature near Scott, AB, 2019. The average (Ave) daily temperature (˚C), maximum (Max), minimum (Min) and daily relative humidity (%) The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate), although no flowering dates were recorded the lower seeding rate for Scott, AB.

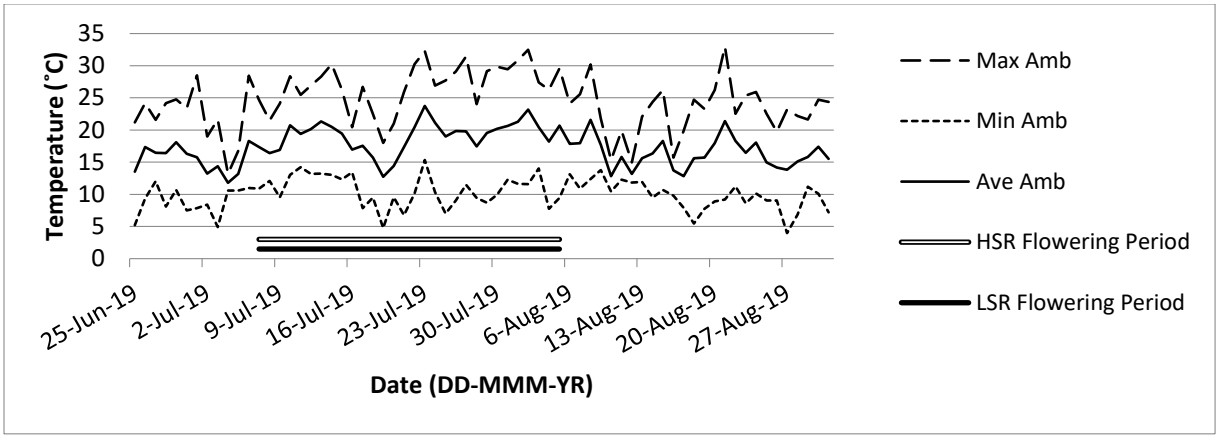

Figure A. 49 Temperature near Brooks, AB, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured above the crop canopy (Amb) with a HOBO U23-002 Temperature and Relative Humidity unit. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

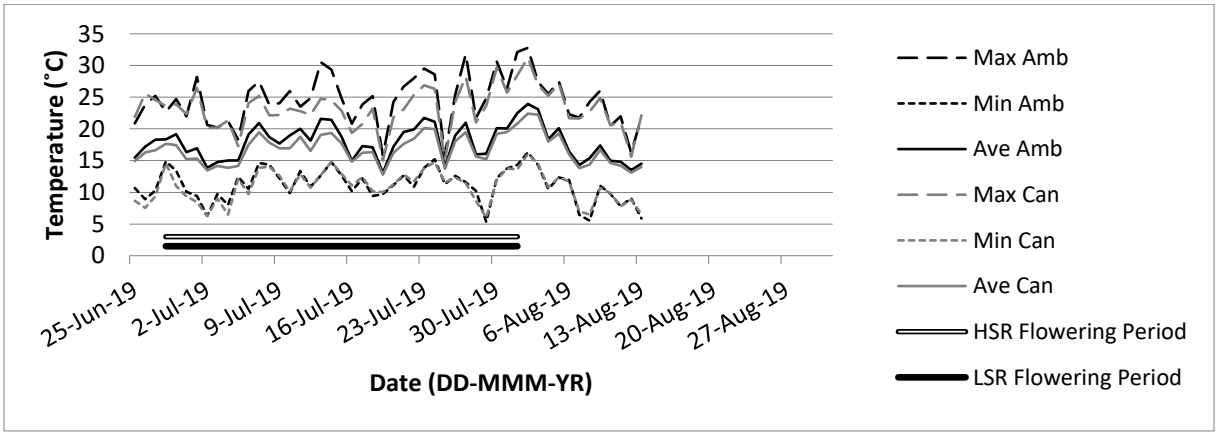

Figure A. 50 Temperature near Outlook, SK, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

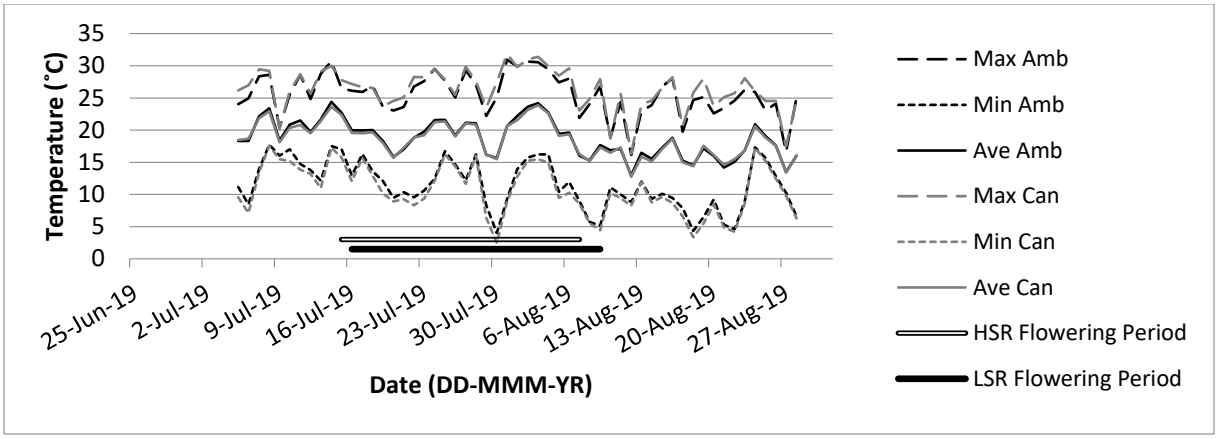

Figure A. 51 Temperature near Brandon, MB, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

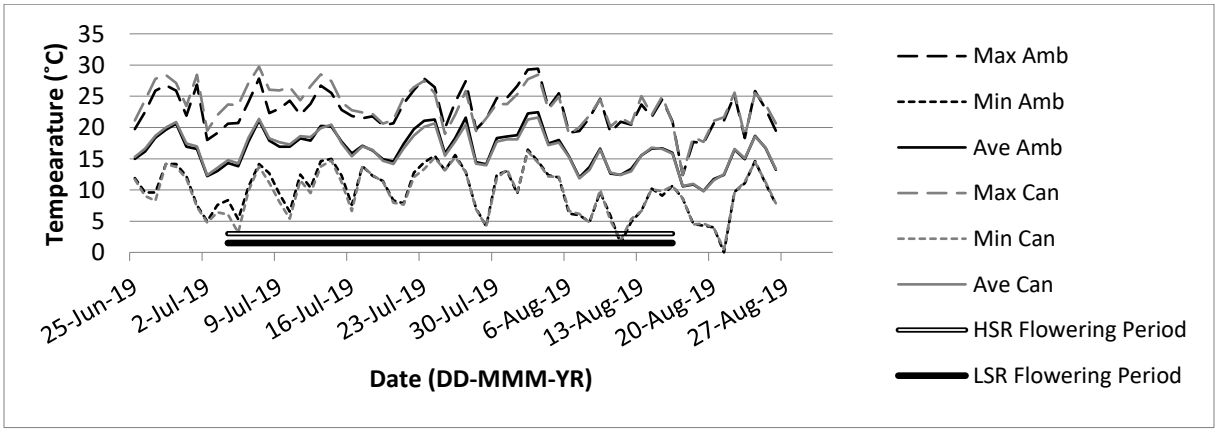

Figure A. 52 Temperature near Normandin, QC, 2019. The average (Ave) daily temperature (˚C), maximum (Max) and minimum (Min) values were measured under the canopy (Can) and above the crop canopy (Amb) with two HOBO U23-002 Temperature and Relative Humidity units. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate) and LSR (Low seeding rate).

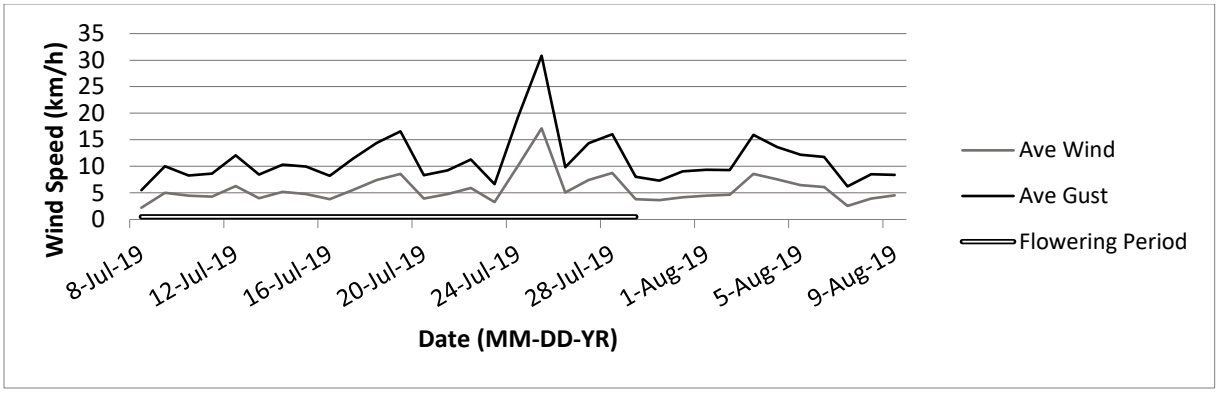

Figure A. 53 Wind speed in field 1 (F1) near Oliver, AB, 2019. Wind (km/h) was measured with a HOBO Davis Wind Speed and Direction Smart Sensor (S-WCF-M003).

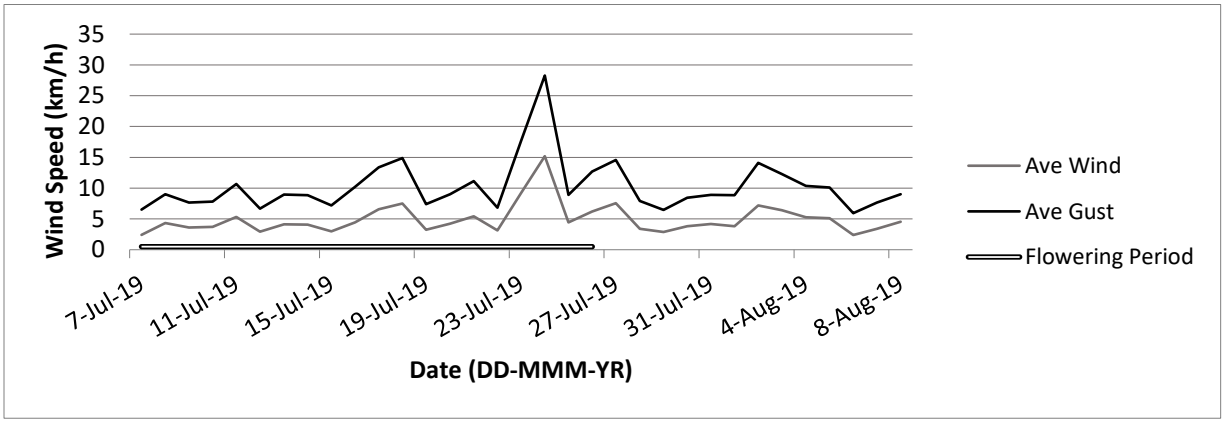

Figure A. 54 Wind speed in field 2 (F2), near Namao, AB, 2019. Wind (km/h) was measured with a HOBO Davis Wind Speed and Direction Smart Sensor (S-WCF-M003).

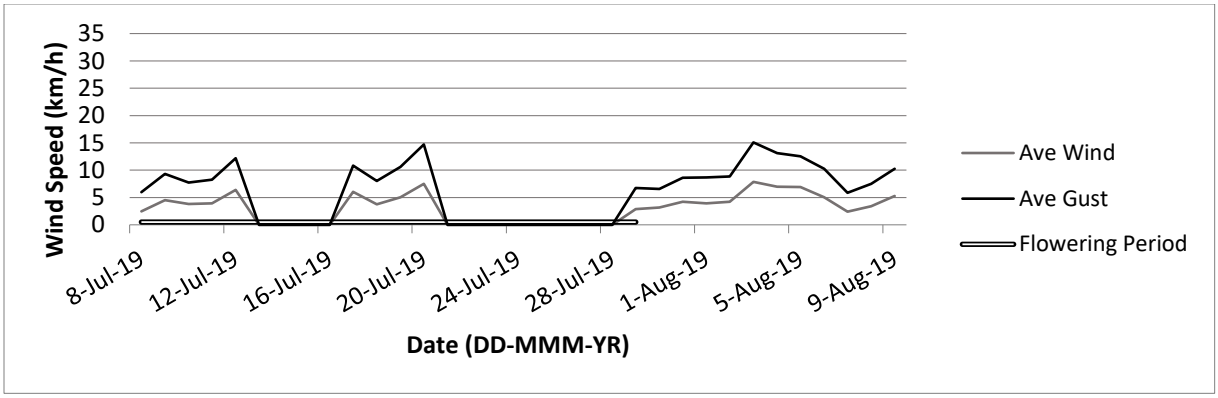

Figure A. 55 Wind speed in field 3 (F3), near Namao, AB, 2019. Wind was (km/h) measured with a HOBO Davis Wind Speed and Direction Smart Sensor (S-WCF-M003).

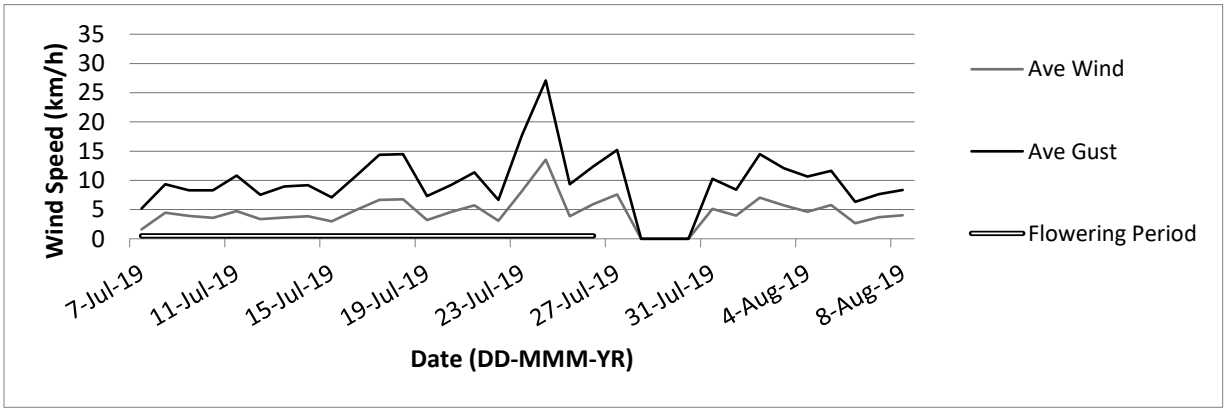

Figure A. 56 Wind speed in field 4 (F4), near Oliver, AB, 2019. Wind (km/h) was measured with a HOBO Davis Wind Speed and Direction Smart Sensor (S-WCF-M003).

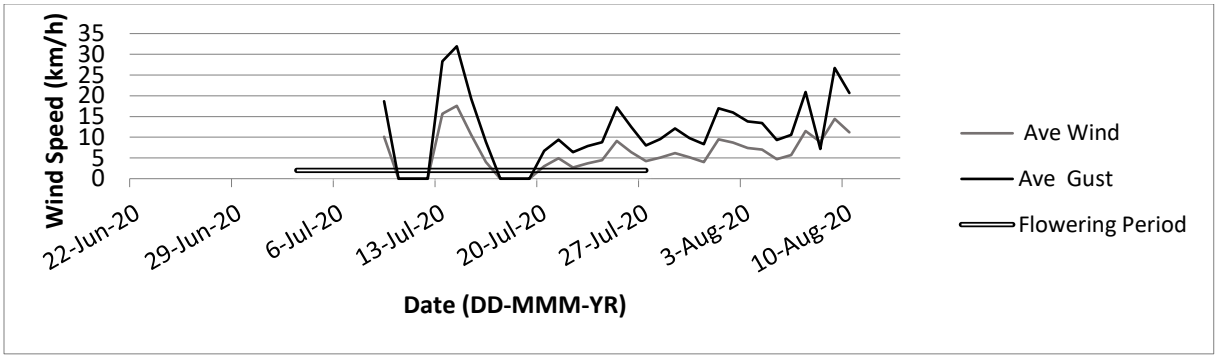

Figure A. 57 Wind speed in field 1 (F1), near Radway, AB, 2020. Wind (km/h) was measured with a HOBO Davis Wind Speed and Direction Smart Sensor (S-WCF-M003.

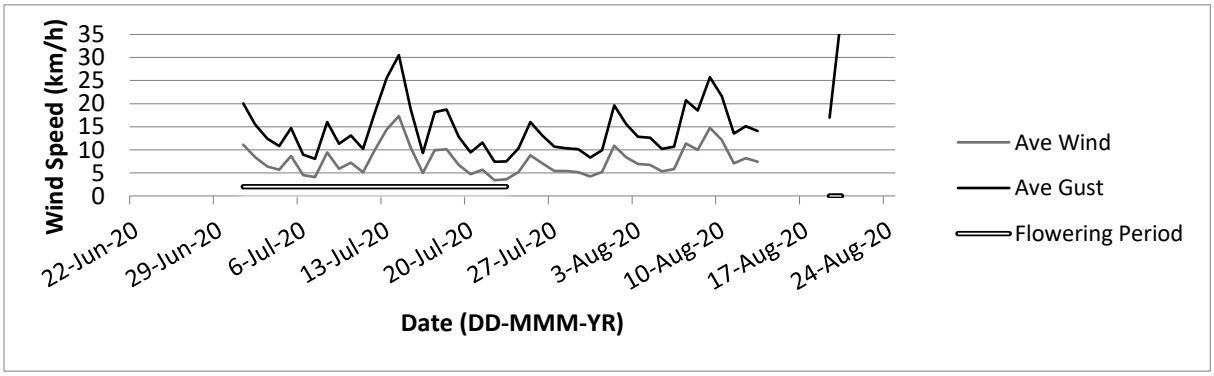

Figure A. 58 Wind speed in field 5 (F5), near Legal, AB, 2020. Wind (km/h) was measured with a HOBO Davis Wind Speed and Direction Smart Sensor (S-WCF-M003).

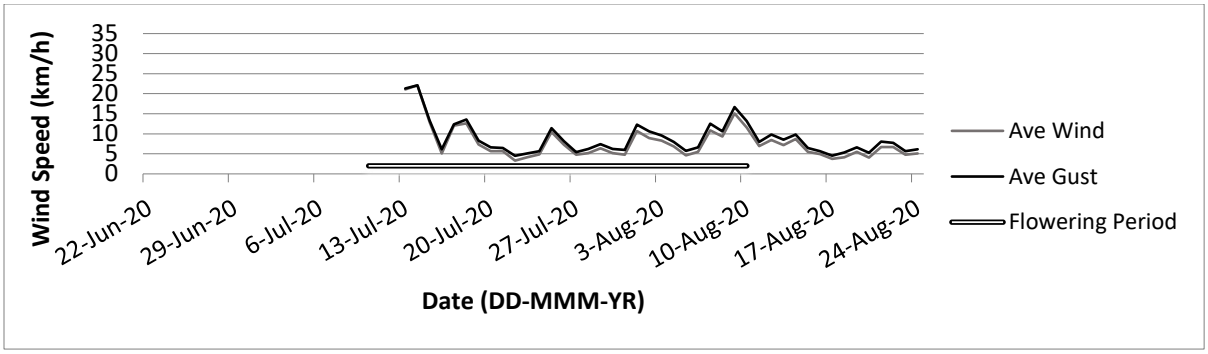

Figure A. 59 Wind speed in field 2 (F2), near Radway, AB, 2020. Wind (km/h) was measured with a HOBO Davis Wind Speed and Direction Smart Sensor (S-WCF-M003).

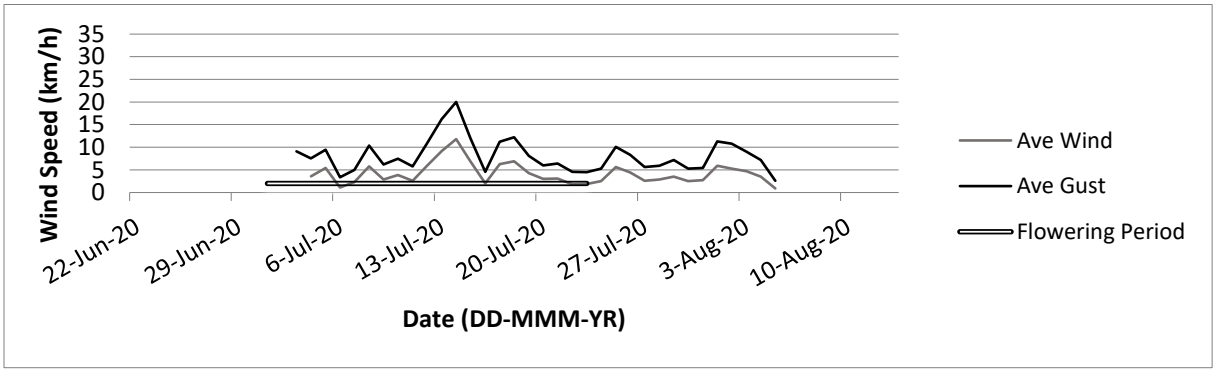

Figure A. 60 Wind speed in field 3 (F3), near Oliver, AB, 2020. Wind (km/h) was measured with a HOBO Davis Wind Speed and Direction Smart Sensor (S-WCF-M003).

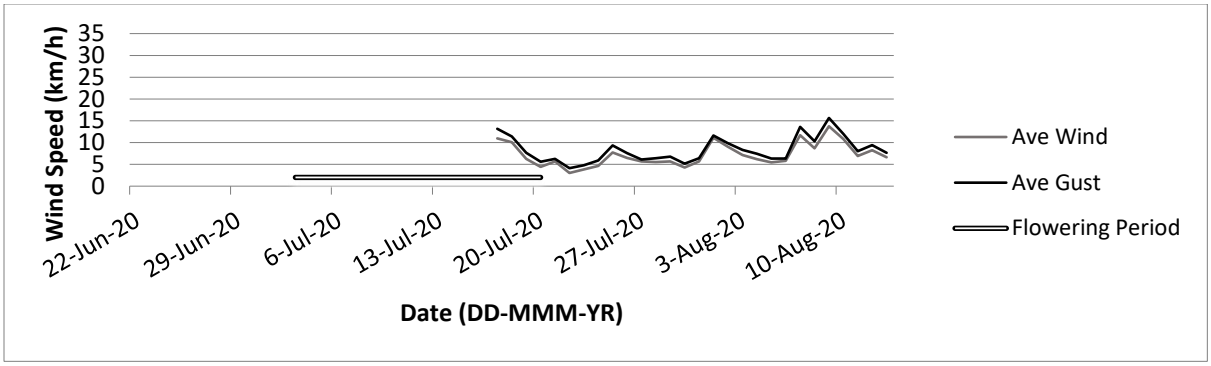

Figure A. 61 Wind speed in field 4 (F4), near Legal, AB, 2020. Wind (km/h) was measured with a HOBO Davis Wind Speed and Direction Smart Sensor (S-WCF-M003).

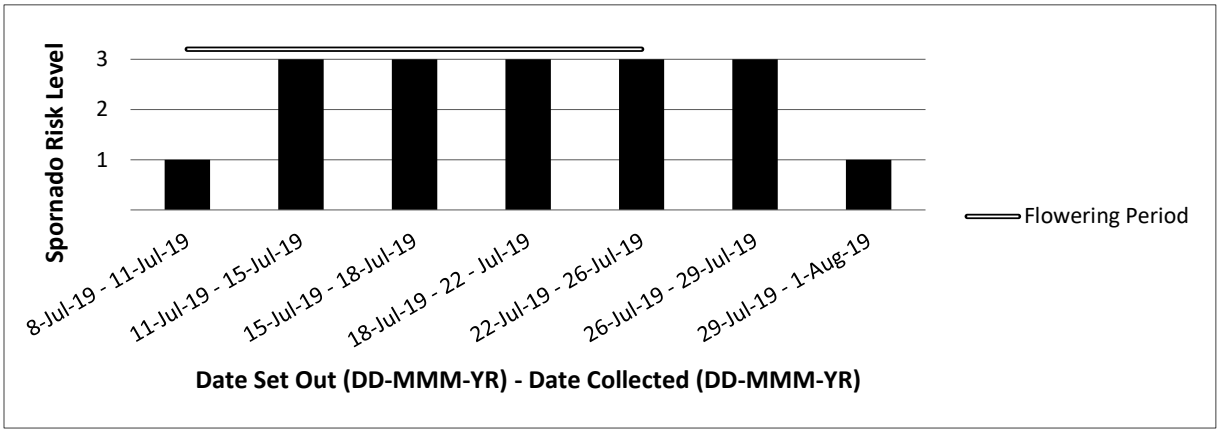

Figure A. 62 Spornado ratings for Field 2 (F2), near Namao, AB, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. Spornado cassettes were changed twice per week for about five weeks in 2019 and 10 weeks during 2020.

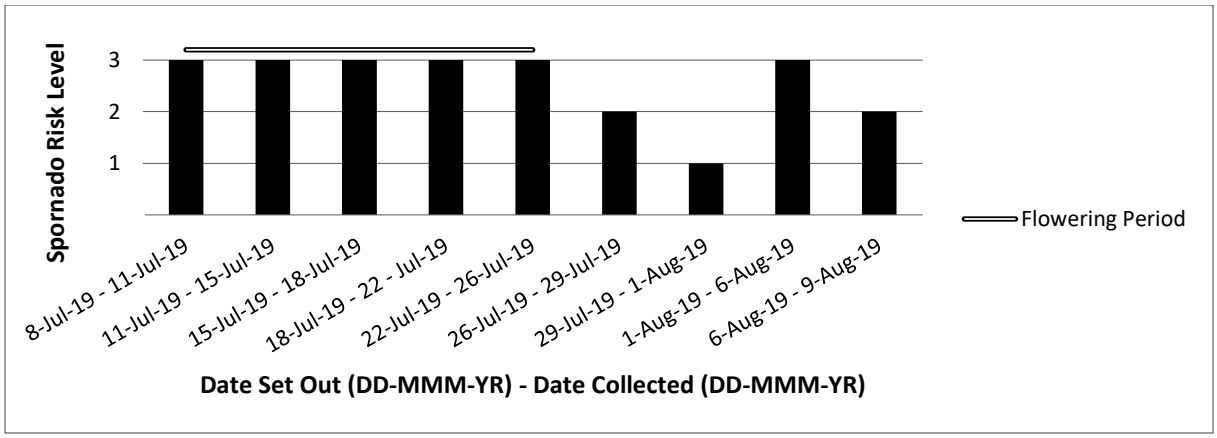

Figure A. 63 Spornado ratings for field 4 (F4), near Oliver, AB, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. Spornado cassettes were changed twice per week for about 5 weeks in 2019 and 10 weeks during 2020.

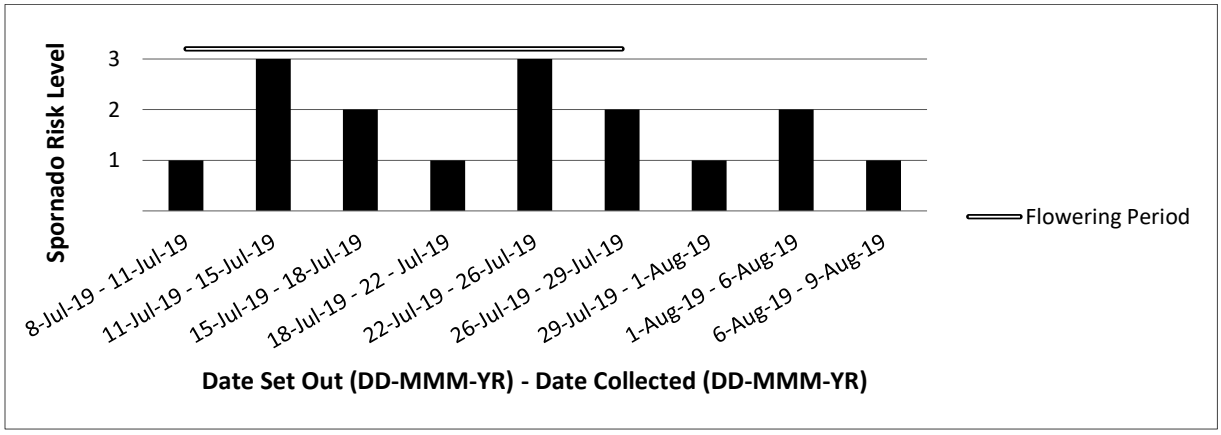

Figure A. 64 Spornado ratings for Field 1 (F1), near Oliver, AB, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA), and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. Spornado cassettes were changed twice per week for about five weeks in 2019 and 10 weeks during 2020.

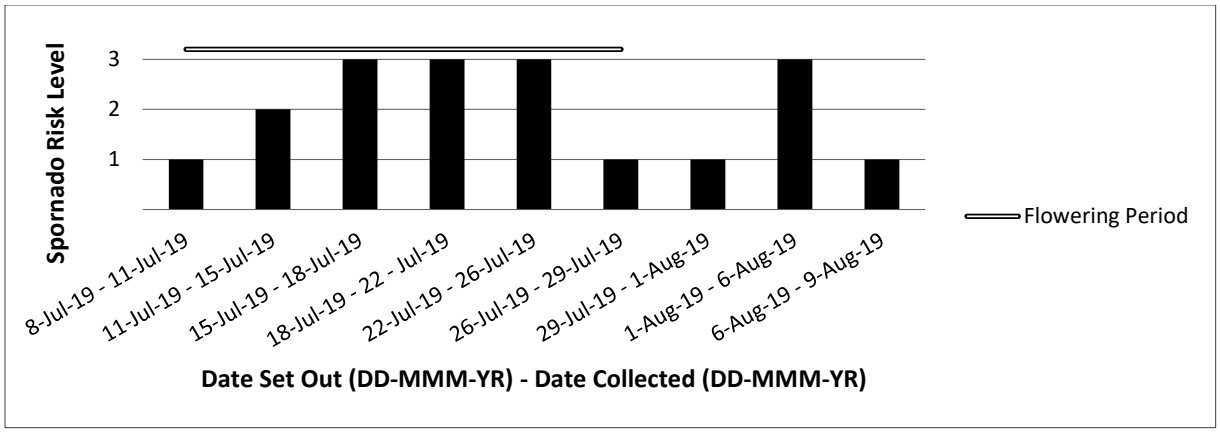

Figure A. 65 Spornado ratings for field 3 (F3), near Namao, AB, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. Spornado cassettes were changed twice per week for about five weeks in 2019 and 10 weeks during 2020.

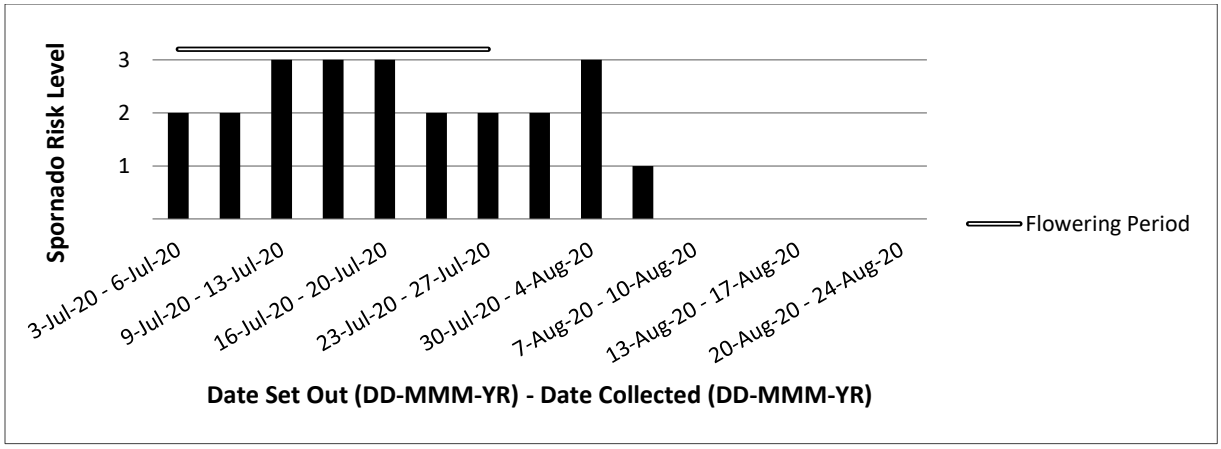

Figure A. 66 Spornado ratings for field 1 (F1), near Radway, AB, 2020. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. Spornado cassettes were changed twice per week for about 5 weeks in 2019 and 10 weeks during 2020.

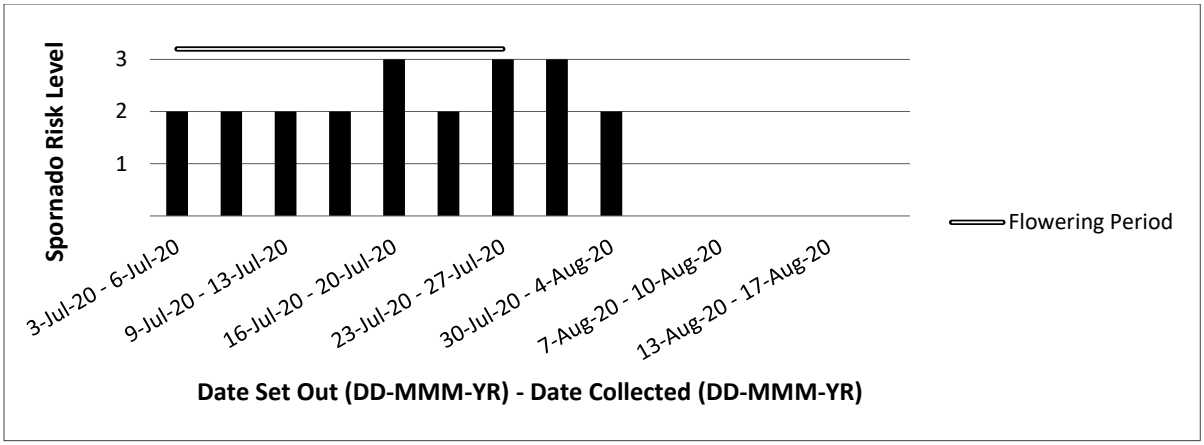

Figure A. 67 Spornado ratings for field 3 (F3), near Oliver, AB, 2020. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. Spornado cassettes were changed twice per week for about 5 weeks in 2019 and 10 weeks during 2020.

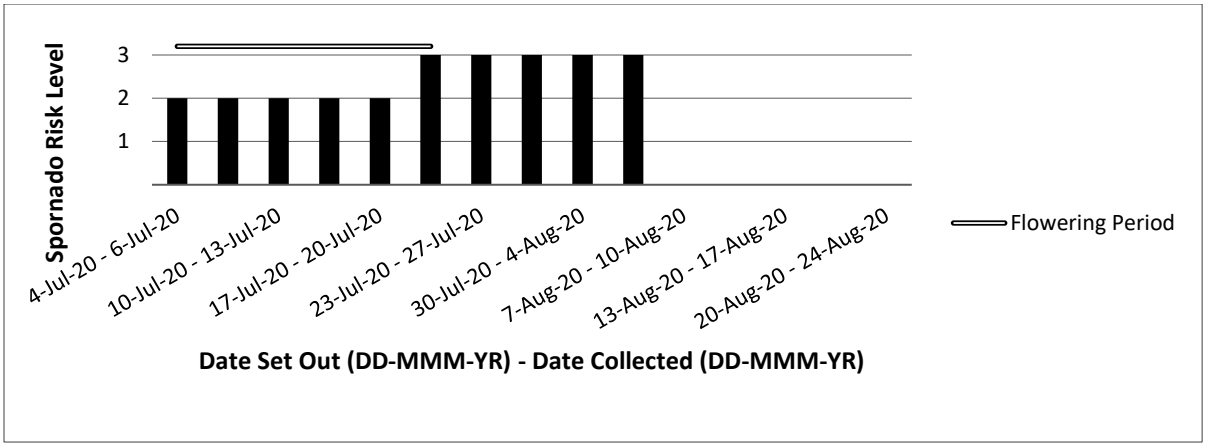

Figure A. 68 Spornado ratings for field 5 (F5), near Legal, AB, 2020. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. Spornado cassettes were changed twice per week for about 5 weeks in 2019 and 10 weeks during 2020.

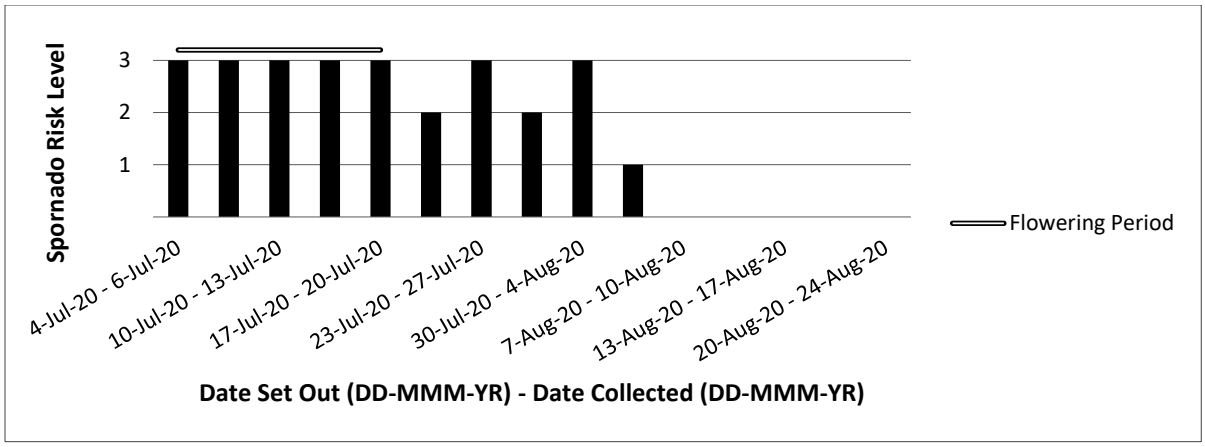

Figure A. 69 Spornado ratings for field 4 (F4), near Legal, AB, 2020. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. Spornado cassettes were changed twice per week for about 5 weeks in 2019 and 10 weeks during 2020.

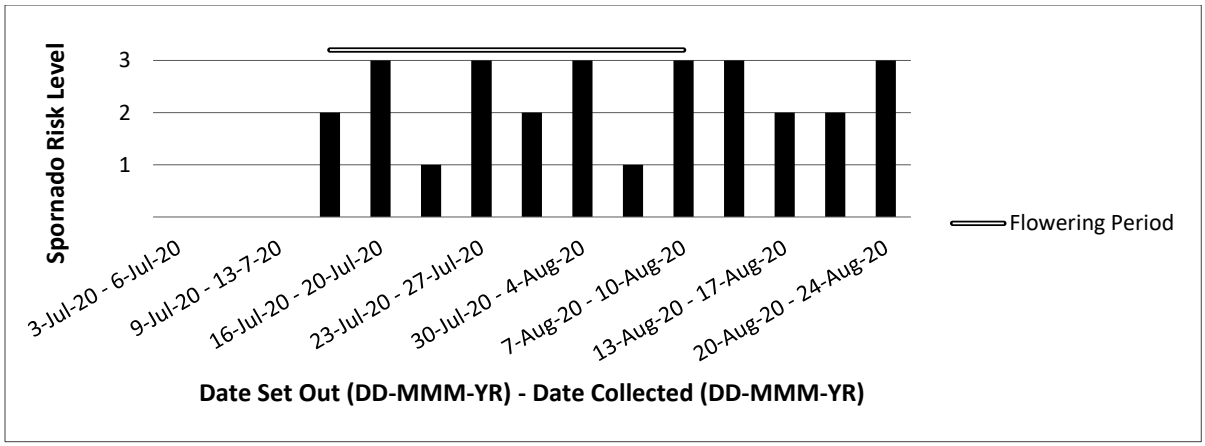

Figure A. 70 Spornado ratings for field 2 (F2), near Radway, AB, 2020. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. Spornado cassettes were changed twice per week for about 5 weeks in 2019 and 10 weeks during 2020.

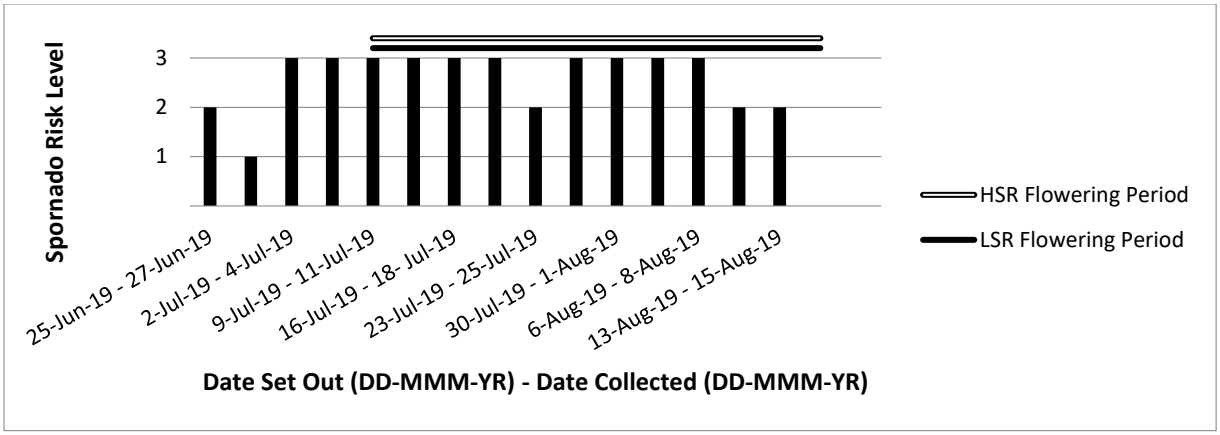

Figure A. 71 Spornado ratings for Beaverlodge, AB, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

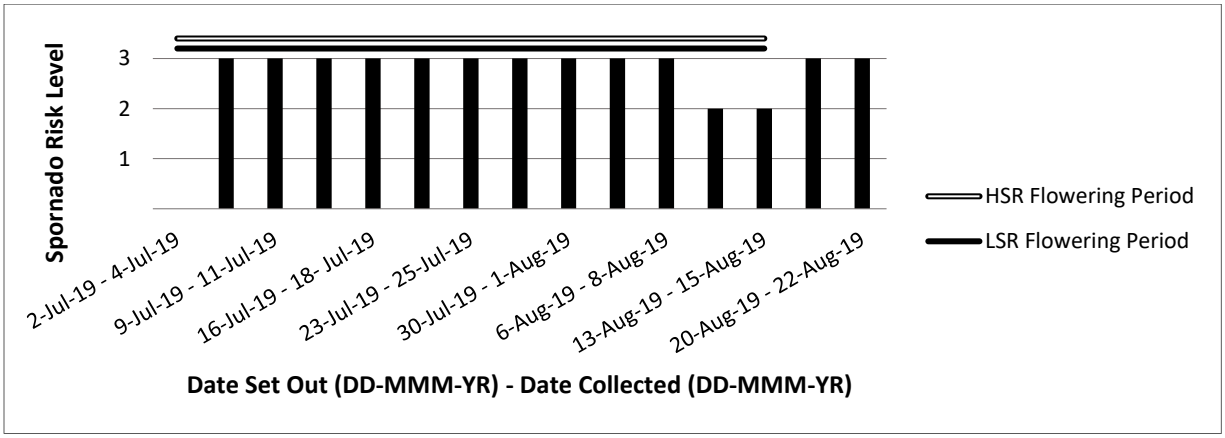

Figure A. 72 Spornado ratings for Melfort, SK, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

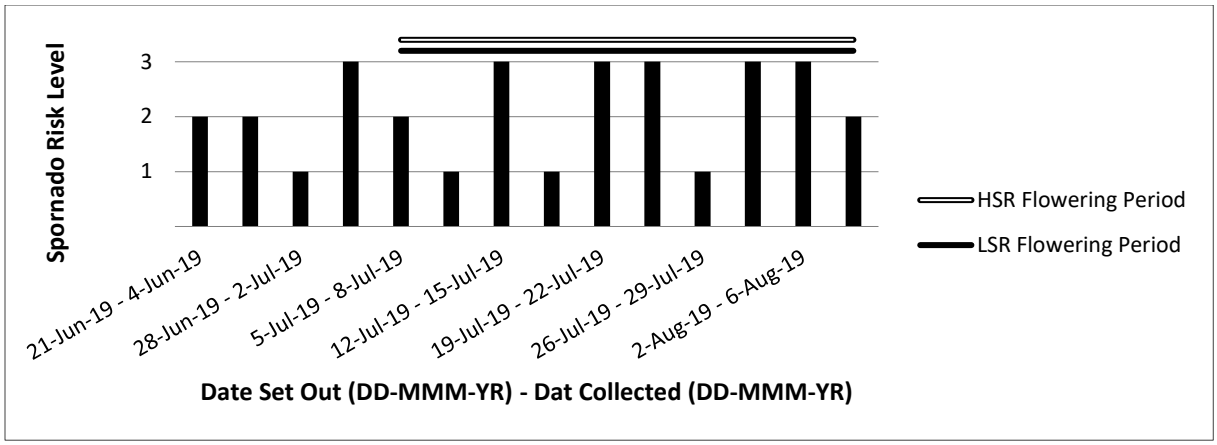

Figure A. 73 Spornado ratings for Brooks, AB, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

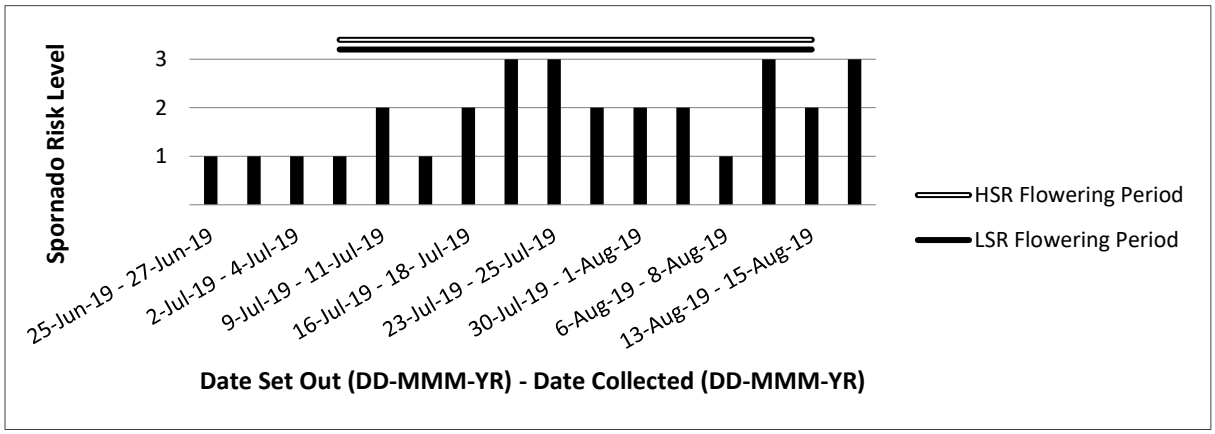

Figure A. 74 Spornado ratings for Lacombe, AB, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

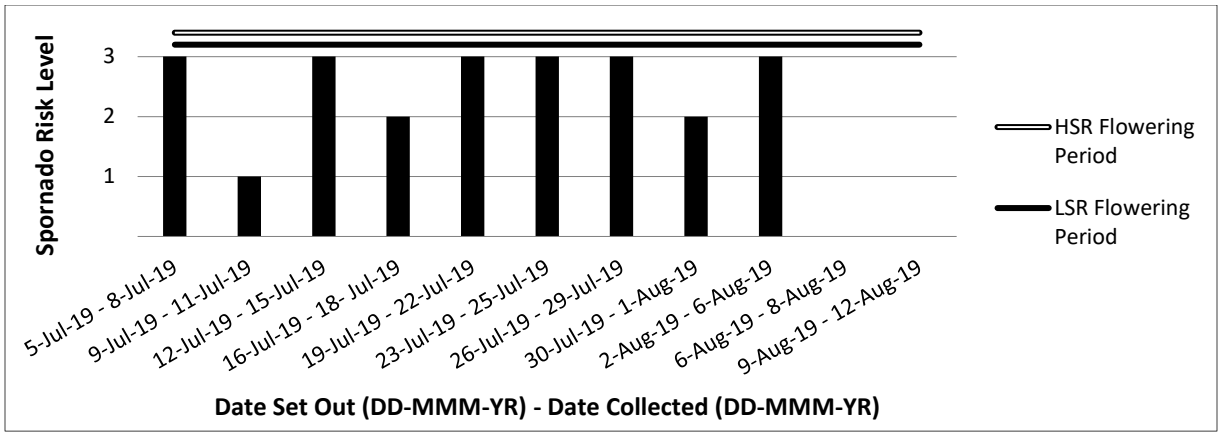

Figure A. 75 Spornado ratings for Indian Head, SK, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

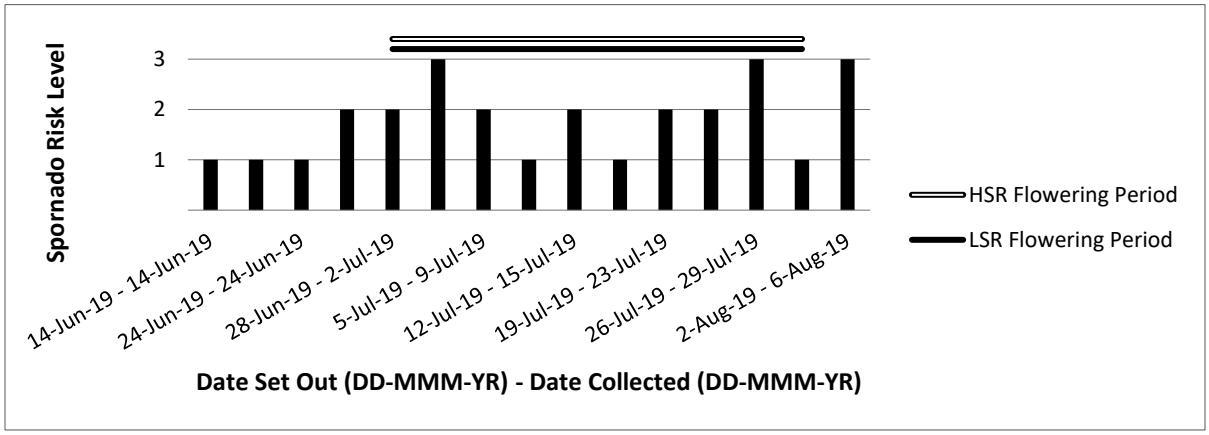

Figure A. 76 Spornado ratings for Outlook, SK, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

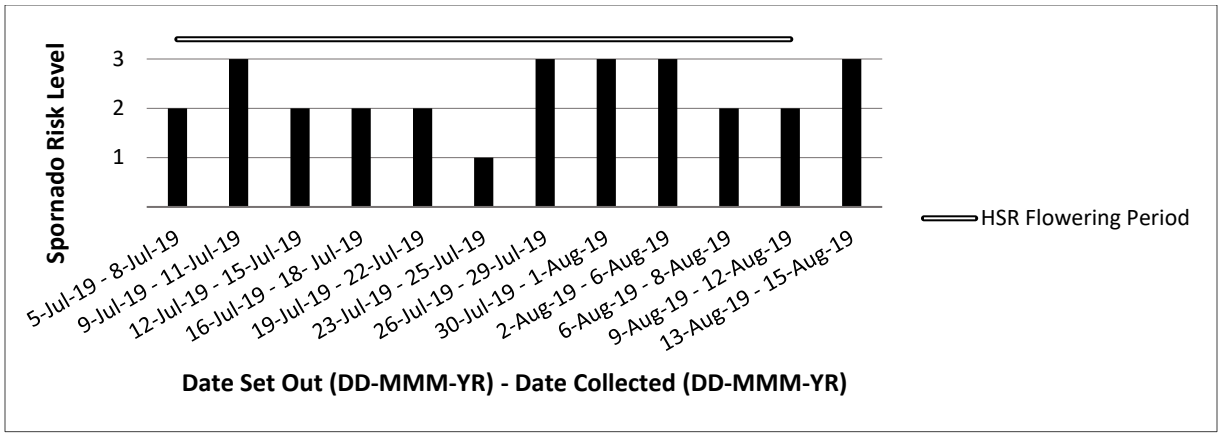

Figure A. 77 Spornado ratings for Scott, SK were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada.

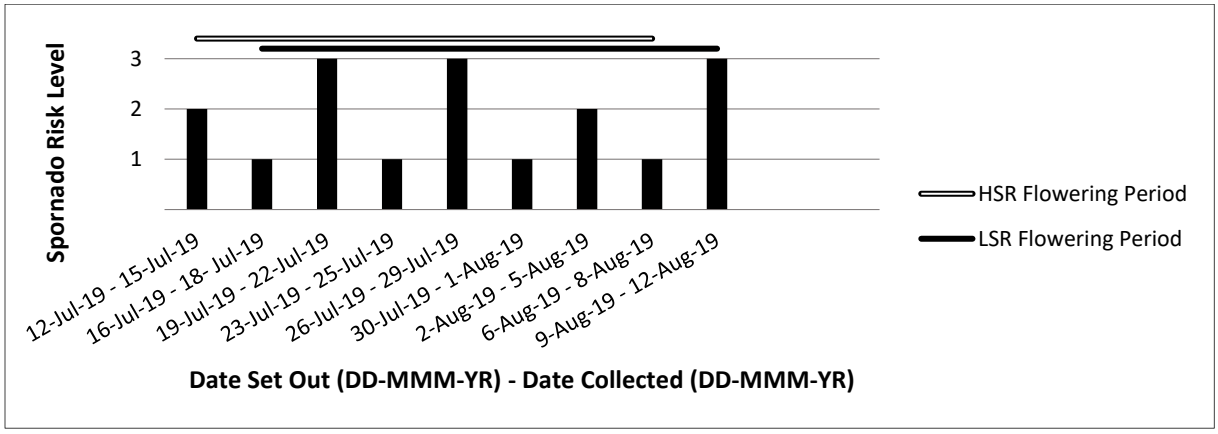

Figure A. 78 Spornado ratings (a) and petal infestation (b) for Brandon, MB, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

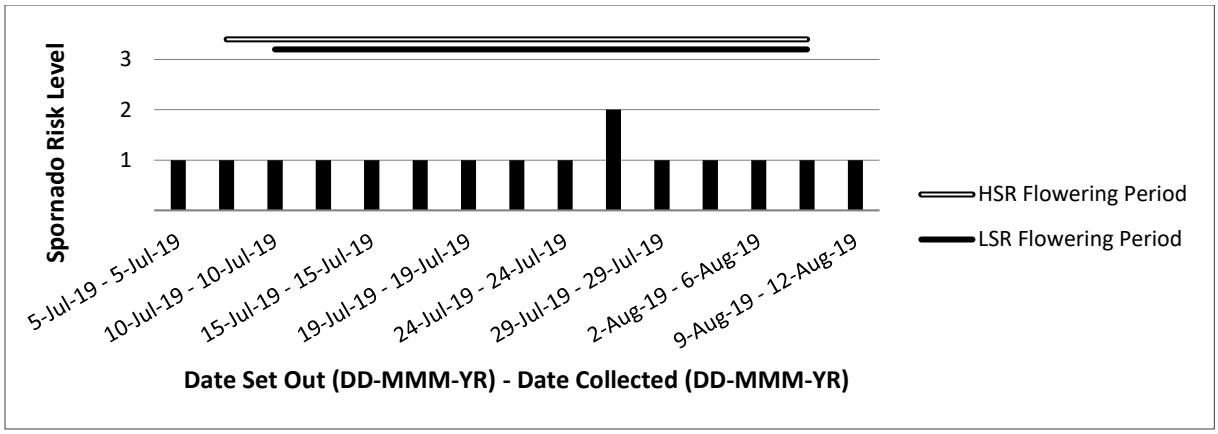

Figure A. 79 Spornado ratings for Lethbridge, AB, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

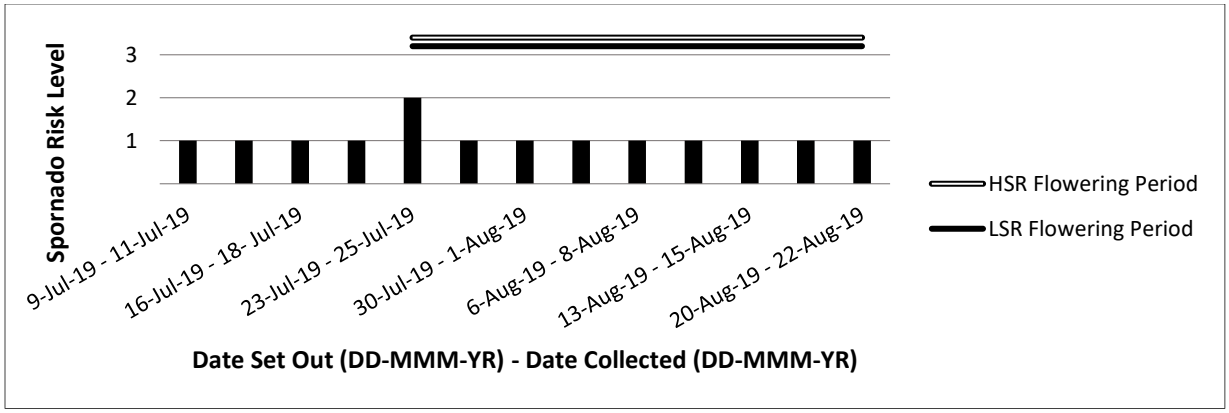

Figure A. 80 Spornado ratings for Normandin, QC, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

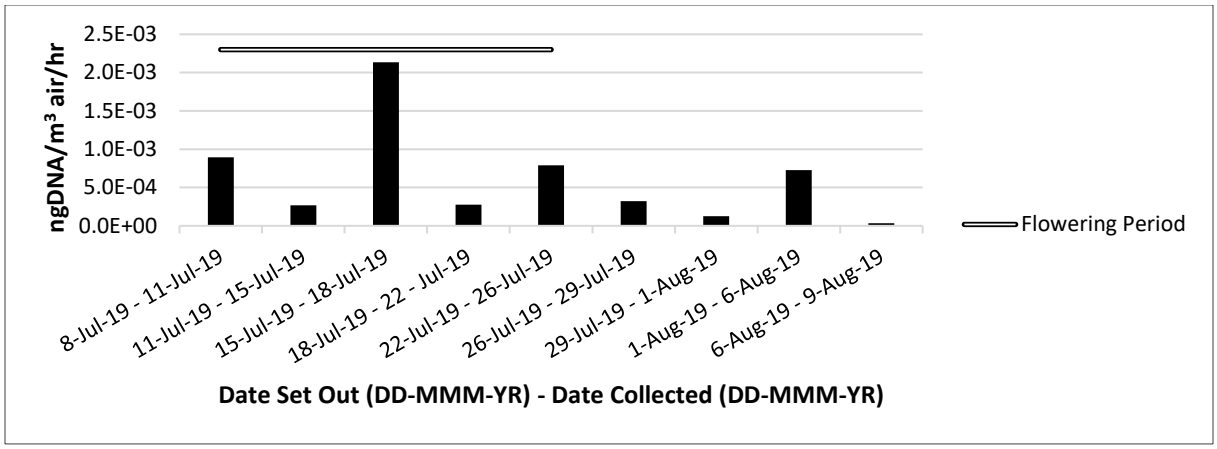

Figure A. 81 *Sclerotinia sclerotiorum* airborne ascospores in field 4, near Oliver, AB, 2019 were captured by a GRIPST – 2009 Rotation Impact Sampler (ngDNA/m^3air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods were changed out twice per week during the canola flowering period.

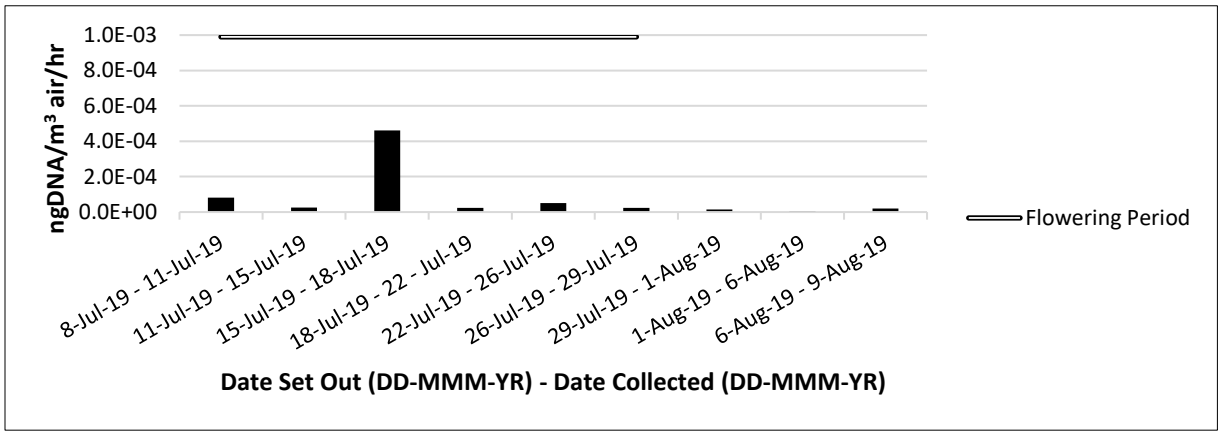

Figure A. 82 *Sclerotinia sclerotiorum* airborne ascospores for Field 1 (F1), near Oliver, AB, 2019 were captured by a GRIPST – 2009 Rotation Impact Sampler (ngDNA/m^3air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods were changed out twice per week during the canola flowering period.

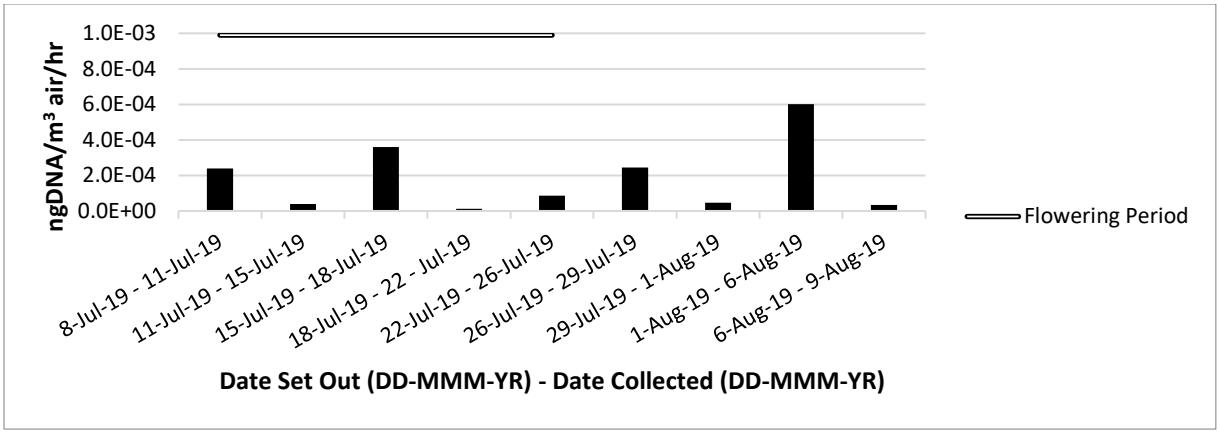

Figure A. 83 *Sclerotinia sclerotiorum* airborne ascospores for Field 2 (F2), near Namao, AB, 2019 were captured by a GRIPST – 2009 Rotation Impact Sampler (ngDNA/m^3air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods were changed out twice per week during the canola flowering period.

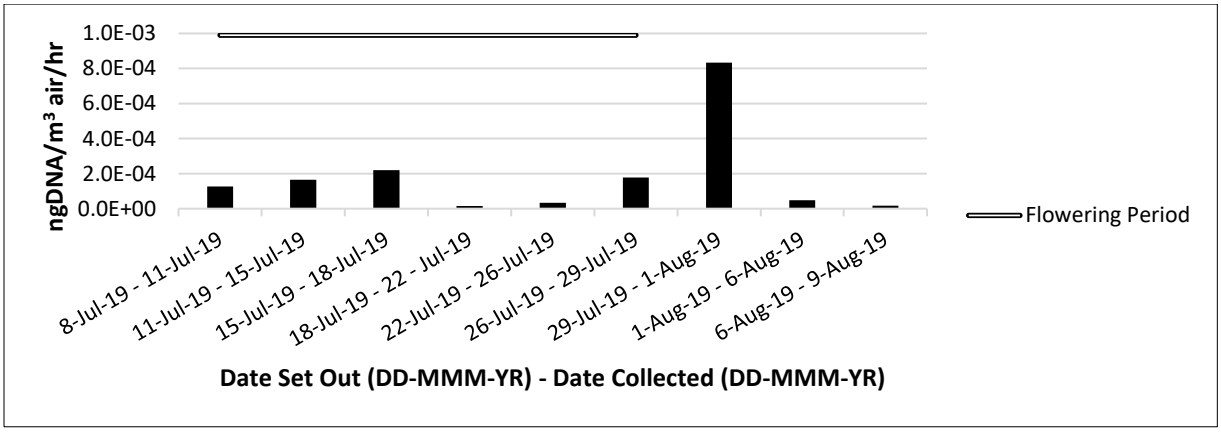

Figure A. 84 *Sclerotinia sclerotiorum* airborne ascospores for Field 3 (F3), near Namao, AB, 2019 were captured by a GRIPST – 2009 Rotation Impact Sampler (ngDNA/m^3air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods were changed out twice per week during the canola flowering period.

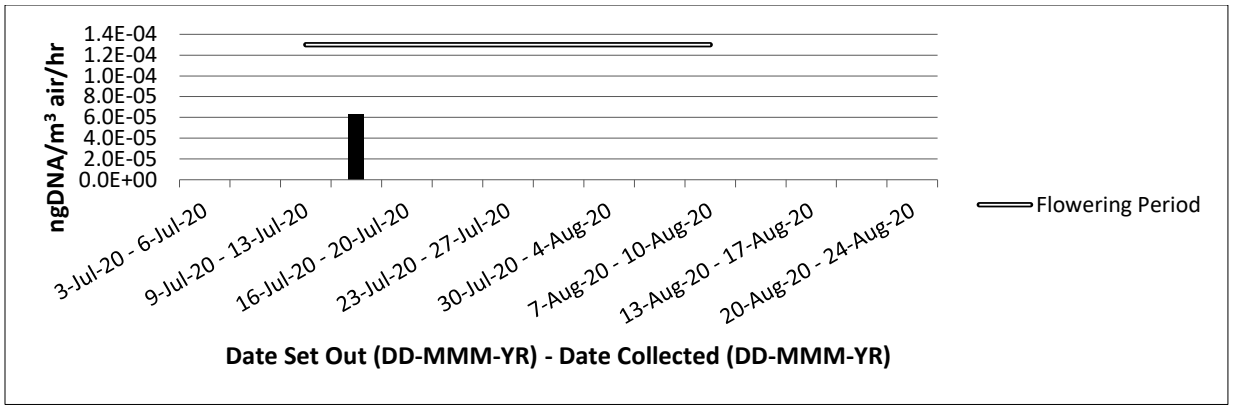

Figure A. 85 *Sclerotinia sclerotiorum* airborne ascospores for Field 2 (F2), near Radway, AB, 2020 were captured by a GRIPST – 2009 Rotation Impact Sampler (ngDNA/m^3air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods were changed out twice per week during the canola flowering period.

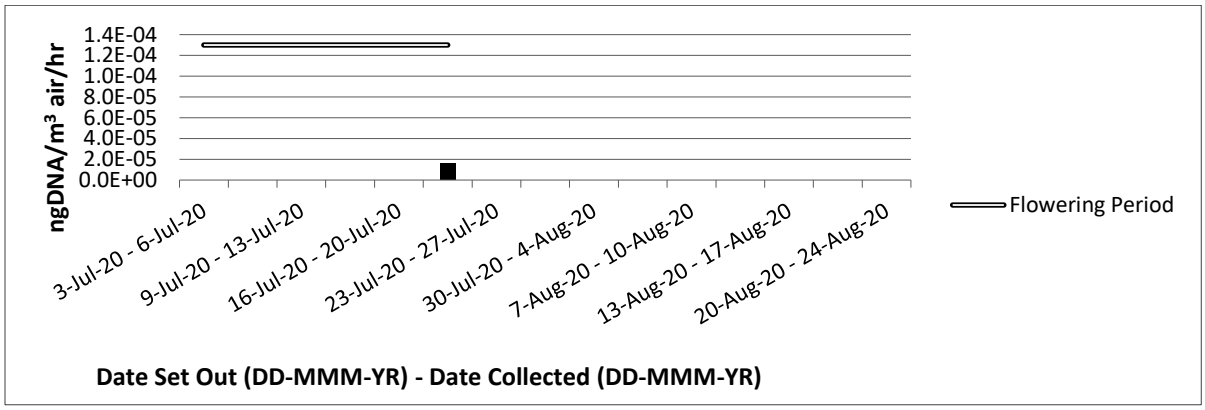

Figure A. 86 *Sclerotinia sclerotiorum* airborne ascospores for Field 3 (F3), near Oliver, AB, 2020 were captured by a GRIPST – 2009 Rotation Impact Sampler (ngDNA/m^3air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods were changed out twice per week during the canola flowering period.

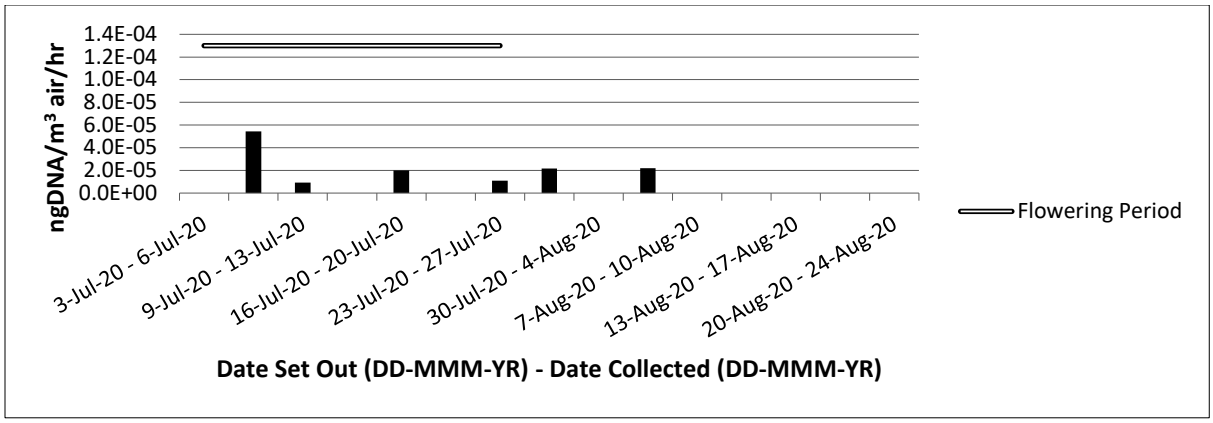

Figure A. 87 *Sclerotinia sclerotiorum* airborne ascospores for Field 1 (F1), near Radway, AB, 2020 were captured by a GRIPST – 2009 Rotation Impact Sampler (ngDNA/m^3air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods were changed out twice per week during the canola flowering period.

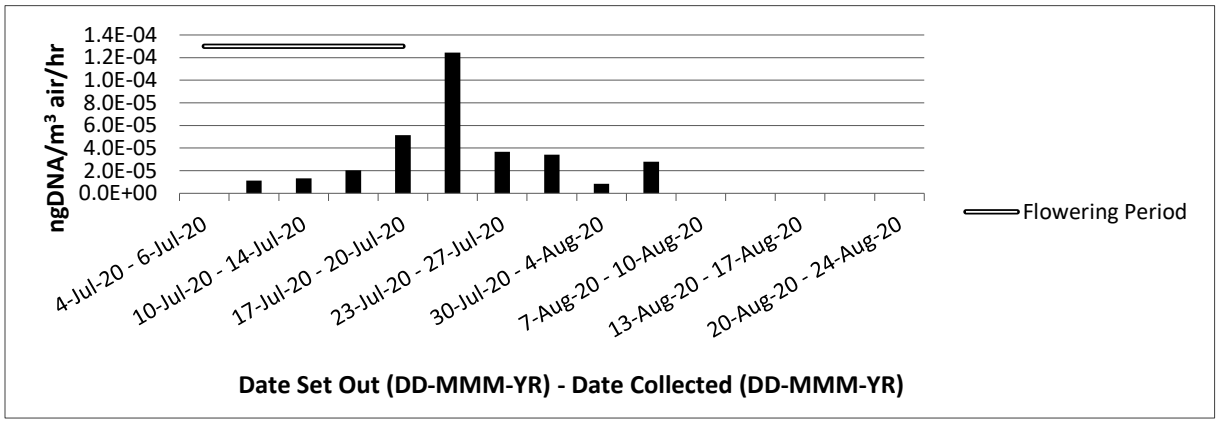

Figure A. 88 *Sclerotinia sclerotiorum* airborne ascospores for Field 4 (F4), near Legal, AB, 2020 were captured by a GRIPST – 2009 Rotation Impact Sampler (ngDNA/m^3air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods were changed out twice per week during the canola flowering period.

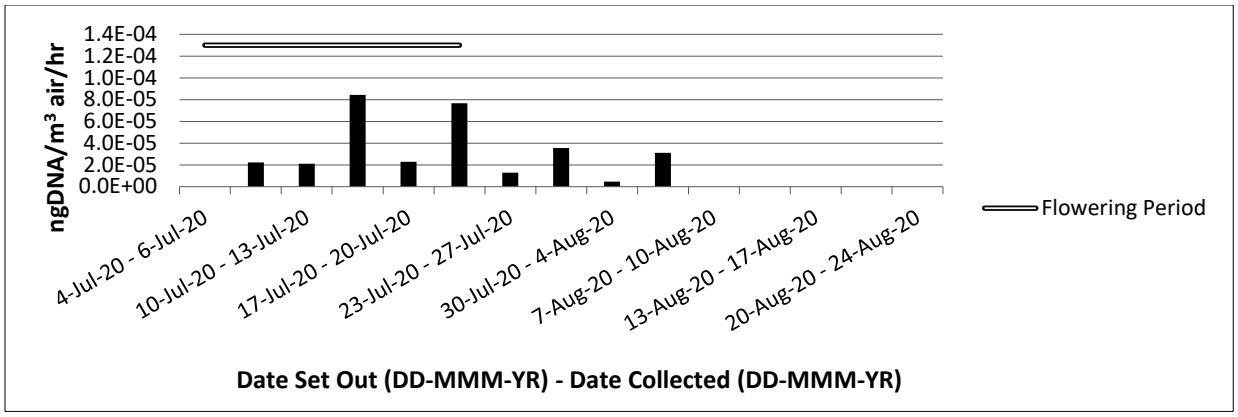

Figure A. 89 *Sclerotinia sclerotiorum* airborne ascospores for Field 5 (F5), near Legal, AB, 2020 were captured by a GRIPST – 2009 Rotation Impact Sampler (ngDNA/m^3air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods were changed out twice per week during the canola flowering period.

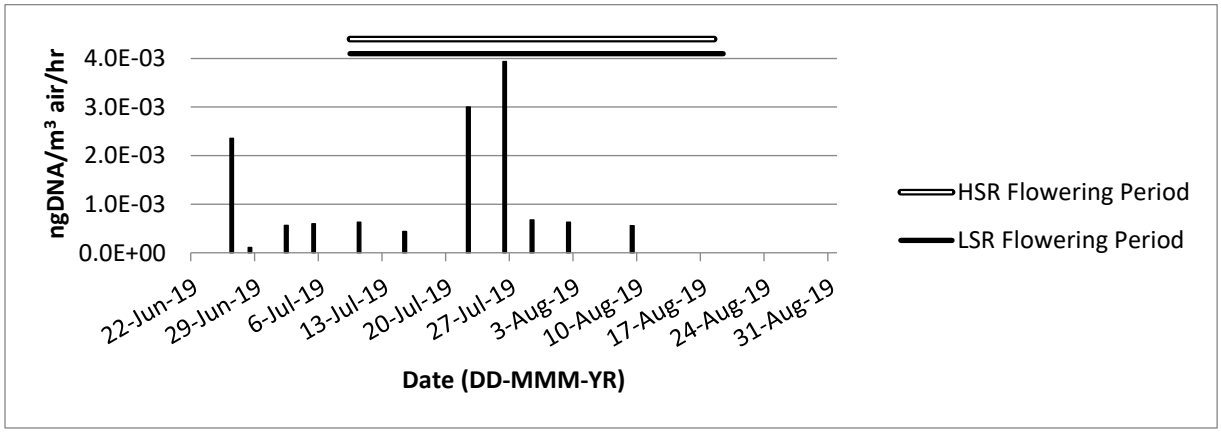

Figure A. 90 *Sclerotinia sclerotiorum* airborne ascospores for Beaverlodge, AB, 2019 were captured by a GRIPST – 2009 Rotation Impact Sampler (ngDNA/m^3air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods were changed out twice per week during the canola flowering period.

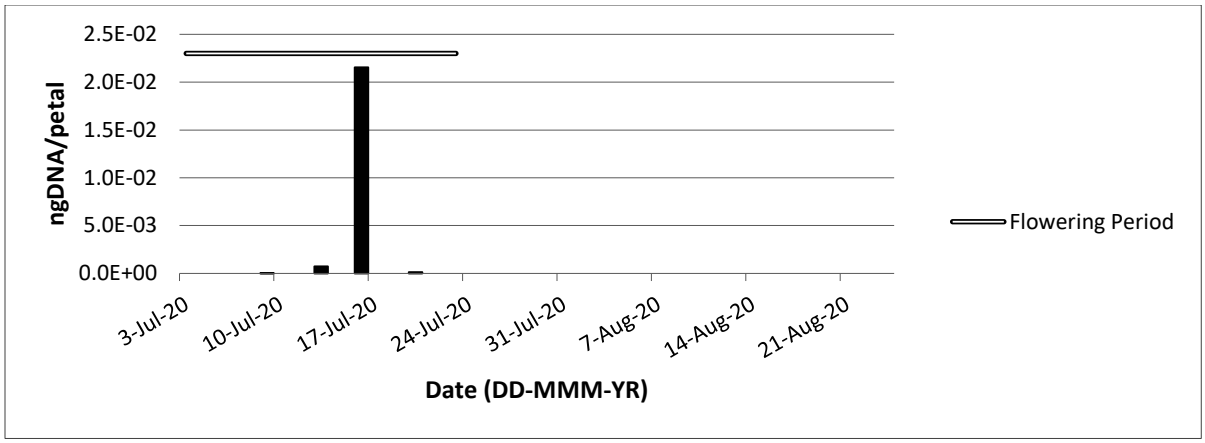

Figure A. 91 Canola petal infestation levels of *Sclerotinia sclerotiorum* in field 3 (F3), near Oliver, AB, 2020. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016).

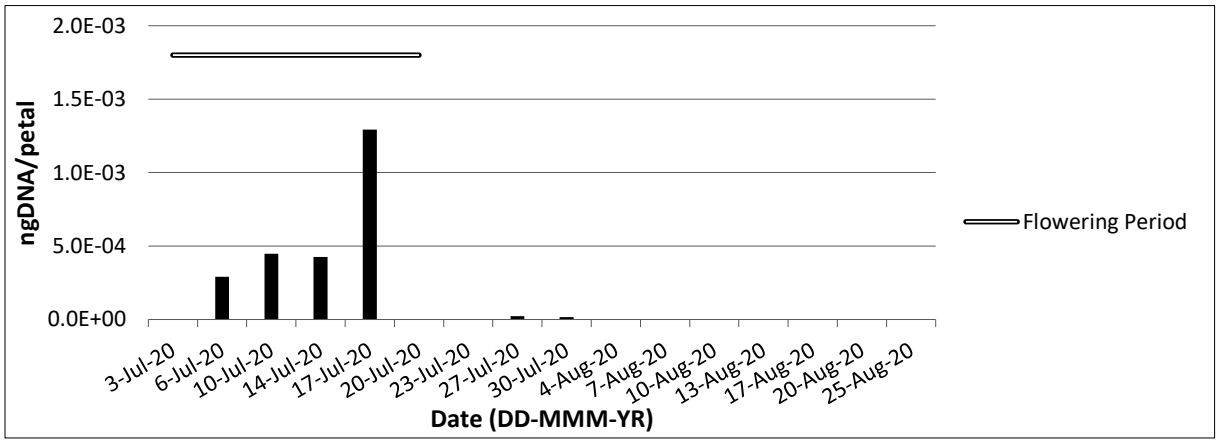

Figure A. 92 Canola petal infestation levels of *Sclerotinia sclerotiorum* in field 4 (F4), near Legal, AB, 2020. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016).

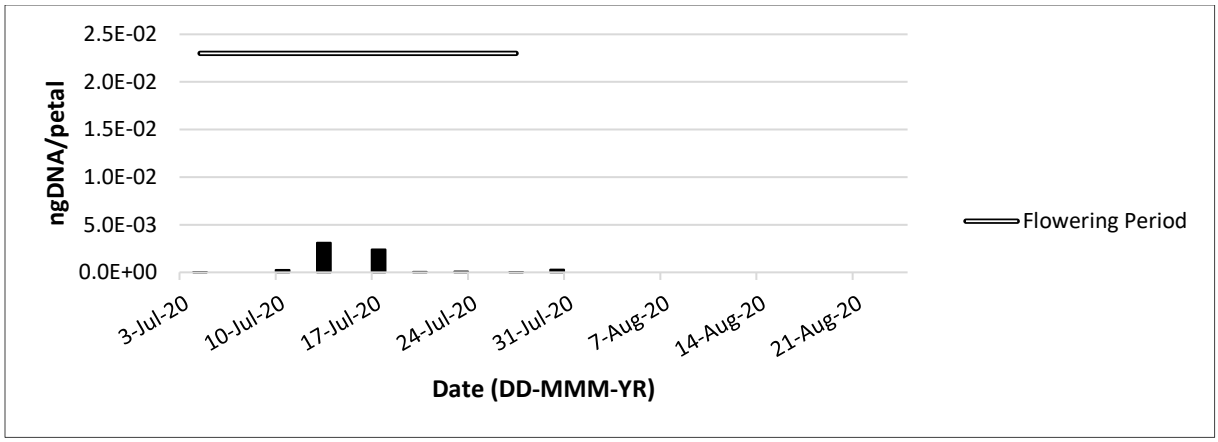

Figure A. 93 Canola petal infestation levels of *Sclerotinia sclerotiorum* in field 5 (F5), near Legal, AB, 2020. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016).

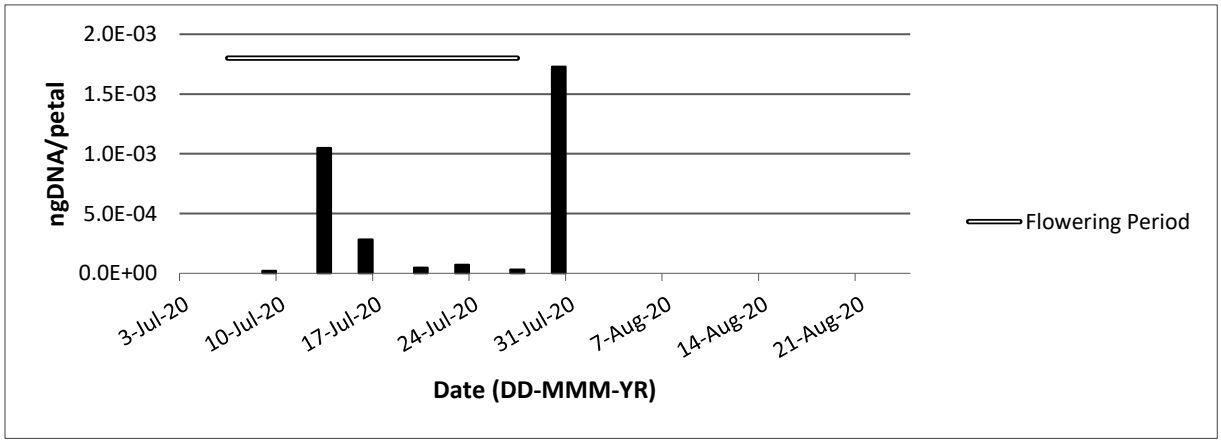

Figure A. 94 Canola petal infestation levels of *Sclerotinia sclerotiorum* in field 1 (F1), near Radway, AB, 2020. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016).

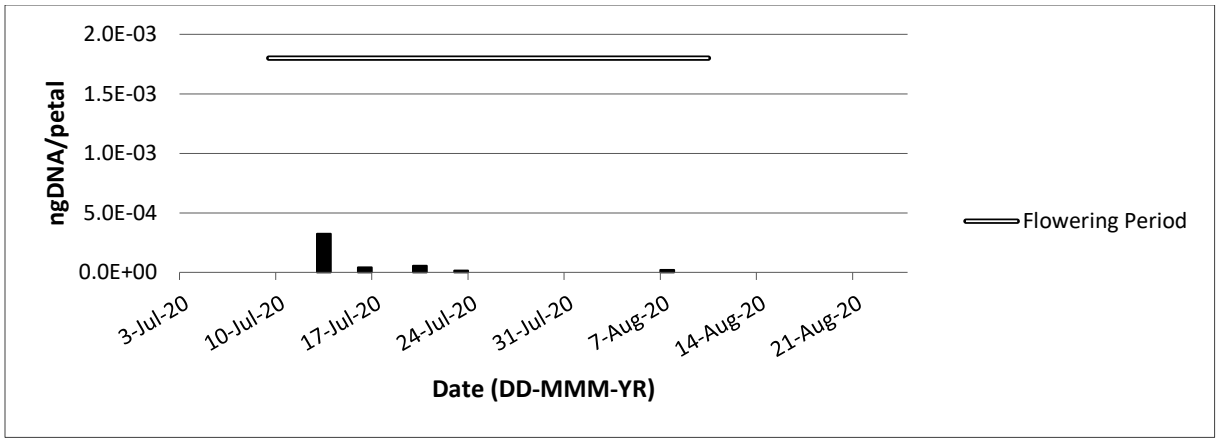

Figure A. 95 Canola petal infestation levels of *Sclerotinia sclerotiorum* in field 2 (F2), near Radway, AB, 2020. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016).

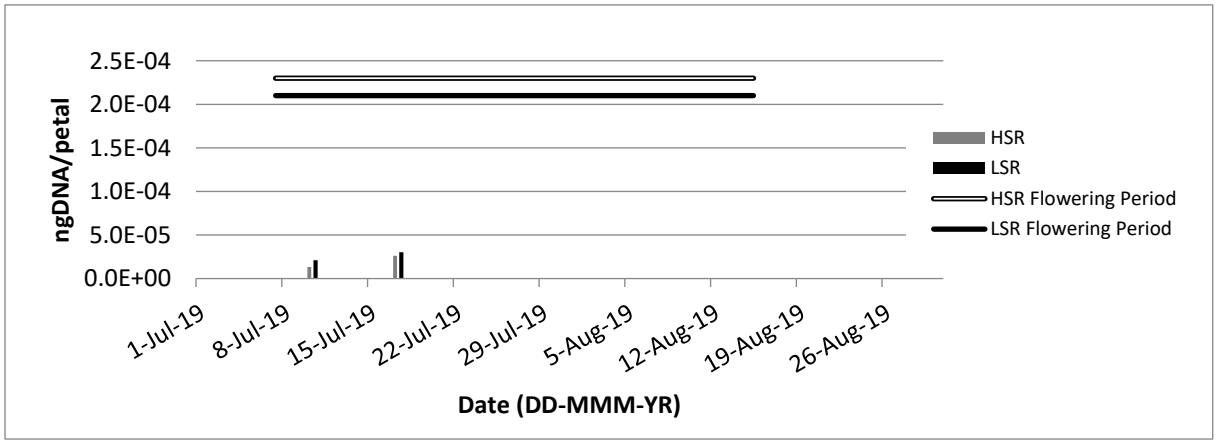

Figure A. 96 Canola petal infestation levels of *Sclerotinia sclerotiorum* for Lacombe, AB, 2019. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate**,** 120 seeds/m^2) and LSR (Low seeding rate**,** 60 seeds/m^2).

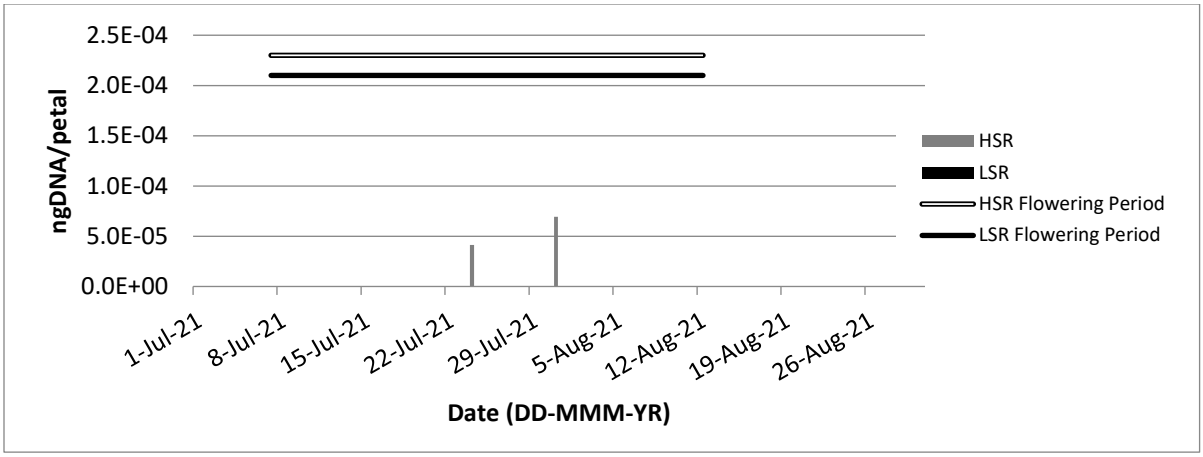

Figure A. 97 Canola petal infestation levels of *Sclerotinia sclerotiorum* for Indian Head, SK, 2019. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

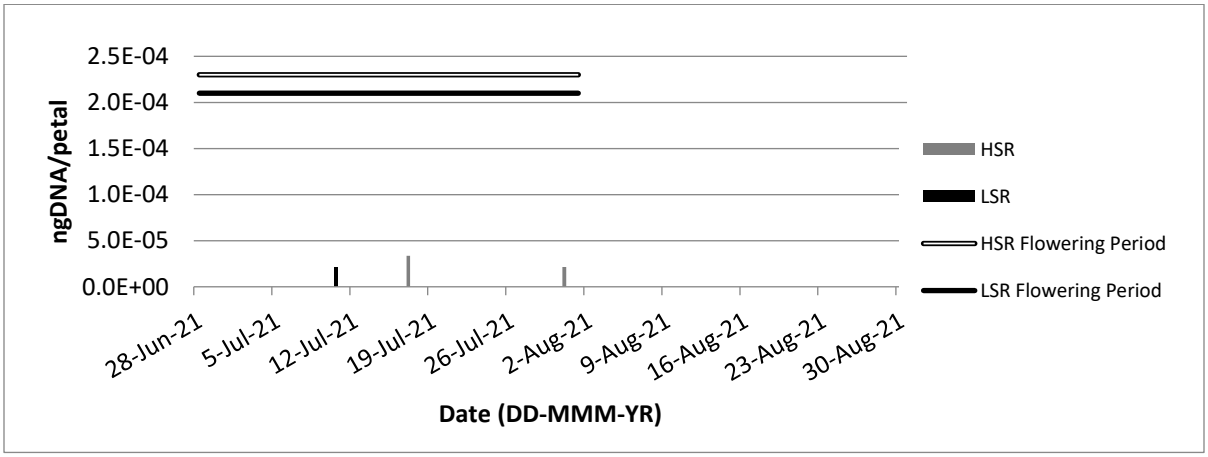

Figure A. 98 Canola petal infestation levels of *Sclerotinia sclerotiorum* for Outlook, SK, 2019. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

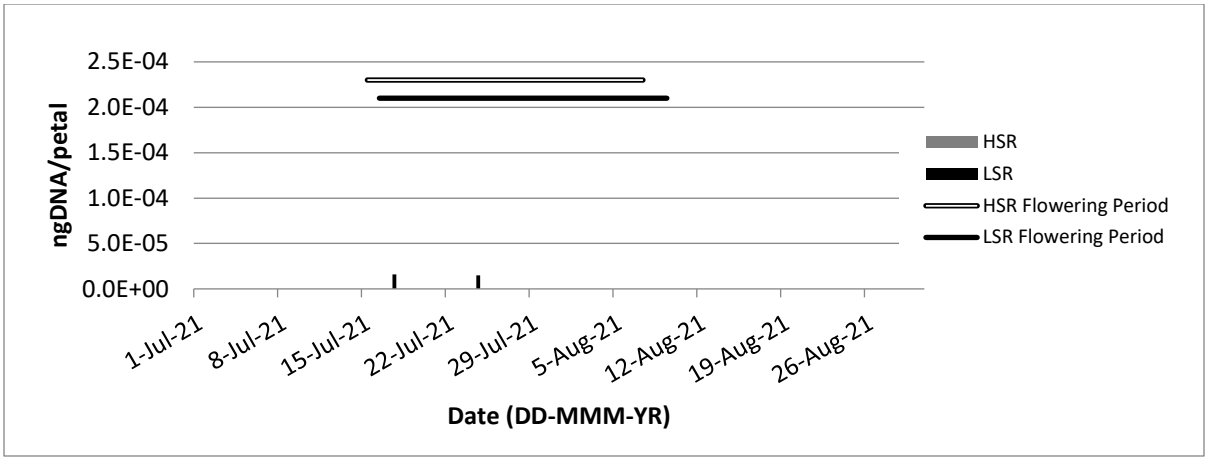

Figure A. 99 Canola petal infestation levels of *Sclerotinia sclerotiorum* for Brandon, MB, 2019. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

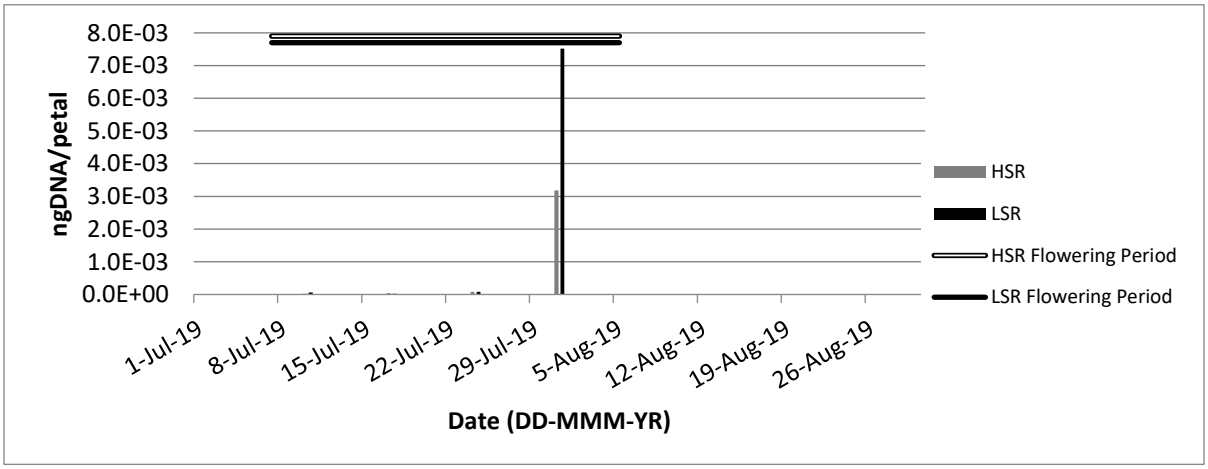

Figure A. 100 Canola petal infestation levels of *Sclerotinia sclerotiorum* for Brooks, AB, 2019. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

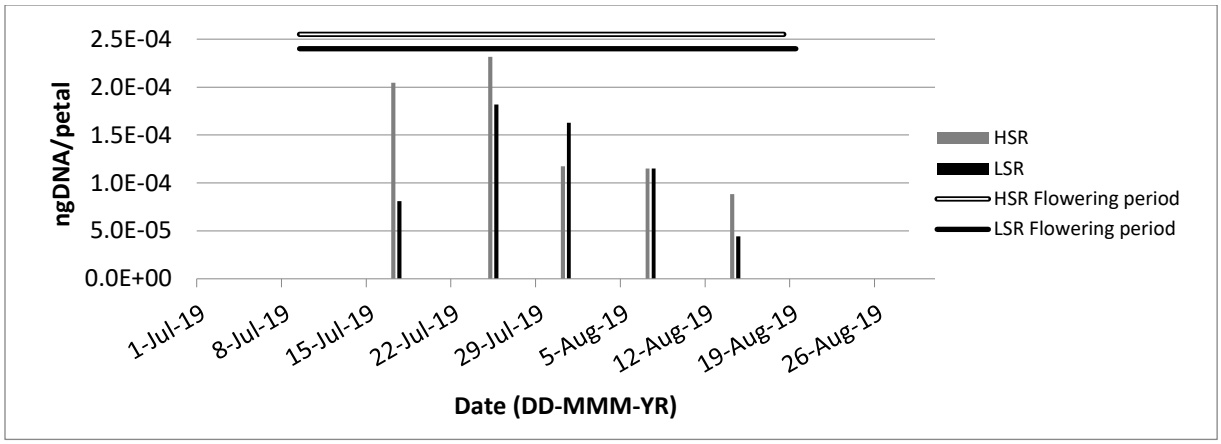

Figure A. 101 Canola petal infestation levels of *Sclerotinia sclerotiorum* for Beaverlodge, AB, 2019. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

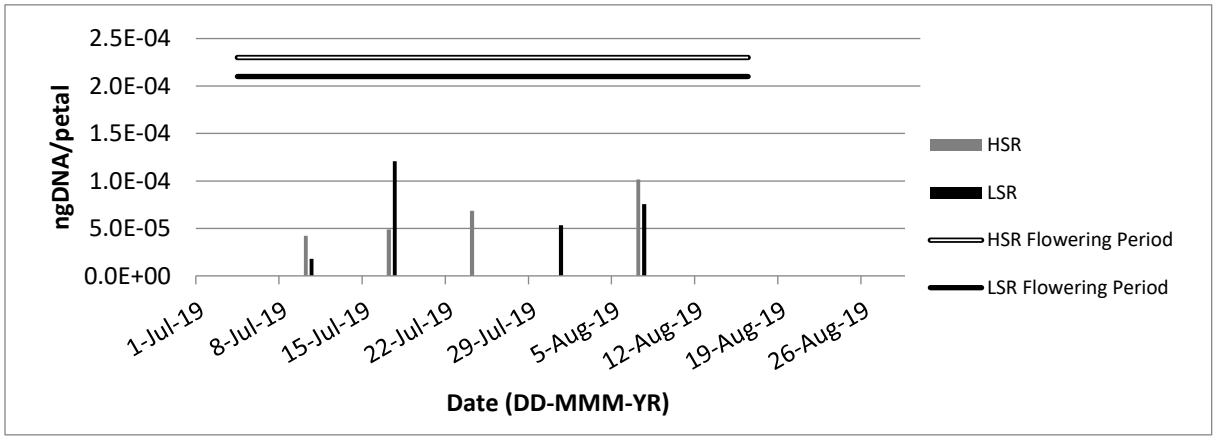

Figure A. 102 Canola petal infestation levels of *Sclerotinia sclerotiorum* for Melfort, SK, 2019. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).

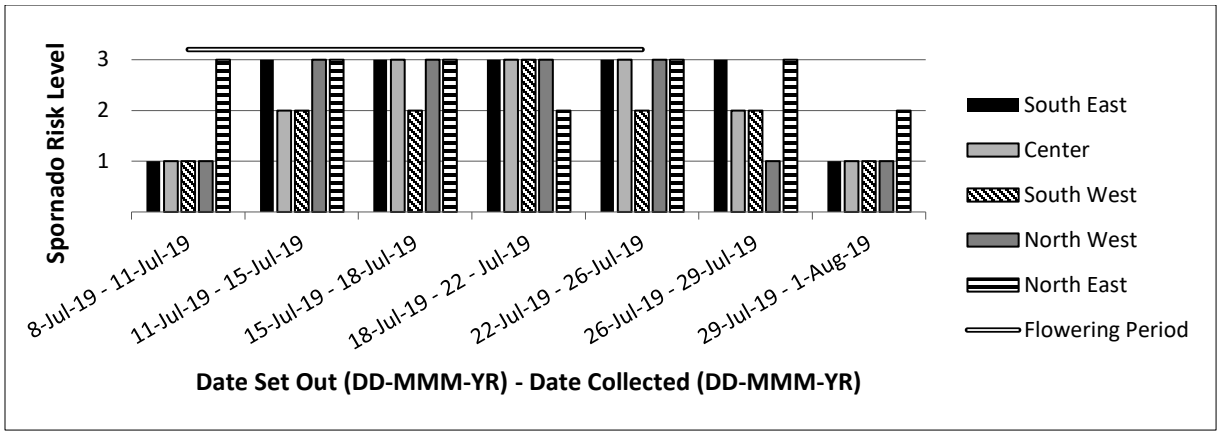

Figure A. 103 Spornado ratings in 5 locations in field 2 (F2, "Grid Field"), near Namao, AB, 2019. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. Spornado cassettes were changed twice per week for about 5 weeks in 2019 and 10 weeks during 2020.

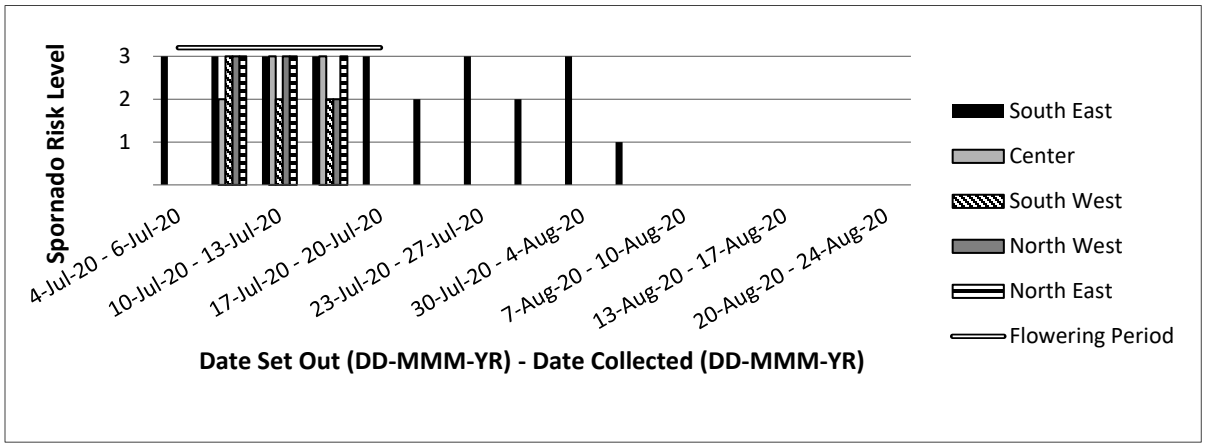

Figure A. 104 Spornado ratings in separate locations of field 4 (F4), near Legal, AB, 2020. Spornado ratings were categorized by T. K. Turkington and Eleanor McBain (personal communication) as 1 = no risk (no *S. sclerotiorum* DNA detected), 2 = low risk (limit of detection of *S. sclerotiorum* DNA) and 3 = moderate-high risk (*S. sclerotiorum* DNA detected). Spornado cassette testing for *S. sclerotiorum* DNA was done by 2020 Seed Labs, Nisku, Alberta, Canada. Spornado cassettes were changed twice per week for about 5 weeks in 2019 and 10 weeks during 2020.

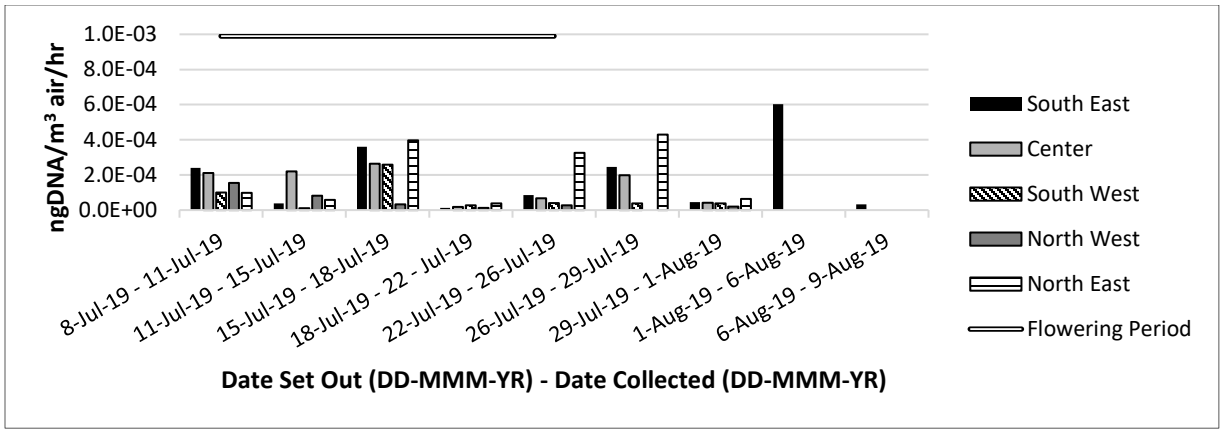

Figure A. 105 Rotorod spore samples for 5 locations in field 2 (F2), near Namao, AB, 2019. *Sclerotinia sclerotiorum* airborne ascospores were captured by a GRIPST – 2009 Rotation Impact sampler (ngDNA/m^{3}air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods and cassettes were changed out twice per week during the canola flowering period.

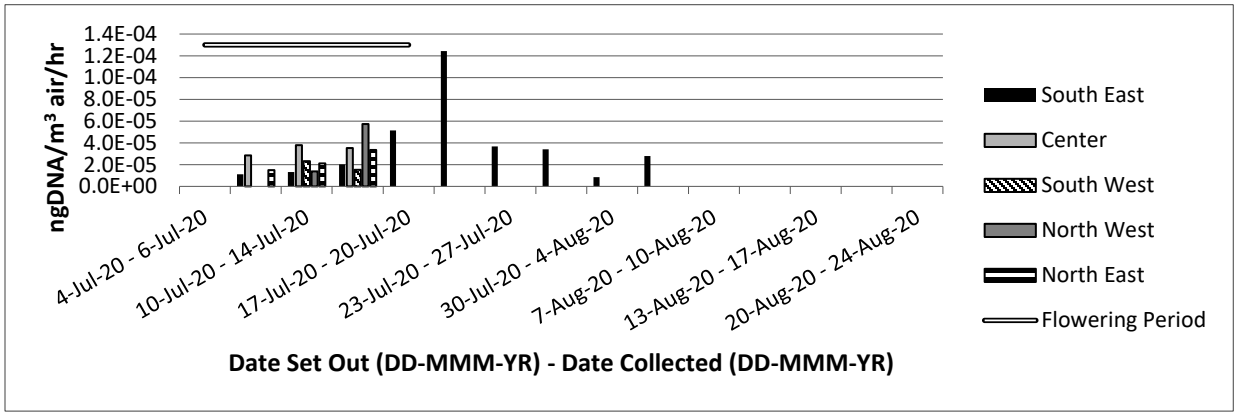

Figure A. 106 Rotorod spore samples for 5 locations in field 4 (F4), near Legal, AB, 2020. *Sclerotinia sclerotiorum* airborne ascospores were captured by a GRIPST – 2009 Rotation Impact sampler (ngDNA/m^{3}air/h) set at a 10% duty cycle placed in the field near the Spornado. Rods and cassettes were changed out twice per week during the canola flowering period.

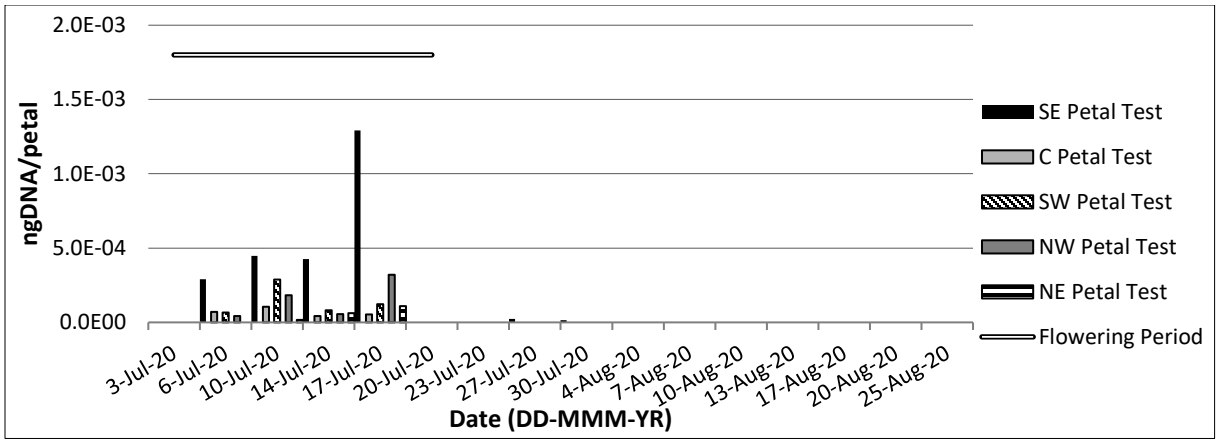

Figure A. 107 Canola petal infestation levels of *Sclerotinia sclerotiorum* in five locations within field 4 (F4), near Legal, AB, 2020. Petal infestation (ngDNA/petal) was measured by collecting approximately 15 fully open canola flower petals from around the spore traps which were subjected to qPCR analysis by AAFRED plant health lab using the protocol developed by Ziesman (2016) and Ziesman et al. (2016). The different seeding rate treatments and their corresponding flowering dates are represented as HSR (High seeding rate, 120 seeds/m^2) and LSR (Low seeding rate, 60 seeds/m^2).