Weather, Microclimate, Canopy Density and Neighbouring Non-Host Crop Impacts on Sclerotinia Stem Rot Disease in Canola

by

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ABSTRACT

Pernerowski, Reanne. M.Sc., The University of Manitoba, January 2014. Weather, microclimate, canopy density and neighbouring non-host field impacts on sclerotinia stem rot disease in canola. Major Professors; Paul R. Bullock and W.G. Dilantha Fernando.

Sclerotinia stem rot (SSR) disease is one of the most devastating diseases of canola in the Canadian prairies caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. Yield losses ranging between 5 to 100 percent can be experienced as a result of this disease. This study evaluated the impacts of weather and microclimate on SSR development in canola with varying canopy density. Ascospore dispersal and disease incidence were compared under modified canopy densities and misting regimes to alter microclimate. The effectiveness of crop rotation and the influence of neighbouring non host crops were also analyzed in this study. A randomized complete block design was used to compare values for canopy density, microclimate and disease development under 3 seeding rates and 3 fertilizer treatments. This design was implemented over 4 site-years, in Winnipeg and Carman during 2011 and 2012. Weather stations were installed to monitor environmental conditions at each site and compare these to disease. At each site, a wheat plot was created to examine ascospore release under a non-host crop to determine the influence such a crop may have on neighbouring canola fields. Results of this study showed that peaks in ascospore concentrations occurred simultaneously between Winnipeg and Carman fields during both years indicating that regional weather conditions are important for ascospore release. Disease development in canola fields

occurred where adequate precipitation and relative humidity were present prior to ascospore release and dispersal. A decrease in relative humidity and an increase in temperature were required for spore release from apothecia. Disease development was greater in Carman, where relative humidity values overall were higher and temperatures remained lower compared to those in Winnipeg in 2011 and 2012. Ascospore release did occur under the wheat canopy and ascospores were dispersed to a distance of at least 7 meters from the plot.

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1. INTRODUCTION

1.1 Importance of Canola in Canada

Canola is a highly profitable crop grown in Canada, contributing $19.3 billion to the Canadian economy each year and generating one fourth of all farm cash receipts through sales and exports to the US and other countries worldwide (Canola Council of Canada, 2011); yield losses in this crop can be very costly to the Canadian economy. Increasing demands for canola encourage the need for higher yielding varieties and increased acreage. Various products are manufactured from harvested canola seed including oil for human consumption (44% of harvested canola) as well as meal for livestock (56% of harvested canola) and recently for biodiesel (Canola Council of Canada, 2011). Canola is mostly grown in Alberta, Saskatchewan and Manitoba, although a substantial amount of crop is also grown in British Columbia, Ontario and Quebec (Canola Council of Canada, 2013). Canola is particularly important in Manitoba and due to increased seeded acreage, the frequency of canola in rotations is increasing (McLaren et al., 2012). Specifically in Manitoba, 3.5 million acres of canola were seeded in 2012 to meet increasing demand for the crop (McLaren et al., 2012).

Several fungal diseases posing major concerns in canola are prevalent in Canada, including sclerotinia stem rot (SSR), blackleg, alternaria blackspot, seedling disease complex and root rot complex (Canola Council of Canada, 2011). In Western Canada, SSR is one of the most serious diseases in canola. It is caused by the fungus *Sclerotinia*

sclerotiorum (Lib.) de Bary and has been present in Manitoba and Saskatchewan for the past 25 years (Manitoba Agriculture, 2013). Sclerotinia has been identified worldwide, however it is more prevalent in temperate and subtropical regions with cool and wet climates (Purdy 1979). Sclerotinia stem rot is capable of affecting over 400 host crops worldwide including dry beans, field peas, lentils, mustard, potatoes, sunflower and canola (Manitoba Agriculture, 2013). Sclerotinia has been known to reduce yields in canola by an average of 0.4 to 0.5 times the percentage of infection, causing losses ranging from 5-100% in the Canadian prairies (Manitoba Agriculture, 2013). Sclerotinia has been the most prevalent disease in canola in surveys conducted annually to determine impacts to canola crops (McLaren et al., 2012).

1.2 Sclerotinia Infection

Sclerotinia sclerotiorum and *Sclerotinia minor* are the two sclerotinia species present in Canada, with *S. sclerotiorum* capable of infecting 408 species of crops (Boland and Hall 1988) and *S. minor* capable of infecting 94 species (Melzer et al., 1997). *Sclerotinia* species can infect plants by either carpogenic germination resulting in the production of ascospores infecting above ground tissues (*Sclerotinia sclerotiorum*) or myceliogenic germination infecting the roots of host plants (*Sclerotinia minor*) (Bardin and Huang, 2001). Ascospores are the primary source of inoculum; mycelial infections are rare in nature (Abawi and Grogan, 1979). Myceliogenic germination causes plant wilt in various plant species (Huang and Erikson, 2002). Diseases caused by carpogenic germination include white mold and pod rot of bean, stem blight and leaf blight of canola, pod rot of peas, head rot of sunflower, lettuce drop and blossom blight of alfalfa (Huang and Erikson, 2002; Fernando et al. 2004). The mechanism by which the pathogen infects

plants depends on the host crop and environmental conditions at that particular location. Canola crops in Western Canada are typically only infected by ascospores of *Sclerotinia sclerotiorum* (Bardin and Huang, 2001).

The infection process for *S. sclerotiorum* begins in the spring or summer (Figure A.1). *Sclerotinia sclerotiorum* begins its life cycle as dormant sclerotia; irregularly shaped fungal structures capable of surviving in the soil for prolonged periods of time, generally up to four years (Manitoba Agriculture, 2013). In Manitoba, sclerotia are present in the soil throughout the winter when they are exposed to low temperatures allowing for conditioning of the fungal body for future stimulation or acceleration of apothecia production (Clarkson et al., 2007). During the spring or early in the summer, the sclerotia germinate through asexual reproduction and produce apothecia, which appear as mushroom-like structures at the soil surface (Manitoba Agriculture, 2010). Apothecia produce asci from which airborne ascospores are produced asexually and are forced into the atmosphere (Harthill and Underhill, 1976). In order for germination and infection to occur on the plant, the ascospores require a readily available food source by initially landing on canola flowers, fallen petals and pollen located on the stems and leaves (Manitoba Agriculture, 2010). Infection occurs when the petals fall on the stems and leaves of the canola plant (Government of Saskatchewan, 2009). Once deposited onto the leaf, ascospores are capable of surviving for up to 12 days depending on their position in the canopy and environmental conditions (Caesar and Pearson, 1983). Once established, mycelium releases oxalic acid and acidic enzymes into the plant, disrupting vascular tissue and killing the plant (Boland and Hall, 1988). Secondary spread from

diseased to healthy tissue is possible through direct contact (Huang and Hoes, 1980). Sclerotia are newly formed in and on infected tissues (Huang and Erikson, 2002).

Initial symptoms of sclerotinia stem rot disease in canola appear as water-soaked spots followed by pale grey to white lesions on stems. Infection then spreads to branches and pods several weeks after the onset of flowering (Manitoba Agriculture, 2014). Infected stems appear bleached and hard black fungal bodies are often found within shredded stems, branches or pods (Manitoba Agriculture, 2014). Canola infected with sclerotinia will ripen prematurely, wilt and lodge much more easily (Manitoba Agriculture, 2010). Individual plants can be rated for disease severity using a 0-5 scale, where 0 indicates no disease and 5 indicates a plant infected completely. Fields and plots can also be rated for percentage of infection by counting the number of infected plants over the total number of plants. This can be done in small sample units and extrapolated over entire plots or fields.

1.3 Sclerotinia Control

Management of sclerotinia stem rot is increasingly important due to the severe economic losses experienced by canola crops exposed to the disease. Very few commercially available canola crop varieties are resistant to the pathogen and breeding is difficult due to the need for multiple genes (Fuller et al., 1984). Canola varieties that resist lodging or reduce canopy density are beneficial in sclerotinia control (Canola Council of Canada, 2011). Canola varieties with some physiological tolerance have been recently introduced by DuPont Pioneer with 45S54 marketed in 2013, and by Bayer Crop Science with their sclerotinia tolerant InVigor variety that will be launched in 2014.

Sclerotinia resistant varieties are only effective in reducing severity and do not always eliminate the need for fungicide use.

1.3.1 Fungicides:

Sclerotinia stem rot disease is primarily controlled by the use of fungicides, although this method is not always recommended due to the costs associated with the purchase of fungicides and sporadic nature of the disease making it difficult to effectively time fungicide applications (Bradley et al., 2006). Current registered fungicides include Astound (cyprodinil and fludioxonil), Lance (boscalid), Overall (iprodione), Proline (prothioconaole), Quadris (azoxystrobin) and Rovral Flo (iprodione) (Manitoba Agriculture, 2013). Generally, chemical fungicides are applied at 20-50 percent bloom stage before symptoms are visible (Manitoba Agriculture, 2013). Once symptoms are present, fungicide application will not help in the management of the disease (Manitoba Agriculture, 2013).

Although the main method to reduce infection of SSR is through fungicide application, other control methods have been proven to reduce infection as well. Seeds infected by *S. sclerotiorum* generally fail to germinate or seedlings die upon germination then become rotted with sclerotia forming in the seed (Fernando et al., 2004). The fungus can be removed from the infected seed through the use of seed treatment.

1.3.2 Biological Control:

Due to environmental concerns associated with the use of chemicals in agricultural crops, biological control is an attractive alternative. This can be accomplished through the presence and addition of biological control agents including fungal and bacterial antagonists. These agents are capable of reducing sclerotia populations before the formation of apothecia and release of ascospores (Fernando et al., 2004). *Coniothyrium*

minitans is an example of a widely studied fungal antagonist capable of reducing airborne ascospore infections (Fernando et al., 2004). *C. minitans* is applied prior to planting a spring crop in a solution called Contans (Manitoba Agriculture, 2013). Bacterial antagonists include *Bacillus* and *Pseudomonas* species, however little research has been conducted on the use of these species in SSR management. Organic and inorganic soil ammendments can also be added to the soil in order to promote the growth of soil-borne micro-organisms to reduce sclerotia germination and suppress *S. sclerotiorum* germination (Bardin and Huang, 2001).

1.3.3 Cultural Methods:

Cultural methods are also used instead of or in conjunction with chemical applications. These methods include tillage, flooding, reduced irrigation, rotation with non-host crops and biological control agents (Bardin and Huang, 2001). The development of environmentally-friendly management strategies is required to effectively control the infection of sclerotinia on canola. This can be more easily addressed through understanding the causes of the disease and its rate of infection and severity as a response to microclimate and standard weather conditions. Management can be further enhanced by early identification of crops at risk and proper timing of fungicide applications. Microclimate modifications can also be managed to create conditions unfavourable to disease development via seeding rate and row spacing modifications.

Burial depth is an important factor affecting the germination and viability of sclerotia in field settings (Duncan et al., 2005). Stipes can generally grow no longer than 3 cm in the field; therefore the only functional sclerotia are found in the top 2 to 3cm of the soil column (Abawi and Grogan, 1979; Wu and Subbarao, 2008). Tillage, as a method to control sclerotial germination may also be effective as a management practice. Tilling

sclerotia-containing soils effectively buries the sclerotia deeper into the soil profile, reducing their ability to receive sunlight and germinate (Wu and Subbarao, 2008); however resurfacing of the fungal bodies is possible. Other studies have shown that a zero tillage method could be beneficial in reducing future sclerotia inoculum levels in the soil. Mycoparasites are present in the top soil and are capable of deteriorating the fungal bodies (Tu, 1989). Survival is therefore prolonged in tilled soil where mycoparasite numbers may be reduced.

Crop rotation strategies are implemented to reduce sclerotia populations in fields and alleviate disease history. The Canola Council of Canada (2011) recommends at least 1 year between canola crops; 2 to 3 years are preferred. Although this strategy is widely used to increase canola yields and reduce pressures from pests and disease (Canola Council of Canada, 2013), it may not always benefit the farmer. Limitations for rotating with non-susceptible crops include; the limited availability of non-host crops (Manitoba Agriculture, 2010), duration of rotations required to deplete sclerotia levels (4 years) (Bradley et al., 2006) and disease pressures from neighbouring fields. Williams and Stelfox (1979) found that sclerotia germination increased under consecutive years of rapeseed (host) crops. Under non-host crops, germination rates remained the same each year; therefore inoculum depletion did not occur (Schwartz and Steadman, 1978; Williams and Stelfox, 1979). According to Williams and Stelfox (1979), ascospores are capable of travelling a distance of 150 m and up to 7m high from the apothecia source, Additional study on sclerotia longevity and depletion rates in non-host crops is required. Ascospore release and dispersal is more defined under specified environmental conditions in a wheat (non-host) crop in chapter 3.

1.4 Weather Impacts on Sclerotinia

Microclimatic conditions are influenced by plant and canopy density which is altered through the modification of seeding rates as well as row spacing. Jurke and Fernando's (2008) study demonstrated that plant density does affect sclerotinia stem rot incidence in canola. They compared several seeding rates over three years with resulting sclerotinia disease incidence. Dense canopies have been shown to create conditions where relative humidity is higher with longer periods of leaf wetness (Turkington, 1991) and temperatures tend to be lower (Blad et al., 1978). With certain cultivars, lodging also became more apparent where higher density crops were situated creating microclimatic conditions where development of sclerotinia stem rot was increased (Jurke and Fernando, 2008). Where lodging is observed, there is generally an increase in soil surface moisture under the canopy and there is also more contact between plants both creating conditions conducive for SSR development and allowing for the disease to spread among plants (Jurke and Fernando, 2008). A study on soybean also demonstrated that wider row spacing decreased the incidence of disease (Steadman et al., 1973). Additional studies focusing on the effects of canopy density on microclimate is essential for a better understanding of the impact of canopy density on disease.

Researchers have attempted to determine critical conditions required for sclerotia germination, ascospore release and infection (Table 1.1). Soil moisture is the most common limiting factor on germination (Matheroon and Porchas, 2005; Mila and Yang, 2008). Germination increases with additional moisture supply during prolonged periods of time and are destroyed under saturated conditions. Soil moisture may not be required

for sclerotia germination in dry regions such as Arizona (Matheroon and Porchas, 2005). With respect to temperature, optimum sclerotia germination rates are variable depending on the geographical region from which sclerotia are collected. Temperature requirements also vary depending on the presence of light and moisture. Precipitation and irrigation favour sclerotia germination by reducing temperatures and increasing moisture supply to the canopy (Gugel and Morall, 1986; Teo et al., 1989). Light, temperature and moisture regimes influence the appearance of stipes (Sun and Yang, 2000). Researchers have found that ascospore release has occurred under both light and dark conditions (Clarkson et al., 2003), contrary to the discovery by Harthill (1980) that light was involved in the process. This finding leads to the assumption that other factors including temperature or relative humidity are responsible for daytime release of spores (Clarkson et al., 2003). Qandah and Mendoza (2011) attempted to validate several findings by relating environmental factors to the daily and seasonal release and dispersal patterns of ascospores into the atmosphere. Their three year study (2005 to 2007) during canola flowering suggests that temperature and relative humidity have a significant impact on ascospore release. Hourly observations showed that ascospore dispersal occurs in a single event lasting between four to six hours no more than once daily, either at night or during the day depending on the year. Although these weather variables have been extensively studied, McCartney and Lacey (1991) have shown that ascospore concentrations in the air were more closely related to the number of apothecia produced than to weather variables. Spore survival may also be a limiting factor in the infection by ascospores on various crops under conditions of high temperature and relative humidity (Clarkson et al., 2003). Precipitation and irrigation are responsible for infection as heavy irrigation practices increased disease severity in a study on dry edible beans (Weiss et al., 1980). Spring rainfall also leads to

ideal conditions for disease development, favouring sclerotia germination (Kirkegaard et al., 2006). Plant wetness is also required for infection of ascospores on nutrient-containing tissues such as petals for further development of stem rot of canola (Bardin and Huang, 2001). A disease prediction model in canola that can relate weather conditions such as rainfall to soil moisture content and leaf wetness would create a better understanding of the development of sclerotinia stem rot and also allow for better prediction of disease occurrence (Table 1.1).

Table 1.1 Factors influencing disease development (including sclerotia germination, ascospore release and infection) of sclerotinia stem rot disease according to past research.

Factor	Value	Influence on disease	Location of experiment	Reference
Sclerotia Germination				
Temperature, soil moisture	15-40°C , ≥ 0.02 Mpa	decrease sclerotia germination with increasing temperature in wet soil	growth room (Arizona)	Matheroon and Porchas, 2005
Temperature	25°C	Highest apothecia production	growth room (Iowa)	Sun and Yang, 2000
Soil temperature	20°C and fluctuating temperature	similar effects on sclerotia germination	growth room (Iowa)	Mila and Yang, 2008
	15°C	highest germination rates from in sclerotia from california	growth room (California)	Wu and Subbarao, 2008
	reduction by 15°C	favourable for sclerotia germination	Field (Saskatchewan)	Teo et al., 1989
Soil moisture	at or above -0.4 MPa to -0.5 MPa	above these levels sclerotia germination is increased	growth room (Saskatchewan); growthroom (Ontario)	Teo and Morall, 1985a; Boland and Hall, 1987
	0 Mpa (saturation)	sclerotia rot and are destroyed	growth room (Saskatchewan)	Teo and Morall, 1985b
	continual -0.001 Mpa	favours sclerotia germination over fluctuating moisture	growth chamber (Iowa)	Mila and Yang, 2008
	10 days moist soil	sclerotia germination and apothecia production	Manitoba	Manitoba Agriculture, 2013
	increased soil moisture	increase in sclerotia germination	growth room (California)	Wu and Subbarao, 2008
Precipitation	10 mm	appearance of appothecia 3-7 days after precipitation	Field (Saskatchewan)	Gugel and Morall, 1986
Light, temperature and soil moisture	120 - 190 mol/m/s, 20°C to 25°C	require saturated soil for production of short, thick stipes	growth room (Iowa)	Sun and Yang, 2000
	80 - 120 mol/m/s, 6-12°C	require partially (50%) saturated soil for production of long thin stipes	growth room (Iowa)	Sun and Yang, 2000
Ascospore Release and Dispersal				
Light	light, dark	light involved in ascospore release	lab (New Zealand)	Harthill, 1980
		spores released in light and dark conditions	lab (Worcester, UK)	Clarkson et al., 2003
light, temperatureand moisture	high light, high temperature, high relative humidity	large daytime ascospore release during wet years at increasing temperaute and decreasing relative humidity, following a period of high relative humidity	field (North Dakota); United Kingdom	Qandah and Mendoza, 2011; McCartney and Lacey, 1991
	no light	small ascospore release during dry years at night	field (North Dakota)	Qandah and Mendoza, 2011
temperature, relative humidity	high temperature, high relative humidity	reduction in spore survival	lab (Worcester, UK)	Clarkson et al., 2003
Infection				
Precipitation	5.5 cm every 10 days versus 5.5 cm every 5 days (irrigation)	increased disease severity		Weiss et al., 1980
	550 - 650 mm rain	high levels of sclerotinia stem rot incidence	Field (Australia)	Kirkegaard et al., 2006

1.5 Sclerotinia Disease Models

Models focusing solely on relationships between *S. sclerotiorum* development stages have been created. Disease development originates with sclerotia germination followed by ascospore dispersal and subsequent petal infestation, followed by disease occurrence as fallen petals land on canola leaves and stems. Gugel and Morall (1986) have attempted to relate sclerotia germination and petal infestation to disease incidence. A positive relationship exists between sclerotia germination and disease incidence in large scale sampling regimes (Gugel and Morall, 1986). Better relationships were found between petal infestations and disease incidence where petal samples were obtained in early bloom stages; petal infestations at this stage have the best opportunity to cause disease because they have a longer period for establishment on the main stem (Gugel and Morall, 1986). Although petal sampling is a simple and cost effective way to assess disease risk, monitoring ascospore dispersal is beneficial in assessing disease risk prior to flowering and ascospore colonization; however this method is not cost effective for farmers due to the price of spore capturing devices. The simplest and most effective way to assess risk prior to flowering is to relate disease development to weather conditions.

Researchers have attempted to develop models relating microclimatic and weather variables to disease incidence. The purpose of the model would be to inform farmers on the need for and the timing of fungicide application for more cost-effective sclerotinia control. Risk point systems and disease prediction models were created by Twengstrom et al. (1998) and Bom and Boland (2000) respectively to provide information on the need for spraying. They also suggested and modeled factors responsible for disease incidence

such as rainfall, soil moisture, canopy density, crop height, growth stage and apothecia counts (Bom and Boland, 2000). This shows that in addition to the presence of pathogen and host crop, a favorable environment is necessary for disease to occur. Since the environment plays a key role in completing the disease cycle, more studies are required to better quantify the environmental factors that cause sclerotinia stem rot disease in canola. In doing so, disease prediction will also be enhanced, which, in turn, will facilitate management decisions about both if and when fungicide application can most effectively control the disease.

Evaluation of potential disease risk can be accomplished through the use of various methods; however some are more practical and economical than others. In fields with unknown history of disease, monitoring of germinated sclerotia can be useful in determining inoculum levels present within the field and forecasting disease (Gugel and Morall, 1986; Boland and Hall, 1988). Apothecia counts do not directly represent inoculum levels as sclerotia germination may be limited by environmental conditions. In commercial fields, great efforts are required to complete apothecia counts over large scales but inoculum in the form of ascospores arising from neighbouring fields is also not taken into consideration. Spore trapping devices used to monitor atmospheric ascospore concentrations as an indicator of spore release patterns and to forecast disease are extremely expensive and require frequent visits to the field and timely analysis to complete spore counts. Use of petal infestations to evaluate risk of sclerotinia stem rot was suggested by Gugel and Morall (1986) and Turkington (1991). Monitoring of petal infestations can be accomplished by collecting petals in the field and plating petals on a nutrient based agar. For large scale sampling in commercial fields, this method can be

quite costly. It takes approximately 7 days to observe and identify *S. sclerotiorum* mycelium from a plated petal containing ascospores; which is often too late for chemical applications. The relationship between petal infestation and disease incidence was often weak and therefore not a useful indicator in forecasting (Turkington, 1991). Several studies have proven that microclimate can be a predictor of disease development, however microclimate stations are costly and difficult to use compared to weather stations. A link between weather, microclimate and disease development is required to predict sclerotinia stem rot risks. Weather data is widely available and has the potential to be of great benefit in determining the need for chemical applications based on risks associated with weather.

1.6 Research Objectives

The specific objectives of this study were:

1) To relate weather to sclerotinia stem rot disease and determine the impact of microclimatic conditions in varying canola canopy densities on *S. sclerotiorum*
2) To determine the impact of a sclerotinia infected field containing a non-host wheat crop on sclerotinia disease development and spread by spore dispersal

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2. CANOPY DENSITY, MICROCLIMATE AND WEATHER IMPACTS ON SCLEROTINIA STEM ROT DISEASE IN CANOLA

2.1 Abstract

Due to the lack of commercially available resistant cultivars, management of sclerotinia stem rot (SSR) disease by fungicide application and through cultural practice is essential. Fungicide application must be properly timed through disease forecasting which can be accomplished through the understanding of weather impacts on SSR. More importantly, microclimate more closely affects *S. sclerotiorum* development and can be modified through canopy density alterations. Above and below canopy conditions were evaluated for their impact on SSR from ascospore release and dispersal during flowering to disease incidence at harvest. Three seeding rates were chosen to create variation in canopy density and microclimate in a randomized complete block design in two Manitoba locations (Winnipeg and Carman) during 2011 and 2012. Although cooler, moist microclimates were experienced under denser canopies, ascospore release was not affected. Percentage of infection was higher among denser canopies only in 1 of the 4 site-years. Ascospore concentrations were elevated almost simultaneously at both sites in both years indicating that the regional weather conditions play an important role considering the similar climates experienced throughout Manitoba. Peaks in daily mean ascospore concentrations occurred after a precipitation event following conditions of prolonged elevated relative humidity. Reduced temperatures and increased relative humidity were responsible for the increased mean ascospore concentrations over the sampling period and percentages of infection in Carman compared to Winnipeg. Results

of this study indicate that the environment plays a role in disease development, however slight canopy density modifications are not sufficient to create favorable or unfavorable microclimates for SSR.

2.2. Introduction

In western Canada, sclerotinia stem rot (SSR) is one of the most serious diseases of canola, caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary (Manitoba Agriculture, 2013). Considerable variation in disease incidence is evident among spring-sown canola crops, especially in this region (Turkington and Morall, 1993) ranging from 5 to 100% (Manitoba Agriculture, 2014). Premature ripening, wilting and lodging occur as a result of this pathogen in canola crops (Manitoba Agriculture, 2014). Due to severe economic losses to canola crops exposed to SSR, management of the disease is increasingly important. Primary control of SSR is through the use of fungicides, although this method can be quite costly, especially where risks are low. It is suggested that fungicides are to be applied once in a single event during full flowering at growth stage 65 (Lancashire et al., 1991;Twengstrom et al, 1998), however it is not always beneficial to apply fungicides at this stage due to the sporadic nature of the pathogen (Jurke and Fernando, 2008). To improve risk evaluation in order to better time fungicide applications and in order to decide when fungicide application is cost effective, several researchers have attempted to develop, evaluate and improve predictive models (Turkington et al., 1988; Turkington et al., 1990, Turkington et al., 1991, Turkington and Morall, 1993; Twengstrom et al., 1998; Koch et al., 2007; Bom and Boland, 2000). Better understanding of the influence of weather factors is required to increase fungicide application efficiency. Cultural control methods are also available as alternatives to the use of fungicides, however their ability to reduce or control disease remains debatable.

Forecasting models for SSR in canola by use of petal infestations, crop density and weather have been used in Canada (Turkington et al., 1991; Turkington and Morall, 1993). Factors such as disease incidence in the last host crop, sclerotia germination, petal infestation, crop height, precipitation, soil moisture, leaf wetness weather forecast and regional risk are all correlated to disease incidence (Twengstrom et al., 1998; Bom and Boland, 2000; Clarkson et al., 2003). Gugel and Morall (1986) found a significantly positive relationship between sclerotia germination and disease incidence in western Canada. When weather is similar across various treatments, petal infestation is the factor accounting for the majority of variation in disease incidence (Bom and Boland, 2000). Petal infestations during early bloom stages have a long period for establishment on the main stem and therefore relate to disease incidence more closely (Gugel and Moral, 1986).Weather variables that were positively correlated with high disease incidence (>20%) were soil moisture, crop height, rainfall and presence of apothecia on the soil surface (Bom and Boland, 2000). Precipitation occurring at the end of flowering also increases disease incidence (Bom and Boland, 2000). Sclerotia germination has also been modeled based on temperature to predict disease on lettuce, however sclerotia germination was the only phase of the disease life cycle examined and therefore does not accurately predict disease incidence on its own (Clarkson et al., 2003). In several crops, including beans, leaf wetness and prolonged high relative humidity is required for infection and disease development (Torés and Moreno, 1991; Abawi and Grogan, 1979). The highest values for disease incidence in canola tend to occur during years with extremely wet springs and early summers (Koch et al., 2007). Lower July and August temperatures have also been linked to increased disease prevalence as sclerotinia thrives

in cool climates (Workneh and Xuang, 2000). Most rapid SSR development occurred at 16°C to 22°C on winter oilseed rape stems (Koch et al., 2007). Prevailing dry weather throughout the season can be responsible for low sclerotinia incidences due to the dependency of the disease on moisture (Morall and Dueck, 1982).

Under warm, dry conditions, misting may increase SSR disease incidence and severity. Reduced temperature (approximately 15°C) and increased soil moisture and leaf wetness in irrigated plots (Weiss et al., 1980) favour sclerotia germination (Teo and Morall, 1985; Boland and Hall, 1987; Teo at al., 1989; Matheroon and Porchas, 2005; Mila and Yang, 2008; Wu and Subbarao, 2008) and enhance development of white mold disease in dry edible beans in semi-arid regions (Blad et al., 1978). Leaf wetness is required for colonization, germination and infection of ascospores on nutrient containing petal tissues for development of stem rot disease in canola (Bardin and Huang, 2001; Huber and Gillespie, 1992). At high temperatures, reduction in spore survival on plant tissue is possible and can reduce infection as a result (Clarkson et al. 2003). As a result of misting, canopy structures are modified to increase crop height and leaf area index creating denser canopies and favorable microclimatic conditions for disease development (Blad et al., 1978).

To date, few studies have been completed on the effects of plant density and misting on SSR in canola crops. Canopy density alterations have been proven to relate to sclerotinia stem rot incidence in other crops including beans (Blad et al., 1978; Weiss et al., 1980), rapeseed (Nordin et al., 1992) and several other crops (Krupinsky et al., 2002). Proven reduction in disease incidence as a result of decreasing canopy densities was

demonstrated by both reducing seeding rates (Jurke and Fernando, 2008) and increasing row spacing (Steadman et al., 1973). Lodging is increased under dense canopies favouring within canopy moisture and contact between plants (Jurke and Fernando, 2008). Variety selection is also important in the modification of crop density and height which are both proven to relate to disease incidence (Turkington and Morall, 1993). Canopy density has also been included as a predictive factor in several disease forecasting models as a contributor to disease development (Twengstrom et al., 1998; Bom and Boland, 2000; Government of Saskatchewan, 2009; Government of Alberta, 2010). In contrast, plant density and disease incidence were not correlated in a study conducted by Nordin et al. (1992) in Sweden and by Koch et al. (2007) in Germany. In Koch et al. (2007) , the number of oil seed rape plants seeded had no significant effect on disease due to the capability for plants to fill the void space where seeding rates were decreased. Additional study is required on the effects of misting and modified canopy densities on disease incidence.

Release of airborne ascospores from apothecia is a critical stage in the disease cycle. It has been debated by Harthill (1980) and Clarkson et al. (2003) whether ascospore release occurred mainly during the day or at night. Light was required in Harthill's (1980) study and spore release occurred in both light and dark conditions in Clarkson et al.'s (2003) study. Hourly spore release observations conclude that ascospore dispersal occurs in a single event lasting between four to six hours daily (Qandah and Mendoza, 2011). During wet years larger ascospore releases occurred during the day, whereas in dry years smaller releases occurred at night (Qandah and Mendoza, 2011). Similar to previous findings (McCartney and Lacey, 1991), ascospore release was

preceded by an increase in temperature (above 20°C) and drop in relative humidity (below 80%) following periods of elevated relative humidity during wet years (Qandah and Mendoza, 2011). Ascospore release and dispersal is largely influenced by precipitation as shown by Qandah and Mendoza (2012), where shallower spore dispersal gradients were generated as ascospores are capable of travelling further distances in wet years. No spore production occurred during a dry year indicating that moisture is a major requirement for spore release (Qandah and Mendoza, 2012). Further, mean percentage of petal infestations determined through petal samples obtained from canola crops showed higher petal infestations mid-day compared to the morning (Turkington et al, 1991), which may be explained by elevated ascospore release occurring in the afternoon (Harthill, 1980; Qandah and Mendoza, 2008). Airborne ascospore concentrations are closely associated with the number of apothecia produced (McCartney and Lacey, 1991). Below canopy ascospore concentrations were also positively associated with disease incidence (Qandah and Mendoza, 2012). Above canopy ascospore concentrations have not yet been evaluated against disease incidence and severity, nor has the influence of microclimate on ascospore release been studied.

The objectives of this study were to determine the influence of weather on disease development as well as to examine the impact of modified canopy density on microclimate, ascospore release and disease incidence. This study focuses on the modification of several microclimates using three seeding rates and three fertilizer treatments measured to compare ascospore release and disease incidence. Ascospore release was also compared daily during varying weather conditions during two growing

seasons under both misted and non-misted canola plots. Disease was then correlated to weather and microclimatic conditions.

2.3 Methods

2.3.1 Study Sites

Two plot trials were established in this study during the 2011 and 2012 growing seasons at two locations in southern Manitoba, Canada. The plots at the Carman Field Station were situated on a well-drained clay loam soil (Manitoba Agriculture, 2013). The plots at "The Point", University of Manitoba, Winnipeg were on clay soil (Manitoba Agriculture, 2014).

The history of each of the sites used in this study is important as the type of crops grown and past disease incidence is essential in understanding the results from the study. The Carman 2011 plot had been seeded with soybean in 2010 and sunflower in 2009, both of which are susceptible to *Sclerotinia sclerotiorum*. Sclerotinia stem rot disease was found throughout the field in 2010 and was therefore likely still present in 2011. The Winnipeg 2011 plot had been seeded with canola in 2008, flax and winter wheat in 2009 and winter wheat in 2010. No sclerotia were identified. Flax and wheat are non-host crops of *Sclerotinia sclerotiorum*. In 2012, the plots used in both locations had been planted to non-host crops in 2011, flax in Carman and wheat in Winnipeg.

2.3.2 Field Preparation

2.3.2.1 2011 Growing season

In 2011, seeding in Winnipeg took place on May 26th. The portion of the field being used was approximately 34 metres wide by 120 meters long. Prior to seeding, the field was sprayed with glyphosate to eliminate weeds and the seeds were treated with Helix XTra ® treatment to control flea beetles (*Phyllotreta spp.*). The field received no

tillage. Fungicides were not applied to ensure the presence of sclerotinia. The south portion of the field (34m wide by 60m long) was seeded with *Brassica napus* canola (Westar). The canola was divided into 18 equal-sized plots that were 10m by 10m, with 2m gaps between each plot running from west to east (Figure A.4). The plots contained 3 seeding density treatments in 3 replicates in a randomized-complete block design and used two precipitation environments (natural rainfall only and wet continuously with a misting system). Seed and fertilizer rates are shown in Table 2.1. Fertilizer rates applied to the soil were determined based on recommendations provided by Agvise Laboratories Inc. Soil samples were taken from 0 to 6 inches and 6 to 24 inches on May 6th, 2011 and sent to Agvise for analysis of soil nutrient status.

Due to technical difficulties, the south 2m of plots 7 (low seeding) and 8 (medium seeding) in Winnipeg were incorrectly seeded with a high seeding rate in the plot allocated as "low seeding" and no seed in the plot allocated as "medium seeding". Plot 8, allocated as medium seeding was seeded by hand on June 10th, 2011. These modifications should not have impacted the overall analysis.

In 2011, the Carman plot was seeded June 8th. The site was approximately 54 by 60 metres in size. Prior to seeding, the field was conventionally tilled. No herbicides were applied to the field prior to seeding and the seeds were treated using Helix XTra treatment. No fungicides were applied to ensure the presence of disease. A 30m wide by 70m long area was seeded to Westar, which was similarly divided into 18 equal plots (10 m by 10m). Two meter gaps separated each plot. Fertilizer rates applied were similar to

the rates applied in Winnipeg and based on recommendations provided by Agvise Laboratories Inc. (Table 2.1).

No seed was applied to a 2 m wide strip in plot 1 (high seeding) and a very high seeding rate was applied to a 2m strip in plot 6 (medium) in Carman because the cone on the seeder was incorrectly tripped. These changes should not have impacted the overall analysis.

Table 2.1 Seeding and fertilizer treatment rates for Winnipeg and Carman field sites in 2011 and 2012.

	Canopy Density Treatment	**Seeding Rate (lb/ac)**	**Fertilizer Type**	**Fertilizer Rate (kg/ha)**
Canola	Low	5.53	22-0-0-24 (Ammonium Sulphate)	63
			11-52-0 (MAP)	25
			46-0-0 (Urea)	25
	Medium	11.06	22-0-0-24 (Ammonium Sulphate)	63
			11-52-0 (MAP)	25
			46-0-0 (Urea)	84
	High	16.59	22-0-0-24 (Ammonium Sulphate)	63
			11-52-0 (MAP)	25
			46-0-0 (Urea)	168

2.3.2.2 2012 Growing Season

Fall field preparation for both sites for the 2012 growing season was completed by November 2nd, 2011. Carman and Winnipeg field layouts were similar to those in 2011 at each location. Pre-emergent Edge was applied to the soil at both the Carman and Winnipeg canola portions of the fields on October 19th and 11th respectively for weed control in 2012. A harrow was then used to trap granules at the soil surface directly after the application of Edge. One week later, the fields were harrowed again at a 90 degree angle to further incorporate the herbicide.

During the spring of 2012, roundup was applied to the field in Winnipeg for weed control and Carman received tillage during the spring prior to the growing season. Similar to 2011, seeds were treated with Helix seed treatment and seeding and fertilizer rates remained the same (Table 2.1). Winnipeg and Carman were seeded on June 6th and May 23rd respectively. Equinox EC with merge surfactant was applied to the canola in Carman on June 22nd to eliminate grassy weeds.

Due to technical difficulties operating the misting system, no misting occurred in 2012 at either field location. Thus, there were two identical sets of plots at each location, each with 3 replications of high, medium and low crop densities following a randomized-complete block design under natural environmental conditions (Figure 2.1).

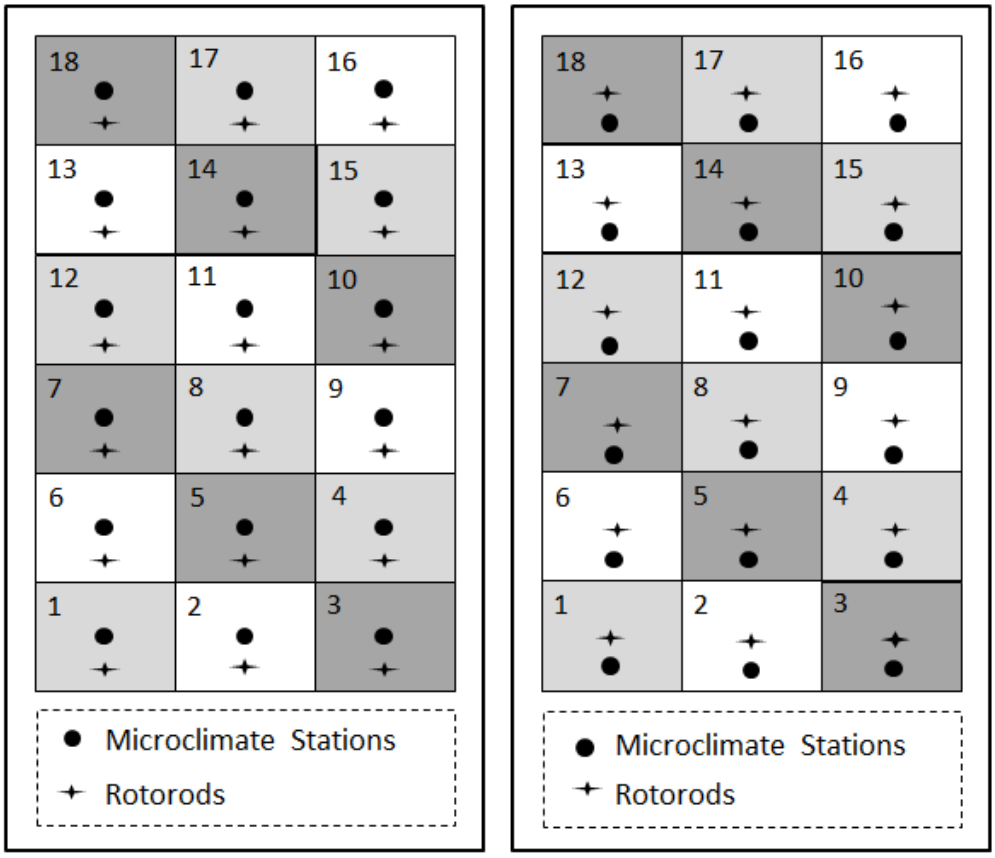

Figure 2.1 Canola field set up for Winnipeg and Carman in 2011 (left) and 2012 (right). High, medium and low seeding and fertilizer rates are represented by dark grey, light grey and white shades within canola plots.

2.3.3 Inoculation

Both the Carman and Winnipeg sites were inoculated with sclerotia after seeding to ensure the presence of the pathogen. Since sclerotia require conditioning throughout the winter, the inoculum was placed outside in January, 2011 inside nylon mesh bags and then covered by snow for the duration of winter. Upon snowmelt, the mesh bags were removed from the soil and placed in a refrigerator to avoid sclerotial germination.

On June 6th 2011, sclerotia were taken out of the refrigerator and weighed. The experiment at The Point in Winnipeg was inoculated with 60 grams of sclerotia placed on each of the 18 canola plots. The sclerotia were spread by hand by tossing the inoculum into each of the plots. There was no known amount of sclerotia present within this field from previous years. Inoculation of sclerotia in Carman occurred on June 15th, 2011 with 60 grams of sclerotia placed within each of the 18 canola plots. There were sclerotia present in this field from the 2010 growing season, however the amount of sclerotia from the previous year was unknown.

For the 2012 growing season, fall field preparation in 2011 included the inoculation of sclerotia onto each of the Winnipeg (November 2nd, 2011) and Carman (October 26th, 2011) field sites. Equal amounts of sclerotia were applied by hand to both fields in the fall to allow conditioning to occur throughout the winter. In both fields, 66g of sclerotia was applied to each of the 18 canola plots. During the spring of 2012, an additional 40g of sclerotia was applied to each of the 18 canola plots in Winnipeg and Carman on June 5th and June 14th respectively.

2.3.4 Microclimate Monitoring

Microclimate was monitored throughout each individual plot during the two growing seasons using individual HOBO microclimate stations in each plot (Figures A.3, A.4 and A.5). Microclimate measures included air temperature, relative humidity, leaf wetness and surface soil temperature and moisture. One microclimate station was placed in each of the plots situated within the canola fields (Figure 2.1). In 2011, the microclimate stations were placed in the fields in Winnipeg and Carman on June 6th and June 17th respectively, once the crops had emerged and were at the seedling stage. In 2012, stations were installed in the Winnipeg and Carman fields on June 26th and June 13th respectively. Soil moisture and temperature probes were placed in the soil vertically using a trenching shovel to dig the holes to 8 cm and the surrounding area was replaced with soil. The radiation shield, containing the relative humidity and air temperature probe, was placed facing northwards at approximately 10 cm above the soil surface to account for microclimate conditions near the surface at both Carman and Winnipeg locations. Microclimate stations were placed in the center of each plot in 2011 and a quarter way into the plot in 2012 (Figure A.4).

Leaf wetness sensors and relative humidity and temperature sensors within the solar radiation shield were adjusted throughout both growing seasons. Leaf wetness, temperature and relative humidity sensors were placed at 12 cm in Winnipeg and Carman on June 28th and July 17th respectively in 2011 and on June 26th and June 13th respectively in 2012. In 2011, leaf wetness, temperature and relative humidity sensors were raised to

24 cm on July 7th (Winnipeg) and July 21st (Carman). In 2012, sensors were placed at 24 cm on July 4th (Winnipeg) and June 21st (Carman).

The presence of weeds within each plot affected canola growth and may also have impacted microclimate. In 2011, at Winnipeg, weeds were present in strips within the plots running through the microclimate stations locations in several plots. Plots were characterized as having high, medium and low weed presence. Plots with heavy weed presence included plots 2, 5, 6, 8, 14 and 18. Some weeds were found in plots 1, 7, 9, 11, 12, 16 and 17 while little to no weeds were found in plots 3, 4 and 15. During the flowering period, weeds were less apparent. In Carman 2011, weeds were heavily present in plots 11, 13 and 15 while some weeds were present in plots 1 and 10. Little to no weeds were observed among the remainder of the plots in Carman. Due to the presence of heavy weed infestations in Carman in some plots where microclimate stations were situated, stations were moved on July 12th, 2011 at 10:00 am to better represent microclimatic conditions in canola among the plots. In 2012, weeds in Carman were sufficiently controlled to avoid any impacts to canola growth and microclimate monitoring. In Winnipeg, the majority of weeds were present in plots 13, 14, 16, 17and 18 and were characterized as having a medium weed presence.

Surface soil volumetric water content (m^3/m^3) was measured using soil moisture smart sensors (S-SMC-M005) that work with HOBO data loggers to determine the dielectric constant in the soil.

In order to calibrate the soil moisture smart sensors, soil samples were taken during four sampling periods at each site throughout each growing season. In 2011, during a soil sampling period, several gravimetric and volumetric soil samples were taken throughout the field. Three samples were taken in every plot next to each soil moisture smart sensor. A volumetric soil surface sample was taken by scooping soil into a labelled aluminum tin and covering the sample with a lid to avoid evaporation. A gravimetric soil surface sample was taken by scooping a sample of surface soil into a labelled plastic bag using a small shovel. The number of volumetric and gravimetric samples taken per plot varied due to the amount of aluminum tins available, although at least one volumetric sample was taken per plot. Samples were then placed in a cooler and brought to the lab for further analysis. Soil samples were weighed and dried in an oven at 105 degrees Celsius for 24 hours, then weighed again to get a measure for gravimetric water content. Volumetric water content was also determined for some of the samples by incorporating the known volume of the aluminum tin into the calculation. In 2012, only volumetric samples were taken using aluminum cores. Cores containing the samples were placed in a larger tin with a lid and brought back to the lab in a cooler. Volumetric soil moisture content was also calculated using the weighing and drying method as described above. The volume of the core was incorporated in the calculation.

Calculations for gravimetric and volumetric soil moisture content are as follows:

Gravimetric moisture content

$$GM = (WS(g) – DS(g)) / DS(g) \quad (1)$$

Where GM is gravimetric moisture content, WS is the wet soil before drying in grams and DS is the dry soil after drying in grams. All three gravimetric values per plot were averaged to get average gravimetric soil moisture content values per plot.

Bulk Density (g/cm^3)

$$BD(g/cm^3) = DS(g) / V(cm^3) \quad (2)$$

Where BD is bulk density V is the volume of the aluminum tin or core containing the soil. An average bulk density (g/cm^3) was obtained for the entire field.

Volumetric Moisture Content

$$VM(g/cm^3) = BD(g/cm^3) \times GM \quad (3)$$

$1\ g/cm^3 = 1m^3/m^3$

2.3.5 Weather Station Monitoring

Standard weather data was monitored at both locations throughout the growing season. Weather data was recorded at The Point in Winnipeg from June 2nd, 2011 through August 8th, 2011, and from June 1st, 2011 through August 26th, 2011 in Carman at the Ian N. Morrison Field Station. A Campbell Scientific Inc. weather station was set up on the southwest corner of field 8 located in Winnipeg on June 1st, 2011. Air temperature (˚C),

relative humidity (%), wind speed (km/h), solar radiation (W/m^2 and MJ/m^2) and precipitation (mm) were measured through this station. The air temperature and relative humidity probe was enclosed in a radiation shield and was placed at a height of 1.5m above the soil surface. The anemometer was placed at a height of 3m and pyranometer at 2m above the soil surface. A tipping bucket rain gauge was placed alongside the weather station at a height of 30cm above the surface. Each of the instruments was hooked up to a data logger (10X) which averaged data hourly and daily. A watchdog station was also placed alongside the weather station as a backup and to obtain additional data for wind direction (degrees) through the use of a wind vane. In Carman, similar weather data was obtained online from the Carman Field Station, less than 2 km from the field through a weather station that was previously set up by Manitoba Agriculture, Food and Rural Development (MAFRD). A tipping bucket rain gauge was also placed alongside the field at 30cm above the soil surface in Carman to serve as backup for precipitation data.

Due to technical issues with the Watchdog Station at The Point in Winnipeg, Watchdog data is only available from June 2, 2011 through June 29th, 2011 and July 27th, 2011 through August 8th, 2011.

2.3.6 Ascospore Dispersal

2.3.6.1 Rotorod Spore Sampler

The release and dispersal of spores by apothecia was monitored through the use of rotorods (Multidata LLC) (Figure A.6). The rotorod spore sampler is a device containing a sampling head which holds 2 polystyrene collector rods. The sampler was operated on a timer that intermittently spun the rods in a clockwise direction at a speed of 2400 RPM

capturing airborne spores. The rods spin for 1 minute and rest for 9 minutes, therefore collecting fungal spores 10 percent of the time. The surface of the rod facing the direction of the wind while spinning was marked as the leading edge and pre-greased with a silicon solution to provide a "sticky" surface for the collection of spores. Rods were greased in the laboratory by holding one end of the rod (the end being placed into the spinning head) and applying the silicon grease to the one side by hand ensuring the silicon was spread as evenly and thinly as possible. Each rotorod was interconnected by 10 and 12 gauge electrical wiring and hooked up to a timer, which is connected to a 12 volt battery. The battery was also connected to a solar panel which provided additional power and recharged the battery.

In each field, one spore sampler was placed in each of the 18 canola plots to monitor spore dispersal variation among the various crop densities. Rotorods placed within canola plots were elevated directly above the crops. All rotorod samplers placed in Winnipeg contained retracting heads that exposed the spore collecting rods only when the heads were spinning. Rotorods placed in Carman contained fixed heads in which the spore collecting rods are always facing downwards. Rods were replaced daily and placed in centrifuge tubes for further analysis. In 2011, rotorods were set up in Winnipeg on June 20th and in Carman on July 5th. In 2012, rotorods were set up and monitored beginning on June 27th in Carman and July 7th in Winnipeg.

2.3.6.2 Collector Rod Sample Assessment

The rods removed from the field were analyzed under a microscope at 400x magnification and fungal ascospores of *S. sclerotiorum* were counted. Identification was

based on ascospore morphology. Ascospores are single-celled, hyaline in appearance, bi-nucleate and are ellipsoid in shape and 4-6 x 9-14 µm in size (Kohn, 1979). Spores were not counted if they could not be identified. Since the collector rods recovered so many spores and other particles, analyzing an entire rod was impractical therefore an abbreviated analysis was done. A blank slide was first prepared by permanently marking a solid line at each 1.375 mm, creating sixteen 2.09 mm^2 sampling areas. *Sclerotinia sclerotiorum* ascospores were then counted within the circular lens view area once in each sampling area. Only ten of sixteen sampling areas were used because the first six were generally those with the black mark that was placed and held within the sampling head of the rotorods. The total number of ascospores per rod was then calculated based on the counts obtained in each lens view by the following calculations:

Circular lens view area of microscope formula:

$$A = л\,(r)^2 \qquad (4)$$

$A_1 = л\,(0.275\ mm)^2 = 0.238\ mm^2$ (Olympus BX51)

$A_2 = л\,(0.24\ mm)^2 = 0.181\ mm^2$ (BH-2)

$A_3 = л(0.24\ mm)^2 = 0.181\ mm^2$ (Hund Wetzlar Typ H 600/12)

Where "A_1, A_2 and A_3" are the lens view areas of the circle for microscope 1 (Olympus BX51), microscope 2 (BH-2) and microscope 3 (), "л" is pi (3.14159265) and "r" is the radius of the lens view.

Total area for all 10 spore counts:

$A_1(10) = 0.238\ mm^2\ (10) = 2.38\ mm^2$ (Olympus BX51) (5)

$A_2(10) = 0.181\ mm^2\ (10) = 1.81\ mm^2$ (BH-2)

$A_3(10) = 0.181\ mm^2\ (10) = 1.81\ mm^2$ (Hund Wetzlar Typ H 600/12)

Where "A(10)" is the total area for all 10 spore counts.

Multiplication factor for entire rod for each microscope:

$M_1 = 22\ mm^2 / 2.38\ mm^2 = 9.24$ (Olympus BX51) (6)

$M_2 = 22\ mm^2 / 1.81\ mm^2 = 12.15$ (BH-2)

$M_3 = 22\ mm^2 / 1.81\ mm^2 = 12.15$ (Hund Wetzlar Typ H 600/12)

Where M_1, M_2 and M_3 are multiplication factors used to calculate total spore counts. The spore counts for each area were then multiplied by the multiplication factors (M_1, M_2 and M_3) to get the total number of spores for each rod.

$TS = S(10) \times M_1$ (Olympus BX51) (7)

$TS = S(10) \times M_2$ (BH-2)

$TS = S(10) \times M_3$ (Hund Wetzlar Typ H 600/12)

Where TS is the total spore count for a rod, S(10) is the spore count over 10 lens view areas.

The total spore count was then divided by the volume of air sampled ($3.12\ m^3$). Occasionally samples were taken slightly before or after one day therefore the number of

minutes during the sampling period was divided by the total minutes in a day (1440) and multiplied with the volume of air sampled. The counts for each rod were then divided by the resulting volume of air sampled.

The values obtained for each of the two rods were then averaged to get a mean value of ascospores per metre cubed per rotorod per day:

$$\text{Ascospores/m}^3 = (\text{TS(rod 1)} + \text{TS(rod 2)}) / 2 \quad (8)$$

Occasionally rods could not be counted or contained no spores for several reasons. Rods that could not be counted properly had lots of dirt on them, large sections of the rod were covered by a bug or large sections of the rod contained no spores. Rods containing either no spores or dirt on them were generally those that had been placed in the rotorod backwards and also could not be counted. Some rods were also missing meaning that they may have been dropped in the field and not found. Where only one of the two rods from a rotorod was counted, the value from that rod alone was counted as the mean daily ascospore concentration. Where neither of the two rods could be counted, gaps in the data resulted.

2.3.7 Petal Sampling

Canola petal samples were taken twice throughout the flowering period, at early and late bloom stages, at both locations to determine whether ascospores were present on the petals. In 2011, samples were taken at 30 and 70% bloom, in Winnipeg on July 6th and 14th and in Carman July 18th and 25th. Sixteen flowers were sampled at random in

each plot, from plots 1 through 18 at both locations. The lowermost flowers were taken in eight of the sixteen flower samples in each plot, while the uppermost flowers were taken for the remaining eight samples. In 2012, nine random samples were taken per plot at each site during each sampling period; at 15%, 30% and 70% bloom. Samples were taken on July 5th, July 9th and July 18th in Carman and on July 13th, July 19th and July 24th in Winnipeg. Each flower was cut from the stems using scissors, and placed into a small labelled freezer bag within a cooler containing ice, then brought to the lab to be plated.

In 2011, a Rose Bengal Agar was used as the medium for plating petals. One petal from each flower was plated on Rose Bengal Agar (Bom and Boland, 2000) immediately after sampling. As per Bom and Boland (2000), Rose Bengal Agar was prepared by first mixing 20 grams per litre (g/L) potato dextrose agar (Difco) with 30 parts per million (ppm) Rose Bengal. The mixture was then autoclaved for 15-20 minutes. 20 parts per million (ppm) of streptomycin sulphate was added after cooling. In 2012, a semi-selective media containing bromophenol blue was used for the petals to improve the ability to identify ascospores of *S. sclerotiorum* (Steadman et al., 1994). At a pH of 4.7 or lower, bromophenol blue changes from blue to yellow; a color change caused by the presence of *S. sclerotiorum* due to the production of oxalic acid. A solution of 39g/L potato dextrose agar (Difco), 25 mg/L of 75% pentachlorobenzene (PCNB),150 mg/L of penicillin G, 150 mg/L of streptomycin sulfate and 50 mg/L of bromophenol blue was made. Potato dextrose agar was autoclaved for 15-20 minutes before the remaining sterilized ingredients were added. The prepared solutions were then poured into plates and left to cool. Within a fume hood, four individual petals were cultured on each labelled plate using forceps to pull the petals from each flower. Petals were then placed on the medium

while ensuring that the entire surface of each petal was touching the agar medium. Prior to plating each petal, forceps were dipped in an alcohol solution and flamed to remove any contamination. Plates were left at room temperature for 14 days. Observations were made after 14 days and colonies of *S. sclerotiorum* were identified.

For petals plated in 2011 on Rose Bengal Agar, petals containing *S. sclerotiorum* ascospores were described as having a colony of white loosely interwoven mycelium filaments, large black fruiting bodies (sclerotia) produced above the mycelium and adjacent agar medium turning from pink to clear. Petals that did not contain ascospores were frequently not diseased and only the petal on the rose Bengal agar was observed. Sometimes yellow, green or white fluffy mycelium was present which represented other fungi and were not characteristic of *Sclerotinia* ascospores. Several plates that did not contain ascospores also contained small fruiting bodies as well as adjacent agar medium turning from pink to orange. White-grey or colonies present on the agar plates did not originate from the petals, and were contaminants likely introduced while plating the petals.

Petals plated on the semi-selective media in 2012 containing *S. sclerotiorum* ascospores were identified by observing a color change in the medium from blue to yellow. Since other fungal pathogens also create this color change including *Penicillium* and *Aspergillis*, sclerotia must also be found on the plates in order to positively identify *S. sclerotiorum*.

2.3.8 Misting

During the 2011 growing season, half of the canola plots in each field were misted to ensure continual moisture for disease development and to provide a range of microclimatic conditions. The remaining plots were not misted to represent standard weather conditions. Misting occurred in Winnipeg plots 10 through 18, 1 through 9 in Carman. Misting in 2011 began at the 20% bloom stage on July 5th and July 15th in Winnipeg and Carman respectively. Ten days after the final canola petal had fallen, misting ceased on July 31st (Winnipeg) and August 10th (Carman). Misting began daily at 4 pm and was left on until the 1,200 gallons (4,542 L) emptied. Approximately 26,250 gallons (99,367 L) of water was irrigated onto the misted field portions in total.

The misting system at each site consisted of a tank capable of holding 1,250 gallons (4,732 L), a pump and a generator operated with fuel to provide energy to the pump. The pump contained a timer which allowed for misting to occur for 5 minutes out of every hour. The system was set up so that piping ran from the pump to the misted field portions. Each misting head was secured to a pole, 4.5 feet (1.37 metres) high, which was placed in the soil where misting was required. A single misting head was capable of misting up to a radius of 2.5 meters, covering an area of approximately 19.6 square meters total. Four misting heads were placed at equal distance in each plot, each being 5 meters apart.

Neither the Winnipeg nor Carman plots were misted in 2012 due to technical issues and availability of generators and pumping systems. This created two sets of completely randomized block designs in each of the Winnipeg and Carman fields in 2012.

2.3.9 Crop Development

Crop development of canola was observed throughout each growing season. The development stage and the date at which each stage was observed in the field are listed in Table 2.2 and illustrated in Figures 2.2 and 2.3. Development stages are approximated to when the fields were visited. There was slight variation among growth stages within different canopy densities in each field. Growth stages for canola were based on those described by the Canola Council of Canada (2011).

Table 2.2 Growth stages of Canola and date at which these stages were initially observed at Carman and in Winnipeg.

		Winnipeg	**Carman**
Year	**Development Stage**	**Day**	**Day**
2011[z]	Seedling Cotyledon	June 8th	June 15th
	Rosette Stage (with Cotyledons and Second True Leaves)	June 13th	June 20th
	Rosette Stage (4th Leaf)	June 15th	June 29th
	Budding stage	June 29th	July 9th
	Flowering stage	July 4th	July 14th
	End of Flowering stage	July 21st	August 6th
2012[y]	Seedling Cotyledon	June 10th	May 31st
	Rosette Stage (with Cotyledons and Second True Leaves)	June 22nd	June 9th
	Rosette Stage (4th Leaf)	June 26th	June 16th
	Budding stage	July 6th	June 26th
	Flowering stage	July 10th	July 1st
	End of Flowering stage	Aug 1st	July 22nd

z Winnipeg and Carman sites were seeded on May 26th and June 8th respectively.
y Carman and Winnipeg sites were seeded on May 23rd and June 6th respectively.

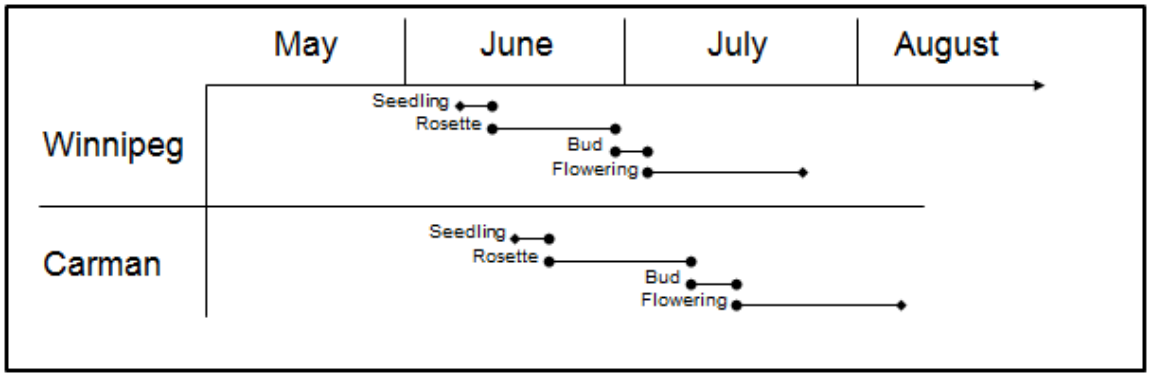

Figure 2.2 Diagram of canola growth stages in Winnipeg and Carman in 2011.

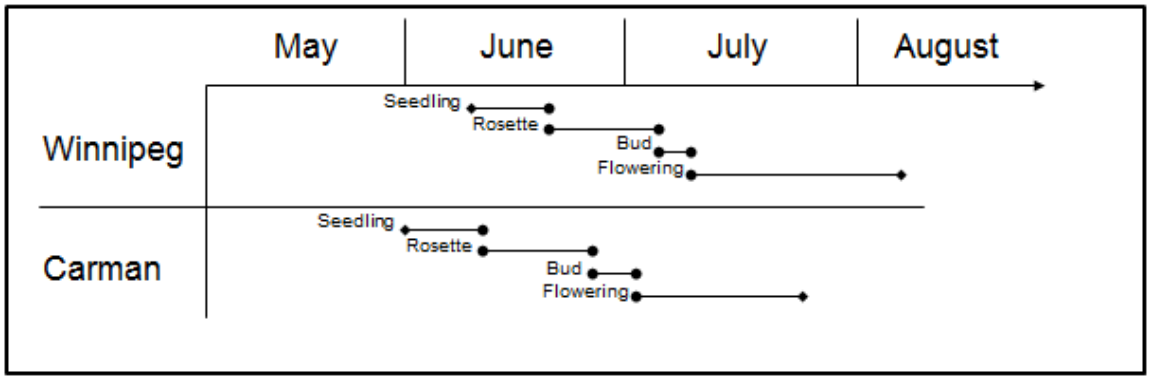

Figure 2.3 Diagram of canola growth stages in Winnipeg and Carman sites in 2012.

2.4.0 Canopy Density

Canopy thickness or density was determined using an LAI 2200 meter and by counting plants within $1m^2$ quadrats at both the Winnipeg and Carman locations. Average canopy densities were also compared between misted and non-misted field portions in 2011.

The LAI 2200 Plant Canopy Analyzer was used throughout the growing season to determine leaf area index (LAI). Leaf area index (LAI) is a measure of the leaf surface area (1 side) per unit area of the ground with values typically ranging from 0 to 6 for bare ground to very dense canopy, respectively. Each of the 18 canola plots at both locations were monitored four and five times in Winnipeg and Carman respectively in 2011. In Winnipeg, measurements were taken on June 30th, July 5th, July 12th and July 21st 2011. Measurements were taken on July 8th, July 11th, July 19th, July 27th and August 3rd, 2011 in Carman. In 2012, four measurements were taken in Winnipeg and Carman. Winnipeg measurements were taken on July 9th, July 13th, July 19th and July 26th and Carman measurements were taken on June 25th, June 29th, July 10th and July 17th.Within each canola plot, one LAI measurement was taken above the canopy, and four LAI measurements are taken below the canopy. In each plot one measurement was taken approximately 2.5m inside from each of the plot corners. An average LAI was then calculated based on the four below canopy measurements and one above canopy measurement. Measurements were generally taken before 11:30 to ensure the sensor was shaded by the sampler while taking individual measurements. Each measurement was taken at the same angle, with the sampler blocking the sun from the sensor and no view caps were used. The plant canopy analyzer sensor was placed horizontally on the surface

below the canopy for each below canopy reading, while ensuring that there were no leaves directly above the sensor.

Four $1m^2$ quadrats were placed in each plot at both locations prior to swathing in order to compare canopy densities. The total number of plants per metre square was counted in each of the quadrats. The four quadrats in each plot were then averaged to get a value of plants per meter squared representative of each plot. Plants were counted on August 5th, 2011 and August 7th, 2011 in Winnipeg and Carman respectively and on August 13th, 2012 and August 7th, 2012 in Winnipeg and Carman respectively. An additional plant count was completed on August 22nd, 2012 where two quadrats were placed in each plot.

2.4.1 Disease Incidence

Disease incidence was evaluated in Carman and Winnipeg prior to swathing by determining the percentage of *S. sclerotiorum* infected canola plants to the total number of canola plants. In 2011, disease assessments were done on August 5th and August 22nd in Winnipeg and Carman while 2012 disease assessments were done on August 13th and August 7th, respectively. Four one meter square quadrats were placed in each plot at random locations and disease counts were made. The total number of canola plants and number of plants infected by *S. sclerotiorum* were counted in each quadrat. The mean of all four quadrats in each plot was determined to get a representative percentage value for disease incidence (Jurke and Fernando, 2008). An additional abbreviated disease assessment was done on August 22nd, using two quadrats in each plot.

Diseased canola plants were identified as having bleached stems with pale grey and white lesions on stems, branches and pods. Canola plants that contained the above symptoms were also counted if they were lodging. Infected plants were also checked to determine if they were brittle and shredded (Manitoba Agriculture, 2013). Blackleg was another canola disease present at both the Winnipeg and Carman sites. Plants with blackleg were buff coloured, contained spots and small black fruiting bodies in the stems (Manitoba Agriculture, 2014). Fusarium wilt causes canola to have purple, grey or brown streaks running throughout the plant. Generally only 1 side of the plant or several branches will show symptoms of Fusarium Wilt. Only plants with symptoms for Sclerototinia stem rot disease were counted.

2.4.2 Data Analysis

Average, total, minimum and maximum values for canopy density, weather (precipitation, temperature, relative humidity and solar radiation), microclimate (temperature, relative humidity and leaf wetness) and disease development parameters were tabulated and summarized for comparison among site-years. Comparisons between Winnipeg and Carman for parameters including ascospore concentrations, precipitation, relative humidity, temperature, incoming solar radiation and wind speed were made based on timely trends observed in figures. Precipitation and ascospore concentrations were accumulated during the flowering period and compared. The effects of precipitation and misting were analyzed against ascospore concentration and disease development. Other weather and microclimate factors including temperature (above and below canopy), relative humidity (above and below canopy), solar radiation, wind speed, soil temperature and leaf wetness were also compared with ascospore concentrations and disease

incidence. Ascospore concentrations that fell between certain thresholds were averaged and graphed to demonstrate weather impacts on atmospheric spore concentrations.

A factorial analysis of variance where all cell means are compared simultaneously through a 2 x 2 x 3 design was used to examine the effects of year, location and seeding rate on the dependent variables. Dependent variables include canopy density (LAI and plant counts), disease development (ascospore concentration and percentage of infection) and microclimatic measurements. Dependent variables were also compared in a factorial analysis with location, misting and seeding rate as independent variables using data obtained from 2011. Statistical Analysis System 9.2 (SAS Institute Inc. Cary, NC), was used for the analysis of variance (PROC MIXED) for all 8 dependent variables at $p = 0.05$. Custom comparisons were made using contrasts. Tukey's HSD (honestly significantly different) test using a pair-wise comparison of differences in least squares means was used for comparison of treatment means for equal sample sizes. A Tukey-Kramer test was used for unequal sample sizes where data were missing and individual plots were removed from the analysis.

2.4 Results

2.4.1 Summary of Results

The growing season, sampling period, bloom stage and ripening period dates for each site year are outlined in Table 2.3. Monthly mean temperature and total precipitation compared to monthly means from 1981 to 2010 are shown in table 2.4. Temperatures were above normal throughout the season in all 4 site years. Precipitation was below normal in all four site years during all months except for June in Winnipeg 2011 and Carman 2012. Measurements for canopy density, weather, ascospore concentrations as well as percentage of infection are summarized in Table 2.5. Overall, highest LAI values were in Carman 2011. Generally early bloom LAI was higher compared to late bloom with the exception of Carman 2011. The highest mean plant counts were obtained in Winnipeg in 2011 while higher plant counts occurred in 2011 within each plot at both Carman and Winnipeg. Average mean infection percentages were higher in 2011 overall compared to 2012, with the highest percentages occurring in Carman compared to Winnipeg during both years. Average mean ascospore concentrations were higher in 2012 overall, with significantly higher values in Carman than Winnipeg in 2012. Higher maximum mean daily ascospore concentrations were found in Carman during both years. The late bloom stages in all site years showed higher average, minimum and maximum mean daily ascospore concentrations than early bloom stages. During the growing season, total precipitation showed the highest values in Carman 2012. Higher values were reported for Winnipeg in 2011 than in 2012. The 2012 sampling period received more rain than 2011 with higher values in Winnipeg during both years. Total precipitation

during late bloom was higher than in early bloom during all site years. Precipitation was higher during 2012 towards harvest compared to 2011. Average mean daily air temperatures were relatively consistent throughout the growing season however, in 2012 Winnipeg experienced slightly higher temperatures than Carman. During late bloom and ripening, Winnipeg also experienced higher temperatures than Carman with minimum and maximum values also being higher. Average values for daily mean relative humidity were higher in Carman than Winnipeg throughout each growing season during both years. There was no consistent difference in relative humidity between early and late bloom stages. During the growing season, average daily mean wind speed was lowest in Winnipeg in 2012 compared to other site-years when a maximum mean daily speed of only 3.3 m/s was achieved. Average total daily incoming radiation was relatively similar across all site years. Slightly higher incoming solar radiation was experienced in Winnipeg during late bloom compared to Carman during both years.

Table 2.3 Dates for growing season, sampling period, ripening, early and late bloom stages during all four site years.

	Growing Season	Sampling Period	Early Bloom	Late Bloom	Ripening
Carman 2011	June 8 - August 22	July 11 - August 17	July 14 - July 25	July 26 - August 6	August 6 – August 22
Winnipeg 2011	May 26 - August 5	July 4 - July 29	July 4 - July 12	July 13 - July 21	July 21 – August 5
Carman 2012	May 23 - August 7	July 8 - August 6	July 1 - July 11	July 12 - July 22	July 22 – August 7
Winnipeg 2012	June 6 - August 13	June 27 - July 24	July 10 - July 20	July 24 - August 1	August 1 – August 13

Table 2.4 Monthly mean temperature and total precipitation and long term monthly means in Winnipeg and Carman in 2011 and 2012.

			2011		2012	
Site	**Month**	**Long-term mean[z]**	**Monthly**	**Percent of Mean (%)**	**Monthly**	**Percent of Mean (%)**
		Temperature (°C)				
Winnipeg	**May**	11.6	-	-	-	-
	June	17	18.2	1.2	19.1	2.1
	July	19.7	22.2	2.5	24	4.3
	August	18.8	-	-	-	-
Carman	**May**	11.6	-	-	12.5	0.9
	June	17.2	17.3	0.1	18.4	1.2
	July	19.4	21	1.6	22.6	3.0
	August	18.5	-	-	19.4	0.9
		Precipitation (mm)				
Winnipeg	**May**	54.1	-	-	-	-
	June	90	114.3	127	68.3	76
	July	79.5	37.3	47	24	30
	August	77	-	-	-	-
Carman	**May**	67.7	-	-	31.6	47
	June	96.4	81.2	84	110	114
	July	78.6	45.2	58	35	45
	August	74.8	-	-	56.2	75

[z] Government of Canada. 2014. Climate.1981-2010 Climate Normals and Averages. Climate.weather.gc.ca/climate_normal/index_e.html

Table 2.5 Summary of measurements for canopy density (LAI, plant counts), weather parameters, ascospore concentrations and percentage of infection during the growing season, sampling period, ripening, early bloom and late bloom for all four site years.

Year	Location	Period	Total Precipitation (mm)	Mean Daily Air Temperature (°C)	Growing Degree Days	Mean Relative Humidity (%)	Mean Wind Speed (m/s)	Total Solar Radiation (kW/m^2)	Mean LAI	Mean Plant Counts (Plants/m^2)	Mean Ascospore Concentrations (ascospores/m^2)	Mean Percent Infection (%)
2011	Carman	Growing Season	135[z]	20 (12,26)	1441	72 (54,92)	2.4 (1.0,5.4)	6.1 (1.0,8.5)	3.46	92	-	22.5
		Sampling Period	24[z]	-	767	-	-	-	-	-	595 (64,2760)	-
		Early Bloom	5[z]	22 (15,26)	257	74 (54,92)	2.4 (1.1,3.7)	6.4 (3.5,8.1)	3.81	-	398 (64,1332)	-
		Late Bloom	11[z]	21 (18,23)	247	73 (64,83)	2.0 (1.0,3.3)	6.3 (3.9,7.9)	3.91	-	765 (203, 2760)	-
		Ripening	8	19 (15,22)	-	71 (59,81)	-	-	-	-	-	-
2011	Winnipeg	Growing Season	162[z]	20 (10,28)	1398	64 (40,90)	2.6 (0.6,6.0)	6.1 (1.6,8.5)	3.04	141	-	5.5
		Sampling Period	32[z]	-	572	-	-	-	-	-	543 (35,1988)	-
		Early Bloom	27[z]	22 (18,25)	192	61 (51,71)	2.1 (1.5,2.9)	7.1 (5.3,8.4)	3.18	-	251 (35,640)	-
		Late Bloom	34[z]	25 (19,28)	217	62 (45,74)	2.4 (0.6,4.8)	6.8 (5.2,8.4)	2.13	-	486 (55,1201)	-
		Ripening	14	22 (16,25)	-	61 (45,71)	-	-	-	-	-	-
2012	Carman	Growing Season	208[z]	19 (6,26)	1458	70 (46,90)	2.4 (0.8,5.1)	6.0 (0.9,9.0)	3.05	111	-	15.1
		Sampling Period	35[z]	-	656	-	-	-	-	-	1086 (24,5195)	-
		Early Bloom	14[z]	23 (21,26	255	69 (63,77)	1.6 (0.8,2.4)	6.4 (2.6,8.7)	3.44	-	669 (24,2685)	-
		Late Bloom	21[z]	22 (17,25)	245	75 (62,87)	2.0 (1.1,3.2)	6.0 (2.9,8.4)	3.17	-	1961 (127,5195)	-
		Ripening	27	21 (16,25)	-	94 (91,98)	-	-	-	-	-	-
2012	Winnipeg	Growing Season	129[z]	22 (11,27)	1459	64 (46,87)	1.8 (0.6,3.3)	6.1 (1.7,8.8)	3.08	76	-	3.6
		Sampling Period	53[z]	-	666	-	-	-	-	-	720 (32,2850)	-
		Early Bloom	7[z]	24 (19,27)	261	64 (53,81)	2.0 (0.8,2.9)	5.7 (1.2,8.0)	3.34	-	569 (32,1169)	-
		Late Bloom	10[z]	24 (20,26)	285	60 (53,71)	1.7 (0.8,2.8)	6.5 (4.1,7.8)	2.86	-	801 (111,2850)	-
		Ripening	39	20 (16,23)	-	64 (52,78)	-	-	-	-	-	-

[z]Represents total values

Values in parenthesis represent min and max respectively

2.4.2 Weather Impacts on Ascospore Concentrations

2.4.2.1 General Weather Patterns

Comparisons of ascospore concentrations and weather parameters were made between the Carman and Winnipeg plots in 2011 and 2012 (Figures 2.4 and 2.5). Ascospore concentrations were noticeably similar between these sites, with peaks occurring at approximately the same dates during 2011 and 2012. Weather parameters in Winnipeg and Carman including daily mean temperature, daily mean relative humidity and daily mean wind speed were similar throughout the season in 2011 and 2012. Comparisons among total daily solar radiation in Winnipeg and Carman showed similarities during 2011, but were different in 2012. Precipitation occurred on similar days in Winnipeg and Carman during both years. Higher relative humidity and lower temperatures were apparent in Carman during both years compared to Winnipeg. Increases in atmospheric relative humidity tended to occur during precipitation events in all site years.

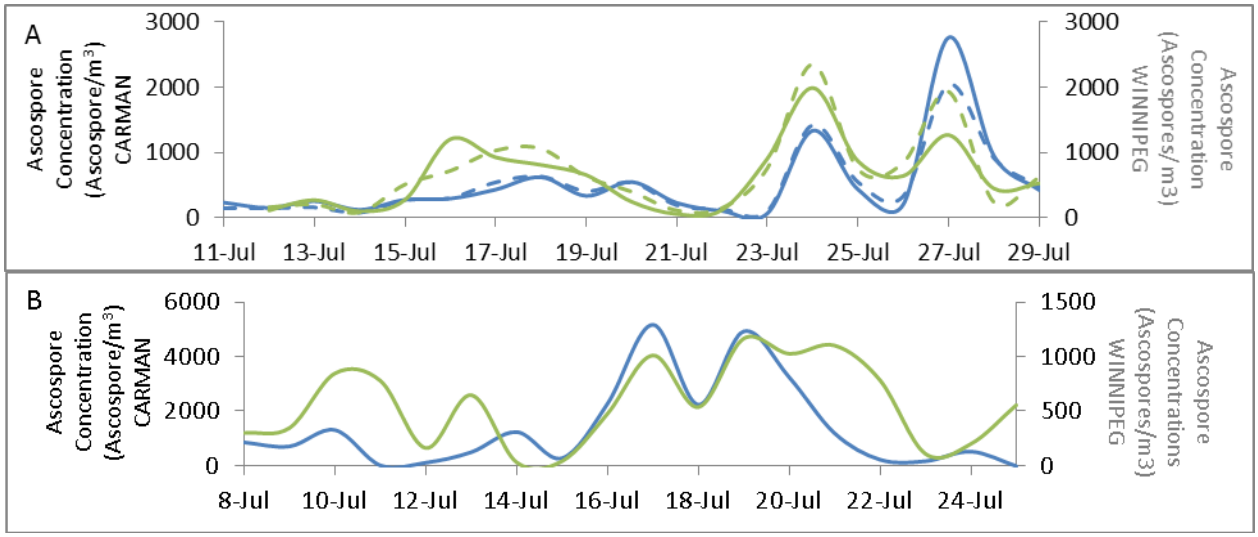

Figure 2.4 Comparison of average daily mean ascospore concentrations over the sampling period in misted (dashed lines) and non-misted (solid lines) plots in Winnipeg (green lines) and Carman (blue lines) in A) 2011 and B) 2012.

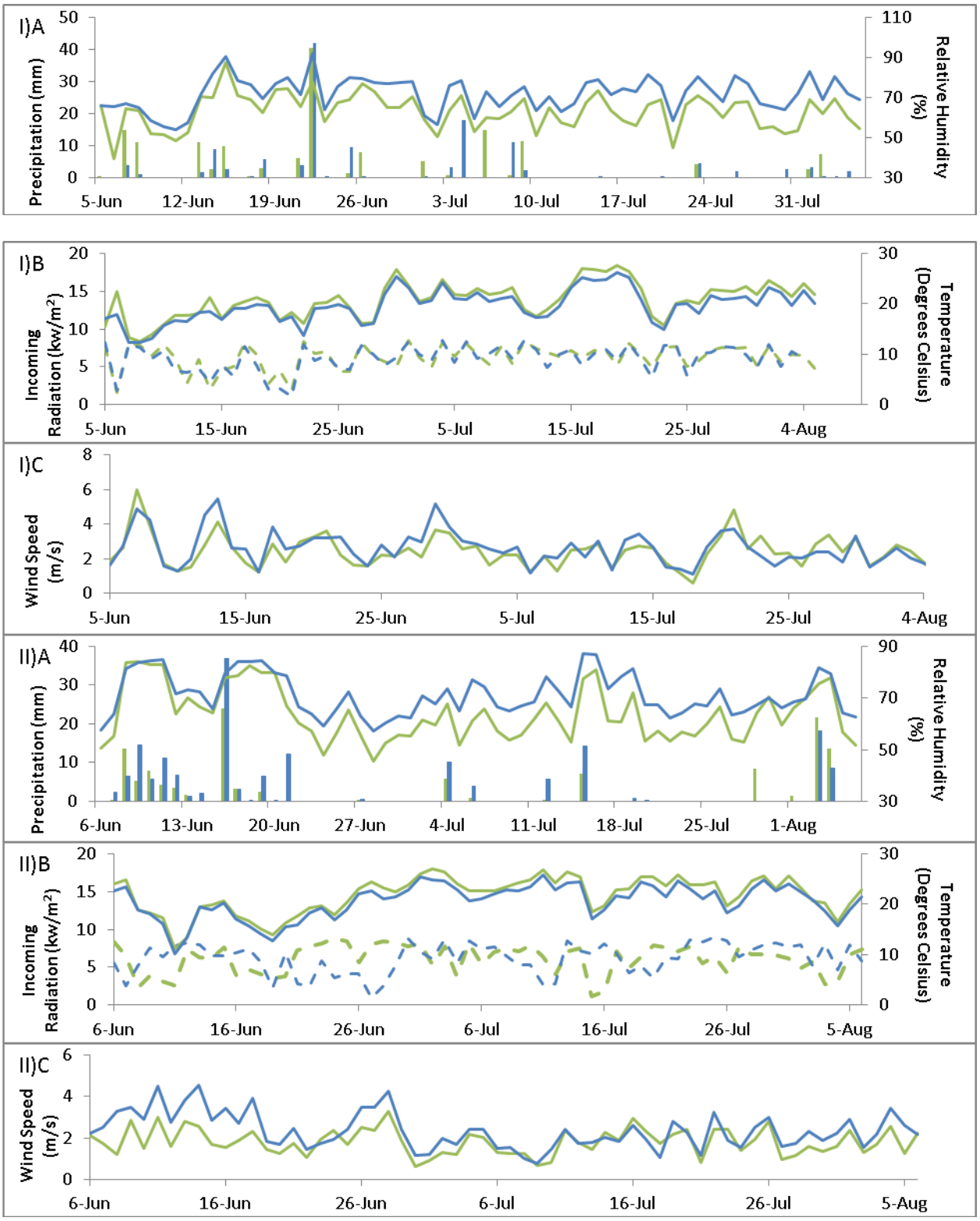

Figure 2.5 Seasonal comparisons between Winnipeg (green lines) and Carman (blue lines) in I) 2011 and II) 2012 among A) precipitation (mm) (bars), relative humidity (%) (solid lines), B) temperature (°C) (solid lines), solar radiation (kw/m^2) (dashed lines) and C) wind speeds (m/s) (solid lines).

2.4.2.2 Precipitation and Misting Effects

Cumulative precipitation and ascospore concentrations during the flowering period were closely matched based on the site-year (Figure 2.6). Precipitation and ascopsore concentrations were not cumulated in Winnipeg during 2012 due to missing data at the beginning of flowering. In Carman during 2011, accumulation of ascospore concentrations and precipitation appear to be somewhat correlated. The 2012 ascospore concentrations and precipitation accumulations were both slightly higher at the end of flowering than those for Carman 2011. Carman 2012 had the highest accumulated ascospore concentrations and precipitation totals overall at the end of the flowering period.

In 2011, in Carman especially, major precipitation events occurred just prior to an increase in ascospore concentration above the canopy (Figure 2.7). In Winnipeg (2011), only a few precipitation events occurred during the ascospore sampling period whereas during two events, peak ascsopore concentrations were reached. Another large precipitation event occurred where spore concentrations are unknown. In Winnipeg in 2012, increases in ascospore concentrations followed precipitation events, with some peaks occurring one day after rain. Carman 2012 also showed peaks following most precipitation events, with some peaks occurring during a precipitation event. During 2011, misted plots did not show large differences in ascospore concentrations compared to the non-misted plots. There appears to be higher concentrations in non-misted plots in Carman and slightly higher concentrations among misted plots in Winnipeg. Peaks in ascospore concentration occurred at similar times between misted and non-misted plots.

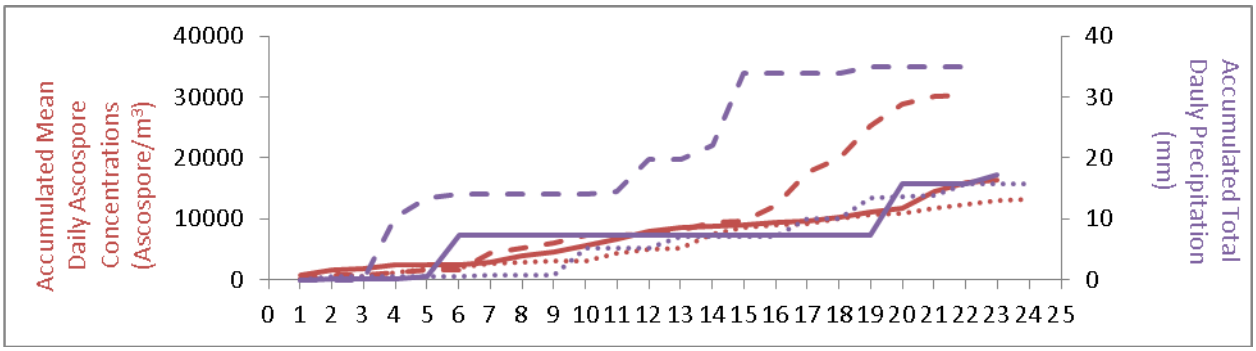

Figure 2.6 Cumulative mean daily ascospore concentrations (red) and total daily precipitation (purple) from the 1st to the 24th day of flowering in Carman 2011 (dotted line), Winnipeg 2012 (solid line) and Carman 2012 (dashed line).

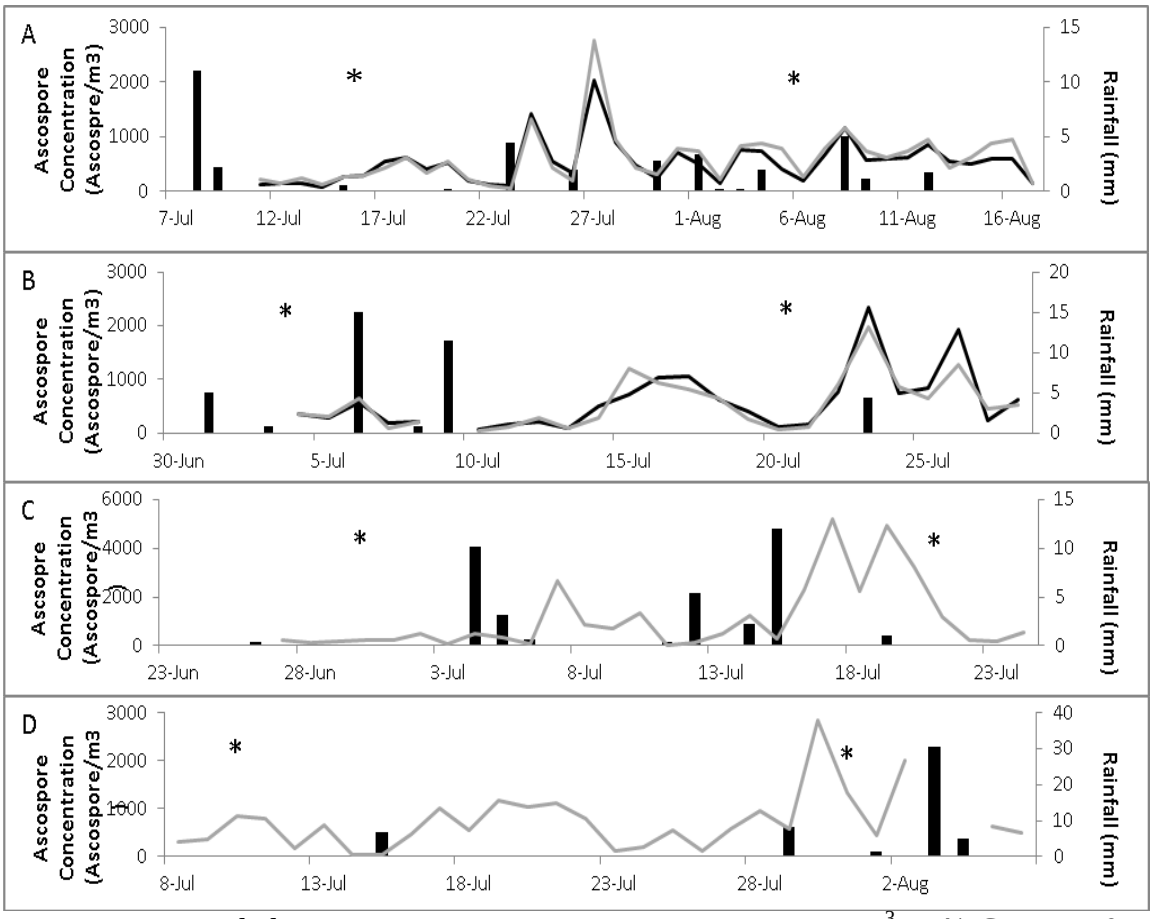

Figure 2.7 Mean daily ascospore concentrations in ascospores per m^3 in A) Carman 2011 in misted (black lines) and non-misted (grey lines) plots B) Winnipeg 2011 in misted (black lines) and non-misted (grey lines) plots C) Carman 2012 in non-misted (grey lines) plots and D) Winnipeg 2012 in non-misted (grey lines) plots plotted with total daily rainfall in mm (black bars) in A) Carman 2011 B) Winnipeg 2011 C) Carman 2012 and D) Winnipeg 2012. The flowering period is represented by the time period between the * symbols**.**

2.4.2.3 Temperature, Relative Humidity, Solar Radiation and Wind Speed Effects

In all four site years, the majority of ascospore concentration peaks were either preceded by peaks in relative humidity or occur simultaneously (Figure 2.8). However these trends did not occur consistently throughout the sampling periods. Winnipeg 2011 tended to show increasing ascospore concentrations with increased relative humidity during most peaks. Mean daily temperatures tended to increase during days with decreasing relative humidity in the majority of cases. Thus, increases in temperatures tended to occur simultaneously with peaks in ascospore concentrations but, again these trends were not consistent. No trends were apparent among daily mean wind speed and daily mean ascospore concentrations in all site years (Figure 2.9). Similarly, total daily solar radiation and daily mean ascospore concentrations showed no apparent correlation.

During 2011, higher spore releases occurred on days with mean temperatures between 18-22 °C in Carman (Figure 2.10). In Winnipeg, concentrations were higher on days with mean daily temperatures from 14 to 18 °C. During 2012, highest ascospore releases occurred on days with mean temperatures between 18-20 °C in Carman and 20-22 and 26-28 °C in Winnipeg. Overall, highest ascospore releases occurred between 18 to 22 °C. Ascospores were released during daily mean relative humidity between 50 to 90%. Highest concentrations were released from 74-78% and 62-66% mean daily relative humidity in Winnipeg during 2011 and 2012 respectively with an overall range of concentrations occurring between 50 to 78% in Winnipeg. In Carman, highest ascospore concentrations occurred at daily mean relative humidity of 74-78 % in 2011 and 78-82%

in 2012 and all concentrations were within the range from 58 to 90% mean daily relative humidity. Overall, highest daily ascospore concentrations occurred approximately from 62 to 82% mean daily relative humidity. In Winnipeg, highest values occurred during days with 30 to 35% and 40-45% minimum relative humidity in 2011 and 2012 respectively. In Carman, days with minimum relative humidity at 50 to 55% and 60 to 65% achieved the highest ascospore concentrations during 2011 and 2012 respectively. Days that experienced greater than a 20 percent decrease in relative humidity from 8 am to 2 pm showed highest ascospore concentrations overall. Low daily mean ascospore concentrations were experienced during days with increases below 20 percent. During days where no precipitation occurred, daily mean ascospore concentrations were highest overall, especially during 2012. Very little ascospore release occurred during large precipitation events exceeding 6mm/ day. Overall, ascospore concentrations were highest during days with mean wind speeds between 0.5 to 3.0 m/s and at wind speeds above 3 m/s ascospores release was either very low or spores were not captured in the device.

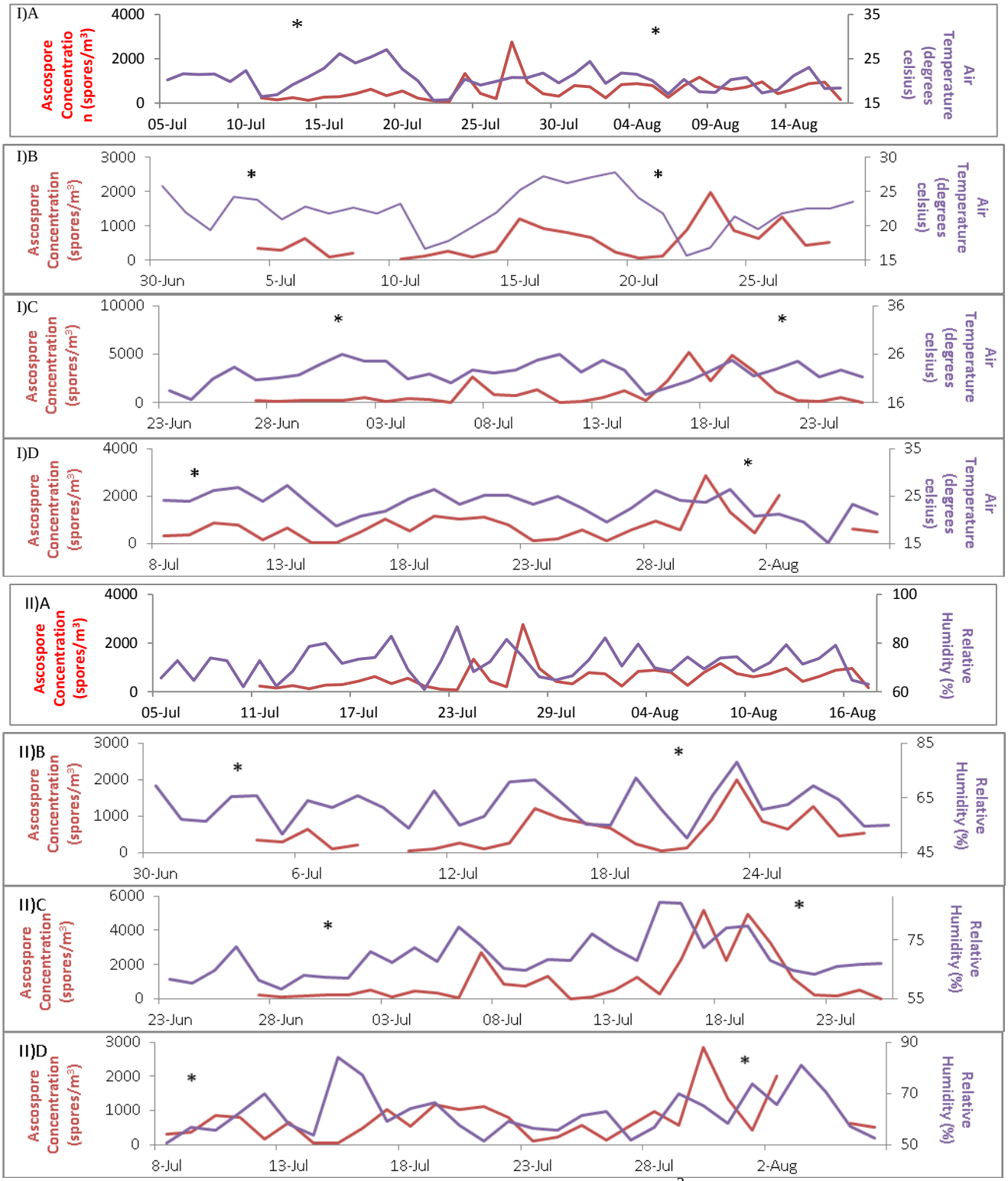

Figure 2.8 Mean daily ascospore concentrations in ascospores per m^3 (red lines) plotted with average daily I) temperatures in degrees celsius (purple lines) and II) relative humidity in percentage (purple lines) for A) Carman 2011 B) Winnipeg 2011 C) Carman 2012 and D) Winnipeg 2012. The flowering period is indicated by the area within the * symbols.

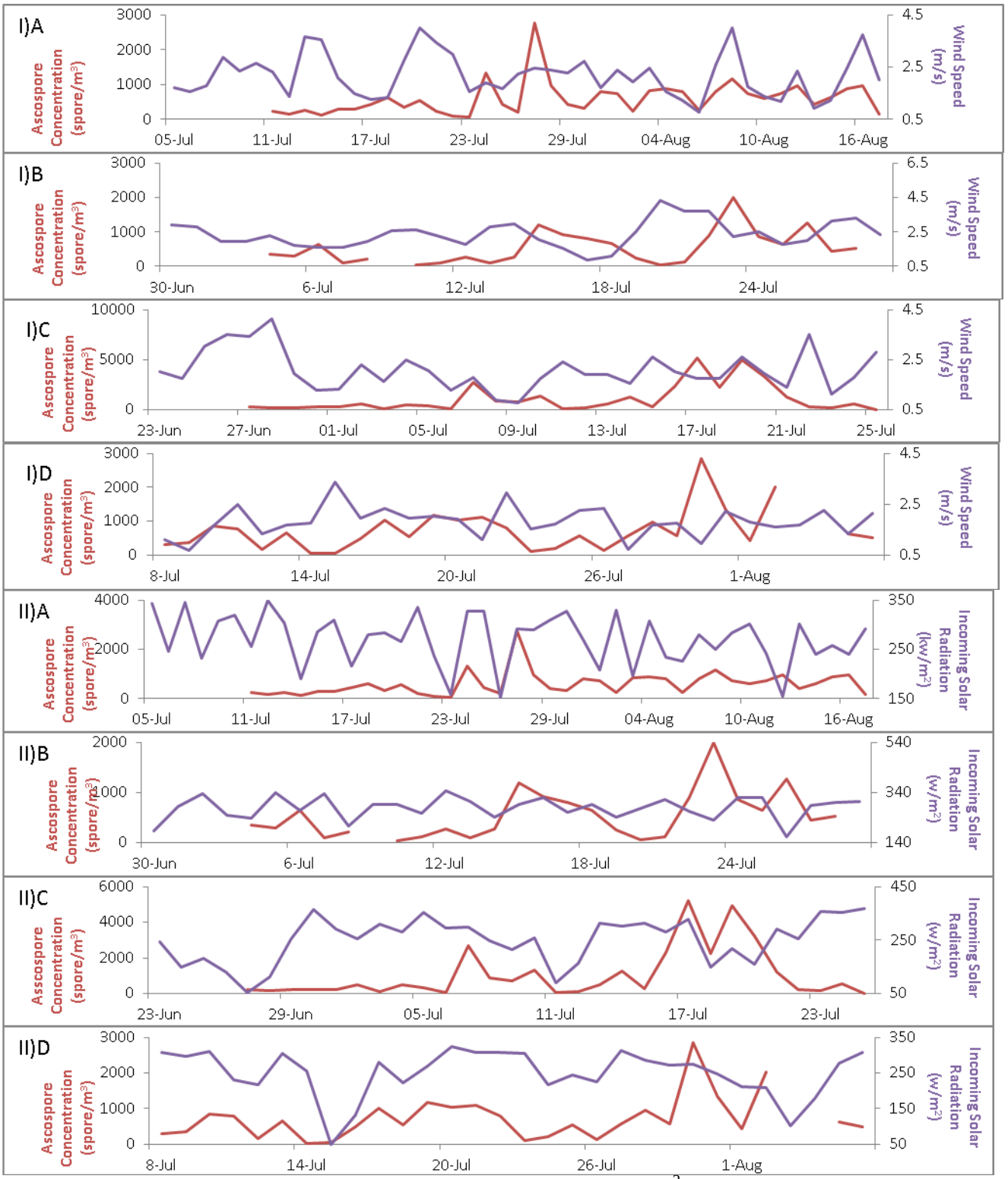

Figure 2.9 Mean daily ascospore concentrations in ascospores per m^3 (red lines) plotted with I) mean daily wind speed (purple lines) and II) total daily solar radiation (purple lines) for A) Carman 2011 B) Winnipeg 2011 C) Carman 2012 and D) Winnipeg 2012.

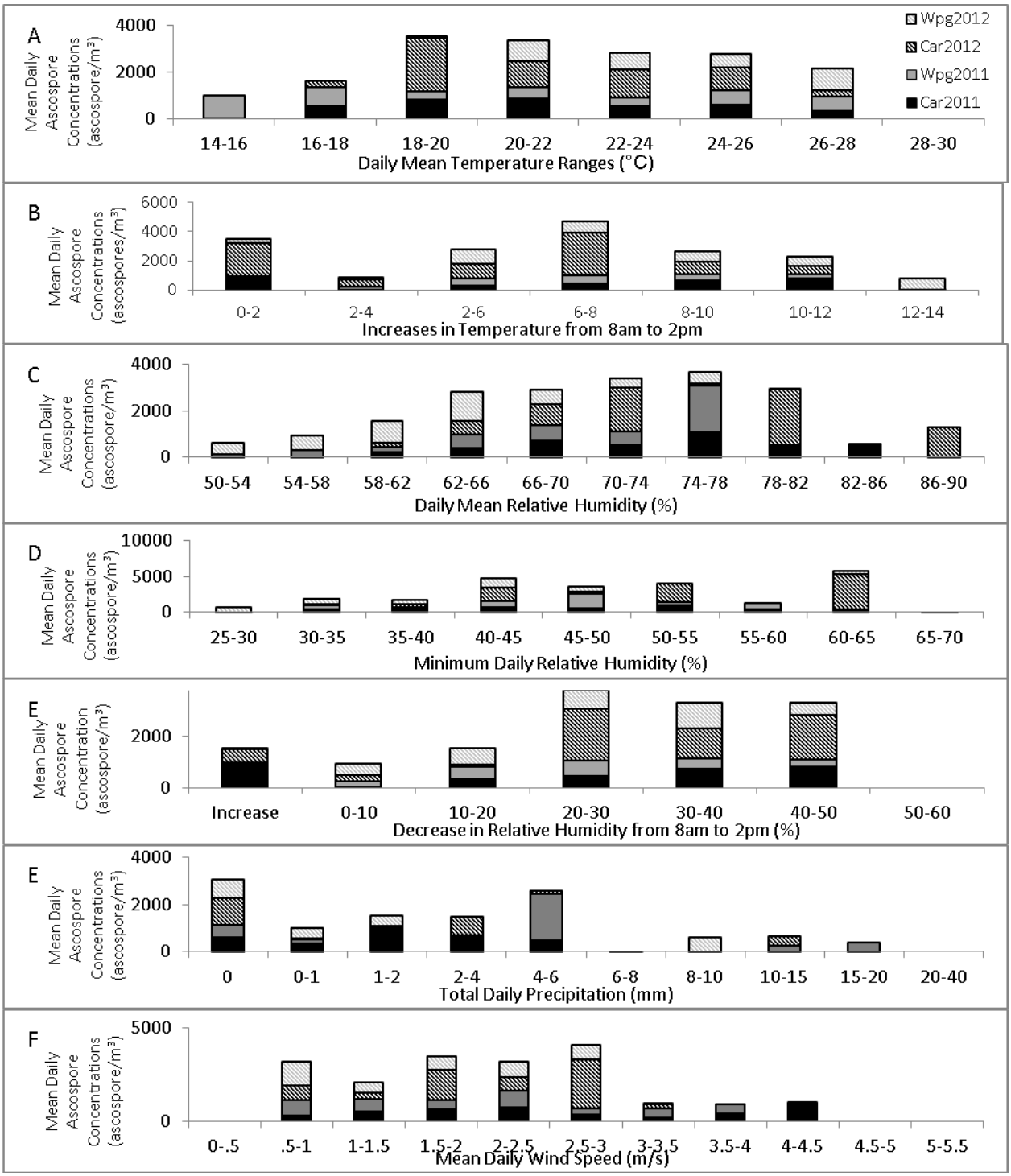

Figure 2.10 Average of daily mean ascospore concentrations for non-misted plots in Carman 2011, Winnipeg 2011, Carman 2012 and Winnipeg 2012 occurring within specified intervals for factors including A) mean daily air temperature (°C), B) mean daily relative humidity (%), C) minimum daily relative humidity (%), D) daily decrease in relative humidity from 8am to 2pm (%) E) total daily precipitation (mm) and F) mean daily wind speed (m/s).

2.4.3 Weather Impacts on Disease Incidence

According to the daily values computed for daily mean temperature, daily mean relative humidity and total daily precipitation, it is evident that during a precipitation event relative humidity increases slightly and temperatures decrease slightly. During 2011, little precipitation occurred during the period just prior to harvest when disease was assessed in both Carman and Winnipeg. Winnipeg received slightly more rain at harvest, however disease incidence at harvest was lower than Carman. During 2012, more precipitation occurred before harvest, especially in Carman and percentage of infection was approximately 15%. In Winnipeg, percentage of infection was low at harvest followed by a small precipitation event. A large precipitation event occurred after harvest and an increase in percentage of infection was determined post-harvest.

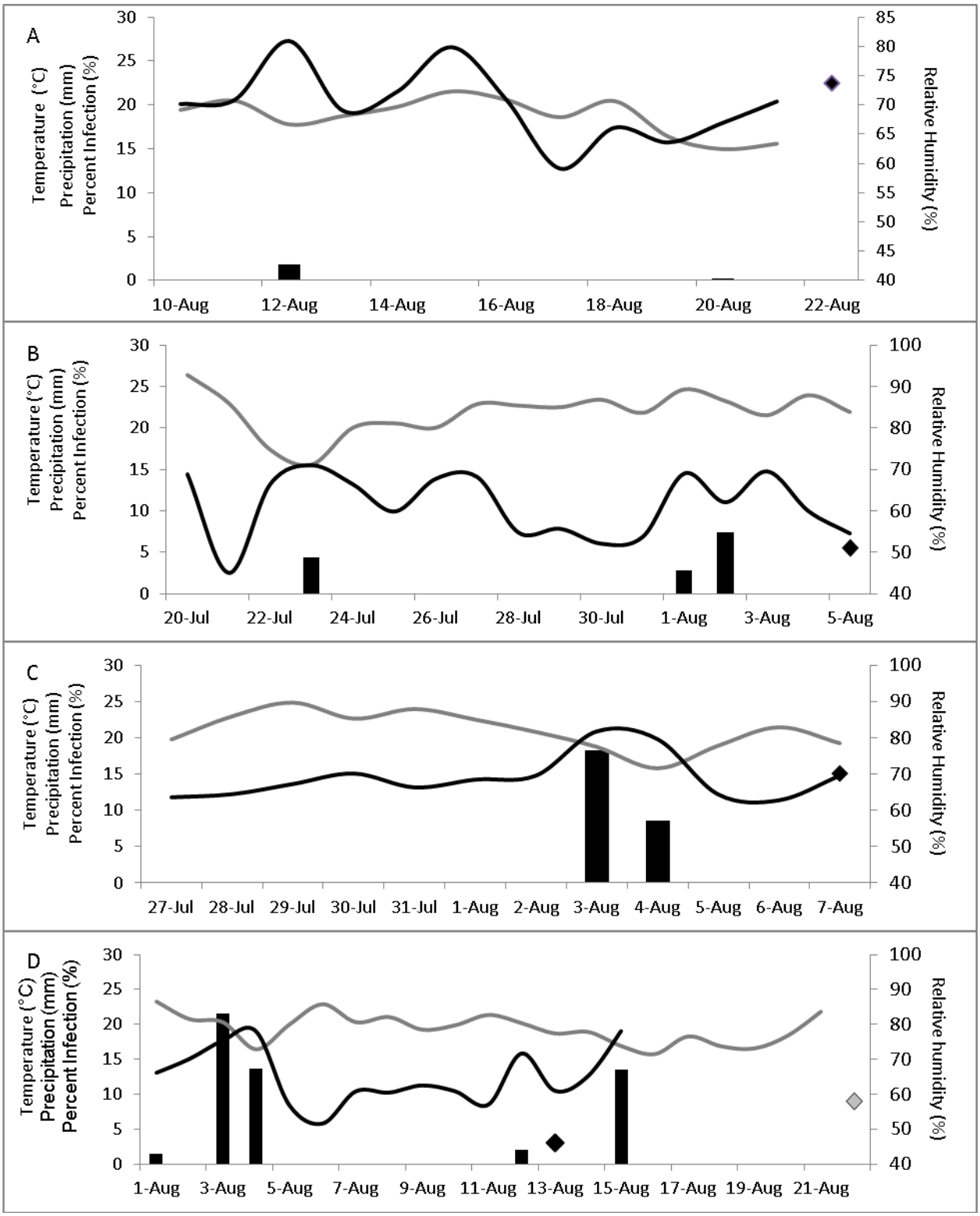

Figure 2.11. Percent infection counts during harvest (black diamond) and after harvest (grey diamond) in A) Carman 2011, B) Winnipeg 2011, C) Carman 2012 and D) Winnipeg 2012. Weather parameters include total daily precipitation in mm (black bars), temperature in °C (grey lines) and relative humidity in % (black lines).

2.4.4 Misting and Seeding Rate Treatment Effects on the Canola Canopy

2.4.4.1 Canopy Density

Based on three different seeding rates and fertilizer requirements, three canopy densities resulted. In 2011, at both locations there appears to be a difference between high, medium and low seeding rates as represented by the LAI values in each of the 18 plots, although the distinction is more apparent at the Winnipeg location (Figure 2.12). In 2012, there was a clear difference between high and low seeding rates and a slight difference between high, medium and low seeding rates at both locations. In 2011, from budding to the end of flowering, a decreasing trend in LAI in Winnipeg was apparent, although this trend did not occur in Carman where LAI increased. In 2012, no trends were apparent in the Winnipeg plot due to the LAI sampling period which began just prior to flowering to nearly the end of flowering. From the rosette stage to the end of flowering in Carman (2012), an increasing trend was apparent.

The ANOVA for LAI revealed significant differences among all high and low plots, with highest LAI under high seeding rate treatments (Table 2.6). In Winnipeg during both years, high versus medium, high versus low and medium versus low plots contained significantly different LAI. In 2011, Winnipeg contained significantly higher LAI than Carman while in 2012, LAI values were not significantly different among locations. No significant differences in LAI were apparent between years. According to the ANOVA, plant counts were significantly lower in low versus high and low versus medium plots in all site-years (Table 2.6). No significant differences in plant count occurred among high and medium plots in any year except for Winnipeg 2011. Plant counts were significantly higher in Winnipeg in 2011 and in Carman in 2012. Plant

counts in 2011 were significantly higher than 2012 overall. Distinguishable canopy densities were also apparent in the field in both years, with plots seeded at high rates being visibly dense, and plots appearing sparse at low seeding rates.

The ANOVA results for misting and seeding rate treatments on LAI revealed significant differences in Winnipeg for high versus medium, high versus low and medium versus low seeding rate treatments in both misted and non-misted plots (Table 2.7). In Carman, high seeding rate treatment plots contained significantly higher LAI than low plots and higher LAI under medium versus low plots under both misted and non-misted plots. LAI and plant counts were significantly higher overall in Carman compared to Winnipeg. Significantly higher plant counts occurred in misted plots in Carman, with no significant effects of misting in Winnipeg (Table 2.7). High seeding rate treatments yielded significantly higher plant counts compared to low seeding rates treatments in Carman, while in Winnipeg high versus low and medium versus low seeding rate plots showed significant differences. There were significant differences among LAI and plant counts in Winnipeg and Carman where both misted and non-misted plots were included in the ANOVA. Combined effects of misting treatments and seeding rate treatments did not significantly affect LAI or plant counts at either location.

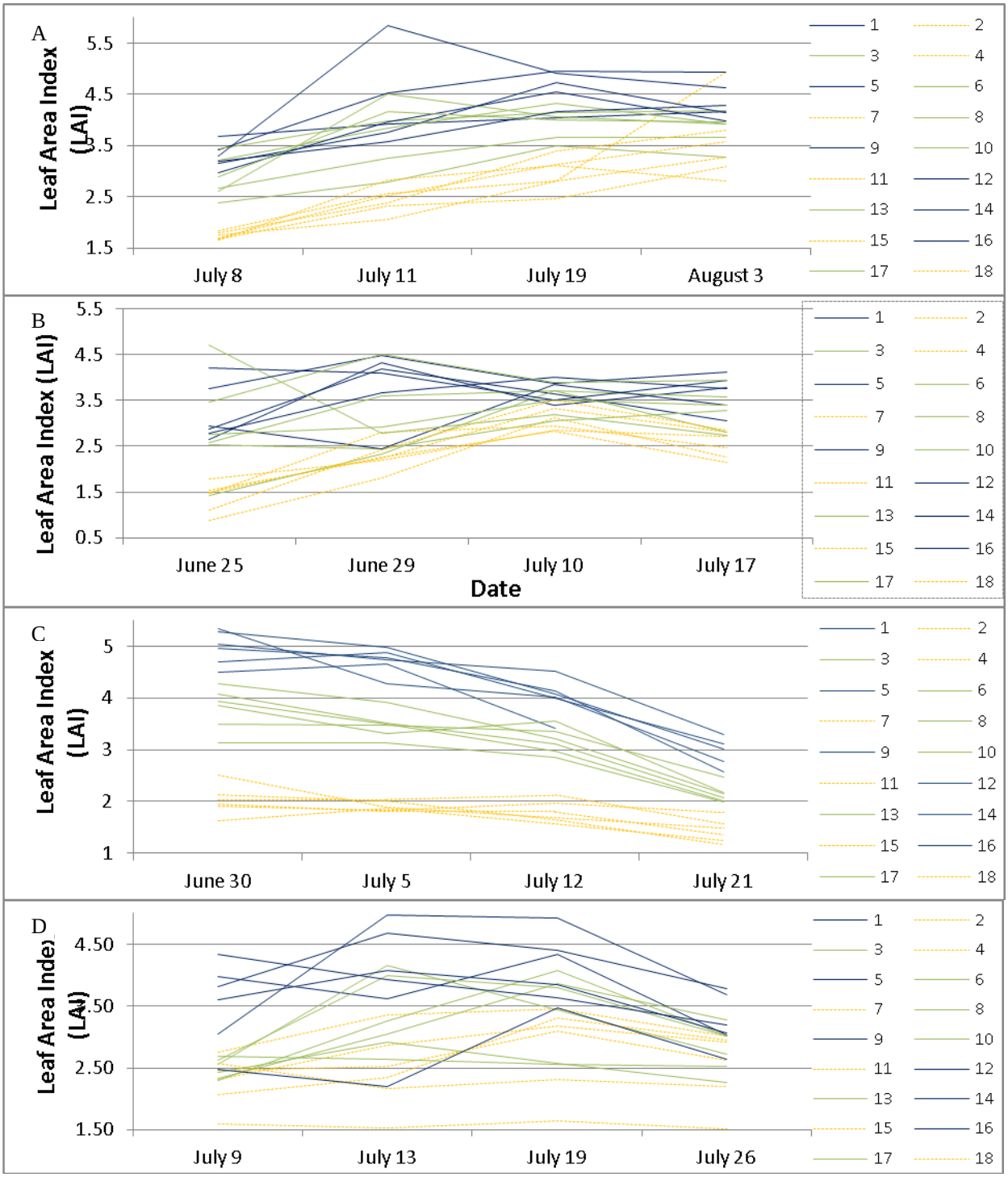

Figure 2.12 Leaf area index values taken from plots 1-18 throughout the A) 2011 growing season in Carman (plots 1-9 are misted) B) 2011 growing season in Winnipeg (plots 10-18 are misted) C) 2012 growing season in Carman and D) 2012 growing season in Winnipeg. Blue, green and dotted yellow lines represent plots seeded at high, medium and low seeding rates respectively.

Table 2.6 Significance levels from analysis of variance on the effects of seeding rate on leaf area index (LAI) and plant counts from Winnipeg and Carman in 2011 and 2012 under natural weather conditions (non-misted plots). Words in brackets indicate which selection (year or location) is higher.

	Winnipeg 2011	**Carman**	**Winnipeg 2012**	**Carman**
	Non-misted	**Non-misted**	**Non-misted**	**Non-**
Effects	**Pr>f**	**Pr>f**	**Pr>f**	**Pr>f**
Year on LAI	**0.5998 (2012)**			
Location on LAI	**0.0142 (Wpg)**		**0.8884 (Wpg)**	
Seed Rate on LAI	**<.0001**	**<.0001**	**<.0001**	**<.0001**
High vs. Low	<.0001	<.0001	<.0001	<.0001
High vs. Medium	<.0001	0.1667	<.0001	0.0074
Medium vs. Low	<.0001	0.0001	0.0026	<.0001
Year on Plant Count	**0.0028 (2011)**			
Location on Plant Count	**<.0001 (Wpg)**		**<.0001 (Car)**	
Seed Rate on Plant Count	**<.0001**	**0.0148**	**0.0017**	**0.0001**
High vs. Low	<.0001	0.0088	0.0005	<.0001
High vs. Medium	0.0371	0.8261	0.2037	0.1118
Medium vs. Low	0.0068	0.0153	0.0162	0.0044

Table 2.7 Significance levels from analysis of variance on the effects of seeding rate and misting on leaf area index (LAI) and plant counts in Winnipeg and Carman in 2011. Words in brackets indicate which selection (location, misting) is higher.

	Winnipeg 2011		**Carman 2011**	
	Misted	**Non-misted**	**Misted**	**Non-misted**
Effects	**Pr>f**	**Pr>f**	**Pr>f**	**Pr>f**
Location on LAI	**<.0001 (Car)**			
Misting on LAI	**0.1020 (Mist)**		**0.2318 (NonMist)**	
Seed Rate on LAI	**<.0001**		**<.0001**	
Seed Rate on LAI	**<.0001**	**<.0001**	**<.0001**	**<.0001**
High vs. Low	<.0001	<.0001	<.0001	<.0001
High vs. Medium	<.0001	<.0001	0.1631	0.0025
Medium vs. Low	<.0001	<.0001	0.0002	0.0009
Misting*Seeding Rate on LAI	**0.6817**		**0.5811**	
Location on Plant Count	**<.0001 (Wpg)**			
Misting on Plant Count	**0.2475 (NonMist)**		**0.0295 (Mist)**	
Seeding Rate on Plant Count	**0.0023**		**0.0036**	
Seed Rate on Plant Count	**0.0202**	**0.0027**	**0.0059**	**0.0784**
High vs. Low	0.0007	0.0080	0.0424	0.0016
High vs. Medium	0.1062	0.5254	0.8646	0.0481
Medium vs. Low	0.0363	0.0340	0.0604	0.1519
Misting*Seeding Rate on Plant	**0.8209**		**0.0598**	

2.4.4.2 Canola Microclimate

Several observations can be made based on the test for analysis of variance (ANOVA) on microclimatic parameters under all three treatments for non-misted conditions in 2011 and 2012 (Table 2.8). In 2011, there were significantly lower relative humidity, temperatures and leaf wetness periods. In Carman, relative humidity was significantly higher during both site years and as a result leaf wetness was also higher. Temperatures were significantly lower in Carman compared to Winnipeg in 2011. Significant differences occurred between high versus low seeding rate treatments with respect to relative humidity and temperature during three site-years including Winnipeg 2011, Carman 2012 and Winnipeg 2012. In these cases, temperatures were higher among low density plots and relative humidity was higher under high density plots. Soil temperatures were significantly higher in low versus high density plots at both sites in both years and significantly higher values were observed in medium versus low plots in all but the Carman 2011 location.

Misted plots contained significantly higher relative humidity and leaf wetness values, but lower temperatures (Table 2.9). Soil temperatures were not significantly affected by misting. Relative humidity, temperature, as well as leaf wetness parameters were significantly different among high and low plots in most site-years. Thus, misting and variable seeding rates did create significant difference for all microclimate parameters.

Table 2.8 Significance levels from analysis of variance on the effects of seeding rate on microclimatic parameters including relative humidity (RH) in %, temperature (T) in °C, growing degree days (GDD) in days and leaf wetness (LW) from Winnipeg and Carman in 2011 and 2012 under natural weather conditions (non-misted plots) during the flowering period. Words in brackets indicate which selection (year or location) is higher.

	Winnipeg	**Carman**	**Winnipeg**	**Carman**
	Non-misted	**Non-misted**	**Non-misted**	**Non-misted**
Effects	**Pr>f**	**Pr>f**	**Pr>f**	**Pr>f**
Year on RH	**0.0053 (2012)**			
Location on RH	**<.0001 (Car)**		**0.0003 (Car)**	
Seed Rate on RH	**<.0001**	**0.1248**	**0.0006**	**0.0001**
High vs. Low	<.0001	0.0456	0.0002	<.0001
High vs. Medium	0.0349	0.4583	0.1330	0.4392
Medium vs. Low	0.0002	0.1923	0.0098	0.0007
Year on T	**<.0001 (2012)**			
Location on T	**0.0002 (Wpg)**		**0.2023 (Wpg)**	
Seed Rate on T	0.0057	0.5068	0.0081	0.0011
High vs. Low	0.0016	0.2831	0.0021	0.0007
High vs. Medium	0.0980	0.8703	0.1080	0.7362
Medium vs. Low	0.0532	0.3605	0.0800	0.0018
Year on LW	**0.1135 (2011)**			
Location on LW	**0.0004 (Car)**		**<.0001 (Car)**	
Seed Rate on LW	0.8387	0.6229	0.8993	0.1916
High vs. Low	0.6684	0.3379	0.6531	0.2964
High vs. Medium	0.8922	0.6930	0.7691	0.4275
Medium vs. Low	0.5736	0.5688	0.8755	0.0726
Year on Soil T	**0.0010 (2012)**			
Location on Soil T	**0.0656 (Car)**		**0.0082 (Car)**	
Seed Rate on Soil T	0.0006	0.1212	0.0053	0.0192
High vs. Low	<.0001	0.0024	0.0004	0.0086
High vs. Medium	0.0674	0.0903	0.0815	0.8130
Medium vs. Low	0.0005	0.1283	0.0301	0.0153

Table 2.9 Levels of significance from analysis of variance on the effects of seeding rate treatment and misting on microclimate parameters in Winnipeg and Carman in 2011 during the flowering period. Words in brackets indicate which selection misting or location) is higher.

	Winnipeg 2011		**Carman 2011**	
	Misted	**Non-misted**	**Misted**	**Non-misted**
Effects	**Pr>f**	**Pr>f**	**Pr>f**	**Pr>f**
Location on RH	**<.0001 (Car)**			
Misting on RH	**0.0003 (Mist)**		**0.0003 (Mist)**	
Seed Rate on RH	**<.0001**		**0.0041**	
Seed Rate on RH	**<.0001**	**<.0001**	**0.0424**	**0.0477**
Low vs. High	<.0001	<.0001	0.0158	0.0206
Medium vs. High	0.0045	0.0111	0.3583	0.7386
Medium vs. Low	<.0001	<.0001	0.1095	0.0426
Misting x Seeding Rate on	**<.0001**		**0.0006**	
Location on T	**<.0001 (Wpg)**			
Misting on T	**0.0002 (NonMist)**		**0.0013 (NonMist)**	
Seed Rate on T	**<.0001**		**0.0003**	
Seed Rate on T	**<.0001**	**<.0001**	**0.0020**	**0.0375**
Low vs. High	<.0001	<.0001	0.0176	0.0006
Medium vs. High	0.0003	0.0038	0.7038	0.2556
Medium vs. Low	<.0001	<.0001	0.0405	0.0106
Misting x Seeding Rate on T	**<.0001**		**0.0003**	
Location on Leaf Wetness	**<.0001 (Car)**			
Misting on Leaf Wetness	**<.0001 (Mist)**		**<.0001 (Mist)**	
Seed Rate on Leaf Wetness	**0.0006**		**0.0006**	
Seed Rate on Leaf Wetness	**0.0003**	**0.2468**	**0.0021**	**0.0308**
Low vs. High	0.2219	<.0001	0.0093	0.0102
Medium vs. High	0.6956	0.2427	0.2591	0.2799
Medium vs. Low	0.1119	0.0019	0.1077	0.0007
Misting x Seeding Rate on	**<.0001**		**<.0001**	
Location on Soil T	**0.3356 (Car)**			
Misting on Soil T	**0.9989 (NonMist)**		**0.1180 (NonMist)**	
Seed Rate on Soil T	**<.0001**		**0.0005**	
Seed Rate on Soil T	**<.0001**	**0.0002**	**0.0045**	**0.0315**
Low vs. High	0.0019	0.0023	0.0415	0.2288
Medium vs. High	0.9958	0.8851	0.7313	0.9281
Medium vs. Low	0.0926	0.0926	0.8221	0.9653
Misting x Seeding Rate on	**<.0001**		**0.0018**	

2.4.4.3 Microclimate and Disease Development

Canopy density, disease development and microclimatic variable comparisons among the 4 site-years are shown in Table 2.10. LAI values were comparable among all site years, with the highest values occurring in Carman (2011). Highest plant counts were observed in Winnipeg in 2011, with the lowest counts in Winnipeg in 2012. Carman contained higher plant counts in 2012 compared to 2011. With respect to disease, mean ascospore concentrations were highest in Carman (2012). Percentages of infection at harvest were highest in 2011, with higher infection percentages in Carman than Winnipeg in both years. Carman also experienced higher within canopy leaf wetness and relative humidity values and lower air temperature values during both years. In 2011, relative humidity and leaf wetness were higher and temperatures were lower under the canopy compared to 2012.

Misted plots in Winnipeg 2011 showed higher overall LAI and lower plant counts, but higher plant counts and lower LAI values were recorded in Carman 2011 (Table 2.10). Both ascospore concentrations and percentages of infection were higher among misted plots in Winnipeg 2011 than non misted plots, however in Carman 2011 the non-misted plots contained higher values than misted plots. Significant differences were apparent among microclimates in misted plots at both locations with increased values for relative humidity and leaf wetness, and decreased air and soil temperatures.

Table 2.10 Summary of whole plot mean ascospore concentrations (ascospores/m^3), disease incidences (%) and microclimate parameters. 2011 misted and non-misted mean ascospore concentrations, disease incidences and microclimatic parameters are listed. Data was used only for days where all values are present and for over lapping Winnipeg and Carman days only.

	Winnipeg			Carman		
Variable	Misted	Non-Misted	All	Misted	Non-Misted	All
	2011					
Leaf Area Index	3.2	3.0	3.1	3.4	3.5	3.5
Plant Counts (plants/m^3)	133.1	148.3	140.7	107.2	77.5	92.4
Ascospore Concentration (ascospores/m3)	705	628	671	569	663	616
Percent Infection (%)	6.0	5.5	5.8	20.7	22.5	21.6
Relative Humidity (%)[x]	78.2	76.6	77.4	84	82.2	83.1
Leaf Wetness[x]	32.9	24.4	28.7	47.8	41	44.4
Air Temperature (°C)[x]	20.6	20.8	20.7	20	20.4	20.2
Soil Temperature (°C)[x]	20.4	20.4	20.4	20.4	21.2	20.8
	2012					
Leaf Area Index		3.1	3.1		3.0	3.0
Plant Counts (plants/m^3)		75.9	75.9		111.0	111.0
Ascospore Concentration (ascospores/m3)		694	694		2170	2170
PercentInfection (%)		3[z], 9[y]	3[z], 9[y]		16	16
Relative Humidity (%)[x]		76.3	76.3		80.7	80.7
Leaf Wetness[x]		25.9	25.9		39.1	39.1
Air Temperature (°C)[x]		21.9	21.9		21.1	21.1
Soil Temperature (°C)[x]		21.3	21.3		20.9	20.9

[z] Percentf Infection was counted at harvest
[y] Percent Infection was counted post-harvest
[x]Data obtained from 4th leaf to harvest

2.4.4.4 Ascospore Concentrations and Disease Incidence

No significant impacts ($p > 0.05$) were found on ascospore concentrations among the three seeding rate treatments in any site-year (Table 2.11). Significantly higher ascospore concentrations occurred in 2012 overall, with highest concentrations in Carman. There was significantly higher disease incidence in 2011 overall. At Carman, there was significantly higher infection than in Winnipeg during both years. The only significant differences in percentage infection occurred between high and low seeding rate treatments in Carman in 2011, where high seeding rates produced more disease. Total daily mean ascospore concentrations and disease levels were most affected by seeding rate as shown in Figures 2.13 and 2.14 with the exception of Carman 2011 where seeding rate treatments appeared to influence percentage of infection (Figure 2.14).

A comparison of the accumulated ascospore concentrations between high, medium and low density plots showed no consistent differences among the three treatments (Figure 2.13). In the Carman 2011 and Winnipeg 2012 plots, accumulated daily mean ascospore concentrations at the end of flowering are highest among high density plots and lowest among medium density plots. In Carman 2012, accumulated daily mean ascospore concentrations are highest among low density plots and lowest among medium density plots. Overall, the 2012 sites showed higher accumulated daily mean ascospore concentrations by the end of the flowering period compared to the 2011 Carman site. From 15 to 20 days after flowering, Carman 2012 showed a steep rise in ascospore concentration which did not occur during the other 2 site-years.

Average daily mean ascospore concentrations and percentage of infection were analyzed under misted versus non misted conditions in 2011 (Table 2.12). No significant effects of location, misting or seeding rates were seen among average daily mean ascospore concentrations. With respect to infection percentages, Carman produced significantly higher percentages than Winnipeg. High and low seeding rate treatments showed significantly different disease percentages in both misted and non-misted plots in Carman; no significant differences were observed in Winnipeg among seeding rate treatments. Misting also had no effect on percent sclerotinia infection at either location.

Table 2.11 Significance levels from analysis of variance on the effects of seeding rate on average daily mean ascospore concentrations and disease incidence (DI) from Winnipeg and Carman in 2011 and 2012 under natural weather conditions (non-misted plots). Words in brackets indicate which selection (year or location) is higher.

	Winnipeg 2011	Carman 2011	Winnipeg 2012	Carman 2012
	Non-misted	Non-misted	Non-misted	Non-misted
Effects	**Pr>f**	**Pr>f**	**Pr>f**	**Pr>f**
Year on Spore Concentration	**<.0001 (2012)**			
Location on Spore Concentration		**0.1220 (Car)**	**<.0001 (Car)**	
Seed Rate on Spore Concentration	**0.9146**	**0.5872**	**0.9660**	**0.2494**
High vs. Low	0.7669	0.5230	0.8106	0.4567
High vs. Medium	0.6805	0.7040	0.8344	0.4036
Medium vs. Low	0.8671	0.3115	0.9756	0.0988
Year on DI	**0.0011 (2011)**			
Location on DI		**<.0001 (Car)**	**<.0001 (Car)**	
Seed Rate on DI	**0.8651**	**<.0001**	**0.6004**	**0.6969**
High vs. Low	0.7296	<.0001	0.6446	0.3437
High vs. Medium	0.8563	0.0030	0.3996	0.8514
Medium vs. Low	0.5989	0.0146	0.7006	0.4457

Table 2.12 Significance levels from analysis of variance on the effects of seeding rate and misting on average daily mean ascospore concentrations (Spore Concentration) and disease incidence (DI) in Winnipeg and Carman in 2011. Words in brackets indicate which selection (location, misting) is higher.

	Winnipeg 2011		Carman 2011	
	Misted	Non-misted	Misted	Non-misted
Effects	**Pr>f**	**Pr>f**	**Pr>f**	**Pr>f**
Location on Spore Concentration	**0.5459 (Wpg)**			
Misting on Spore Concentration	**0.1432 (Mist)**		**0.4875 (NonMist)**	
Seed Rate on Spore Concentration	**0.9946**		**0.8138**	
Seed Rate on Spore Concentration	**0.5030**	**0.6824**	**0.9133**	**0.4435**
High vs. Low	0.5744	0.4362	0.4285	0.9706
High vs. Medium	0.3912	0.2564	0.6368	0.7198
Medium vs. Low	0.7015	0.7129	0.2123	0.6954
Misting*Seeding Rate on Spore Concentration	**0.4864**		**0.7770**	
Location on DI	**<.0001 (Car)**			
Misting on DI	**0.8939 (Mist)**		**0.5866 (NonMist)**	
Seeding Rate on DI	**0.9928**		**0.0006**	
Seed Rate on DI	**0.8840**	**0.9338**	**0.0676**	**0.0023**
High vs. Low	0.8129	0.8339	0.0006	0.0229
High vs. Medium	0.9013	0.7787	0.0380	0.3766
Medium vs. Low	0.7186	0.6243	0.0898	0.1390
Misting*Seeding Rate on DI	**0.9946**		**0.0054 (NonMist,High)**	

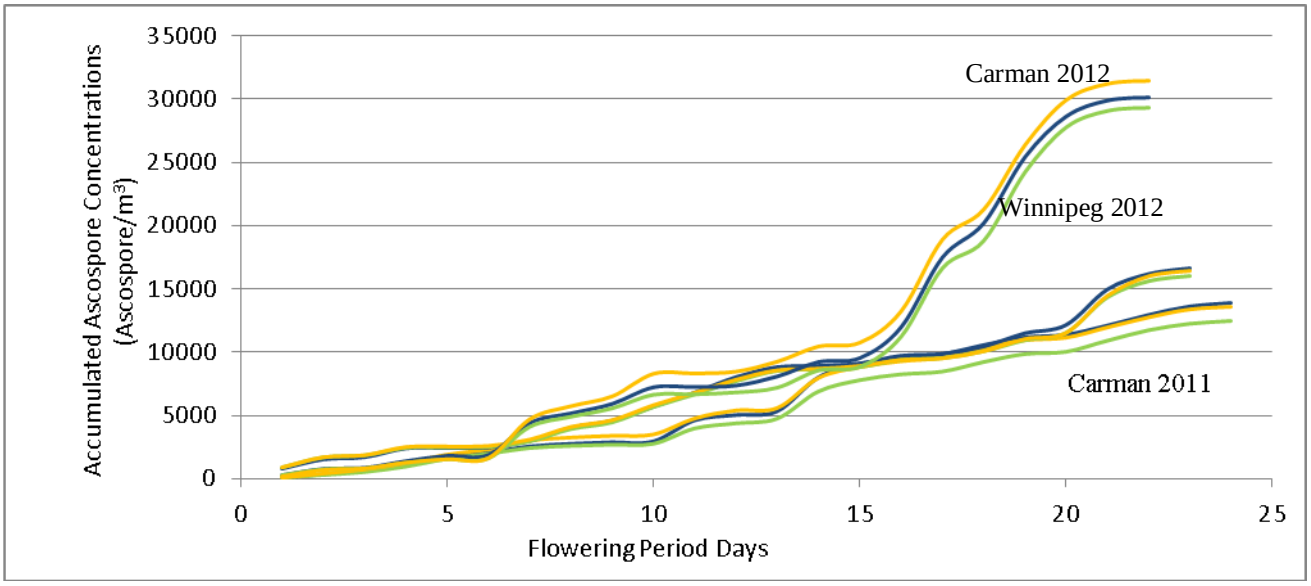

Figure 2.13 Cumulative ascospore concentrations (ascospores/m3) in high (blue), medium (green) and low (yellow) plots in Carman 2011, Winnipeg 2012 and Carman 2012.

Values obtained for mean LAI across the growing season and plant counts in each plot were plotted against percentages of infection (Figure 2.14) and ascospore concentration (Figure 2.15). The coefficients of determination obtained from these correlations indicate that ascospore concentration is not associated with LAI or plant counts during any of the four site-years. Percentage of infection was weakly associated to LAI and plant count in Carman in 2011; however no other site years showed this association..

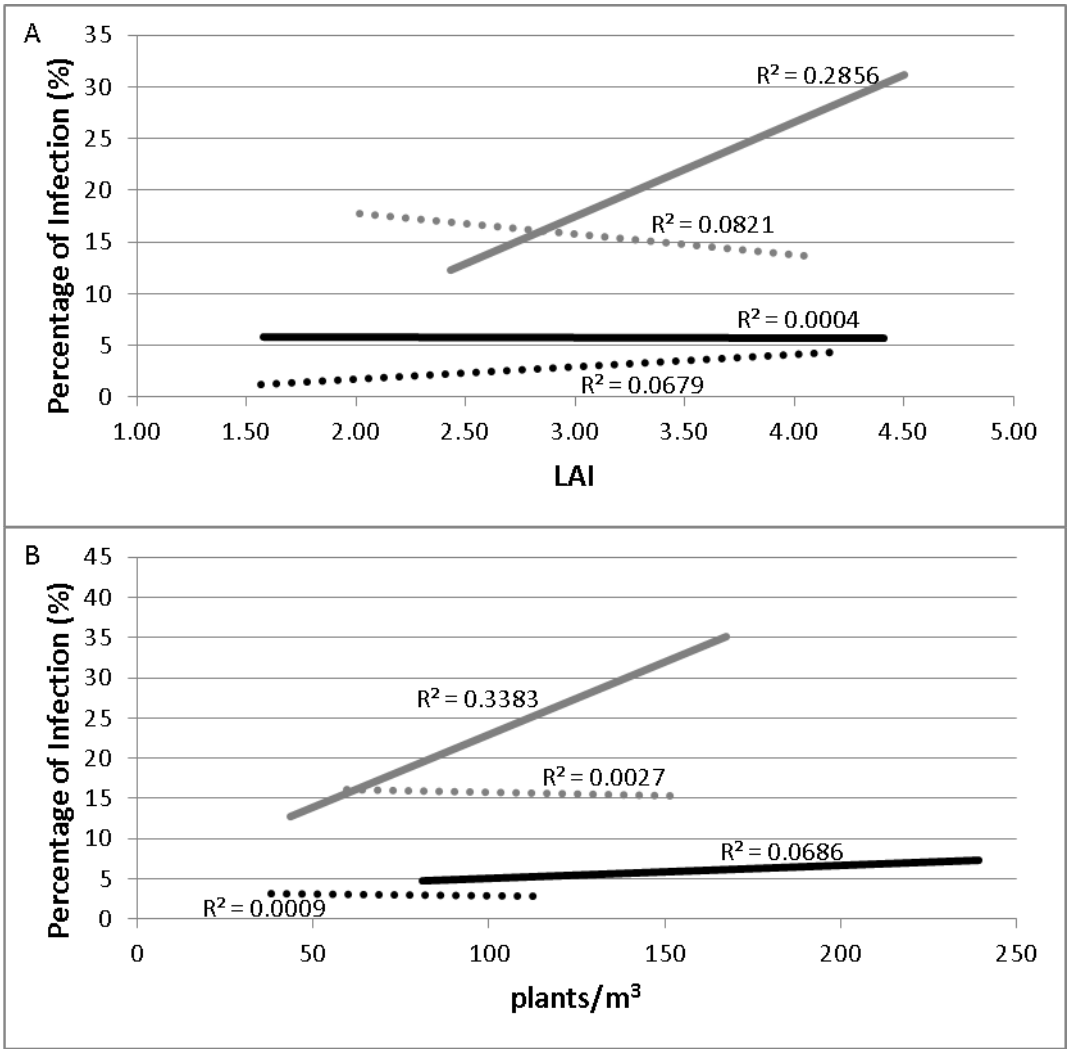

Figure 2.14 Relationship between sclerotinia stem rot disease incidence (%) and a) LAI; b) plant density in Winnipeg 2011 (black line), Carman 2011 (grey line), Winnipeg 2012 (dotted black line) and Carman 2012 (dotted grey line).

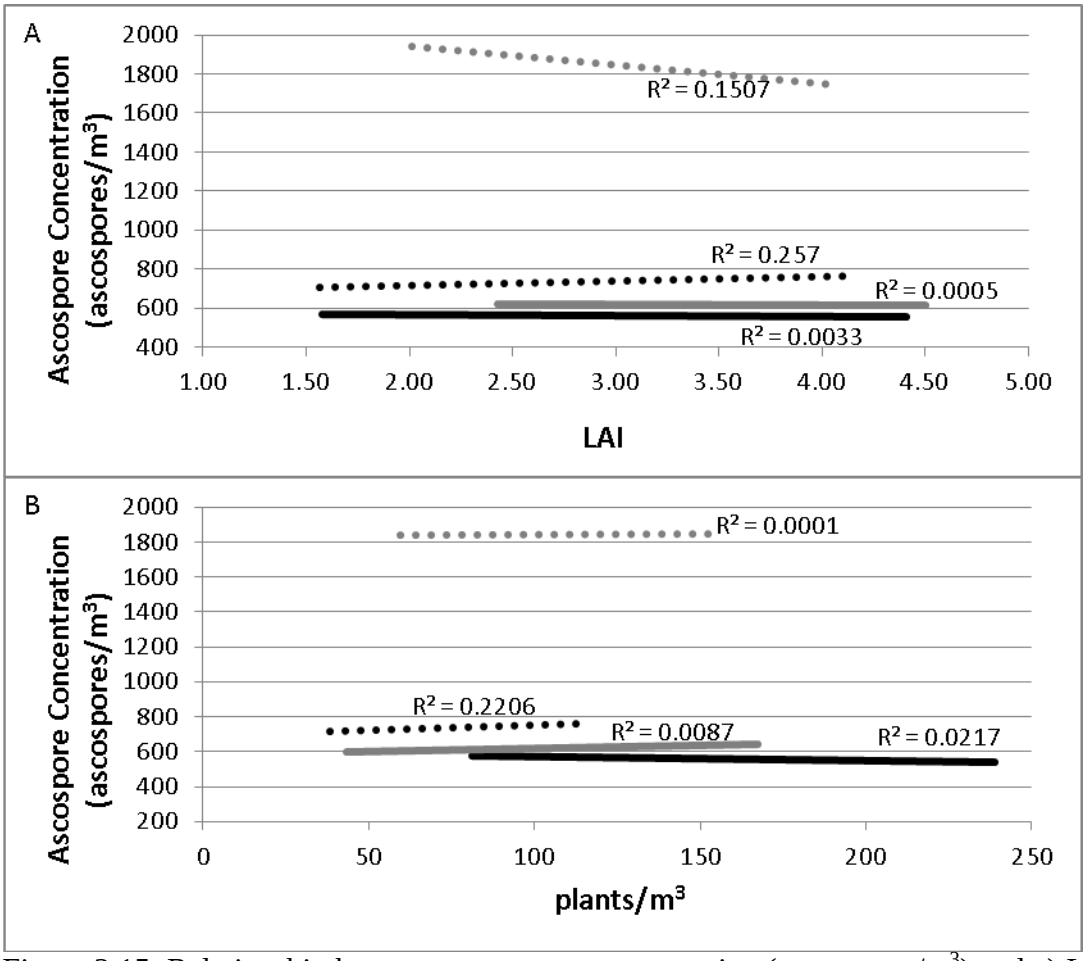

Figure 2.15 Relationship between ascospore concentration (ascospores/m^3) and a) LAI; b) plant density in Winnipeg 2011 (black line), Carman 2011 (grey line), Winnipeg 2012 (dotted black line) and Carman 2012 (dotted grey line).

Correlation between total ascospore concentrations (excluding data gaps) and disease incidence were calculated for Winnipeg and Carman. The regression (R^2) between all total ascospore concentrations (ascospores/m^3) and disease incidences (%) for each canola plot showed no linear relationship between the two variables for either Winnipeg or Carman (R^2 = 0.0314 and 0.0115 respectively). Similarly, in Winnipeg no linear relationships were observed within each of the high, medium and low treatments comparing disease incidence (%) to total ascospore concentration with R^2 values of 0.0076, 0.1041 and 0.0512 respectively. No linear relationships were observed in Carman among the three treatments with R^2 values of 0.6133 (negative), 0.0867 and 0.066 (negative) in high, medium and low treatments respectively.

2.4.4.6 Microclimate Time Series Comparisons to Daily Ascospore Production

Figures 2.16 through 2.19 show no clear correlation between microclimate parameters and ascospore release. Among environmental factors, peaks in relative humidity coincide with peaks in leaf wetness and increasing temperatures within the canopy also lead to increased soil temperatures. Occasionally, peaks in daily mean ascospore concentrations lag peaks in relative humidity.

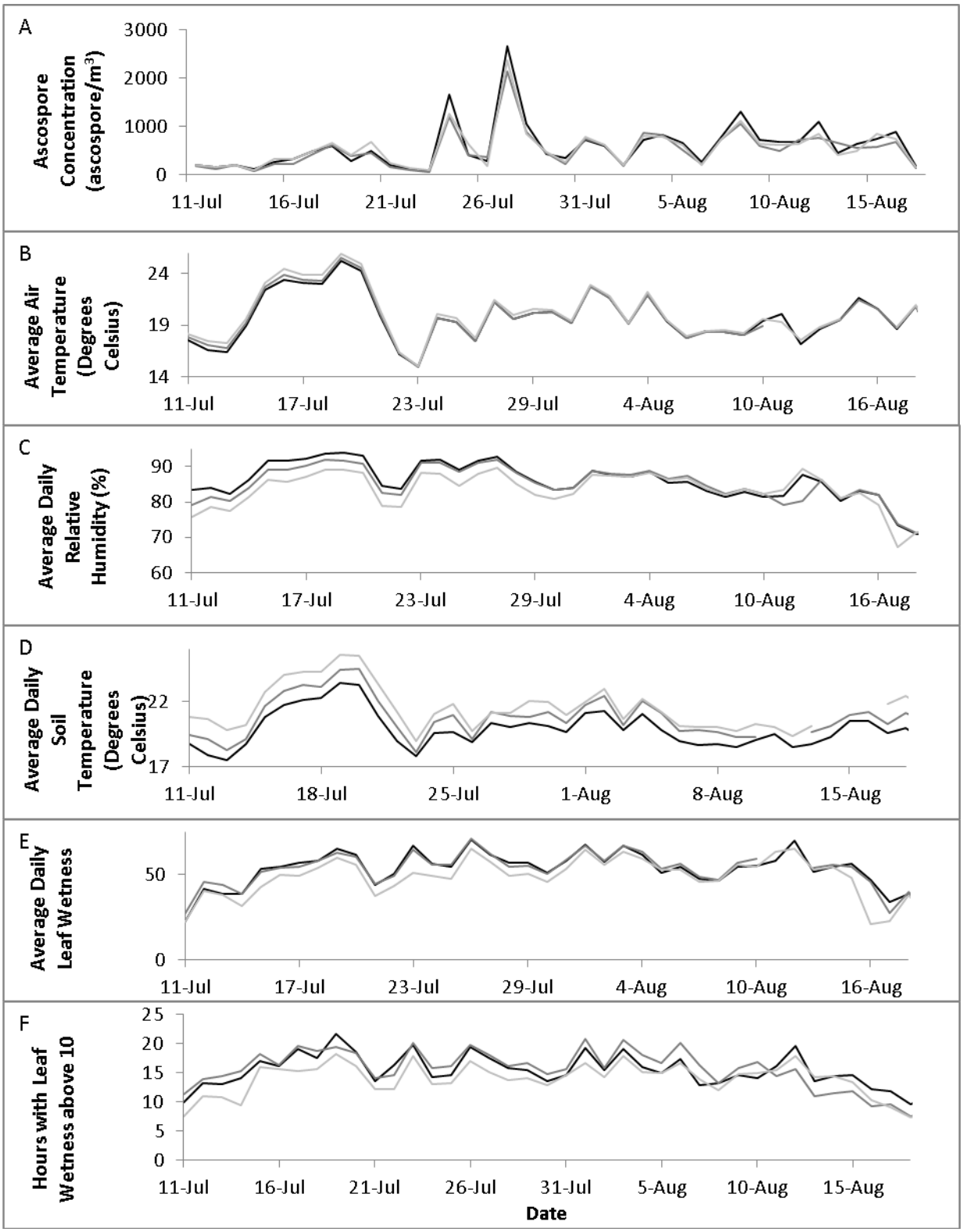

Figure 2.16 Comparisons of A) ascospore concentrations (ascospores/m3) and overall microclimatic averages in high, medium and low plots for B) daily air temperature (˚C), C) daily relative humidity (%), D) daily soil temperature (˚C) and E) daily leaf wetness in Carman, 2011.

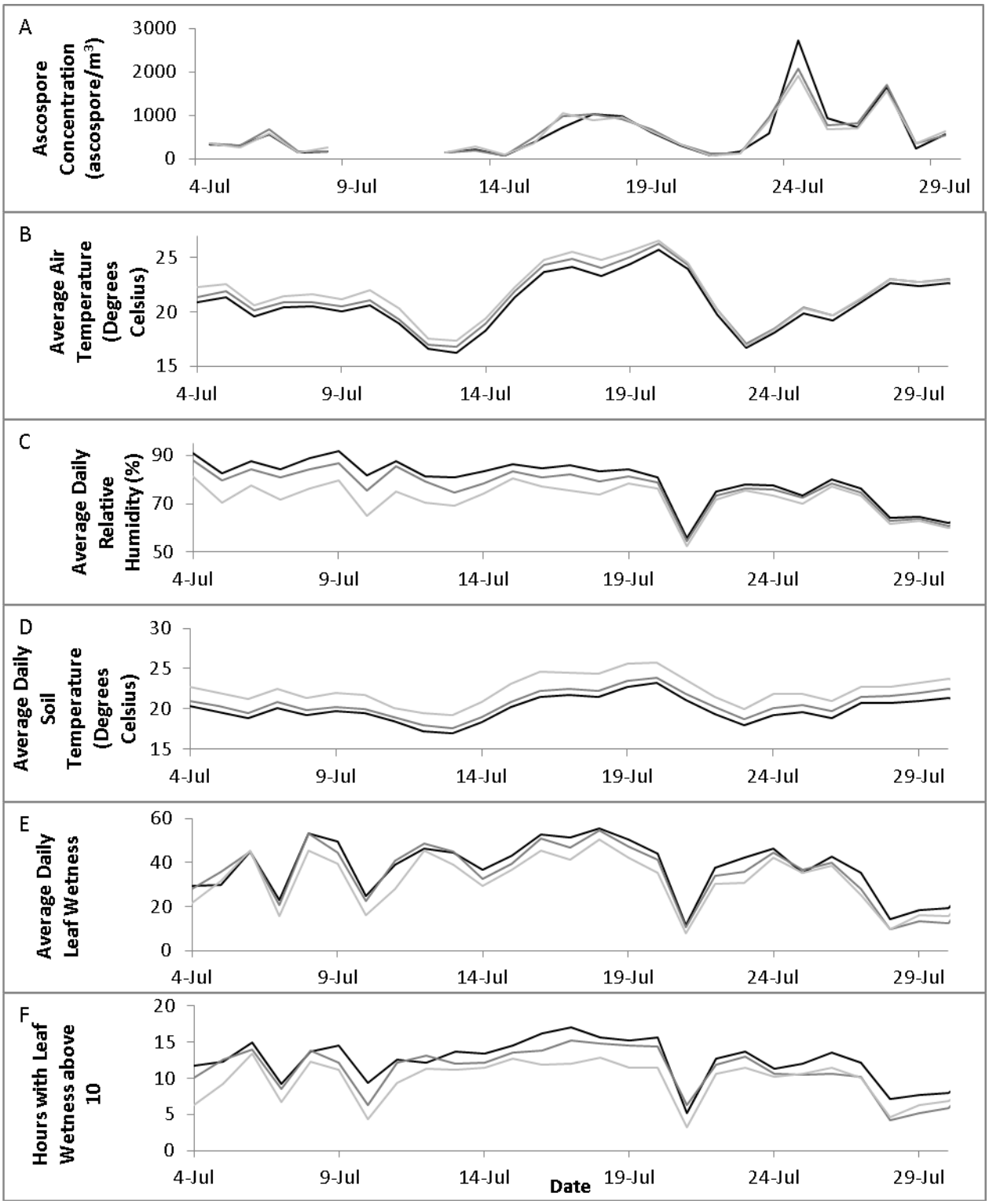

Figure 2.17 Comparisons of A) ascospore concentrations (ascospores/m3) and overall microclimatic averages in high, medium and low plots for B) daily air temperature (˚C), C) daily relative humidity (%), D) daily soil temperature (˚C) and E) daily leaf wetness in Winnipeg, 2011.

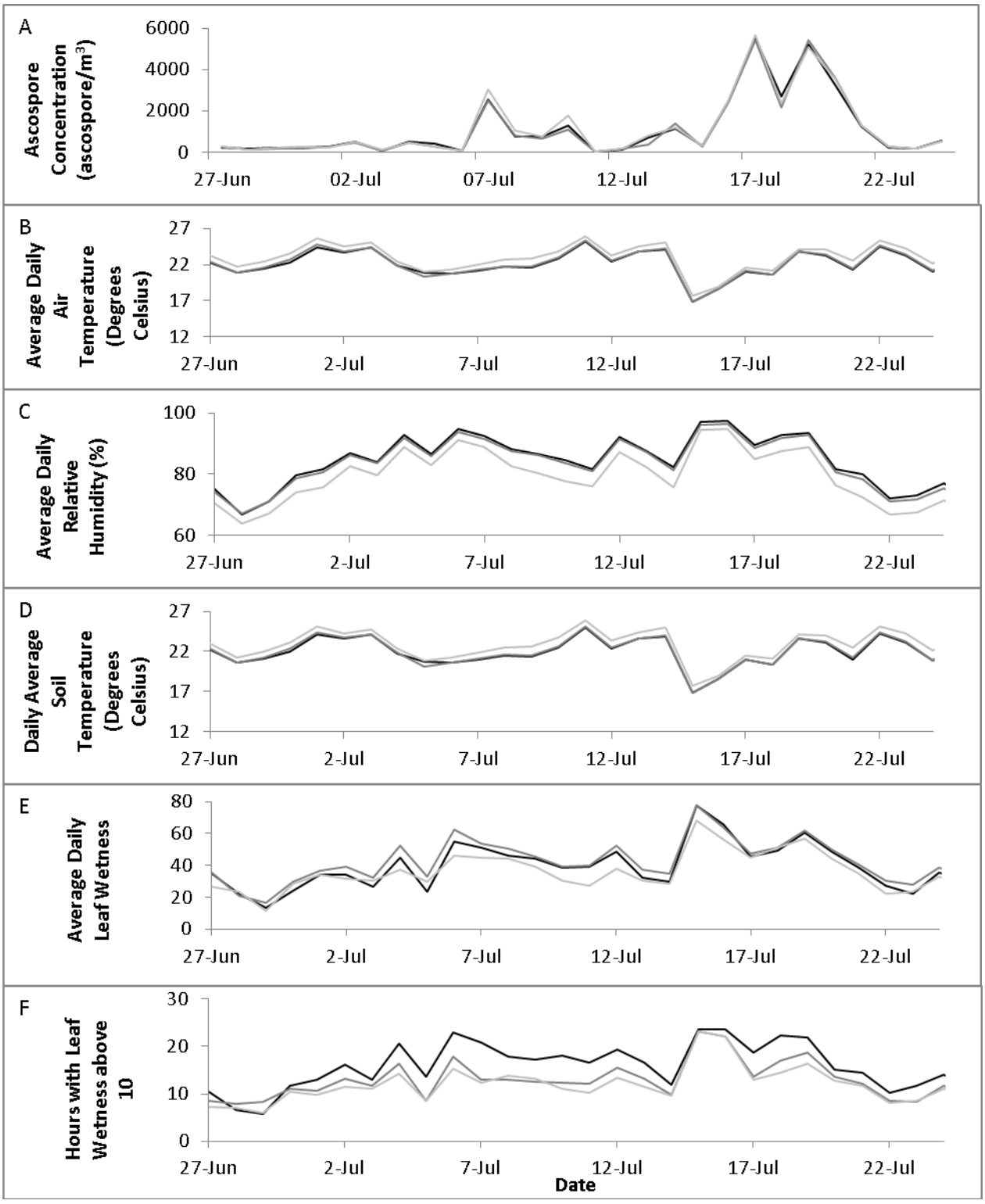

Figure 2.18 Comparisons of A) ascospore concentrations (ascospores/m3) and overall microclimatic averages in high, medium and low plots for B) daily air temperature (˚C), C) daily relative humidity (%), D) daily soil temperature (˚C) and E) daily leaf wetness in Carman, 2012.

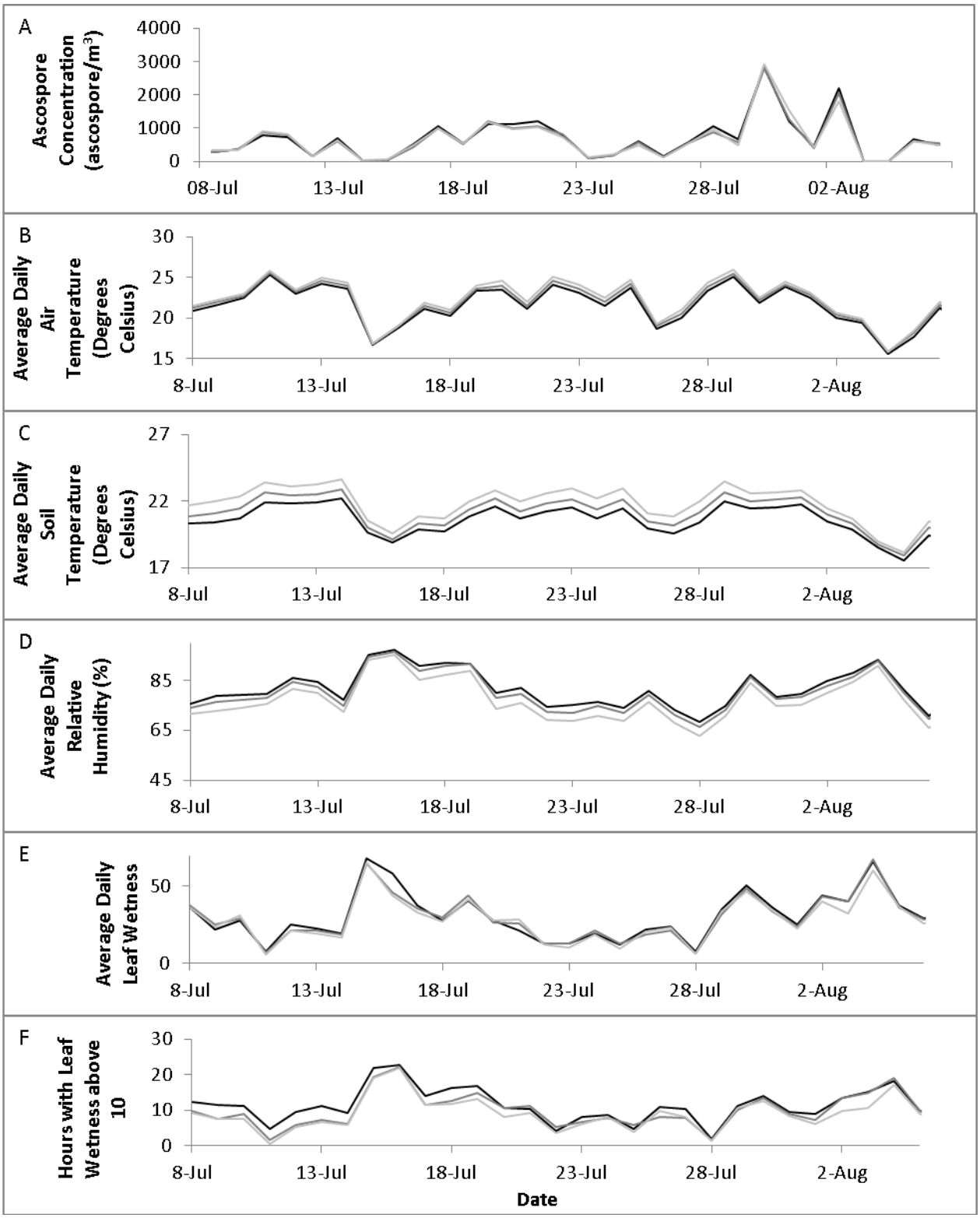

Figure 2.19 Comparisons of A) ascospore concentrations (ascospores/m3) and overall microclimatic averages in high, medium and low plots for B) daily air temperature (˚C), C) daily relative humidity (%), D) daily soil temperature (˚C) and E) daily leaf wetness in Winnipeg, 2012.

2.4.4.5 Petal Test Results

In 2011, petal tests were done in Carman and Winnipeg at 30 and 70 percent bloom stages to ensure the presence of ascospores on petals in each field. In 2012, petal tests were done in Carman and Winnipeg at 15, 30 and 70 percent bloom stages in each of the plots. Based on the observations made approximately 14 days after plating, no consistent results were found (Table 2.13). Very few sampled petals contained ascospores during any of the sampling periods.

Table 2.13. Petal counts containing ascospores within each plot at various bloom stages. Non listed plots contained 0 ascospore colonized petals.

Year	Location	Date	Bloom Stage (%)	Plot #	# Petals with ascospores
2011	Winnipeg	Jul-06	30	18	1
				3	1
		Jul-14	70	12	1
				13	4
				14	1
	Carman	Jul-18	30	8	2
	Carman	Jul-25	70	None	0
2012	Winnipeg	Jul-13	15	17	3
				5	1
		Jul-19	30	14	1
				11	1
		Jul-24	70	None	0
	Carman	Jul-05	15	2	2
				3	1
		Jul-09	30	14	1
		Jul-18	70	12	1

2.5 Discussion

2.5.1 Weather and Disease Development

This study suggests that a higher concentration of inoculum in the form of sclerotia may have been responsible for higher ascospore concentrations in Carman compared to Winnipeg in 2011. In comparing ascospore concentrations between Winnipeg and Carman where equal inoculum was provided to both sites in 2012, higher rates were released in Carman due to the elevated relative humidity, reduced temperatures and additional precipitation. Evidently weather plays a major role in ascospore production due to the similarity in peak spore concentrations between Winnipeg and Carman situated more than 50 km apart. During late bloom, precipitation led to increased airborne ascospore concentrations. Peaks in ascospore concentrations generally followed prolonged periods of increased relative humidity following a precipitation event, similar to observations by Qandah and Mendoza (2011). Higher precipitation rates in Carman during 2012 also correlated to increased infection towards harvest. Decreased temperature along with increased relative humidity and leaf wetness appear to be responsible for higher disease incidence in Carman compared to Winnipeg. Canopy density modifications effectively altered microclimates however, these changes were not sufficient to significantly influence ascospore release and infection. Similarly, disease development was not significantly affected by misting despite the creation of a cooler and moister canopy.

Specific environmental conditions are required for crop and disease development. Crops require temperatures, moisture and nutrients within a given range for survival and growth. Similarly, sclerotinia stem rot is increased under specific weather conditions. For example adequate moisture and cool temperatures have been shown to be required for disease development (Turkington et al., 1991; Manitoba Agriculture, 2013). The first year of this study, 2011, began with seeding occurring later than normal throughout Manitoba in 2011 due to the saturated top soils, heavy snow melt and precipitation during May and June. The rest of the summer was fairly dry and hot compared to normal. Warm dry weather in April preceded a prolonged cool period at the beginning of May in 2012 followed by warm dry conditions during the summer. Slightly warmer temperatures and lower relative humidity were experienced in 2012 reducing time to flowering and affecting seed set and size. Microclimates were also affected by the overall weather creating warmer temperatures and reduced relative humidity below the canopy in 2012.

Some interesting trends were observed among all the variables compared among both years and sites. With respect to canopy density, site years with higher LAI values did not contain the highest plant counts. For example, Winnipeg 2011 showed the highest LAI, however plant counts were below average. One of the reasons for this may be due to the presence of weeds which were competing with canola for available resources and covering additional surface area thus increasing LAI values and decreasing plant counts. Weeds were also abundant in Winnipeg in 2012. Higher LAI measurements generally during early bloom can be explained by the thinning of the canopy from early to full flowering to pod development.

With respect to disease, mean ascospore concentrations were higher in 2012 which may have resulted from the additional inoculum levels in the form of sclerotia placed on the fields that year. However, the increased ascospore concentrations released from apothecia did not result in increased infection. Thus environmental factors influencing disease incidence must have been a limiting factor. Additionally, average, minimum and maximum mean daily ascospore concentrations were higher during late bloom compared to early bloom. This trend may have occurred as a result of the natural timing of ascospore release occurring during the growing season or due to weather conditions during late bloom favoring ascospore release from apothecia. For species *Leptosphaeria maculans* (the pathogen responsible for blackleg in canola), humid conditions with frequent rainfall and prolonged moisture favour ascospore release with air temperatures at approximately 13 to 18°C and relative humidity values above 80 per cent (Canola Council of Canada, 2014). *Leptosphaeria maculans* ascospore dispersal occurs several hours after a precipitation event of greater than 2 mm and can persist for approximately 3 days (Guo and Fernando, 2005). Similarly, the ascospores discharged into the atmosphere by *Fusarium graminearum* (fusarium head blight in wheat) require warm (25 to 28°C) moist conditions during flowering for successful infection (Government of Saskatchewan, 2007). Precipitation may have also influenced *S. sclerotiorum* ascospore release in several ways in this study. Due to different seeding dates among site years, precipitation was variable throughout the growing season where totals were higher in Winnipeg in 2011 and Carman in 2012. Seasonal precipitation was higher under sites with earlier seeding dates. The high precipitation in Carman (2012) increased spore concentrations significantly and also resulted in high disease incidence. In 2012, more precipitation was received overall during the sampling period which may also have contributed to increased

spore concentrations; however overall disease incidence at harvest was not affected. Precipitation was increased during late bloom compared to early bloom which may account for higher ascospore concentrations that occurred during late bloom. Accumulated total precipitation seems to have influenced accumulated ascospore release throughout the flowering period since highest precipitation totals led to higher accumulated ascospore concentrations. Average daily mean relative humidity values that were higher in Carman compared to Winnipeg during both years may be partially responsible for the increased disease and ascospore concentrations occurring in Carman. Several researchers (Tores and Moreno, 1991; Clarkson et al, 2003; Qandah and Mendoza, 2011) have discussed the influence of not only relative humidity on SSR disease development but other weather factors such as temperature, solar radiation and wind speed. The field in Winnipeg in 2012 was located within 30 meters of a fence with trees. These obstructions may be the reason why average daily wind speeds at this location were low in comparison to Carman. Winnipeg 2012 also experienced very low infection levels; however the influence of wind on disease development is not clear. Temperatures were highest during the Winnipeg 2012 growing season compared to other site years which may also have contributed to a reduction in disease. Based on previous research, sclerotia germinate most readily at an optimum temperature of 10°C (Abawi and Grogan, 1975) while ascospore release is optimum at 22°C according to Qandah and Mendoza (2011). Ascospore survival is also important for disease occurrence; survival rates at temperatures at or above 25°C are reduced (Caesar and Pearson, 1983). Koch et al. (2007) showed that most rapid SSR development occurred between 16 and 22°C. During both years at late bloom, Winnipeg showed higher values for average total daily

incoming solar radiation compared to Carman. This may have increased temperatures late in the season and possibly reduced the potential for disease incidence.

Comparisons of ascospore concentrations taken during the sampling period between Winnipeg and Carman plots show similar trends with peaks occurring on the same days. Since values for daily weather parameters plotted for Winnipeg and Carman during the season also show similar trends, we can conclude that daily ascospore release over time was responding to weather conditions. It is evident that with precipitation events, there is an increase in daily mean percent relative humidity percentages. In a study conducted by Qandah and Mendoza (2011), peaks in sclerotinia ascospore concentrations occurred after prolonged periods of high relative humidity followed by a rapid decrease in relative humidity. The result of this study confirms their findings as similar trends were observed. However, this pattern did not occur consistently and thus has limited value for prediction of ascospore release. Peaks in daily mean ascospore concentrations generally occurred following a precipitation event which would have caused an increase in atmospheric relative humidity. The majority of higher relative humidity values occurred slightly before or during increases in atmospheric ascospore concentrations. In most cases, when relative humidity decreased and temperatures increased slightly, ascospore concentration peaks were observed. These trends indicate that prolonged moisture and increased relative humidity prior to ascospore release may be required; however release occurred during decreasing relative humidity while temperatures were increasing and relative humidity was decreasing. Although this phenomenon appears to have occurred during most peak ascospore days, it is not the case throughout the sampling period. Other factors combined with precipitation, relative humidity and temperature need to be examined.

Effects of precipitation and irrigation on sclerotinia stem rot has been studied by several researchers (Blad et al., 1978; Twengstrom et al., 1998; Bom and Boland, 2000; Qandah and Mendoza, 2011), however the effects of misting on *S. sclerotiorum* ascospore release in canola remains uncertain. Natural and artificial precipitation contributes to increased soil moisture, canopy wetness and relative humidity which are considered to be suitable environmental conditions for SSR disease development. Precipitation often also contributes to decreased temperatures under the canopy favoring disease slightly, as shown by Blad et al. (1978) and Weiss et al. (1980) in their studies of irrigation on dry edible beans. Soil moisture is responsible for germination of sclerotia, influencing an important inoculum source (Twengstrom et al., 1998; Bom and Boland, 2000). Relative humidity plays an important role in the release of ascospores from apothecia (Qandah and Mendoza, 2011). Similar results of misting on microclimate were shown in this study. The site-year containing the highest accumulated daily precipitation also showed the highest accumulated mean daily ascospore concentration among all site-years. Thus, a positive correlation between accumulated precipitation and ascospore concentration was evident. During 2011 when plots were misted, Winnipeg showed higher daily mean ascospore concentrations and percent infection on average under misted compared to non-misted plots, however in Carman non-misted plots showed higher ascospore concentrations and higher percentage of infection on average. In Winnipeg, misting may have contributed to increased spore concentrations on average, but other unknown factors may also be responsible. The plot in Carman contained a history of disease in the non-misted portion of the field, therefore even with the addition of equal inoculum levels, the non-misted portion still contained higher levels during the season. Although misting may

contribute slightly to ascospore release, level of inoculum present is also a critical factor in determining ascospore release concentrations and overall disease incidence. There also does not appear to be a combined effect of misting and precipitation on ascospore release as trends in concentrations are similar among misted and non-misted plots. Additional focus needs to be placed on the effects of misting on ascospore release where inoculum levels are similar throughout treatments.

Ascospore release from apothecia is an important part of the disease life cycle as it contributes to the source of inoculum present in the proximate atmosphere available for colonization and infection of the canola crop. Several environmental factors such as precipitation, relative humidity, temperature, wind and solar radiation may affect inoculum levels. As previously mentioned, precipitation contributes to increased atmospheric relative humidity; however ascospore release does not occur during large precipitation events according to this study. Ascospore release may be linked to a series of environmental changes occurring following a rain event as increases in ascospore concentrations tend to occur during the days following rain. Ascospores of *Leptosphaeria maculans* are also released following larger precipitation events (Guo and Fernando, 2005). *Sclerotinia sclerotiorum* ascospore release generally occurs mid-day as shown by several researchers (Harthill, 1980; McCartney and Lacey, 1991). In a study conducted by Qandah and Mendoza (2011), ascospore release generally occured in a single event daily from 8 am to 4pm with highest ascospore concentrations occurring from 10am to 2pm. The increase in ascospore release occurring from 8am to 2pm was reported to be a result of increasing temperature and decreasing relative humidity (Qandah and Mendoza, 2011). This study focuses further on these trends and tries to determine the optimum temperature

and relative humidity changes required to increase ascospore release assuming ascospore release is occurring in a single event from 8 am to 4pm daily. Relative humidity decreases and temperature increases from 8am to 2pm for each day were compared against daily mean ascospore concentrations. This time period was chosen to represent the period at which ascospore concentrations are said to be increasing during the day (Qandah and Mendoza, 2011). In this study, relative humidity decreases computed for each day from 8am to 2pm showed higher daily mean ascospore concentrations where daily decreases exceeded 20%; below that, low concentrations were released among most locations. This pattern may highlight the importance of rapidly decreasing relative humidity required for spore release which is followed by a period of elevated relative humidity. Increases in temperature from 8am to 2pm between 6 to 8°C tended to correlate to highest ascospore release in Carman 2012, however no distinct trends were realized. Daily mean values for several environmental factors were used in comparison to ascospore concentrations. On average, days with mean temperatures between 18 to 22 °C appear to have had the highest average mean daily ascospore release concentrations indicating that ascospores tend to be released in high concentrations during days with approximately average seasonal temperatures in Manitoba. Days with mean percent relative humidity between 62 and 82% produced the highest mean daily ascospore concentrations overall in all site years. Relative humidity in Carman was slightly higher compared to Winnipeg during both years. Higher minimum and mean percent relative humidity was required to increase ascospore release in Carman whereas ascospore concentrations were higher under the increased range of relative humidity compared to Winnipeg. This may be explained by an adaptive behavior of the pathogen which is capable of adapting to regions with varying environments. Although lower relative

humidity in Winnipeg may be responsible for the reduced ascospore concentrations overall, release of spores still occurred under these conditions. Lower daily mean wind speeds ranging from 0.5 to 3.0 m/s contributed to the highest daily mean ascospore concentrations. Slight winds may be required for the release and dispersal of ascospores, especially to a height above the canopy. At higher daily mean wind speeds ascospore concentrations were reduced which could reflect a damaging result of wind on apothecia or the influence wind has on the mechanical ability of the spore capturing device. Higher wind speeds may also remove ascospores from the boundary layer.

Disease incidence is one of the most important indicators of disease at harvest measured as percent infection. It is understood that precipitation alters other atmospheric parameters by increasing relative humidity and decreasing temperature. Combined with the modifications associated with precipitation, they can enhance disease development based on previously completed studies (Twengstrom et al., 1998; Bom and Boland, 2000; Qandah and Mendoza, 2011). Based on observations, precipitation just prior to harvest did not correlate with disease incidence between site years and therefore does not appear to affect disease incidence significantly. Based on the 2011 data from both sites, slightly higher precipitation prior to harvest in Winnipeg did not result in increased disease incidence compared to Carman. However increased disease incidence in Carman may be attributed to the disease history at this location with increased inoculum levels. In 2012 both fields received even inoculum levels and precipitation just prior to harvest were similar which correlated to high percent of disease at harvest in Carman. In Winnipeg (2012), a small precipitation event prior to harvest provided low disease percentage at harvest. An increase in disease incidence occurring post-harvest may be in part due to the

larger rain event occurring before the assessment was complete. Other diseases such as *F. graminearum* requires at least 12 hours of precipitation and high relative humidity for successful germination and infection of ascospores (Government of Saskatchewan, 2007). Another explanation could be that disease is also enhanced over time showing greater symptoms and is easier to identify as it worsens. Similarly, percentage of diseased lettuce plants increased over time in a study conducted by Young et al. (2004). Although precipitation contributed to higher disease percentages in 2012, it does not appear to be a limiting factor for disease incidence since disease incidence was more strongly correlated to inoculum levels and ascospore release loads. It is possible that adequate moisture was supplied to the crops in all site-years and other weather factors may have more influence on disease incidence. Young et al. (2004) studied *S. sclerotiorum* disease development on lettuce and found that disease development was not influenced by leaf wetness duration or relative humidity; however disease developed quickly under optimum temperatures of 15 – 25 °C and continual moisture. Lower July and August daily minimum and maximum temperatures are considered to enhance disease prevalence due to the "cool-climate disease" label sclerotinia has acquired (Workneh and Xuang, 2000). During any given year, maximum and minimum temperatures that were lower than the normal for a specific region received increased disease prevalence (Workneh and Xuang, 2000). On average during late bloom and ripening, mean, minimum and maximum temperatures were highest within Winnipeg plots during both years and lower disease incidence percentages were also observed in Winnipeg.

2.5.2 Effect of Microclimate on Disease Development

Environmental conditions below the canopy are most important for crop and disease development. Temperatures between 10 to 20℃ (Phillips, 1987) and adequate soil moisture (Matheroon and Porchas, 2005) are necessary for sclerotial germination. Ascospore release from apothecia relies on prolonged periods of high relative humidity and followed by decreasing relative humidity and increasing temperatures mid-day (Qandah and Mendoza, 2011). Upon landing on senescent tissue, ascospores require moisture in the form of leaf wetness to germinate and further infect the plant (Bardin and Huang, 2001). In comparing the overall microclimatic conditions between all 4 site-years, relative humidity and leaf wetness were higher and temperatures were reduced in Carman during both years. Overall, 2012 experienced higher temperatures and lower relative humidity and leaf wetness. Below canopy conditions reflect the weather conditions above the canopy. It appears that the microclimatic conditions experienced in Carman during both years were conducive to disease increasing the overall percentage of infection during both years. These conditions may have also created favorable conditions for ascospore release and dispersal based on the high ascospore concentrations observed in Carman during 2012.

Increasing canopy density serves as an important tool for a variety of agricultural improvements, however there may be drawbacks. Increasing canopy density generally provides increased yields and reduces weed pressures though several canola diseases including sclerotinia are shown to thrive under conditions created by denser canopies. A combination of seeding rates and fertilizer treatments has been shown to be effective in the modification of canopy density, microclimate factors, as well as disease severity.

Secondary spread of disease can be reduced in beans and sunflower by increasing row spacing and reducing seeding rates (Hoes and Huang, 1985; Tu and Zheng, 1997). In canola, conflicting results have been found on the effects of canopy density on disease incidence. Turkington and Morall (1990) and Jurke and Fernando (2008) saw an influence of canopy density on disease incidence. Lodging was more prevalent in high density plots, creating conditions conducive to disease development such as increased contact and moisture (Jurke and Fernando, 2008). In other studies, effects of seeding rate on disease incidence were either very low (Turkington et al., 1991) or non-existent (Nordin et al., 1992). In this study, seeding rate and fertilizer treatments were effective in modifying canopy density. Overall, seeding and fertilizer treatments created significantly different LAI values and plant counts among the four site-years, especially among high and low seeding rate treatments. Canopy density treatments were effective in modifying several microclimatic parameters as well. As expected, under denser canopies temperature was decreased and relative humidity was increased significantly among 3 site years. Soil temperatures were also decreased significantly under denser canopies. Leaf wetness was significantly higher during one site-year. These sub canopy modifications had no influence on airborne ascospore concentrations among the three density treatments. Each treatment consisted of a 10 by 10 meter block which may not have been large enough to accommodate spore dispersal distance. According to Williams and Stelfox (1979), ascospores are capable of travelling up to 30 to 50 meters above the surface and 150 meters from the apothecia source. Use of larger plots would allow for a better capture of spores within each individual treatment. Also, because ascospores originate from apothecia present at the soil surface, a denser canopy may not allow spores to travel upwards beyond the foliage. Based on our study, we speculate that increased

relative humidity and leaf wetness and reduced temperatures influenced disease development among denser canopies in Carman 2011; however these results were not consistent throughout the other 3 site-years. As previously mentioned, denser canopies may prevent ascospores from travelling up to the flowers for further colonization of the plant. Additional study needs to be completed on the implication of dense canopies on sclerotinia stem rot development.

Timely comparisons of ascospore concentrations in relation to below canopy conditions yielded inconsistent results; however some trends were worth noting. Peaks in relative humidity preceding peaks in ascospore concentrations demonstrate the importance of humidity prior to ascospore release. Increased within canopy relative humidity also led to higher leaf wetness values. Leaf wetness is important for the deposition and colonization of ascospores on canola petals and further to the development of disease within the entire plant (Bardin and Huang, 2001). High leaf wetness values during and following spore deposition on canola may have led to increased disease incidence among Carman plots during both years. Higher temperatures experienced in Winnipeg may have also led to reductions in ascospore survival as temperature and radiation influence survival rates (Canola Council of Canada, 2013).

Misting provided inconsistent results with respect to canopy density and disease development in both Winnipeg and Carman fields in 2011; however misting did alter microclimates slightly. Under misted plots, relative humidity and leaf wetness values were increased, while soil and air temperature values were decreased slightly at both Winnipeg and Carman locations. As a result of misting, microclimates were modified

mainly through the changes in macroclimate and not entirely due to changes in canopy structure. In Carman, number of plants per square metre was higher in misted plots; however leaf area index values remained lower indicating that although there were additional plants, increased plant cover and densities were not achieved. In Winnipeg, leaf area index was overall slightly higher and plant counts were lower in misted plots indicating that individual plant densities were slightly higher, however fewer plants appeared. Significant changes in canopy structure would have occurred if misting had begun at the beginning of the growing season. Although spore concentrations and disease incidence values were slightly higher among misted plots in Winnipeg, this did not occur in Carman in 2011. Along with even inoculation of the plots, the Carman plot (2011) had a previous history of disease mainly in the north portion of the field (personal communication with Alvin Iverson, Ian N. Morrison Field Station, 2011). Since misting occurred in the south portion, there may be a larger influence from the additional inoculum source to the north creating increased ascospore concentrations and disease incidences in that location of the field regardless of the absence of a misting system. Blad et al. (1978) studied *S. sclerotiorum* in a semi-arid climate, where microclimates are unfavourable for white mold disease in bean. A continuation of this work was completed by Weiss et al. (1980). Both studies determined that, through the use of an irrigation system, disease was most prominent under misted plots due to favorable microclimatic conditions caused by the misting and by the advanced crop development which occurred as a result of misting (Blad et al., 1978; Weiss et al., 1980). Temperatures in the air, on leaves and soils remained cooler under misted plots and leaf wetness periods were longer. The locations at which the fungus *S. sclerotiorum* was studied (eastern Colorado and western Nebraska) have unfavourable conditions for disease with warm arid climates. In

this region, the days that were the hottest showed greater microclimatic changes through the use of a misting system (Weiss et al., 1980). Use of a misting system in these locations is more effective in substantially modifying microclimates and creating an environment where disease can develop compared to Manitoba, which already has the necessary conditions for disease development without the use of a misting system, especially during years with adequate precipitation and cooler growing season temperatures. Changes to the microenvironment may not be substantial enough to elicit significantly more disease incidence in a prairie region such as in the province of Manitoba.

Several confounding variables have made it difficult make accurate comparisons among all four site-years. For instance, inoculation in 2012 occurred during both the fall and spring prior to the growing season, whereas 2011 received inoculation in the spring. Inoculums were also increased in 2012. Fall applied inoculum were able to overwinter in the soil where they would germinate the following season compared to inoculum that were conditioned outdoors in mesh bags and placed in the field during the spring. Both experimental locations contained no history of disease and previously grown crops were not susceptible to sclerotinia in 2012. This is similar for Winnipeg in 2011. Carman (2011) contained history of disease in half the plot, therefore an increased level of inoculum was present at that location which may be responsible for increased SSR disease. Misting was provided to the crop in 2011 only, which may have influenced soil moisture and overall relative humidity and temperature throughout the field making it difficult to compare with 2012. As a result of not misting in 2012, two randomized complete block designs were used in the analysis of natural non misted conditions compared to only one randomized complete block design used in 2011. There was a

switch in the placement of microclimate stations and rotorods from 2011 to 2012. Rotorods were placed at 2.5 meters from the edge of each plot in 2011 and in the middle of each plot in 2012 to better reflect spores being captured mostly from within the plot increasing distance to adjacent plots. Microclimate stations were in the middle of each plot in 2011 and at 2.5 meters from the edge of each plot in 2012 to allow rotorod placement in the center of each plot. Rotorods used in Winnipeg had retracting heads that extended the polysterene rods only when the rods were spinning. Rotorods placed in Carman had polysterene rods that were continually downward facing, which may be responsible for increased spore concentrations found in Carman during both years. In order to make comparisons among the treatments to adequately reflect their impacts on microclimate and disease development, confounding effects need to be eliminated.

2.6 Conclusion

Comparing all four site-years, no correlation was observed between ascospore concentrations and percent infection; however inoculum levels may have been effective in increasing ascospore release. There appears to be an overall influence of weather on ascospore concentrations and percent infection. Peaks in ascospore concentrations occurred simultaneously between the two Manitoba locations separated by more than 50 km. Carman experienced high relative humidity and lower temperatures both above and below canopy which led to increased infection compared to Winnipeg during both years indicating that relative humidity is required for disease development. A precipitation event that resulted in increased relative humidity preceding a decrease in relative humidity and increase in temperature may have contributed to increased ascospore releases, which concurs with previous research.

The misting system used to alter microclimates below the canopy in 2011 was not effective in increasing ascospore release concentrations or percent infection at harvest. Misting was effective in reducing within canopy temperature and increasing relative humidity and leaf wetness. Ascospore concentration and percentage of infection were higher among misted plots in Winnipeg, however this did not occur in Carman which is likely due to the additional inoculum levels present in Carman.

Seeding rate and fertilizer treatments were effective in significantly modifying LAI and plant counts among high versus low density plots. Several microclimatic factors also proved to be significantly different between high and low treatments among all site-years, including air temperature, soil temperature and relative humidity. These

modifications had no influence on airborne ascospore concentrations. Denser canopies showed significantly higher infection values in Carman during 2011; however no significant differences were seen among the other 3-site years.

This study concludes that modifying canopy density and providing additional moisture through misting does alter microclimate, however not enough to significantly affect disease development. The overall seasonal weather will have an impact on disease development; however inoculum concentrations seem to have a greater impact on disease under favourable conditions with adequate moisture and optimum temperatures.

2.7 References

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3. INFLUENCE OF NON-HOST CROPS IN NEIGHBOURING FIELDS ON SCLEROTINIA STEM ROT DISEASE

3.1 Abstract

Crop rotation is a widely used management strategy influencing canola yields and reducing pressures from unwanted pests and diseases including *Sclerotinia sclerotiorum*. Canola fields free of disease history may still be at risk of being infected due to the ability of sclerotinia ascospores to travel from neighboring fields, including fields containing non-host crops. This study compares ascospore release under both wheat and canola canopies and analyzes the influence of weather and microclimate on airborne ascospore concentrations in a wheat field. Gradients were created alongside the wheat field to monitor spore dispersal distance as well as the influence of wind direction on ascospore dispersal. This study demonstrated that sclerotia germinate and ascospores are released from apothecia within a wheat canopy at similar and sometimes greater rates than release under a canola crop. Further, ascospores are capable of traveling at distances of greater than 100 m into neighboring fields. Although crop rotation is an important disease management strategy, its effectiveness in reducing inoculum levels is unclear. Disease forecasting also needs to incorporate information on neighboring fields and their history of disease.

3.2 Introduction

Sclerotinia stem rot (SSR) disease is a serious disease affecting over 400 host crops worldwide caused by the fungus *Sclerotinia sclerotiorum* (Lib.) de Bary. In canola, ascospores are the primary source of inoculum of *Sclerotinia sclerotiorum* (Jamaux et al., 1995) and create most primary infections occurring in the field (Abawi and Grogan, 1979). Ascospore release and dispersal is important due to their impact on SSR disease intensity in canola (Qandah, 2008). During the growing season, ascospores are produced carpogenically from sclerotia, which are overwintering fungal structures resting at or near the soil surface and capable of surviving in the soil for prolonged periods of time. Ascospores are forcibly released into the surrounding atmosphere by asci contained within apothecia which develop from sclerotia (Harthill and Underhill, 1976). SSR is considered to be a monocyclic disease containing only a single infection cycle. During this cycle, several cohorts of airborne ascospores released into the air land on petals which serve as a source of nutrition and the infected petals subsequently fall on nearby stems and leaves and infect and potentially kill entire plants. Timing of ascospore release is critical for disease occurrence; release must occur during the canola flowering stage.

Studies to date have focused mainly on the environmental factors associated with the release of ascospores within SSR sclerotinia stem rot susceptible crops. Light, temperature and relative humidity have been studied extensively by researchers. Harthill's (1980) study indicating that light was involved in the process was later overturned by Clarkson et al (2003) who proved that spores were released under both light and dark conditions. Significant impacts of relative humidity and temperature on

release of ascospores were reported by Qandah and Mendoza (2011). Their study concluded that ascospore release occurs only once daily in a single event and lasts no longer than six hours with the release in wet years occurring only during the day and only during the night in dry years (Qandah and Mendoza, 2011). Ascospores were generally released in periods of increasing air temperatures, and decreasing relative humidity following prolonged periods of high relative humidity (Qandah and Mendoza, 2011). Previous findings also conclude that ascospore release occurs with decreasing relative humidity and increasing temperatures and wind speeds (McCartney and Lacey, 1991). Although these environmental factors affecting ascospore release have been documented, additional emphasis needs to focus on seasonal release and dispersal of spores and effects of additional factors including wind speed and direction.

The few studies focusing on *S. sclerotiorum* ascospore dispersal have found that disease results mainly from inoculum produced within the field (Boland and Hall, 1988; Twengstrom et al., 1998). In contrast, a study specifically focusing on *Sclerotinia* ascospore dispersal in canola was completed by Qandah and Mendoza (2012) in which dishes containing a semi-selective medium were placed throughout a canola field at several distances from a known source of inoculum. Disease and ascospore concentrations declined with distance from the source of inoculum (Qandah and Mendoza, 2012). No ascospores were dispersed in dry years with little precipitation and low relative humidity values (Qandah and Mendoza, 2012). Similar studies have been completed on other fungal pathogens with similar characteristics. Guo and Fernando (2005) conducted a study in Manitoba on ascospore dispersal by *Leptosphaeria maculans* in relation to environmental conditions. Burkard and rotorod spore trapping devices were

placed throughout two fields to capture spores. Hourly and daily ascospore concentrations were recorded from the devices and plotted against weather values. The effect of wind direction on ascospore dispersal was analyzed by monitoring ascospore concentrations at several distances from the inoculated plots. Fungal ascospore concentrations of *L. maculans* peaked during and following rainfall events when relative humidity was elevated and temperature decreased. Ascospore concentrations also decreased with distance from the inoculated source and wind direction had a significant effect on ascospore dispersal where increased concentrations were found downward from the wind source (Guo and Fernando, 2005). Quantification of ascospore concentrations in relation to several weather factors including wind direction was completed successfully for *L. maculans* and a similar analysis needs to be completed for *S. sclerotiorum.*

Previous studies have characterized only a few environmental conditions necessary for release and dispersal of ascospores in crops susceptible to *S. sclerotiorum*. A better understanding of disease development under a crop not susceptible to *S. sclerotiorum* is required to determine the effectiveness of disease management of sclerotinia by crop rotations using non-host crops. An evaluation of wind speed and direction on dispersal of ascospores and their potential to infect neighboring fields is also important. Little is known on the influence of non-host crops and sclerotinia disease. The objective of this study was to determine the influence of a neighbouring non-host wheat crop on sclerotinia stem rot disease in canola, the impact of microclimatic factors within the canopy, as well as wind speed, wind direction and ascospore dispersal above and adjacent to the canopy.

3.3 Methods

3.3.1 Study Sites and Field Layout

Two field sites were used in 2011 and 2012 to study the effects of sclerotia-inoculated wheat fields on ascospore production and dispersal. Fields were located in Winnipeg and Carman. In 2011, the field used in Winnipeg contained no previous history of disease, whereas the field in Carman contained a history of disease in the previous years. Neither field site in 2012 had a previous history of disease and had been seeded with non-host crops in prior years.

In Winnipeg (2011), the south portion of the plot was seeded with 3 density treatments of canola over 18 plots using a randomized complete block design as explained in chapter 2. The remaining field portion to the north, had 'Kane' wheat seeded in a strip running from north to south (10m wide by 50m long), with 10m of bare soil to the east and west of the wheat strip. In 2012, the north portion of the field was seeded with canola, while the remaining south field portion had AC Barrie wheat running from north to south with bare soil to the east and west of the strip. Similarly, in 2011 and 2012 Carman was seeded with a canola field portion containing 3 treatments over 18 plots. The remaining portion of the field to the left contained 'Kane' wheat (10m by 50m) with 10m of bare soil on each side. The canola field portion ran from west to east in Carman in 2011, with the wheat strip to the north. In 2012, the canola portion ran from east to west with the wheat strip located to the south (A.3).

3.3.2 Field Preparation

Wheat (Kane in 2011 and AC Barrie in 2012) and canola seeding in Winnipeg and Carman occurred on May 26th and June 8th in 2011 and on June 6th and May 23rd in 2012 respectively. Carman received tillage in 2011, Winnipeg did not. Wheat was seeded at 130kg/ha and ammonium sulphate (30 kg/ha), MAP (30 kg/ha) and urea (200kg/ha) fertilizers were used for all site years. Field preparation in 2012 was similar to 2011, however pre-emergent herbicide (Edge) was applied and incorporated into the soil on the canola portions of both fields in the fall of 2011 for weed control in 2012. The field in Carman was tilled prior to the 2012 growing season and the field in Winnipeg was not tilled. Post-emergent herbicides (Axial and Infinity) were sprayed to further eliminate grass and broadleaf weeds in Carman.

Inoculum in the form of conditioned sclerotia was prepared by placing the sclerotia within mesh bags outside in snow in January and left covered throughout the winter. Mesh bags were then moved to a refrigerator upon snowmelt to avoid germination. Both the Carman and Winnipeg sites were inoculated with conditioned sclerotia after seeding in 2011. The wheat strip received 360 grams of equally dispersed sclerotia, at the same rate as sclerotia applied to the canola. Fall field preparation in 2012 included the inoculation of 400g sclerotia onto each of the Winnipeg (November 2nd, 2011) and Carman (October 26th, 2011) field sites. An additional 240g of sclerotia was applied to the wheat and canola strips in the spring on June 5th (Winnipeg) and June 14th (Carman), respectively.

3.3.3 Microclimate Monitoring

HOBO microclimate stations were used to monitor microclimate during the growing seasons (2011 and 2012) in both wheat and canola plots. Measures for microclimate included air temperature, relative humidity, leaf wetness, surface soil temperature and surface soil moisture. Two microclimate stations were placed within the wheat strips at each location; one in each of the misted and non-misted wheat portions in 2011 and one in each of the replicated field layouts in 2012. One microclimate station was also placed within each of the 18 canola plots. Placement of the stations in Winnipeg and Carman occurred on June 6th and June 17th, respectively, in 2011. In 2012, stations were placed in the field on June 26th and June 13th in Winnipeg and Carman, respectively. Soil moisture and soil temperature probes were placed in the soil vertically using a trenching shovel to dig holes; the surrounding area was replaced with soil. Air temperature probes and relative humidity sensors were placed at 10 cm above the soil surface.

Leaf wetness sensors and relative humidity and temperature sensors within the solar radiation shield were adjusted throughout both growing seasons. Leaf wetness, temperature and relative humidity sensors were placed at 12 cm in Winnipeg and Carman on June 28th and July 17th respectively in 2011 and on June 26th and June 13th respectively in 2012. In 2011, leaf wetness, temperature and relative humidity sensors were raised to 24 cm on July 7th (Winnipeg) and July 21st (Carman). In 2012, sensors were placed at 24 cm on July 4th (Winnipeg) and June 21st (Carman).

3.3.4 Ascospore Dispersal

Rotorods were installed throughout each field to monitor the dispersal of spores from both canola and wheat field portions. The rotorod collected spores on 2 polystyrene collector rods that spun at 2400 RPM for 1 minute out of every 10 minutes. Rotorod monitoring in 2011 began on June 30th (Winnipeg) and July 11th (Carman). In 2012, rotorods were monitored beginning on June 27th in Carman and July 7th in Winnipeg. Within both fields, one rotorod was placed in each of the canola plots, and 4 rotorods were placed within the wheat strip. In 2011, two were placed in each of the misted and non-misted areas, whereas in 2012 two rotorods were placed in each replicate of the design. Eight rotorods were also placed in the bare soil on each side of the wheat strip. Four rotorods were placed at 3 meters and four at 7 meters from the inoculated wheat strip. The purpose for which spore samplers were placed on the bare soil field portions were to monitor the distance and direction that spores were dispersed on either side of the wheat and to understand disease gradients. All rotorod samplers placed in Winnipeg contained retracting heads, while samplers in Carman contained fixed heads. Rods were replaced daily for further analysis in the lab.

Ascospores of *S. sclerotiorum* on each collector rod were counted in the lab using a microscope at 400x magnification as described in Chapter 2. The total number of ascospores per rod was calculated based on the counts obtained in each lens view as described in Chapter 2.

The values obtained for each of the two rods were then averaged to get a mean value of ascospores per metre cubed per rotorod per day.

Occasionally rods could not be counted or contained no spores for several reasons. Rods that could not be counted properly had lots of dirt on them, large sections of the rod were covered by a bug or large sections of the rod contained no spores. Rods containing either no spores or dirt on them were generally placed in the rotorod backwards and also could not be counted. Some rods were also missing meaning that they may have been dropped in the field and not found. Where only one of the two rods from a rotorod was counted, the value from that rod alone was counted as the mean daily ascospore concentration. Where none of the two rods could be counted, gaps in the data were created.

3.3.5 Misting

During the 2011 growing season, half of the wheat strips and half of the canola field portions in each field were misted to ensure continual moisture for disease development and to provide a range of microclimatic conditions using the system described in Chapter 2. The remaining halves were not misted to represent standard weather conditions. In Winnipeg, plots 10 through 18 and the south half of the wheat strip were misted, and in Carman, plots 1 through 9 and the west side of the wheat strip were misted. Misting in 2011 began at the 20% bloom stage on July 5th and July 15th in Winnipeg and Carman, respectively. Misting was terminated ten days after the final canola petal had fallen on July 31st (Winnipeg) and August 10th (Carman). Misting began daily at 4 pm and was left on until the 1,200 gallons (4,542 L) emptied. Approximately 26,250 gallons (99,367 L) of water was irrigated onto the misted field portions in total.

Neither of the Winnipeg or Carman plots were misted in 2012 due to technical issues and availability of generators and pumping systems. This created two sets of randomized complete block designs in each of the Winnipeg and Carman canola fields.

3.3.6 Growing Season

Length of the growing season from seeding to harvest, the period before flowering from 4th leaf to flowering, flowering stage and the ascospore sampling period dates are outlined below in Table 3.1.

Table 3.1 Start and end dates for specified time periods during the growing season.

Time Period	Carman 2011	Winnipeg 2011	Carman 2012	Winnipeg 2012
Growing season	June 8-Aug 22	May 26-Aug 5	May 23-Aug 7	June 6-Aug 13
Before flowering	June 29-July 14	June 15-July 4	June 16-July 11	June 27-July 10
During flowering	July 14-Aug 6	July 5-July 21	July 1-July 22	July 11-Aug 1
Sampling period	July 11-Aug 17	July 4-July 29	June 27-July 25	July 7-Aug 6

3.3.7 Data Analysis

Recorded weather and microclimate parameters for wheat were summarized for the sampling period and during several growing season intervals for comparison among field locations and between years. Daily microclimate values were plotted with daily mean ascospore concentrations within the wheat for comparison of trends. Comparisons of average daily mean ascospore concentrations and several microclimate parameters including air temperature, soil temperature, relative humidity and leaf wetness were made between misted and non-misted field portions in 2011.

An analysis was completed on wind direction relative to spore dispersal through a series of comparisons of daily mean ascospore concentrations in the direction of the wind. Comparisons of wind direction on ascospore dispersal within the wheat strips were made by comparing ascospore concentrations over days with similar dominant wind directions in Carman in 2011 and 2012 and in Winnipeg in 2012. The wheat strip located in Carman 2011 ran from north to south; daily ascospore concentrations within the wheat strip were compared with wind direction. Ascospore concentrations obtained from both rotorods to the north were averaged to obtain a mean daily ascospore concentration in the north direction and the same was done for the ascospore concentrations within the south of the wheat strip. Some analysis also focuses on distance of dispersal using the data available through the project design.

3.4 Results

3.4.1 Weather and Microclimate Impacts on Ascospore Release in the Wheat Plots

During the rotorod sampling period, daily mean air temperatures were higher overall in 2012 compared to 2011, however growing season temperatures were similar among siteyears (Table 3.2). Daily mean air temperatures in Winnipeg and Carman were comparable during the growing season; however Winnipeg temperatures tended to be slightly higher than Carman temperatures during the sampling period in 2011. Higher maximum temperatures were attained in Winnipeg compared to Carman during each year. Mean relative humidity was higher in Carman in 2011 and 2012 during both time periods with slightly higher percentages occurring in 2011. Total precipitation during the growing seasons was higher in 2012. In 2011, Winnipeg received more precipitation than Carman overall, however in 2012 Carman received more precipitation during the growing season. During the rotorod sampling period, Winnipeg received more in both years. Data for dominant wind direction in Winnipeg in 2011 are not available.

Below canopy daily mean air temperatures within the wheat strip were higher in Carman compared to Winnipeg throughout most of the season in 2011, whereas daily mean air temperatures were higher in Winnipeg in 2012 overall (Table 3.3). Carman showed higher maximum temperatures in 2011, and Winnipeg showed higher maximum temperatures overall in 2012. Relative humidity under the wheat canopy was higher in Carman during both years, with increasing relative humidity during the flowering stages during most site-years. Overall, relative humidity was higher during the growing season at both locations in 2011. Leaf wetness was consistently higher in Carman during both

seasons; with higher values for average leaf wetness during the flowering stages in all site-years.

Average daily values for relative humidity and leaf wetness followed a similar trend throughout the spore sampling period during all site years (Figures 3.1 and 3.2). Air temperature and soil temperature were also similar throughout each period. There appears to be no correlation between average daily temperature and relative humidity values. In most cases, peaks in ascospore concentrations tended to occur following increases in within canopy relative humidity with peaks in spore concentrations occurring 1-2 days after peaks in relative humidity values. Canopy temperature and ascospore concentrations did not show any temporal correlation. Average mean daily ascospore concentrations in Winnipeg and Carman peaked during similar periods in 2011 and 2012. In 2011, major peaks occurred around July 24th and July 27th at both sites; major peaks occur around July 17th and July 20th in 2012.

Table 3.2 Rotorod sampling period and growing season weather parameters above the canopy for Winnipeg and Carman in 2011 and 2012. Maximum and minimum temperatures are indicated in brackets beside mean temperature values.

	Daily mean air temperature (˚C)		Mean relative humidity (%)		Total precipitation (mm)		Dominant wind direction	
	Winnipeg	Carman	Winnipeg	Carman	Winnipeg	Carman	Winnipeg	Carman
Time Period				*2011*				
Sampling Period[y]	22 (34, 10)	20 (33, 9)	62	73	31	24	NA[z]	West
Growing Season[x]	20 (34, 0)	20 (33, 5)	65	72	162	135	-	-
				2012				
Sampling Period[y]	23 (35, 10)	23 (34, 12)	62	70	53	35	South	West
Growing Season[x]	20 (35, 1)	20 (34, 3)	64	71	129	208	-	-

[z] Data is unavailable
[y]Sampling Period - Wpg2011 (July 4 – July 29), Car2011 (July 11 – Aug 17), Wpg2012 (July 7 – Aug 6), Car2012 (June 27 – July 25)
[x]Growing Season – Wpg2011 (May 26- Aug 5), Car2011 (June 8 – Aug 22), Wpg2012 (June 6 – Aug 13), Car2012 (May 23 – Aug 7)

Table 3.3 Microclimate parameters within the wheat strips in Winnipeg and Carman in 2011 and 2012 during the rotorod sampling period, from 4th leaf to flowering stage and during the flowering stage for canola. Calculated values are obtained from the average of two microclimate stations placed within the wheat strips.

	Daily mean air temperature (˚C)		Average relative humidity (%)		Average soil temperature (˚C)		Average leaf wetness	
	Winnipeg	Carman	Winnipeg	Carman	Winnipeg	Carman	Winnipeg	Carman
Time Period				*2011*				
Sampling Period[y]	20 (22, 17)	20z (25, 15)	80	88[z]	21	20z	37	56[z]
4th Leaf to Flowering[x]	19 (22, 17)	21 (25, 17)	80	81	20	21	20	24
Flowering Stage[w]	20 (22, 17)	21z (25, 15)	80	90[z]	21	21z	38	58[z]
				2012				
Sampling Period[y]	22 (26, 16)	23 (26, 17)	78	82	22	22	36	45
4th Leaf to Flowering[x]	23 (25, 21)	18 (23, 13)	73	80	23	20	30	39
Flowering Stage[w]	23 (26, 17)	23 (26, 17)	77	85	22	22	32	47

[z] Missing values from one of the rotorods from Jul 28 to Aug 12. Where missing values are present, only one microclimate station represents the average for the wheat strip
[y] Sampling Period - Wpg2011 (July 4 – July 29), Car2011 (July 11 – Aug 17), Wpg2012 (July 7 – Aug 6), Car2012 (June 27 – July 25)
[x] 4th Leaf to Flowering – Wpg2011 (June15 – July4), Car2011 (June29 – July14), Wpg2012 (June27 – July10), Car2012 (June 16 – July 11)
[w] Flowering Stage - Wpg2011 (July5 – July21), Car2011 (July14 – Aug6), Wpg2012 (July11 – Aug 1), Car2012 (July1 – July22)

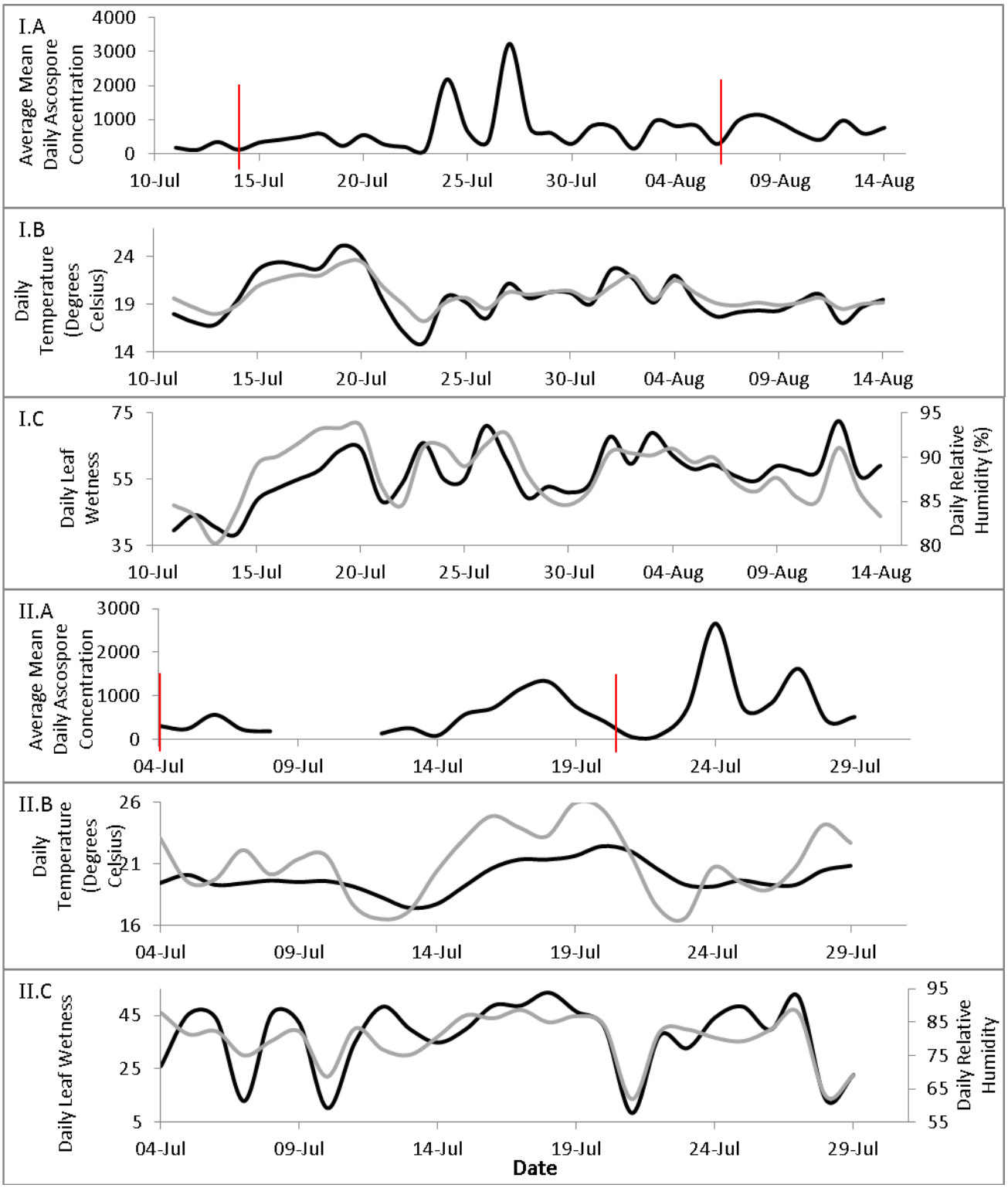

Figure 3.1 Comparison of daily microclimate factors such as B) air temperature in ˚C (black line), and soil temperature in ˚C (grey line) and C) leaf wetness (black line) and relative humidity in percentage (grey line) to A) daily mean ascospore concentrations (ascospore/m^3) in I) Carman and II) Winnipeg in 2011. Red lines indicate the beginning and the end of the flowering period.

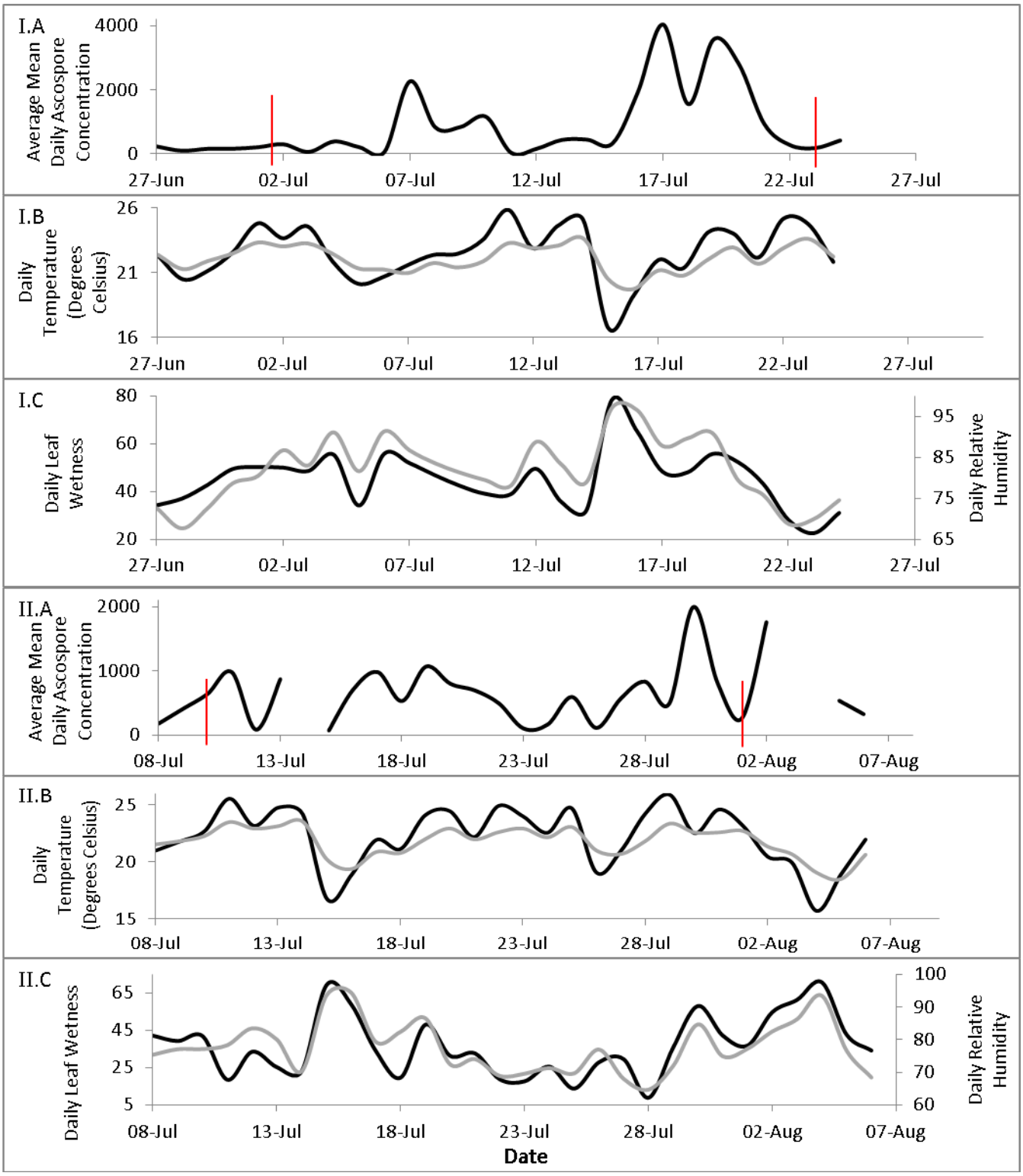

Figure 3.2 Comparison of daily microclimate factors such as B) air temperature in ˚C (black line), and soil temperature in ˚C (grey line) and C) leaf wetness (black line) and relative humidity in percentage (grey line) to A) daily mean ascospore concentrations (ascospore/m^3) in I) Carman and II) Winnipeg in 2012. Red lines indicate the beginning and the end of the flowering period.

A comparison was made between misted and non-misted wheat field portions in Winnipeg and Carman in 2011 using microclimate and daily mean ascospore concentrations (Table 3.4). Calculated values were obtained for the sampling period which began just prior to flowering to several days after flowering at both locations. Compared to non-misted field portions, misted portions contained higher average daily mean ascospore concentrations in Carman, however concentrations were lower among misted plots in Winnipeg. Daily mean air temperatures, relative humidity values and daily mean soil temperatures were very similar between misted and non-misted wheat field portions in both Winnipeg and Carman. Leaf wetness was higher among misted plots at both locations in both wheat and canola.

Table 3.4 Measured values for ascospore concentrations and microclimate parameters in Winnipeg and Carman in 2011 for misted and non-misted wheat field portions during the sampling period.

Location	Sampling Period	Treatment	Average daily mean spore concentration (ascospores/m^3)	Daily mean air temperature (°C)	Relative humidity (%)	Leaf wetness in wheat	Leaf wetness in canola	Daily mean soil temperature (°C)
Carman	Jul 11 – Aug 14	Misted	684[y] (29 days)	20[z]	88[z]	59[z]	62	20[z]
		Non-misted	653[y] (29 days)	20[z]	89[z]	49[z]	47	20[z]
Winnipeg	Jul 4 – Jul 29	Misted	646[x] (20 days)	20	80	40	45	21
		Non-misted	823[x] (20 days)	20	80	35	30	21

[z]missing values during sampling period; only values for days available were used (Jul 11-27:Aug 13-14)
[y]values obtained from 1 rotorod in each treatment
[x]values obtained from 2 rotorods in each treatment

3.4.2 Impact of Wind on Ascospore Dispersal from the Wheat Plots

In Carman in 2011 and 2012 during the sampling period, prevailing winds were predominantly westerly. In Winnipeg in 2012, winds were predominantly southerly; wind direction was not monitored throughout the entire sampling period in 2011 (Figure 3.3). Values for average daily mean ascospore concentrations over the sampling period were generally higher within the wheat strips compared to the bare soil field portions, especially in Carman. There were no other noticeable trends occurring as a result of predominant wind directions during the spore sampling period.

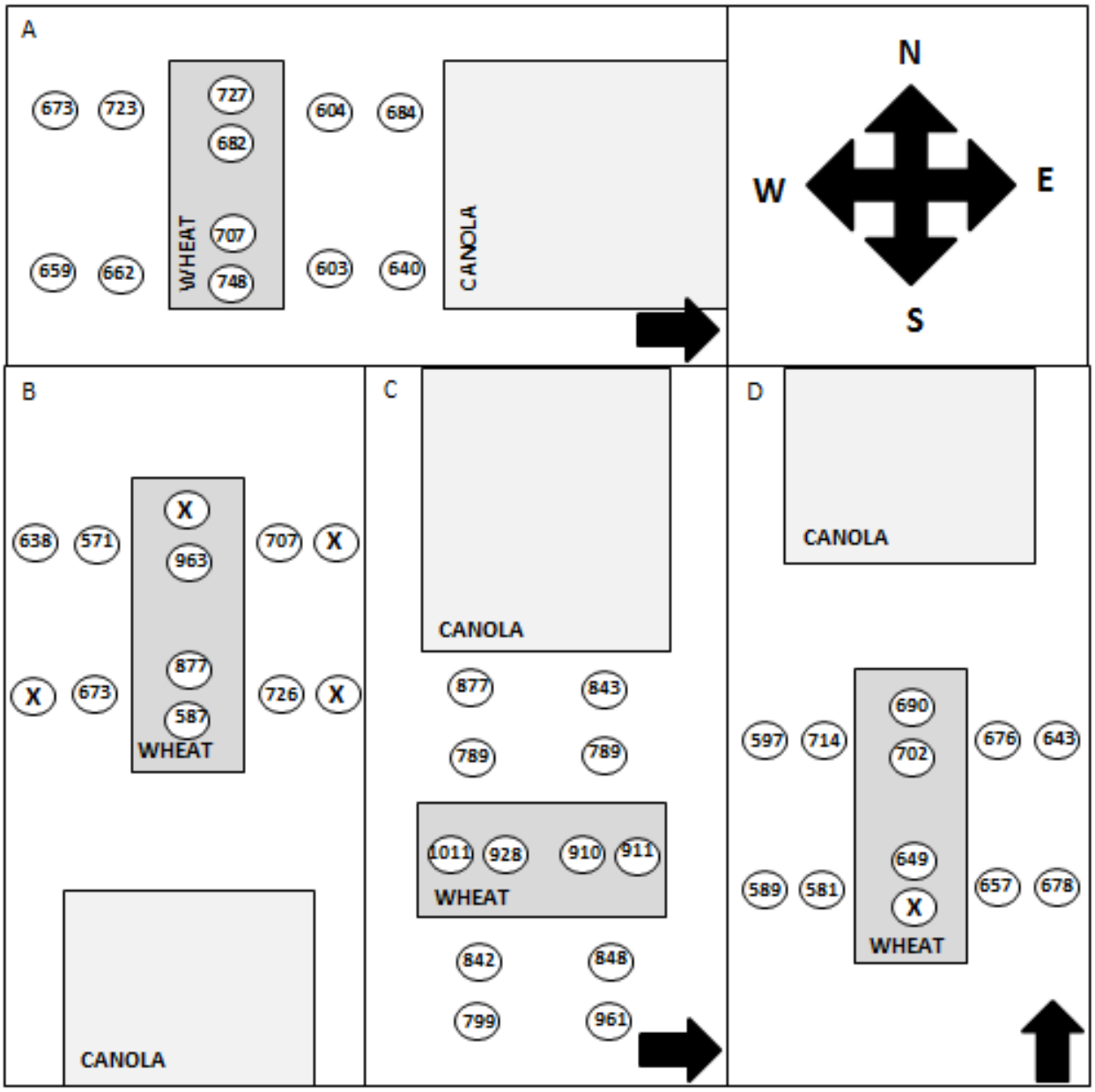

Figure 3.3 Design of bare and wheat field portions for A) Carman 2011 B) Winnipeg 2011 C) Carman 2012 and D) Winnipeg 2012. Circles represent rotorod locations with average daily mean ascospore concentrations within them for the sampling periods at each field at both locations. Black arrows in the bottom right corners represent dominant wind directions during the sampling periods. Rotorods were placed at 3 and 7 metres from inoculated wheat strips in bare soil. Image is not to scale.

In Carman (2011), days with northerly predominant wind directions (including winds blowing towards the south, south-west and south east) or southerly predominant winds (including winds blowing towards the north, north-east and north-west) showed higher daily mean ascospore concentrations in the downwind direction during 50% of the days. In 2012, daily mean ascospore concentrations in the upwind direction within the wheat strip were higher within 64% and 58% of the plots in both Carman (running from east to west) and Winnipeg (running from north to south) respectively. The Winnipeg plot in 2012 contained a canola field portion directly to the north which may have affected spore concentrations in the north portion of the wheat strip.

Ascospore concentrations within the bare soil areas on either side of the wheat strip in Winnipeg, 2012 were also compared based on easterly (including winds blowing from the north-east, south-east and east) and westerly (including winds blowing from the north-west, south-west and west) winds. An average of all ascospore concentrations on the east and west sides were compared based on wind directions. Spore concentrations downwind from the prevailing winds were higher for approximately 71% of the days with easterly or westerly winds.

During certain days in Carman, prevailing winds were from the direction where canola plots were not situated adjacent to the bare soil portion. In 2011 and 2012, these winds were from the west and south respectively. Spore concentrations downward from the prevailing winds were higher for 50% and 40% of the days in 2011 and 2012 respectively.

Distance of dispersal was analyzed by comparing daily mean ascospore concentrations averaged over the sampling period at 3 meters and 7 meters from the inoculated wheat field. In Winnipeg in 2011 and 2012, there is a slight decreasing trend in average daily mean ascospore concentration with increasing distance from the inoculated source (Figure 3.3). A slight decrease in average daily mean ascospore concentration was also apparent in Carman in 2011; however an increasing trend was apparent in 2012. Ascospore concentrations tend to follow a negative exponential relationship with distance away from an inoculated source (Qandah and Mendoza, 2012). This form of model was utilized to analyze the distance relationship in Winnipeg in both years. The Winnipeg plot in 2011 showed a slightly steeper slope than that for 2012 (Table 3.5). A 50 percent percent reduction in daily mean spore concentrations from the inoculum source averaged over the sampling period would occur at approximately 61 and 62 meters in 2011 and 2012, respectively. A 75 percent reduction would occur at approximately 119 and 122 meters in 2011 and 2012, respectively.

Table 3.5 Parameters obtained by an exponential model applied to mean spore concentrations at 3 meters and 7 meters from an inoculum source in Winnipeg. 50% reduction and 75% reduction indicate the distance at which a 50 percent and 75 percent reduction in average mean spore concentration would occur.

Site Year	slope	y-intercept	50% reduction (m)	75% reduction (m)
Winnipeg 2011[z]	-0.01186	693.23	61.4	119.9
Winnipeg 2012	-0.01168	680.44	62.3	121.6

[z] Only 1 value for average daily mean ascospore concentration at 7 m was used

3.43 Dispersal Comparisons among Wheat and Canola Plots

A comparison of average daily mean ascospore concentrations over the sampling period at 3 meters from the inoculated wheat and 3 meters from the inoculated canola fields within the bare soil field portion was done. Consistently higher concentrations were realized for the rotorods within the bare soil portions closer to the canola plots compared to the wheat plot in Carman during both 2011 and 2012 (figure 3.3).

In Carman canola plots (2011 and 2012), average daily mean ascospore concentrations were lowest at 10 to 20 metres from the wheat inoculum source (Table 3.6). Concentrations increased with distance from the wheat inoculum source. There were no consistent trends relating disease incidence to distance from the wheat inoculum source. In 2011, disease incidence was highest among plots at 20 to 30 metres and in Winnipeg disease incidence was highest within plots at 30 to 40 metres.

Table 3.6 Average daily mean ascospore concentrations (ascospores/m3) and disease incidence averages within canola plots at 30-40, 20-30 and 10-20 metres from inoculated wheat source in 2011 and 2012 Carman plots.

	Ascospore Concentration		Disease Incidence	
Distance from wheat source	2011	2012	2011	2012
30-40 metres	655.3	1894.41	18.6042	17.9945
20-30 metres	621.7	1821.87	30.1623	13.5664
10-20 metres	577.21	1821.32	15.9979	15.4809

In 2011 at both sites, average daily mean ascospore concentrations were higher among wheat plots in all plots, misted plots and non-misted plots, whereas in 2012 concentrations were higher within canola (Table 3.7). Mean daily ascospore concentrations under wheat and canola canopies followed similar trends (Figure 3.4). Percent infection at harvest was lower overall in 2011 compared to 2012 with higher values occurring in Carman during both years.

Table 3.7 Whole plot means for average daily mean ascsopore concentrations in wheat and canola plots and percent infection obtained at harvest in canola plots at Winnipeg and Carman in 2011 and 2012. Ascospore concentration data used was taken only for days in which all values were present in both wheat and canola during a specified site year over the sampling period.

Year	Site	Ascospore Sampling Period	Description	Ascospore Concentrations (ascospores/m^3) in Canola	Percenta Infection (%) in Canola	Ascospore Concentrations (ascospores/m^3) in Wheat
2011	Winnipeg	July 6- July 26 (12 days)	All	576	5.8	623
			Non-Misted	571	6.0	609
			Misted	581	5.5	637
	Carman	July 12- August 14 (14 days)	All	655	22	790
			Non-Misted	714	23	789
			Misted	596	21	791
2012	Winnipeg	July 8- August 6 (23 days)	All (Non-Misted)	769	3 and 9[z]	651
	Carman	June 29- July 23 (13 days)	All (Non-Misted)	2008	16	1491

[z] Value was obtained post-harvest

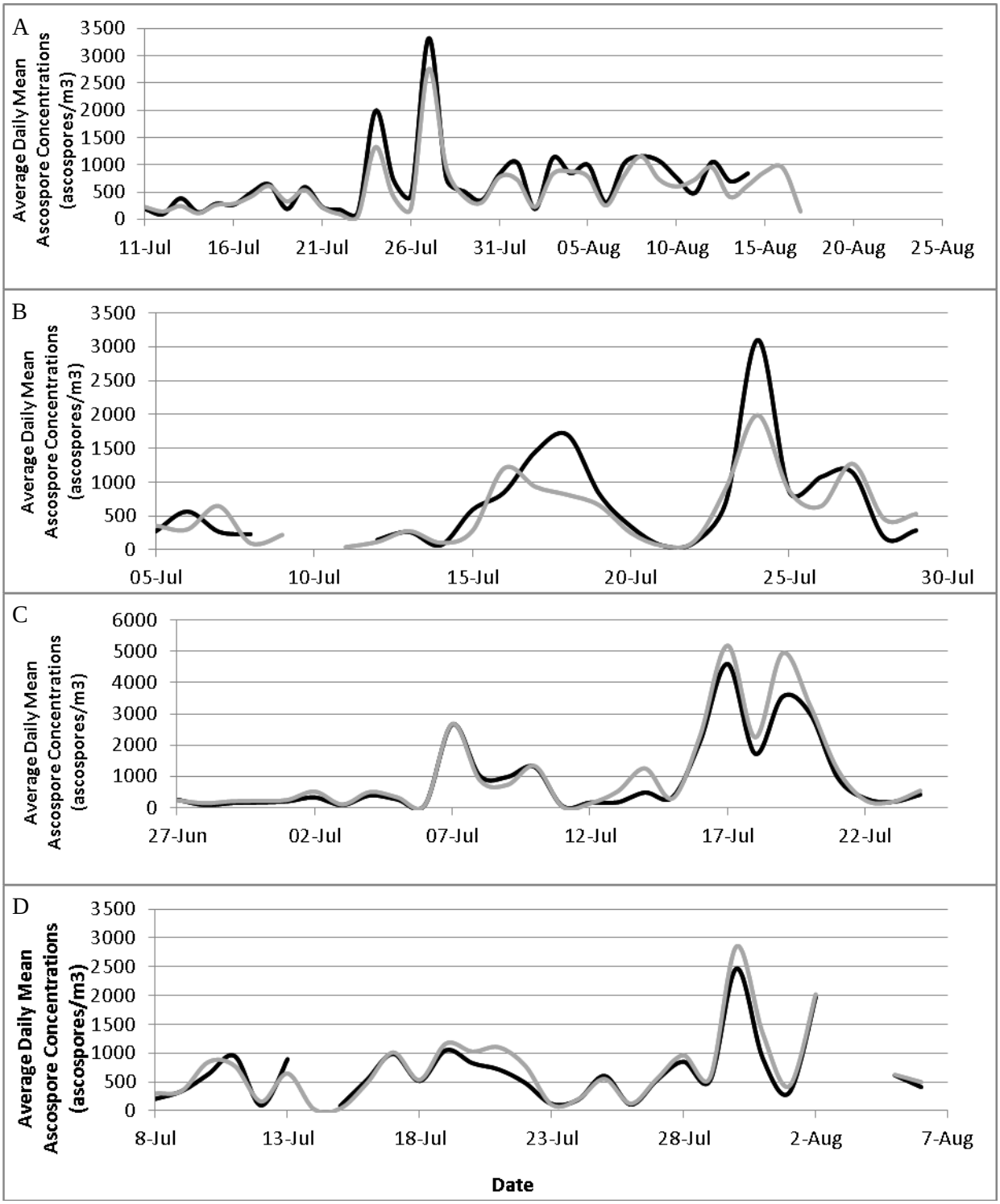

Figure 3.4 Average daily mean ascospore concentrations in wheat (black line) and canola (grey line) throughout the sampling period in A) Carman 2011, B) Winnipeg 2011, C) Carman 2012 and D) Winnipeg 2012.

3.5 Discussion

To the best of our knowledge, this is the first study to investigate the development and spread of *S. sclerotiorum* in a non-host crop. This study has provided evidence that ascospore production is possible under a non-host wheat crop and ascospore release concentrations and timing of peak levels are similar to those under a canola canopy. Ascospores were captured at a distance of 7 meters and it has been reported that travel distance can exceed 60 meters (Qandah and Mendoza, 2012). Climatic conditions necessary for ascospore release and infection were increased relative humidity and leaf wetness and reduced temperature. Wind direction also had a slight impact on ascospore dispersal away from the wheat source. Misting was not effective in increasing ascospore concentrations based on this study.

Weather and microclimate are important factors for both crop and disease development. According to Turkington et al. (2011), risk of stem rot is increased under specific environmental conditions. During the period prior to flowering, adequate rain and moderate temperatures are required to allow for soil surfaces to remain wet for the majority of the day and enhance sclerotial germination (Turkington et al., 2011). These conditions are also necessary under a wheat canopy for successful germination of sclerotia and release of ascospores into the surrounding air. In 2011, seeding occurred later throughout most of Manitoba due to the saturated top soils, heavy snow melt and precipitation occurring throughout May and June. The rest of the summer remained fairly dry and hot. In 2012, warm dry weather in April was followed by a prolonged cool period at the beginning of May. Warm dry conditions ensued throughout the summer. Overall

temperatures were slightly warmer and relative humidity percentages were lower in 2012 which reduced the canola flowering period, reducing seed set and size. Compared to Carman, Winnipeg experienced higher maximum temperatures and lower percent relative humidity during both years due to the urban location of the Winnipeg plots. Below canopy measures for microclimate indicated that soil and air temperatures were higher overall in 2012 and relative humidity values were lower which is expected due to the influence of weather during both years.

The use of a misting system to promote disease development was successful in altering microclimates, however, it did not influence percent infection in this study. Due to the operation of a misting system during canola flowering, relative humidity and leaf wetness were much higher in wheat during the canola flowering period compared to the leaf stages prior to flowering in Carman and Winnipeg in 2011. Relative humidity and leaf wetness were also slightly higher in Winnipeg and Carman in 2012 during the flowering period compared to the leaf stages, however a misting system was not used therefore the increases were not substantial. Moisture is required to increase relative humidity within the wheat canopy during sclerotial germination and spore release; however relative humidity was not higher overall under misted wheat field portions. Ascospore production is therefore not likely increased as a result of misting. In 2011, leaf wetness was the only microclimatic parameter that showed significantly higher values under misted wheat field portions. Similarly in canola, misted plots contained higher values for leaf wetness. Leaf wetness in a canola crop is important for the deposition and colonization of spores, providing an availability of water for pathogens on both flower and leaf surfaces (Huber and Gillespie, 1992). Misted plots within the canola canopy with

consistently higher leaf wetness values could have favoured deposition and development of sclerotinia ascospores into disease epidemics throughout the crop. Greater *S. sclerotiorum* infections would occur where moisture is provided to both host and non-host crops throughout the entire growing season to allow for enhanced germination, spore release, spore deposition and infection.

During the flowering period, predictions of major ascospore release periods in adjacent crops are critical for the determination of necessary fungicide applications within an adjacent susceptible crop. Ascospore release from apothecia within a wheat field is synchronous with release occurring in a canola field due to the influence of weather and microclimate within the canopies. In 2011, major peaks in ascospore concentrations occurred during similar dates in Winnipeg and Carman which is affected by the overall weather conditions throughout Manitoba. These conditions are still not completely understood. In 2012, major peaks also occurred during similar dates in both Winnipeg and Carman. In 2011, both Winnipeg and Carman experienced major peaks in ascospore concentrations towards the middle (50 to 60% in Carman) to end (70% and after flowering in Winnipeg) of the canola flowering stages. In 2012, major peaks in ascospore concentrations in wheat occurred mainly around 30% flowering and with larger peaks at 80 to 90% flowering which would likely have influenced neighbouring canola fields both early in the flowering period and near the end of flowering. Among all microclimatic variables, relative humidity had the greatest impact on ascospore release within wheat canopies. Major peaks in ascospore concentrations mainly preceded prolonged periods of high relative humidity followed by a sudden decrease in relative humidity which agrees with the findings suggested by Qandah and Mendoza (2011). Within susceptible crops

and in adjacent crops with a history of disease, identification of major peaks in ascospore concentrations occurring early in the growing season is important for disease prevention. Recommended fungicide application time in Manitoba is at 20 to 30% bloom (Manitoba Agriculture, 2013), however disease protection is increased when fungicides are applied when apothecia appears in the crop (Mwiindilila and Hall, 1989). The identification process can be facilitated through weather predictions early in the flowering period by assessing the likelihood for precipitation and conditions of increased relative humidity for prolonged periods.

According to Qandah (2008), sclerotinia stem rot incidence is closely associated with airborne ascospore concentrations. This can be applicable to disease incidences observed in canola fields as a result of neighbouring infested fields containing non-host crops. Apothecia are capable of producing spores within non-host crops and releasing them into the surrounding atmosphere as confirmed by this study (by the atmospheric spore counts obtained above wheat canopies), which were consistently higher than the counts obtained above the adjacent bare soil field portions that did not contain inoculum. Twengstrom et al. (1998) suggested that even where external sources of inoculum exist, inoculum originating within the canola field has the greatest influence on disease occurrence. The local inoculum source within each canola plot proved to be a bigger factor contributing to disease incidence and ascospore dispersal within the canola than the nearby wheat canopy in proximity as shown in this study. Adjacent field borne inoculum still however does influence neighbouring fields as established in studies where ascospore dispersal gradients were created. Qandah and Mendoza (2012) were able to collect *S. sclerotiorum* ascospores below a canola canopy at greater than 60 m from an inoculated

source while observing infected plants at 63 meters from the source. Similarly, in a study conducted by Guo and Fernando (2005), *Leoptosphaeria maculans* ascospore concentrations were highest within 10 to 25 m from the source of inoculum. The Canola Council of Canada (2014) recommends field separation of 50 to 100 meters from blackleg infested fields planted to canola the previous year. These findings were further validated within this study since *S. sclerotiorum* ascospores were capable of travelling at least 7 meters along the gradient used to monitor spore dispersal above the canopy. Ascospore dispersal from a source of inoculum tends to follow a negative exponential gradient (Qandah and Mendoza, 2012). The steepness of the gradient can be explained by spore size, nature of the spores dispersal mechanism, sampling method and influence of weather (Qandah and Mendoza, 2012). Using a negative exponential model used by Qandah and Mendoza (2012) and data from 2 sampling locations in the bare soil at 3 and 7 metres from the wheat inoculum source, spore dispersal distances were calculated in Carman. Reduction in spore concentrations by 50 percent would occur at approximately 61 to 62 meters from the inoculum source using the mean values obtained for slope and y-intercept. A 75% reduction would occur at 120-122 meters. This calculation however does not take into consideration the additional contribution from the canola fields located adjacent to the wheat, which also likely impacted spore concentration within bare soil from long distances. Qandah and Mendoza (2012) found that precipitation influences the steepness of ascospore dispersal gradients where gradients become shallower with increasing precipitation. In Winnipeg, the slightly steeper slope in 2011 can be partially explained by the reduced precipitation occurring during the growing season and sampling period compared to 2012. The positive gradient occurring in Carman in 2012 may be explained by strong prevailing winds increasing spore concentrations in the far spore

sampler to the south-east, or by potential presence of inoculum in adjacent fields. Dispersal distances can be determined through the use of prediction models using precipitation as a contributing factor. Growers should be aware of the history of disease and inoculum levels in fields nearby. Fungicide applications may be required on susceptible crops located adjacent to fields with disease history.

Climate is shown to be a major contributor to disease development as established by ascospore concentrations and disease incidence values obtained in this study. Larger inoculum loads were applied to both Winnipeg and Carman plots in 2012, with additional applications in the fall of 2011 for the following year which contributed to the greater airborne ascospore concentrations in both wheat and canola in 2012. Regardless of the source of ascospores present in 2012, disease incidence remained lower in 2012 which is a direct reflection of the influence of microclimate on ascospore germination in canola. Relative humidity and leaf wetness were elevated during the flowering period during both years, with higher values occurring in 2011; both favourable environmental conditions for ascospores to land on canola plants and subsequently germinate and cause infection.

Ascospore release and dispersal was comparable among wheat and canola plots however slight differences may have existed due to weather and microclimatic conditions as well as canopy structures. In 2011 higher ascospore concentrations were noticed above wheat canopies while in 2012 concentrations were slightly higher above canola canopies. Stunted canola growth in 2012 as a result of warm temperatures may have provided an open canopy allowing for ascospores to reach above canopy levels where spore traps were located. Kane wheat used in 2011 provided similar but slightly higher yields than

AC Barrie wheat used in 2012; LAI also appeared noticeably larger in the field in 2011. Microclimatic conditions under the Kane variety may have been more conducive to sclerotia germination and ascospore release due to the increased canopy cover. Use of a misting system in 2011 may also have had an impact on spore release from wheat and canola canopies. Although ascospores were dispersed from within the wheat canopies, ascospore concentrations were generally higher with proximity to canola canopies in the bare soil. Canola field portions were larger than wheat field portions, providing a larger inoculated area for spore dispersal. As a result of the ascospores being produced within an adjacent wheat plot, there were no differences in ascospore concentrations or disease incidences at different distances from the wheat inoculum source in the canola plots. Inoculum present in the canola plots alone likely would have impacted the canola, and any external sources of inoculum did not result in additional disease incidence according to this study. In the case where a canola field without disease is adjacent to a wheat field with presence or previous history of disease there would be an influence from the inoculum in the wheat on the adjacent canola field through ascospore development and dispersal.

Another factor impacting ascospore dispersal and gradients is wind. Predominant wind directions changed often; therefore daily predominant winds were used to analyze spore dispersal in each site-year. Although wind direction had no effect on ascospore movement within the wheat strip, slight effects of wind direction on spore dispersal were observed in the bare soil field portions due to the inoculated wheat strip in Winnipeg in 2012. This agrees with previous findings on the ascospores of *L. maculans* where the

majority of spores are carried in the direction of prevailing winds (Guo and Fernando, 2005). Wind direction affects direction of spore dispersal away from an inoculum source.

Successful disease development requires necessary weather and microclimatic conditions as well as the presence of internally or externally produced inoculum sources. Inoculum producing fields require conditions of elevated moisture for sclerotial germination and ascospore release (Turkington et al., 2011). This is also true for ascospore production among blackleg of canola and fusarium head blight of wheat (Guo and Fernando, 2005; Government of Saskatchewan, 2007). Susceptible crops subject to infection require adequate moisture, especially in the form of leaf wetness for successful spore deposition and colonization (Huber and Gillespie, 1992). The major cause of sclerotinia stem rot is through the presence of inoculum produced within the susceptible field. In the absence of field borne inoculum, ascospores formed in adjacent fields of host and non-host crops are capable of dispersal into susceptible fields. Control of sclerotinia within susceptible crops is of primary concern; however canola growers should be aware of disease history in adjacent crops. Neighbouring non-host crops can be treated using a biological control agent applied to the soil to reduce sclerotial germination and eliminate spore production. Crop rotation with non-host crops during several successive years can also help deplete sclerotia in the field and reduce inoculum levels, however ascospore production continues to occur in these crops as long as scerotia are present. Fungicides are also advised in susceptible crops located in proximity to fields with a history of disease. Timing of application of fungicides can be facilitated through weather predictions focusing on precipitation and relative humidity. Additional studies conducted in larger commercialized fields are required to determine the extent to which ascospores

arising from non-host crops can travel and infect neighbouring host crops and the range of microclimatic and weather conditions required for successful infection in commercial fields.

3.6 Conclusion

Disease development was not significantly altered as a result of varying microclimates. Spore release in wheat and canola occurred during similar times indicating that weather does have an overall influence on ascospore release from apothecia. Major peaks followed prolonged increases in relative humidity which were generally due to precipitation events. This finding agrees with previous literature. Misting did not significantly alter microclimates or ascospore release under the wheat strip. Leaf wetness was the only factor showing significantly higher values under the misting portion in 2011; however no significant increase in ascospore concentration occurred as a result.

This study has not only proven the possibility of spore production within non-host crops, but also that inoculum spread from neighbouring non-host crops in fields containing inoculum from previous years is possible due to the presence of ascospores in similar and greater amounts to those found in the adjacent canola plots. A distance of at least 7 meters was travelled by the spores and much greater travel distances have been reported. High levels of inoculum at 7 meters also provided evidence that the travel distances are substantial. Timing of ascospore release under the wheat field was similar to that of canola with peaks in ascospore release occurring during the canola flowering period.

Wind direction analyzed against ascospore concentrations on either side of the wheat strip showed no effect on ascospore movement within the wheat strip. Ascospore dispersal was slightly impacted by wind direction based on the ascospore counts found within the rotorods placed alongside the wheat strips in Winnipeg during 2012.

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4. OVERALL SYNTHESIS

To my knowledge, this is the first report that attempts to determine the combined impact of varying canopy densities and microclimate on both ascospore release and infection of sclerotinia stem rot disease in Canola in Manitoba. The effects of weather on disease development were analyzed in this study as well. Also, not previously determined was the development, release and spread of *S. sclerotiorum* ascospores in a non-host wheat crop.

Canopy density treatments effectively altered within canopy microclimates, by increasing within-canopy temperatures and decreasing relative humidity under less dense canopies; conditions expected to reduce disease development. Ascospore release was not influenced by canopy density in this study. Percent infection was significantly increased as a result of reduced temperatures and increased relative humidity among higher density plots in Carman during 2011; however no other site-years showed similar results. Jurke and Fernando (2008) found that disease incidence was increased as a result of denser canopies creating favorable microclimatic conditions and more lodging. Although increasing canopy density is useful in increasing yields and reducing weed pressures, diseases such as sclerotinia and blackleg favor moist, humid conditions present under denser canopies. According to this study, the below canopy microclimate modifications may not have been sufficient to effectively alter disease development; however there is some evidence showing otherwise especially in Jurke and Fernando's (2008) study. As a precautionary measure, using average row spacing and normal seeding rates would be

beneficial in slightly controlling lodging and conditions conducive to infection. Recommended rates will also guarantee yields and alleviate excess weed pressures. Further study on the influence of canopy density on disease development is required under various climates including drier locations that would show greater below canopy alterations as a result of canopy density modification.

The seasonal pattern of ascospore dispersal by *S. sclerotiorum* under Manitoba weather and microclimate conditions was determined in this study. Ascospore release concentrations were higher during late bloom compared to early bloom which may be a result of increased precipitation during the latter part of the flowering period. Precipitation plays a key role in the onset of fungal spore release according to research completed on fungal diseases including *S. sclerotiorum, F. Graminearum* and *L. Maculans* (Qandah and Mendoza, 2011; Government of Saskatchewan, 2007; Guo and Fernando, 2005). It is safe to assume that climate is a key driver for ascospore release since peaks occurred simultaneously in Winnipeg and Carman; two locations 50 km apart with similar climatic conditions. Concurrent with the findings of Qandah and Mendoza (2011), this study shows that elevated ascospore concentrations seem to occur as a result of a prolonged increase in relative humidity which occurs as a result of a precipitation event followed by an increase in temperature and decrease in relative humidity. These findings are not completely evident. Although precipitation influenced ascospore concentrations, the effects of misting on ascospore release are still uncertain. Additional focus needs to be placed on the effects of misting on ascospore release, especially in drier regions where misting creates greater changes within a microclimate. In order to better control sclerotinia economically, it is important to focus on several risk management

strategies concerning weather and climate. Forecasting weather conditions, including precipitation, relative humidity and temperature especially just prior to and during the onset of flowering is essential in predicting the need for fungicide applications. A similar study using both rotorods and a burkard spore trap would aid in determining diurnal ascospore release times which would help predict seasonal patterns focusing on a specified period during the day.

Infection was not increased as a result of elevated ascospore concentrations at any of the site-years indicating that the environment may have been more influential than ascospore concentration. Increased relative humidity values and slightly reduced temperatures experienced in Carman compared to Winnipeg may be responsible for the higher infection in Carman. Precipitation only correlated to increased infection where inoculum levels were even among site-years. Even sclerotia dispersal in Winnipeg and Carman during 2012 with no previous history of disease in either field showed an impact of increased precipitation leading to more infection. Inoculum levels most closely correlated to infection, where fields and plots with increased inoculum levels and previous history of disease experienced increased infection. This highlights the importance of knowing the previous crops grown in the field as well as the history of disease in order to better manage a crop susceptible to sclerotinia stem rot disease either through crop rotation or fungicide use.

Under a non-host wheat canopy, sclerotia effectively produced apothecia and released ascospores into the surrounding atmosphere. Timing and quantity of ascospore release in wheat was similar to that in canola. Ascospores were capable of travelling up to

at least 7 meters beyond the inoculum source in this study and records of *S. sclerotiorum* ascospore travel of at least 63 meters has been observed (Qandah and Mendoza, 2012). Wind also played a slight role in the movement of ascospores away from the inoculum source. Due to the ability for ascospore production, release and dispersal under non-host crops, it is important for canola growers to recognize the impact of neighboring fields with a history of disease even when non-host crops are grown. The recommendation by the Canola Council of Canada (2014) to separate fields with previously grown canola containing blackleg in the last year by 50 to 100 meters is also reasonable for sclerotinia. Additional research needs to focus on inoculum persistence in fields containing non-host crops. Knowlege of the annual rotation required to diminish inoculum concentrations would be useful in helping farmers to reduce and prevent infection.

Sclerotinia forecasting models as well as risk point systems provide useful tools for demonstrating known relationships between SSR related factors, and also help canola growers in decision-making for better management practices. This study was not able to further enhance these tools since ascospore release and disease incidence was not significantly affected by the changes in microclimate under the varying canopy density treatments. It is possible that these changes in microclimate were insufficient to impact disease development. Since peaks in ascospore release concentrations occurred on the same days during two consecutive years at two locations more than 50 km apart, there appears to be a combined effect of weather and microclimatic conditions on ascospore release which has yet to be determined.

In summary, the results of this study have clarified the temporal pattern of *S sclerotiorum* ascospore release in canola and wheat. The influence of variable canopy densities on microclimate, spore release and percent infection was also determined and demonstrated that spore release and percent infection were not greatly affected by canopy density alterations. Most importantly, it was discovered that ascospore release occurs under non-host crops and that these ascospores are capable of infecting nearby fields containing host crops.

4.1 References

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Appendix A

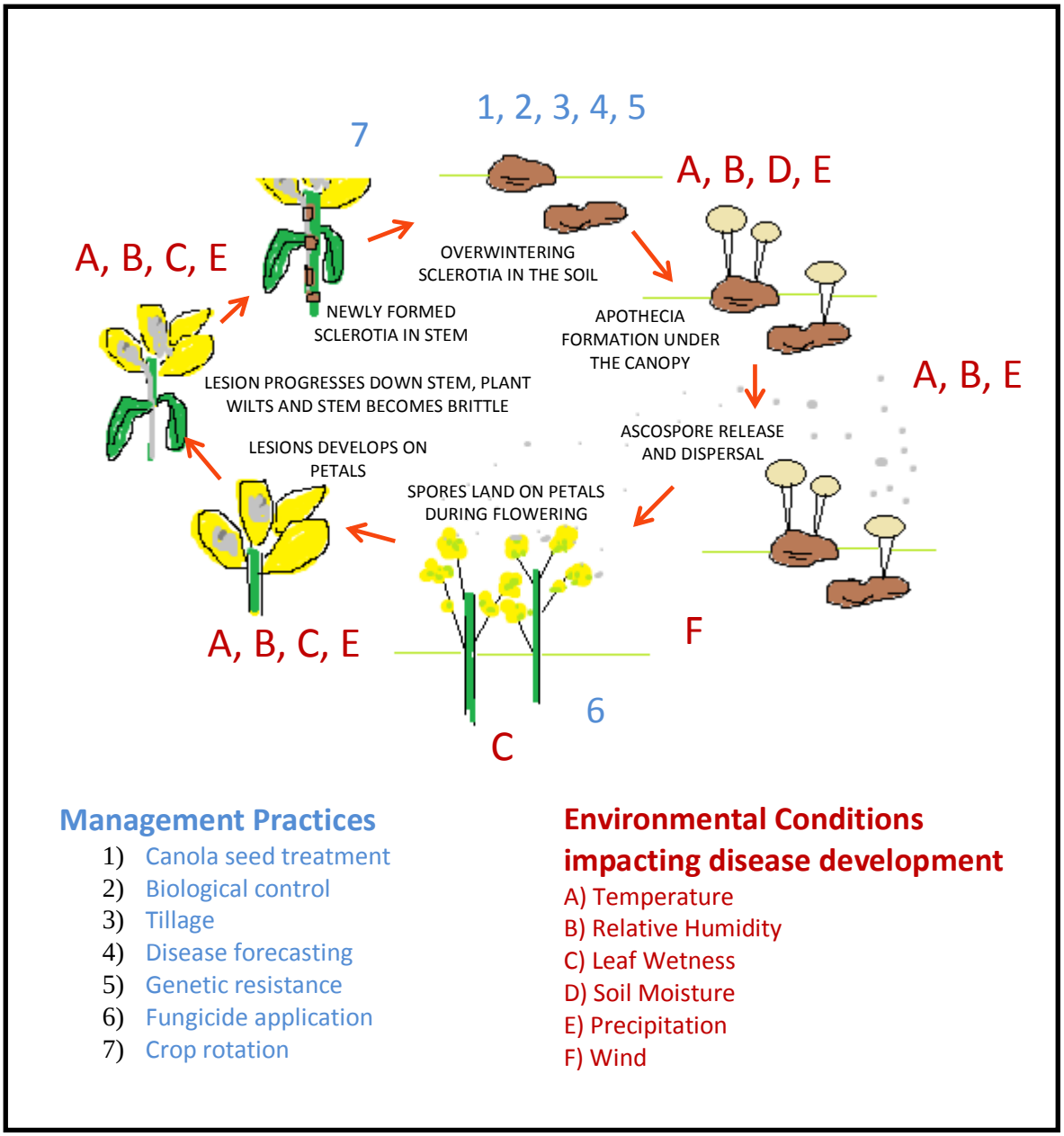

Figure A.1 Sclerotinia stem rot disease life cycle including management practices in blue indicated by the numbers 1 through 7 and environmental conditions impacting disease development in red indicated by letters A through F.

Figure A.2 Canola plots containing 1 microclimate station and 1 rotorod in each plot during flowering in Carman, Manitoba (2012).

Figure A.3 Wheat strip located adjacent to the canola plots with rotorods and microclimate stations in Winnipeg, Manioba (2011).

Figure A.4 Canola plots in Winnipeg (2011) with microclimate stations and rotorods during the leaf stage.

Figure A.5 Hobo microclimate station.

Figure A.6 Rotorod spore sampling device.