Annual Performance Report - Summary of Activity under the Canola/Flax Agri-Science Cluster 2012-2013

Activity Code / Title:	Weather-based Assessment of Sclerotinia Stem Rot Risk
Start Date (yyyy-mm-dd):	2010-04-01
Expected End Date (yyyy-mm-dd):	2013-03-31
Name / Organization of Principal Investigator (PI):	Paul Bullock
Cluster Name:	Canola/Flax Canadian Agri-Science Cluster

Short Executive Summary of report:

In Western Canada, Sclerotinia stem rot continues to be a serious disease of canola with a large acreage affected again in 2012. Very few commercially available canola crop varieties are resistant to the so the disease is primarily controlled by the use of fungicides. This method is not always effective because it is difficult to effectively time fungicide applications. Crop rotation is also not always effective for preventing sclerotinia due to the ability of sclerotia to persist in the soil for long periods of time, the wide range of crops that can be infected and the spread of ascospores from non-host crops. Development of environmentally friendly management strategies to effectively control sclerotinia in canola may be addressed through understanding disease epidemiology and its response to microclimate and standard weather conditions which will facilitate proper timing of fungicide applications and reduce unnecessary fungicide applications.

The objective of this study was to assess the risk of sclerotinia stem rot disease on canola based on standard weather conditions and the canopy microclimate. Dispersal of wind borne spores within the canola field and from a neighbouring wheat field will also be monitored in order to evaluate the impact of spore dispersal on disease risk as well as the potential for infection from a non-host crop.

A plot study was undertaken at two sites (Winnipeg and Carman, MB) during the 2011 and 2012 growing seasons. A high, medium and low density canopy treatment was replicated three times at each site using high, medium and low seeding and nitrogen fertilizer rates. A duplicate set of replicates was also established at each site with one set being misted to maintain wet canopy conditions and one set being rainfed only in 2011. A strip of non-host crop (wheat) was also established at each site in each year, with one half misting and the other half rainfed in 2011. Canopy density was measured using leaf area index and plant count measurements. The weather conditions were monitored at each site. The microclimate for each individual replicate was monitored with a microclimate station in the center of the 10 x 10m or 8 x 8m plots at Winnipeg and Carman, respectively. Rotorods were set up in each individual replicate to collect daily samples of ascospores. Petal samples were taken to determine the presence of sclerotinia in the early and late bloom stage. Disease incidence was quantified in each canola replicate. The effects of wind speed on release and dispersal of airborne ascospores from wheat (non-host crop) were also assessed.

The use of a misting system to promote disease development was successful in altering microclimates, however it did not influence disease incidence. 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 and canola. Petal tests did not show significant results, however in Winnipeg after misting began, ascospores were only found on petals situated in the misted portion of the field indicating that the wet conditions may favor ascospores retention on the petals However, very few ascospores were found in Carman in any of the plots, either misted or non-misted, so there are other factors involved. Ascospore release occurred at similar times in both the canola and the wheat in both 2011 and 2012. Thus, the general overall weather conditions clearly play a critical role in the timing of ascospore release in both host and non-host crops. Although wind direction had no effect on ascospore movement within the wheat, it did on have effects on spore dispersal in the adjacent bare soil. These preliminary results suggest that identification of the controlling weather parameters and key threshold values could provide a general prediction of when to expect high levels of ascospore production in fields that contain inoculum.

A. Research Progress and Accomplishments (to date in relation to expected deliverables / outputs)

- Include brief summary of:
  - Introduction, literature review, objectives and deliverables / outputs.
  - Approach / methodology (summary by objectives).
- Include results and discussion (overview by objectives and deliverables), next steps and references.

Introduction and Literature Review

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 association to sclerotinia stem rot disease intensity in canola (Qandah, 2008). During the growing season, ascospores are produced carpogenically from sclerotia, which are overwintering fungal structures at or near the soil surface 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 are formed by sclerotia (Hartill 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 aimed to determine the environmental factors associated with the release of ascospores within sclerotinia stem rot susceptible crops. Light, temperature and relative humidity have been studied extensively by researchers. The effect of light on ascospore release was studied by Hartill (1980), and Clarkson et al. (2003). Hartill's (1980) study indicating that light was involved in the process was later overturned by Clarkson (2003) who proved that spores were released under both light and dark condition. Qandah and Mendoza (2011) concluded that ascospore release occurs only once daily in a single event and lasts no longer than six hours; whereas release in wet years occurs only during the day and only during the night in dry years. Ascospores were generally released proceeding 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)

Studies have found that disease results mainly from inoculum produced within the field (Boland and Hall, 1988; Twengstrom et al., 1998). A study specifically focusing on Sclerotinia ascospore dispersal in canola found that 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 containing similar characteristics. Guo and Fernando (2005) conducted a study in Manitoba on ascospore dispersal by Leptospharia maculans in relation to environmental conditions. Hourly and daily ascospore concentrations were plotted against weather values. Fungal ascospore concentrations of L. maculans peaked during and following rainfall events and at an elevated relative humidity and decreasing temperature. Ascospores concentrations also decreased with distance from the inoculated source and wind direction had a significant effect on ascospore dispersal with increased concentrations downwind from the 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. The objective of this study was to

assess the risk of sclerotinia stem rot disease on canola based on standard weather conditions and the canopy microclimate. Dispersal of wind borne spores within the canola field and from a neighbouring wheat field will also be monitored in order to evaluate the impact of spore dispersal on disease risk as well as the potential for infection from a non-host crop.

Methods

Study Sites

Two field sites were used in 2011 and 2012 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. Both field sites in 2012 contained no previous history of disease and were seeded with non-host crops in prior years. For each site-year, the site was seeded with 3 density treatments of canola with 3 replicates in a randomized complete block design for 2 different moisture regimes (additional moisture added by misting and natural rainfall only) for a total of 18 canola plots per site year (3 x 3 x 2). In addition, alongside the canola plots, wheat was seeded in a strip 10m wide by 50m long, with 10m of bare soil on either side. One half of the strip was misted and the other half was not misted. 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. In fall 2011, pre-emergent herbicide (Edge) was applied and incorporated into the soil on the canola portions of both fields for weed control in 2012, which was not done in the previous year. 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 for grass and broadleaf weed control in Carman.

Inoculation

Inoculum in the form of conditioned sclerotia was prepared by placing the sclerotia contained 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, and the canola plots received 60 grams. Fall field preparation in 2012 included the inoculation of sclerotia onto each of the Winnipeg (November 2nd, 2011) and Carman (October 26th, 2011) field sites. Four hundred (400) g of equally dispersed sclerotia was placed on the wheat strip; and 66 grams applied to each canola canola plot. An additional 240g of sclerotia was applied to the wheat strip in the spring and the canola plots received 40g on June 5th (Winnipeg) and June 14th (Carman).

Weather and Microclimate Monitoring

Standard weather data was monitored at both locations throughout the growing season (Figure 1). A Campbell Scientific weather station was set up in Winnipeg to measure air temperature (°C), relative humidity (%), wind speed (km h^{-1}), solar radiation (W m^{-2} and MJ m^{-2}) and precipitation (mm). Each of the instruments was connected to a data logger (CR10X) 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 200 m from the field through a weather station set up by Manitoba Agriculture, Food and Rural Initiatives (MAFRI). 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.

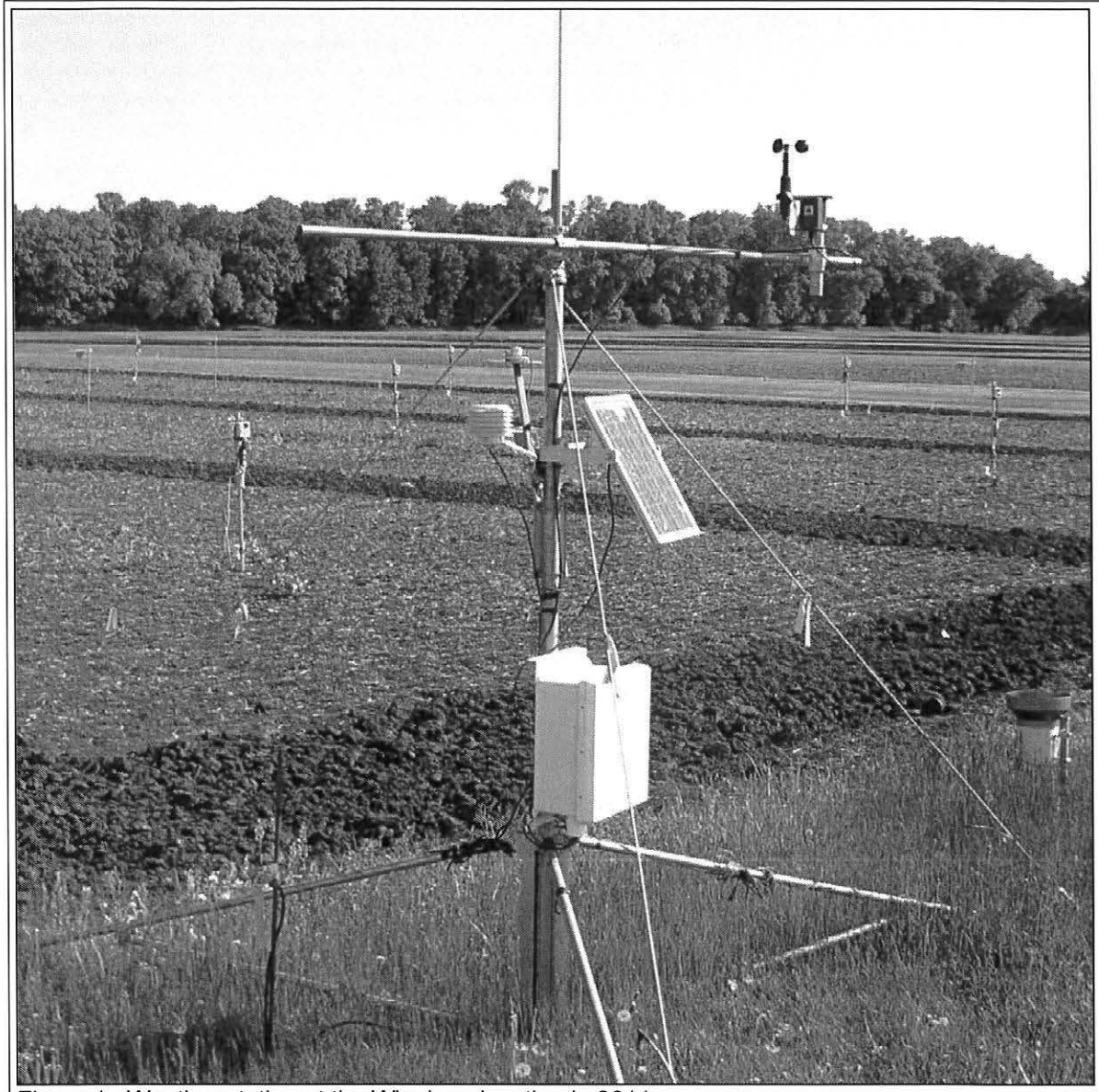


Figure 1. Weather station at the Winnipeg location in 2011.

HOBO microclimate stations were used to monitor microclimate under the crop canopy during the growing seasons (2011 and 2012) in both wheat and canola plots (Figure 2). Measures for microclimate included air temperature, relative humidity, leaf wetness, surface soil temperature and surface soil moisture. One microclimate station was placed in each individual canola plot (18 microclimate stations in total).Two microclimate stations were placed within the wheat strip at each location; one in each of the misted and non-misted portion of the strip. Placement of the stations in Winnipeg and Carman occurred on June 6th and June 17th respectively in 2011 and on June 26th and June 13th, 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. The air temperature probe 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 July4th (Winnipeg) and June 21st (Carman).

Figure 2. Microclimate station deployed in a canola plot.

Figure 3. Rotorod spore trap in the wheat strip.

Ascospore Sampling

Rotorods were installed to monitor the dispersal of spores from individual canola plots and the wheat strip (Figure 3). The rotorod collected spores on 2 polysterene collector rods that spin at 2400 RPM for 1 minute out of every 10 minutes. In 2011, rotorod monitoring 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, two each in the misted and non-misted areas. Eight rotorods were also placed in the bare soil on each side of the wheat strip with 4 placed 3 meters away and 4 placed 7 meters away from the inoculated wheat strip. The purpose for spore sampling on the bare soil was to determine the distance and direction that spores were dispersed on either side of the wheat. All rotorod samplers in Winnipeg contained retracting heads, while samplers in Carman contained fixed heads. Rods were collected and replaced daily.

Ascospores of S. sclerotiorum on each collector rod were counted in the lab using a microscope at 400x magnification, where spores were identified based on their 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 their appearance was uncertain. 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. S. 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 overlaying the black mark from the sampling head of the rotorods. The lens view area differed slightly depending on the specific microscope and lens that was used.

The total number of ascospores per rod was 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
A1 = л (0.275 mm)2 = 0.238 mm2 (Olympus BX51)
A2 = л (0.24 mm)2 = 0.181 mm2 (BH-2)
A3 = л(0.24 mm)2 = 0.181 mm2 (Hund Wetzlar Typ H 600/12)

Where "A1, A2 and A3" 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:
A1(10) = 0.238 mm2 (10) = 2.38 mm2 (Olympus BX51)
A2(10) = 0.181 mm2 (10) = 1.81 mm2 (BH-2)
A3(10) = 0.181 mm2 (10) = 1.81 mm2 (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:
M1 = 22 mm2 / 2.38 mm2 = 9.24 (Olympus BX51)
M2 = 22 mm2 / 1.81 mm2 = 12.15 (BH-2)
M3 = 22 mm2 / 1.81 mm2 = 12.15 (Hund Wetzlar Typ H 600/12)

Where M1, M2 and M3 are multiplication factors used to calculate total spore counts. The spore counts for each area are then multiplied by the multiplication factors (M1, M2and M3) to get the total number of spores for each rod.

TS = S(10) x M1 (Olympus BX51)
TS = S(10) x M2 (BH-2)
TS = S(10) x M3 (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 is then divided by the volume of air sampled during the 24 hour sampling period (3.12 m^3). Occasionally samples were taken slightly before or after one day; therefore the number of minutes during the sampling period varied and the resulting volume of air sampled was slightly different. 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:

Ascospores/m^3 = (TS(rod 1) + TS(rod 2)) / 2

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 had often been placed in the rotorod backwards so that the adhesive side could not capture spores properly. Some rods were also missing usually because 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 used as the mean daily ascospore concentration. Where none of the two rods could be counted, there was a gap in the daily ascospore record for a specific canola plot or wheat sampling location.

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 media for plating petals. One petal from each flower was plated on a Rose Bengal Agar (Bom and Boland, 2000) immediately after sampling. 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). 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 (Figure 4).

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 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.

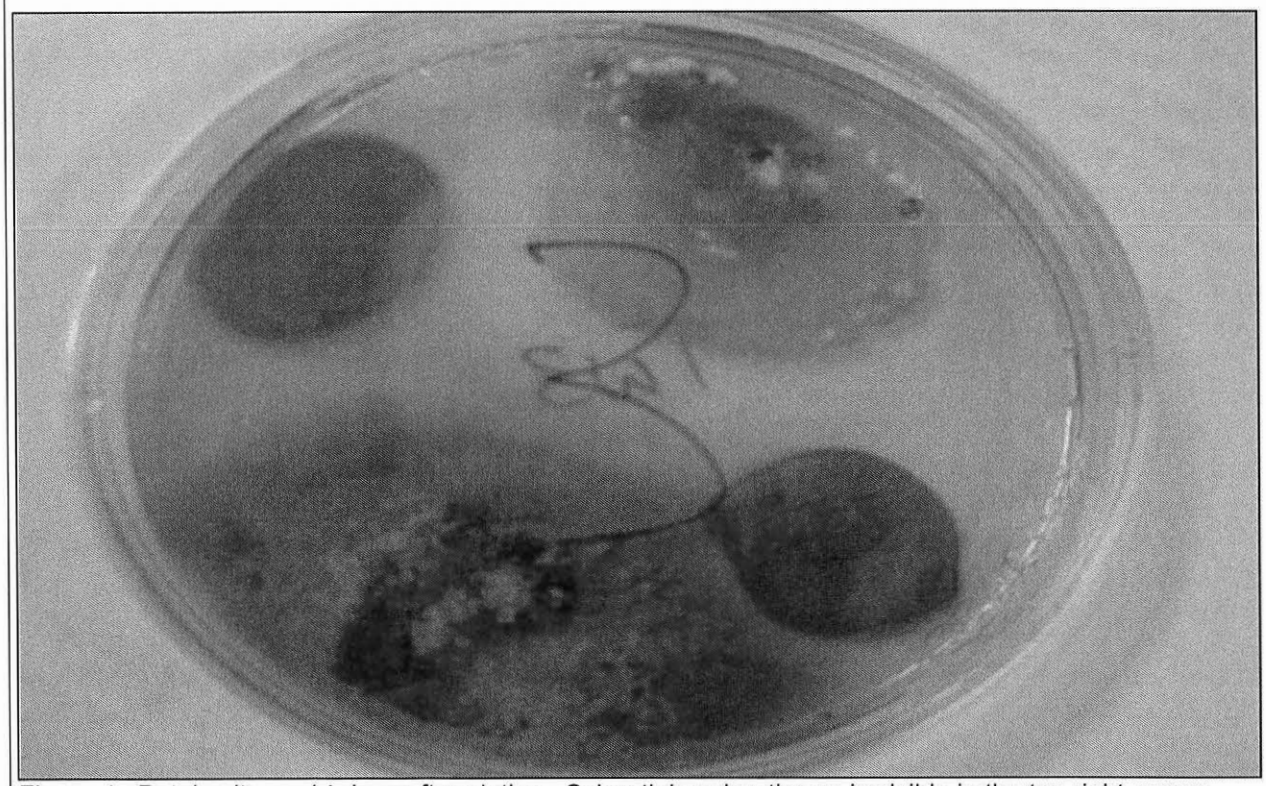


Figure 4. Petal cultures 14 days after plating. Sclerotinia sclerotiorum is visible in the top right corner.

Plot Misting

During the 2011 growing season, half of the wheat strips and half of the canola plots at each site were misted

to ensure continual moisture for disease development and to provide a range of microclimatic conditions. The remaining locations 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. 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 of the Winnipeg or Carman plots were misted in 2012 due to technical issues and availability of generators and pumping systems. Thus, there was no misted treatment at either site in 2012 but rather two sets of randomized complete block designs in each of the Winnipeg and Carman fields.

Crop Development and Canopy Density

Crop development of canola was observed throughout the growing season. Development stages were 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 depicted based on Canola Council of Canada (2011).

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. 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 throughout each growing season. LAI was 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 in each corner at approximately 2.5m away from the corner. An average leaf area index 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 m^2 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 ___, 2012 and August 7th, 2012 in Winnipeg and Carman respectively.

Disease Incidence

Disease incidence was evaluated in Carman and Winnipeg prior to swathing by determining the percentage of S. sclerotiorum infected plants to the total number of canola plants. In 2011, disease assessments were done on August 5th and August 22nd in Winnipeg and Carman, respectively while 2012 disease assessments were done on August ___ and August 7th, respectively. Four $1m^2$ quadrats were placed in each 10 by 10 m plot (1 through 18) at random and canola 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).

Diseased canola plants were identified as having bleached stems with pale grey and white lesions on stems, branches and pods (Figure 5). Canola plants that contained the above symptoms were also counted if they were lodging. Infected plants were also inspected to determine if they were brittle and shredded (Manitoba Agriculture, 2010). Other diseases were also present at both Winnipeg and Carman, the most prominent ones being Blackleg and Fusarium Wilt. Plants with blackleg were buff coloured, contained spots and small black fruiting bodies in the stems (Manitoba Agriculture, 2011). 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.

Figure 5. Canola plot affected by *Sclerotinia sclerotiorum* in Carman 2011.

Data Analysis

The efficacy of the three seeding rate -and fertilizer treatment combinations were evaluated using average seasonal leaf area index (LAI) values as well as the plant counts determined near the end of the growing season. A Shapiro Wilk's test for normality was used to ensure the samples followed a normal distribution. Using Statistical Analysis System 9.2 (SAS Institute Inc. Cary, NC), was used for an analysis of variance (mixed procedure) was used to make the comparison between the three groups for both LAI and plant counts at p =

0.05. Tukey's HSD (honestly significantly different) test was used for comparison of treatment means testing the null hypothesis that all treatment effects are similar. Values for leaf area index (LAI) were also compared over time throughout the growing season and plotted in a a time series plot.

Winnipeg total daily mean concentrations of airborne ascospores (ascospores/m^3) for all canola plots were calculated by taking the totals of all the daily mean concentrations from June 30th to July 30th; from the beginning of flowering to harvest. For the gaps in the data that were present for some single days, all values for that day were not included in the total. Nine out of 26 days were not included in the total due to the presence of gaps. An analysis of variance of the high, medium and low crop density treatments was done using the mixed procedure at $p = 0.05$. Due to unequal sample sizes for high medium and low density plots, Scheffe's test was used for comparison of treatment means. A Shapiro Wilk's test for normality was used to ensure the samples followed a normal distribution.

Percentages of canola plants infected were calculated for each of the plots and an ANOVA was performed to compare high, medium and low density plots in both Carman and Winnipeg. Tests for normality (Shapiro Wilk's) and analysis of variance (ANOVA) were done using Statistical Analysis System 9.2 (SAS Institute Inc. Cary, NC) to determine whether significant differences exist between the percentages of disease incidence among the three canopy density treatments.

Ascospore concentrations were then correlated to disease incidence. Correlation coefficients were calculated to determine the linear relationship between percentage of canola plants infected and disease incidence and average daily mean ascospore concentrations. Correlations were computed for all plots together and for treatments of high, medium and low plant densities separately.

To determine the effect of irrigation on disease incidence, the percentage of plants infected were tested for normality (Shapiro Wilk's) and analysis of variance (ANOVA) using Statistical Analysis System 9.2 (SAS Institute Inc. Cary, NC) to compare the misted and unmisted plots.

Total daily precipitation (mm/day) throughout the growing season was plotted against daily mean ascospore concentrations (ascospores/m^3) to see if any relationship existed among rainfall and peak ascospore concentrations.

The effect of wind speed on release and dispersal of airborne ascospores was analysed by plotting the hours above specified wind speed thresholds for each day and daily mean ascospore concentrations for several plots. Thresholds used in this analysis were 3 m/s, 4 m/s and 4.5 m/s. The closer the threshold for hours above matches the daily mean ascospore concentrations, the more likely that value represents wind speeds that may influence release and dispersal of ascospores. Average daily wind speeds were also compared against daily mean ascospore concentrations.

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. Comparisons of average daily mean ascospore concentrations and several microclimate parameters including air temperature, soil temperature, relative humidity and leaf wetness were also made between misted and non-misted fields for 2011. An analysis was completed on wind direction relative to spore dispersal through a series of comparisons of daily mean ascospore concentrations in the directions 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.

Results

Weather and Microclimate Impacts on Ascospore Release from a Non-Host Crop (Wheat)

During the rotorod sampling period over the 4 site-years, daily mean air temperatures were higher overall in 2012, however temperatures were similar throughout the growing season (Table 1). Daily mean air temperatures in Winnipeg and Carman were similar 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 is 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 2). 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 follow a similar trend throughout the spore sampling period during all site years (Figures 6 and 7). Air temperature and soil temperature are 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 tend 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 do not show any temporal correlation. Average mean daily ascospore concentrations in Winnipeg and Carman peak during similar periods in 2011 and 2012. In 2011, major peaks occur around July 24th and July 27th at both sites; major peaks occur around July 17th and July 20th in 2012.

Table 1. 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	X[z]	West
Growing Season[x]	20 (34, 0)	20 (33, 5)	65	72	168	139	-	-
				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	172	186	-	-

[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 2. 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)

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). 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. 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

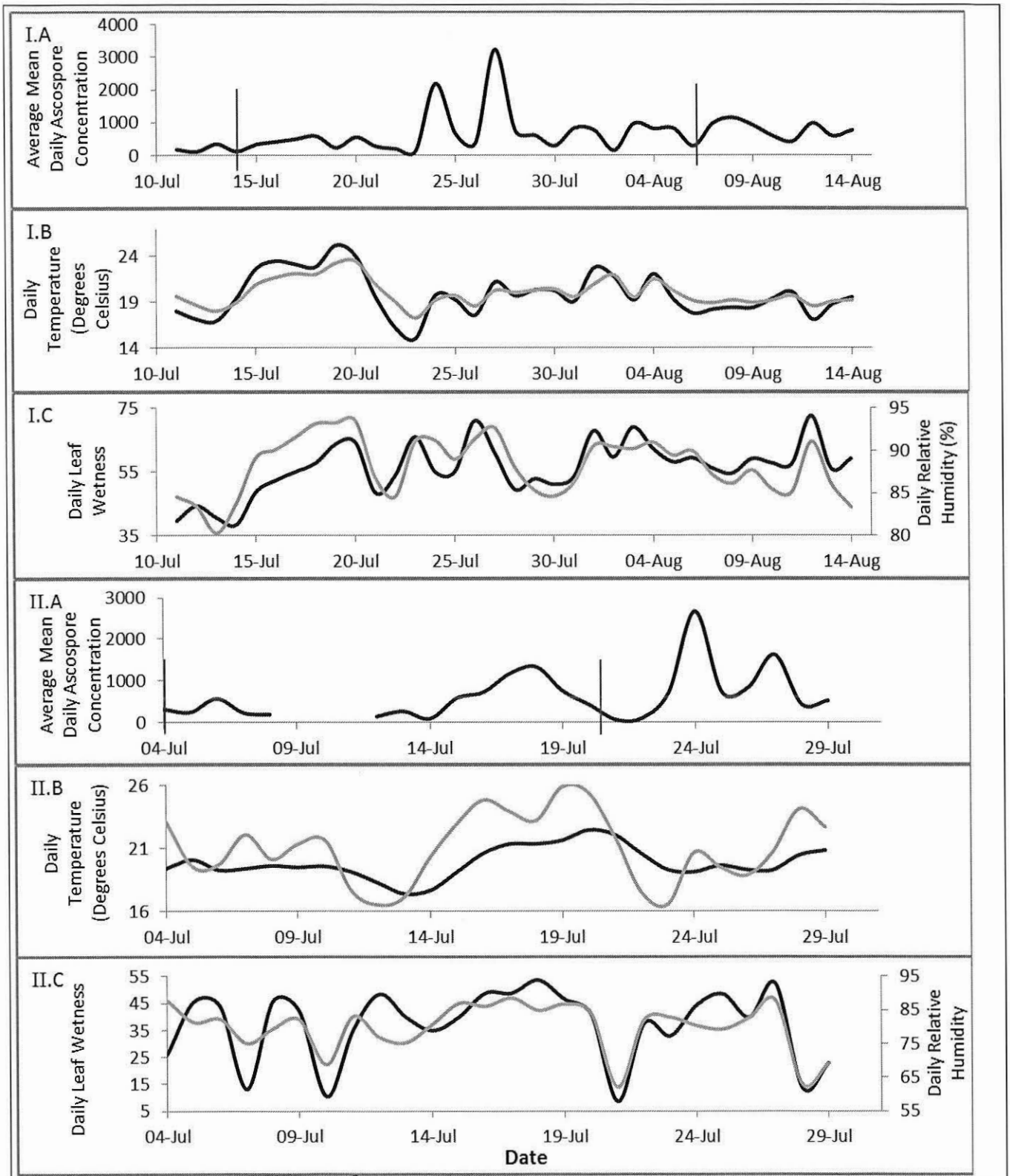


Figure 6. A) Daily mean ascospores/m^3 in I) Carman and II) Winnipeg in 2011 versus B) air temp (black) and soil temp in °C (grey) and C) leaf wetness (black) and RH (%) (grey line). Red lines are start - end of flowering.

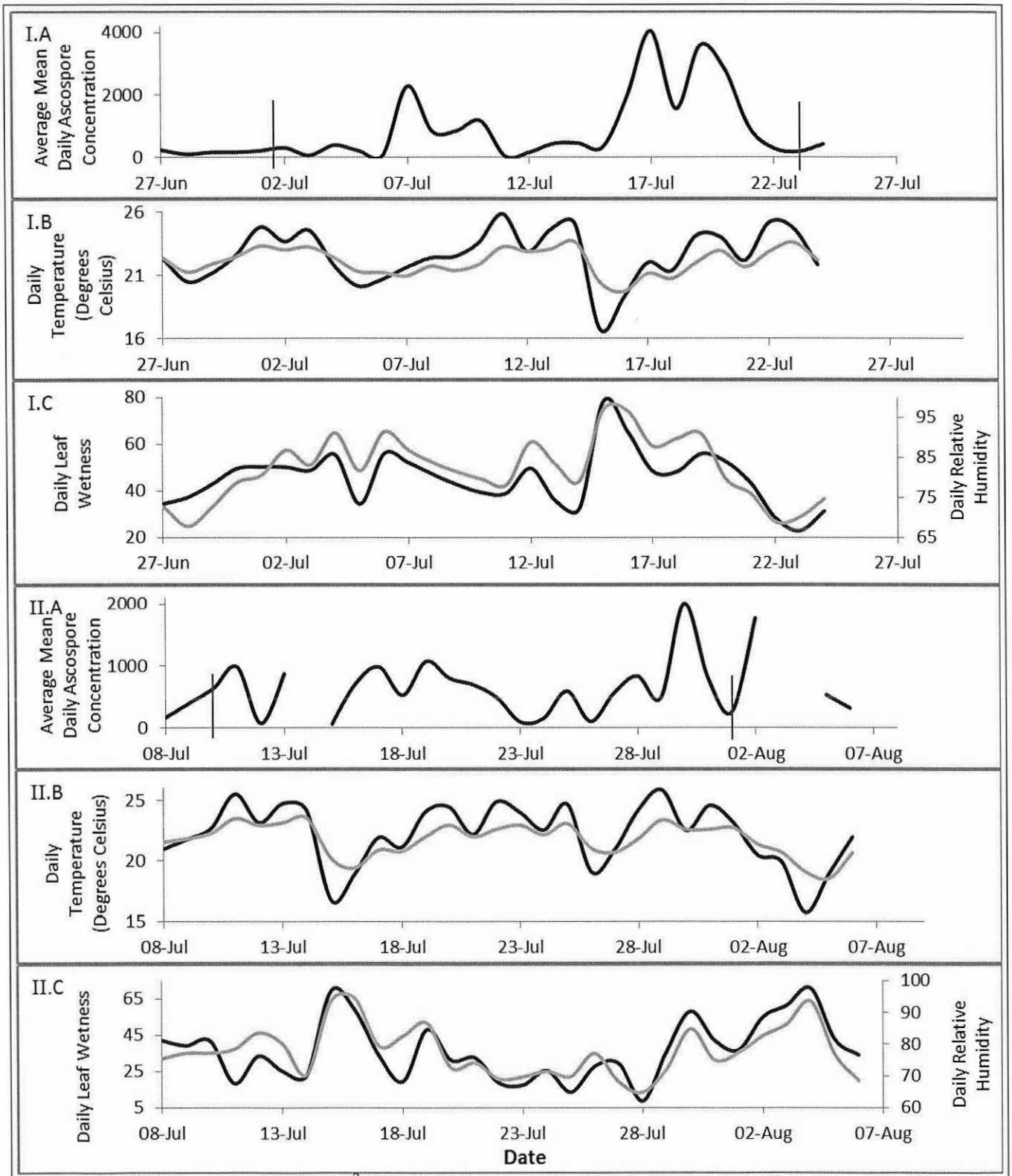


Figure 7. A) Daily mean ascospores/m^3 in I) Carman and II) Winnipeg in 2012 versus B) air temp (black) and soil temp in °C (grey) and C) leaf wetness (black) and RH (%) (grey line). Red lines are start - end of flowering.

Wind Impacts on Ascospore Dispersal

In Carman in 2011 and 2012 during the sampling period, prevailing winds were predominantly west. In Winnipeg in 2012, winds were predominantly south; wind direction was not monitored throughout the entire sampling period in 2011 (Figure 8). 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 are no other noticeable trends occurring as a result of predominant wind directions during the spore sampling period.

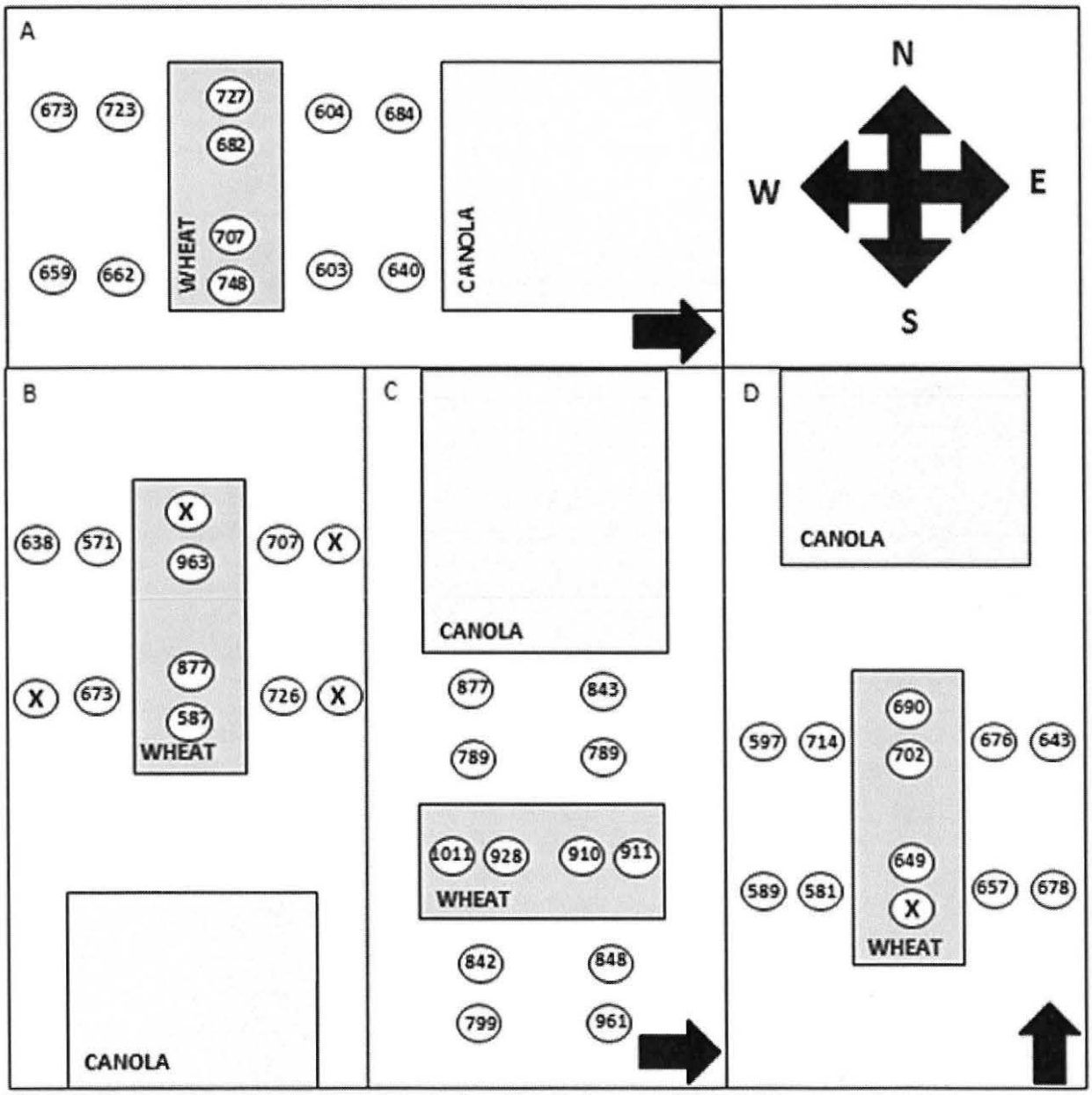


Figure 8. Design of bare and wheat field portions for A) Carman 2011 B) Winnipeg 2011 C) Carman 2012 and D) Winnipeg 2012. Circles represent rotorod locations with average daily mean ascospore concentrations within for the sampling periods at each field at both locations. Black arrows in the bottom right corners represent dominant wind directions during the sampling periods. Rotorods are 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 (see appendices).

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 are 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 analysed 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 8). A slight decrease in average daily mean ascospore concentration is also apparent in Carman in 2011; however an increasing trend is apparent in 2012. Ascospore concentrations tend to follow a negative exponential model with distance from inoculated source (Qandah and Mendoza, 2012). When this model is applied to both years in Winnipeg, the 2011 plot has a steeper slope (Table 4). A 50 percent and 75 percent reduction in daily mean spore concentrations averaged over the sampling period would occur at approximately 61-62 meters and 119-122 meters respectively from the inoculum source.

Table 4. Exponential model of the reduction in mean spore concentrations at 3 meters and 7 meters from an inoculum source in Winnipeg and 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

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 average daily mean ascospore concentrations occurred where rotorods were placed within the bare soil portions closer to the canola field inoculum source compared to the wheat inoculum source in Carman during both 2011 and 2012 (figure 8).

In the Carman canola plots (2011 and 2012), average daily mean ascospore concentrations were lowest at 10 to 20 metres from the wheat inoculum source (Table 5). Concentrations increased with distance from the wheat inoculum source. There are 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 5. Average daily mean ascospore concentrations (ascospores/m^3) 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 under all plots, misted plots and non-misted plots, whereas in 2012 concentrations were higher within canola (Table 6). Mean daily ascospore concentrations under wheat and canola canopies follow similar trends (Figure 9). Percentages of infection at harvest were lower overall in 2011 compared to 2012 with higher values occurring in Carman during both years.

Table 6. Whole plot means for average daily mean ascsopore concentrations in wheat and canola plots and percentages of 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	Percentages of 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

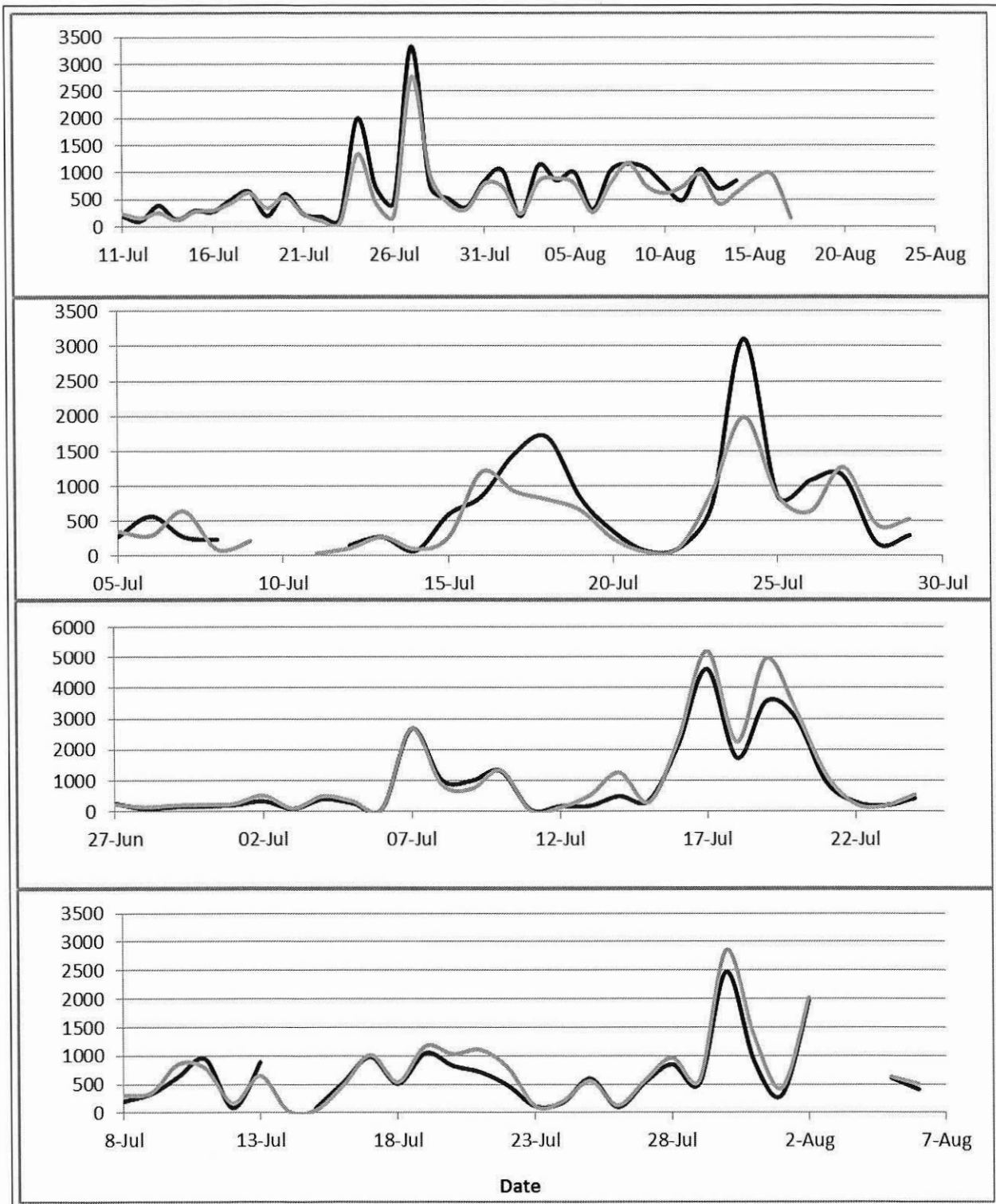


Figure 9. Average daily mean ascospore concentrations in wheat (black line) and canola (grey line) throughout the sampling period in A) Carman 2011, B) Winnipeg 2011, C) Carman 2012 and D) Winnipeg 2012.

Canola Results ***(Note: for 2011 only; 2012 data analysis is not complete)***

Length of the growing season from seeding to harvest, the period from 4th leaf to flowering, flowering and the ascospore sampling period dates are outlined in Table 3. Canola development is also depicted in Figures 6 and 7.

Table 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
4th leaf to flowering	June 29-July 14	June 15-July 4	June 16-July 11	June 27-July 10
flowering	July 14-Aug 6	July 5-July 21	July 1-July 22	July 11-Aug 1
ascospore sampling	July 11-Aug 17	July 4-July 29	June 27-July 25	July 7-Aug 6

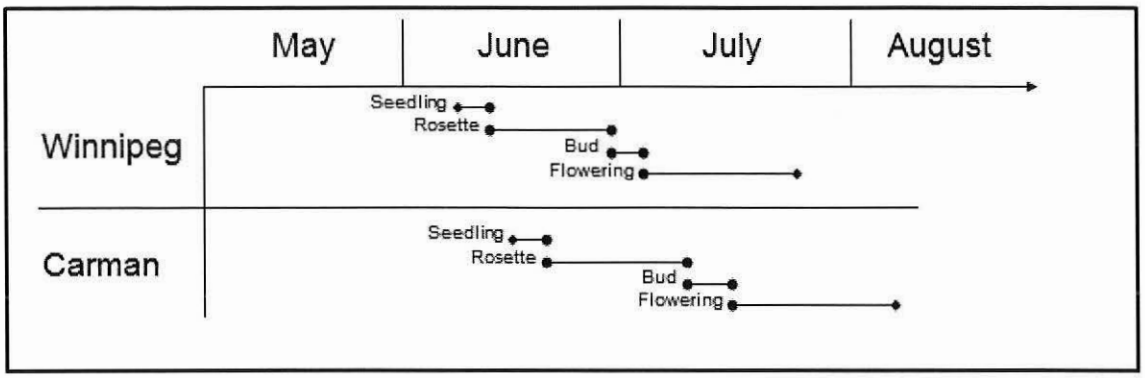


Figure 6. Diagram of canola growth stages in Winnipeg and Carman in 2011.

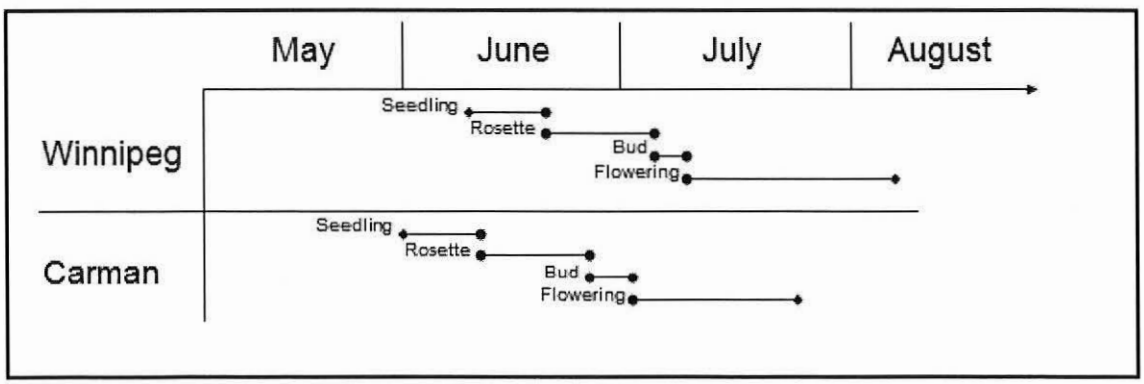


Figure 7. Diagram of canola growth stages in Winnipeg and Carman in 2012.

Canopy Density

Leaf area index (LAI) measurements taken throughout the growing season are represented in figures 8 and 9, for Winnipeg and Carman respectively. At both locations there was a difference in LAI values between high, medium and low seeding rates, although the distinction is more apparent at the Winnipeg location (figures 8 and 9). From budding to the end of flowering, a decreasing trend in LAI was apparent in Winnipeg but not in Carman (figures 8 and 9). The ANOVA revealed a significant difference ($p < 0.05$) in seasonal average LAI values between all seeding rate treatments (table 4). Although there were some significant differences in plant count as

a result of seeding rate, the differences were not as significant as for LAI. Only the high and low seeding rates had significantly different ($p < 0.05$) plant counts in Winnipeg. In Carman, only high and medium seeding rates had significant differences ($p > 0.05$). Distinguishable canopy densities were also apparent in the field, with plots seeded at high rates being visibly dense, and plots appearing sparse at low seeding rates.

Table 4. Significance levels from analysis of variance on the effects of seeding rate on leaf area index (LAI) a from Winnipeg and Carman, MB, in 2011.

	Winnipeg		Carman	
Effects	**F-Value**	**Pr>f**	**F-Value**	**Pr>f**
Seed Rate on LAI	359.07	<.0001	26.71	<.0001
Low vs. High		<.0001		<0.001
Medium vs. High		<.0001		0.0431
Medium vs. Low		<.0001		0.0010
Seed Rate on Plant Count	7.83	0.0047	11.67	0.0009
Low vs. High		0.0039		0.0006
Medium vs. High		0.4000		0.1740
Medium vs. Low		0.0536		0.0279

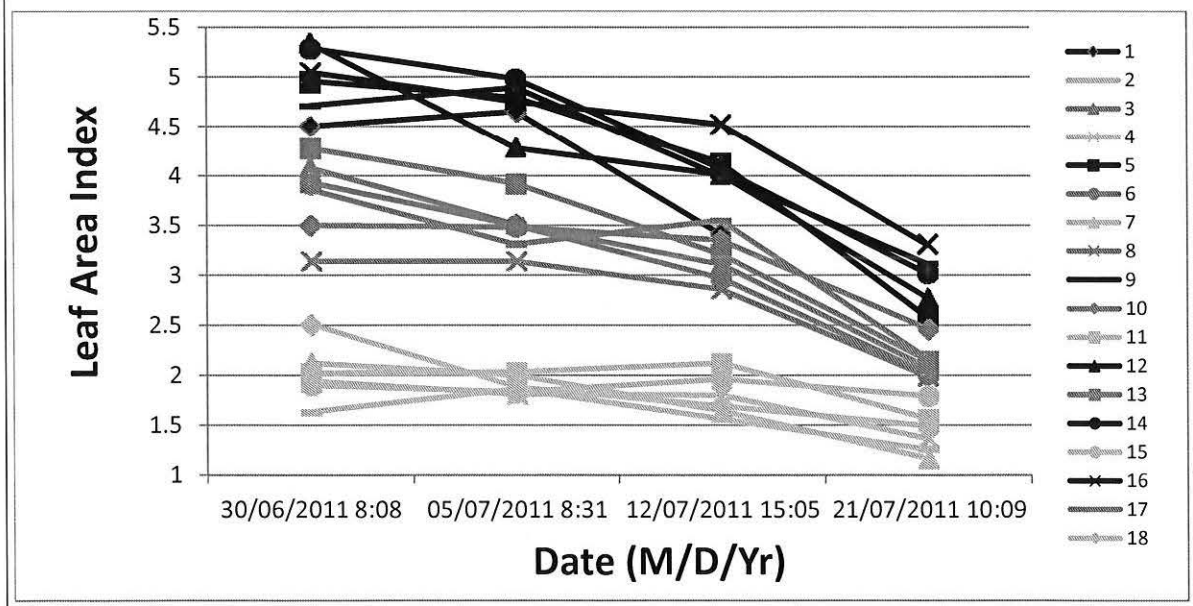


Figure 8: Leaf area index values taken from plots 1-18 throughout the growing season in Winnipeg, Manitoba. Black, grey and light gray lines represent plots seeded at high, medium and low seeding rates respectively.

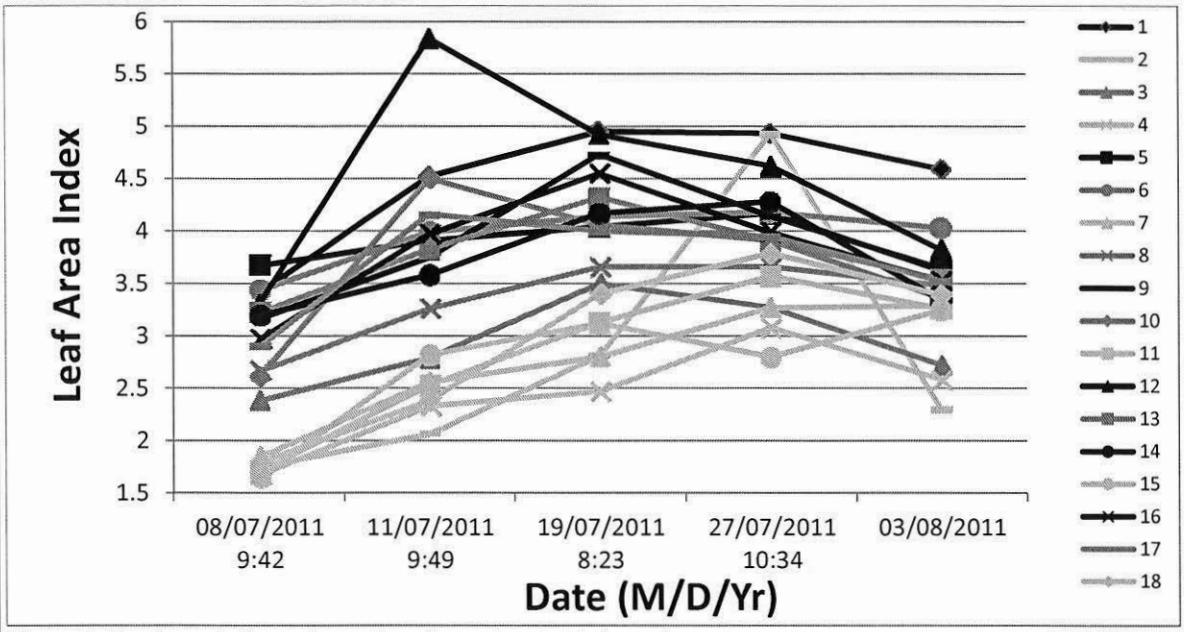


Figure 9: Leaf area index values taken from plots 1-18 throughout the growing season in Carman, Manitoba. E and light gray lines represent plots seeded at high, medium and low seeding rates respectively.

Petal Tests

The results from the petal tests are below.

Winnipeg – July 6, 2011 (30% bloom)

- One bottom petal in plot 18 and one top petal in plot 3 contained sclerotinia

Winnipeg – July 14th, 2011 (70% bloom)

- Plot 12 contained sclerotinia on one top petal
- Plot 13 contained sclerotinia on three top petals and one bottom petal
- Plot 14 contained sclerotinia on one top petal

Carman – July 18th, 2011 (30% bloom)

- Plot 8 was the only location in which sclerotinia was found on 2 petals taken from bottom flowers within the plot

Carman – July 25th, 2011 (70% bloom)

- No Sclerotinia found on any of the petals

Ascospore Concentrations

There were no significant relationships between total daily mean ascospore concentrations (June 30th to July 30th) and high, medium and low seeding rates in Winnipeg (Table 5, Figure 10). The highest values were realized under medium density canopies, with the lowest values in low density canopies. A visual comparison between a high density Winnipeg plot (5w) with high average daily mean ascospore concentration and a low density Winnipeg plot (2w) with low average daily mean ascospore concentration (figure 11) shows similar trends within both plots. The main difference is a higher ascospore concentration near the end of July for the high density plot is only showing increased mean compared to the low density plot.

Table 5. Significance levels from analysis of variance on the effects of canopy density on total daily mean ascospore concentration in Winnipeg and Carman, MB, in 2011.

	Winnipeg	
Effects	**F-Value**	**Pr>f**
Canopy Density on Avg Daily Mean Ascospore Conc.	0.65	0.5386

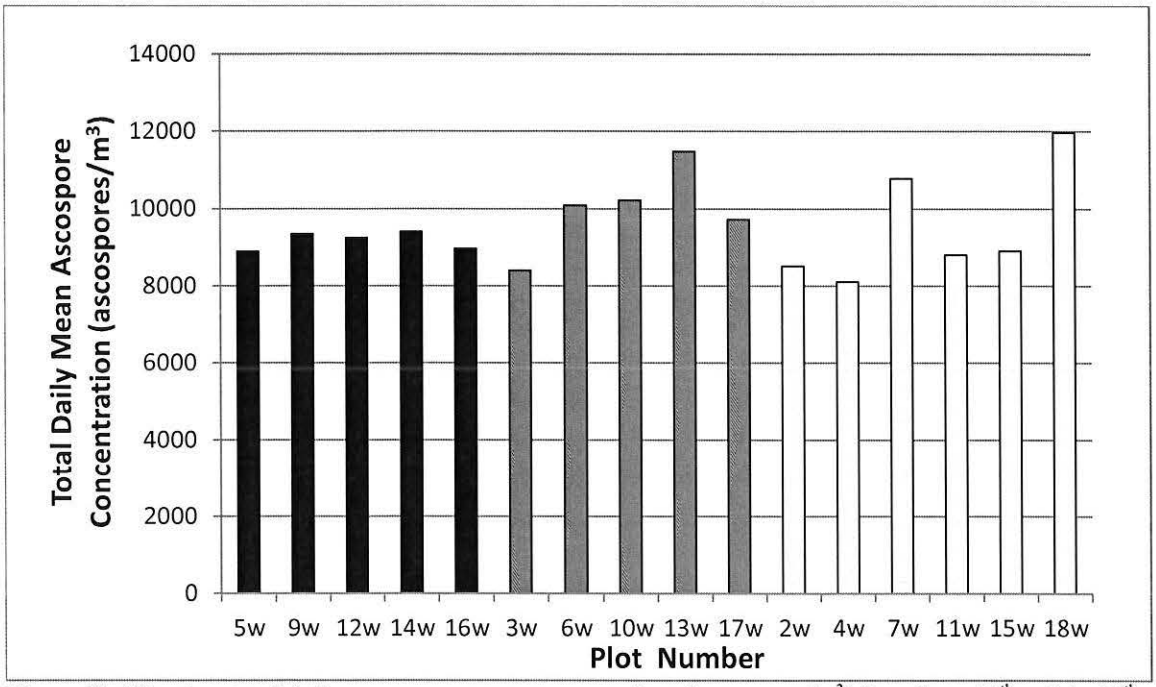


Figure 10. Winnipeg total daily mean ascospore concentrations (ascospores/m^3) from June 30th to July 30th (ascospores/m3). High, medium and low density plots are represented by the black, dark grey and light grey shading respectively.

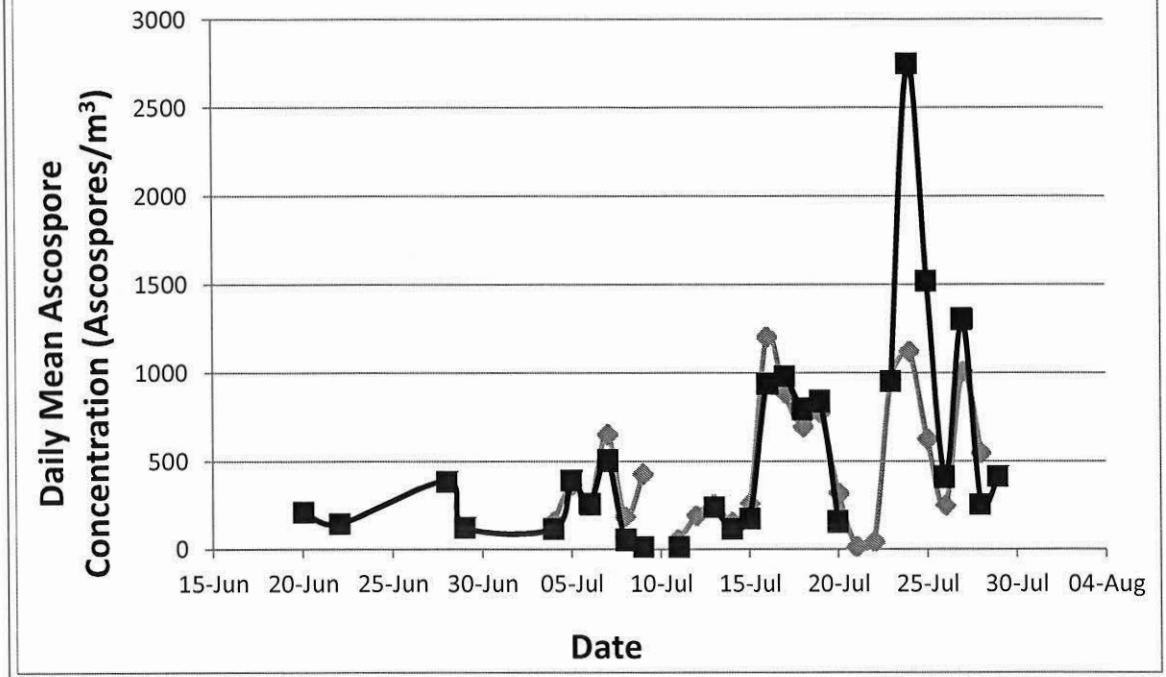


Figure 11. Comparison between Winnipeg daily mean ascospore concentrations (ascospores/m3) between 2w and 5w; a low density (grey) and high density (black) plot.

Disease Incidence

Percentages of infection (%) values determined prior to canola swathing in Winnipeg and Carman are shown in figures 12 and 13. Mean infection percentages for Winnipeg and Carman were 5.8% and 21.6% respectively, with Carman containing the highest values overall. No visible trends were apparent among infection percentages within high, medium and low density plots. The ANOVA also revealed no significant effects ($p > 0.05$) in percentage infection values among canopy densities in Winnipeg (Table 6). The only significant difference in infection percentages was between high and low density plots in Carman.

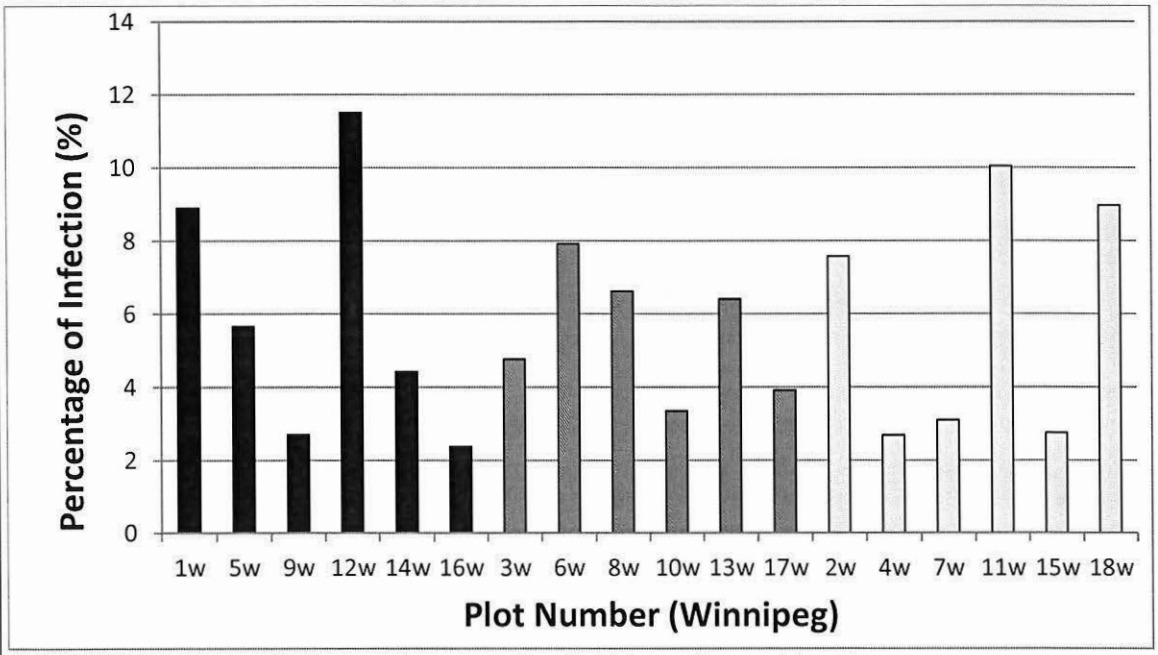


Figure 12. Percentage of Infection prior to swathing in high, medium and low density plots in Winnipeg field in plots 1 through 18 in 2011. High, medium and low density plots are represented by black, dark grey and light grey shading respectively.

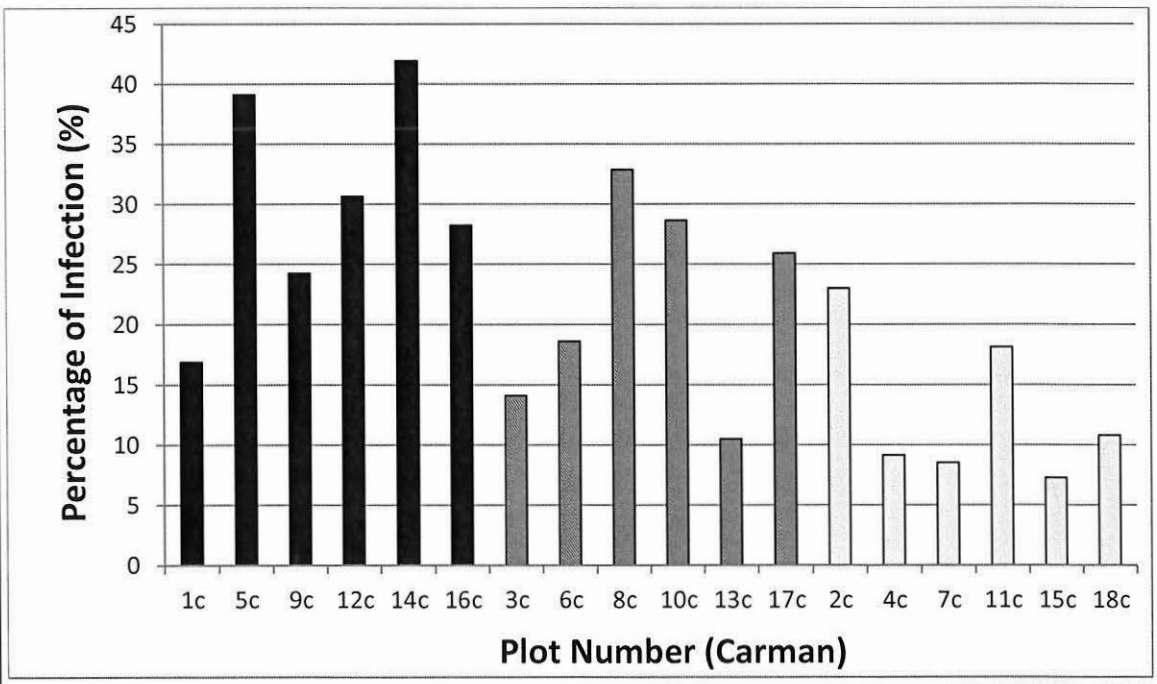


Figure 13. Percentage of Infection prior to swathing in high, medium and low density plots in Carman field in plots 1 through 18 in 2011. High, medium and low density plots are represented by black, dark grey and light grey shading respectively.

Table 6. Significance levels from analysis of variance on the effects of canopy density on percentage of infection fro *sclerotiorum* in Winnipeg and Carman, MB, in 2011.

	Winnipeg		Carman	
Effects	**F-Value**	**Pr>f**	**F-Value**	**Pr>f**
Canopy Density on DI	0.03	0.9660	6.69	0.0084
High vs. Low		0.9990		0.0062
High vs. Medium		0.9672		0.2127
Medium vs. Low		0.9774		0.1768

Correlations between Ascospore Concentrations and Disease Incidence

In Winnipeg, there was no linear relationship between average daily mean ascospore concentrations (ascospores/m^3) and disease incidences (%) (R^2 = 0.0314). Similarly, no linear relationships were observed within each of the high, medium and low treatments comparing disease incidence (%) to average daily mean ascospore concentration with R^2 values of 0.0076, 0.1041 and 0.0512, respectively.

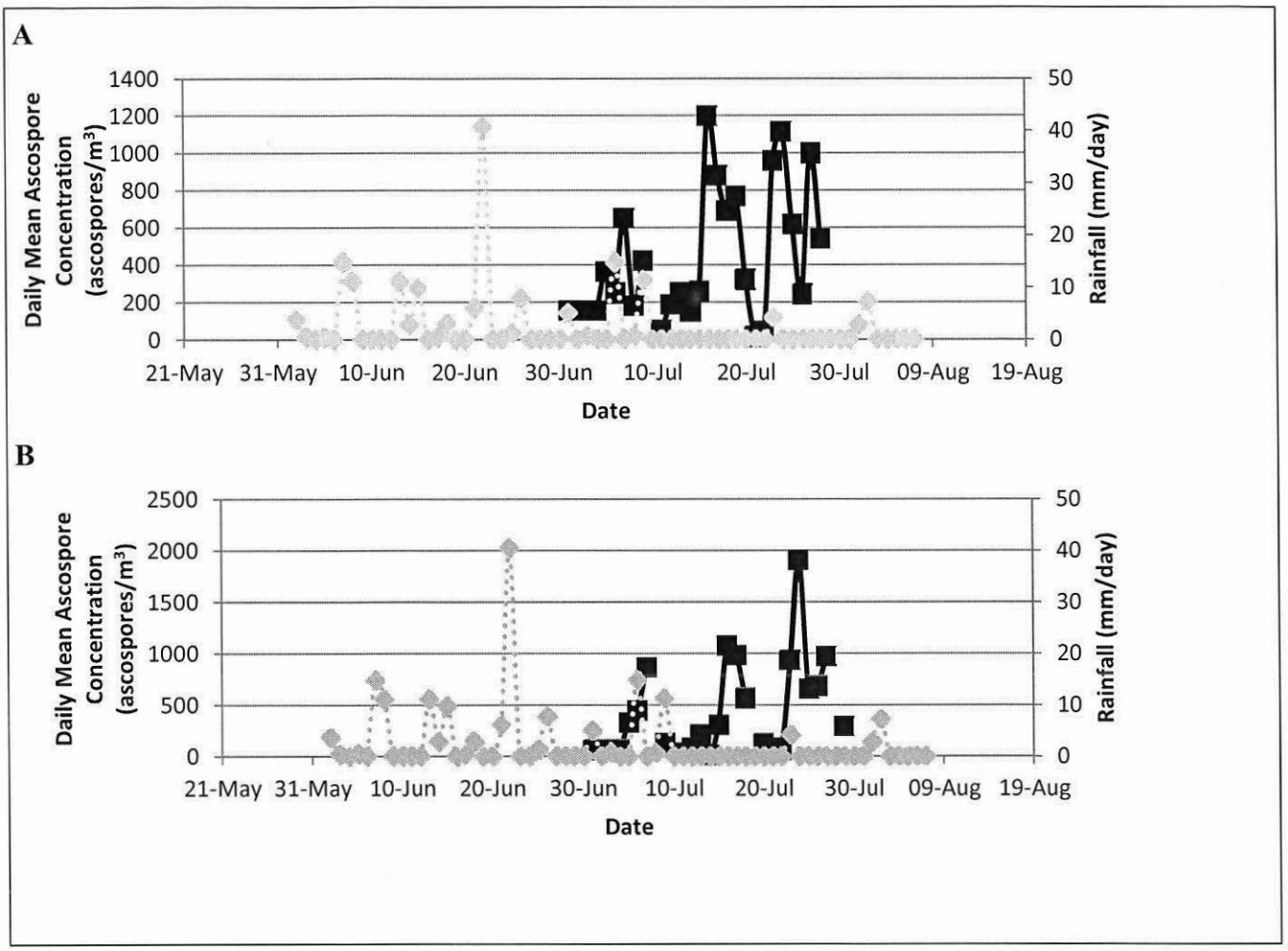
Impacts of Weather

There were no differences in disease incidence (percentage of infection) among irrigated and non-irrigated plots (p>0.05) in either Winnipeg or Carman (Table 7).

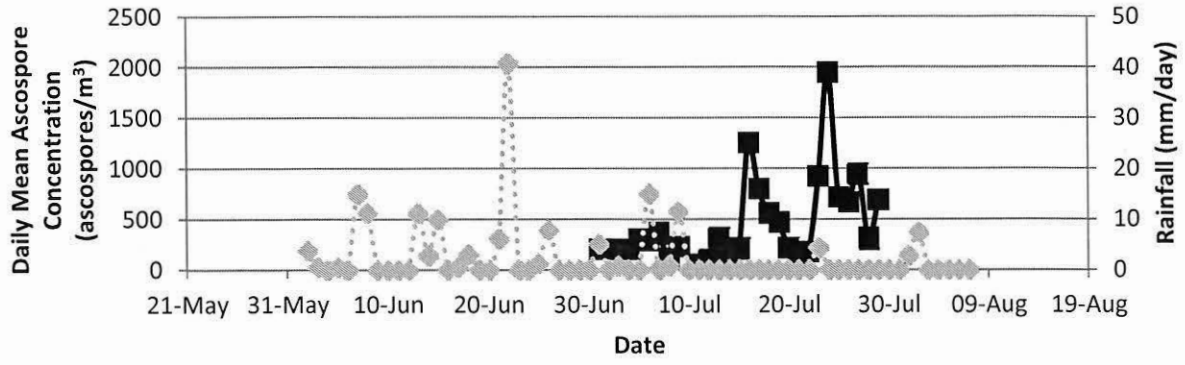
Table 7. Significance levels from analysis of variance on the effects of irrigation on percentage of infection from *S. sclerotiorum* in Winnipeg and Carman, MB, in 2011.

	Winnipeg		Carman	
Effects	**F-Value**	**Pr>f**	**F-Value**	**Pr>f**
Irrigation on DI	0.09	0.7642	0.11	0.7413

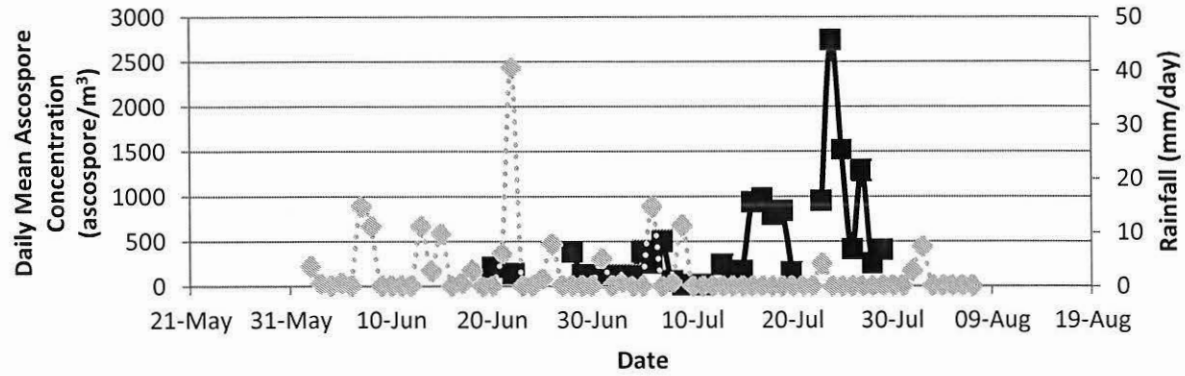
In Figure 14 (A through F), daily mean ascospore concentration is plotted with total daily rainfall. Daily mean ascospore concentrations in each of the plots follow similar trends with a smaller peak around July 7th and several larger peaks occurring on July 17th, July 24th and July 27th. There does not seem to be any link between rainfall and daily mean ascospore concentrations, however some of the peaks occur directly before or after rainfall events.


A
Daily Mean Ascospore Concentration (ascospores/m³)
Rainfall (mm/day)
1400
1200
1000
800
600
400
200
0
50
40
30
20
10
0
21-May
31-May
10-Jun
20-Jun
30-Jun
10-Jul
20-Jul
30-Jul
09-Aug
19-Aug
Date
B
Daily Mean Ascospore Concentration (ascospores/m³)
Rainfall (mm/day)
2500
2000
1500
1000
500
0
50
40
30
20
10
0
21-May
31-May
10-Jun
20-Jun
30-Jun
10-Jul
20-Jul
30-Jul
09-Aug
19-Aug
Date

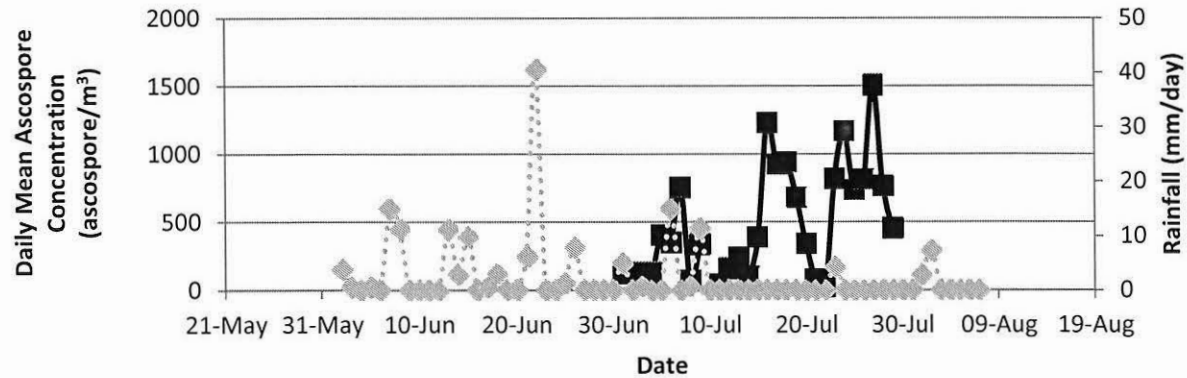
C


Daily Mean Ascospore Concentration (ascospores/m³)
Rainfall (mm/day)
2500
2000
1500
1000
500
0
50
40
30
20
10
0
21-May
31-May
10-Jun
20-Jun
30-Jun
10-Jul
20-Jul
30-Jul
09-Aug
19-Aug
Date

D


Daily Mean Ascospore Concentration (ascospore/m³)
Rainfall (mm/day)
3000
2500
2000
1500
1000
500
0
50
40
30
20
10
0
21-May
31-May
10-Jun
20-Jun
30-Jun
10-Jul
20-Jul
30-Jul
09-Aug
19-Aug
Date

E


Daily Mean Ascospore Concentration (ascospore/m³)
Rainfall (mm/day)
2000
1500
1000
500
0
50
40
30
20
10
0
21-May
31-May
10-Jun
20-Jun
30-Jun
10-Jul
20-Jul
30-Jul
09-Aug
19-Aug
Date

F

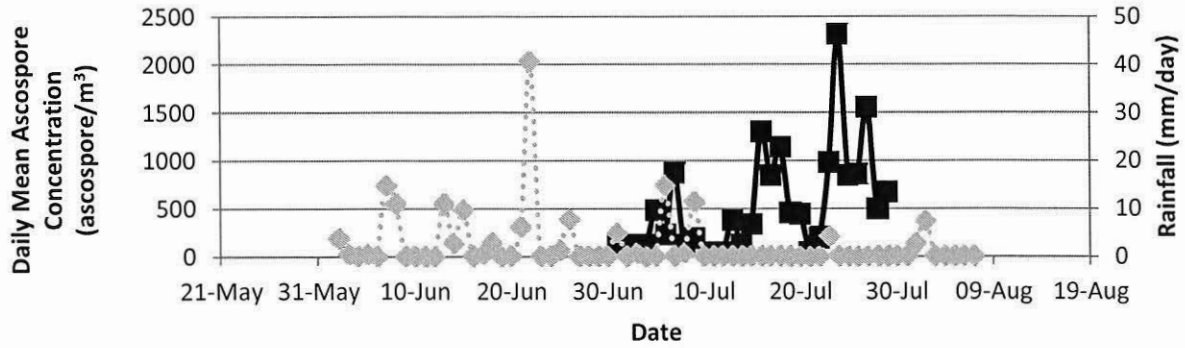
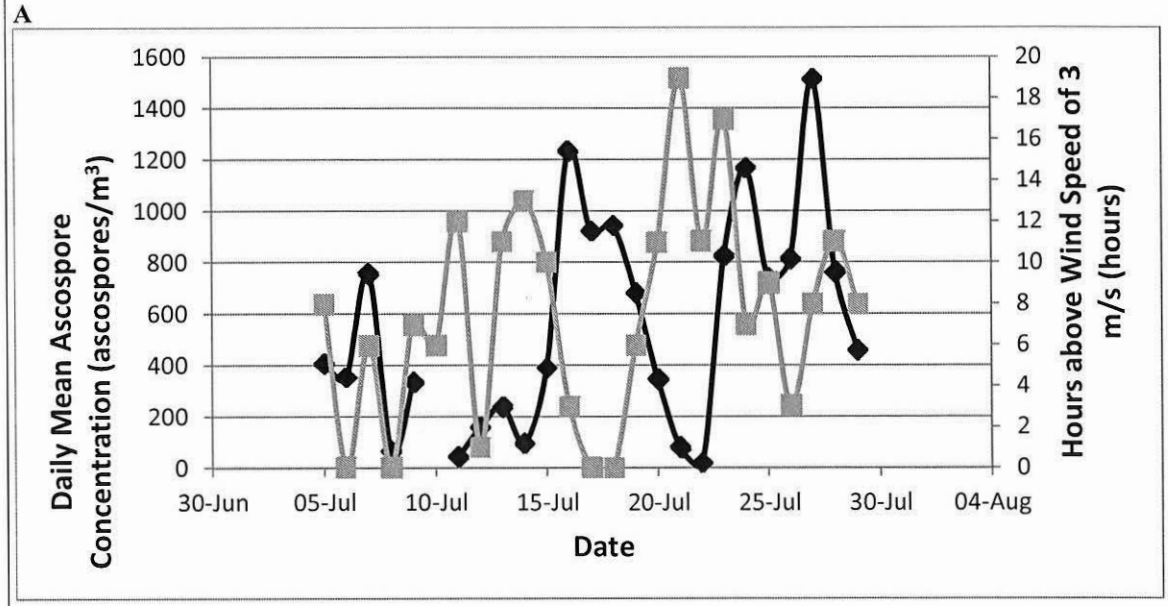


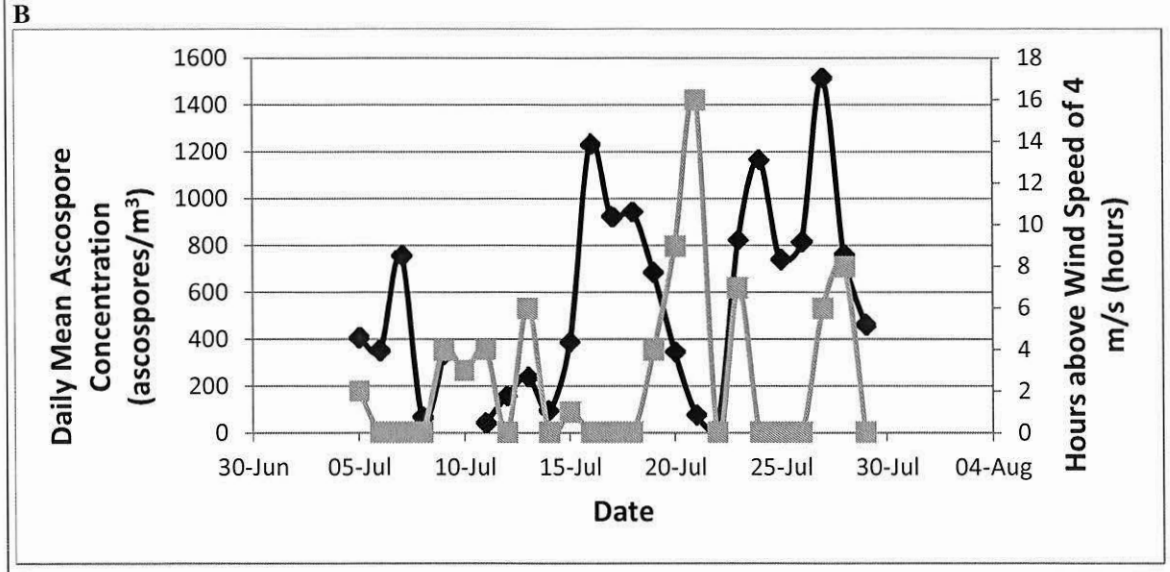
Figure 14. Total daily rainfall and daily mean ascospore concentrations (ascospores/m^3) for each non-irrigated plot (2, 3, 4, 5, 6, 7) throughout the growing season (June 4th to August 8th, 2011) in Winnipeg. **A)** Plot 2 **B)** Plot 3 **C)** Plot 4 **D)** Plot 5 **E)** Plot 6 **F)** Plot 7

Hours per day above three different wind speed thresholds (3 m/s, 4 m/s and 4.5 m/s) were calculated and plotted against daily mean ascospore concentrations for several plots. Since ascospore concentrations contained similar trends across all plots in the non-misted field portion in Winnipeg, an example of plot 6 is shown in Figure 15. Ascospore concentrations in plot 6 tend to increase with greater hours above all three thresholds. There is, however, an increase in ascospore concentration about July 14th that is not explained by hours above each of the thresholds. Average daily wind speeds also show increasing ascospore concentrations in most cases with increasing average wind speeds.

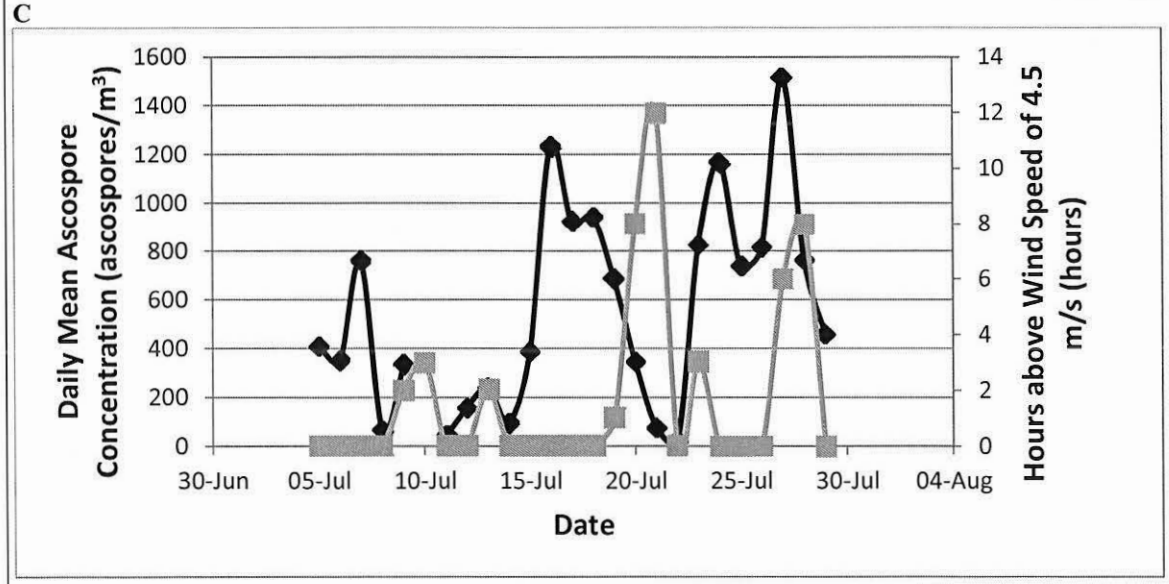
A



B


Daily Mean Ascospore Concentration (ascospores/m³)
1600
1400
1200
1000
800
600
400
200
0
30-Jun
05-Jul
10-Jul
15-Jul
20-Jul
25-Jul
30-Jul
04-Aug
Date
18
16
14
12
10
8
6
4
2
0
Hours above Wind Speed of 4 m/s (hours)

C


Daily Mean Ascospore Concentration (ascospores/m³)
1600
1400
1200
1000
800
600
400
200
0
30-Jun
05-Jul
10-Jul
15-Jul
20-Jul
25-Jul
30-Jul
04-Aug
Date
14
12
10
8
6
4
2
0
Hours above Wind Speed of 4.5 m/s (hours)

D

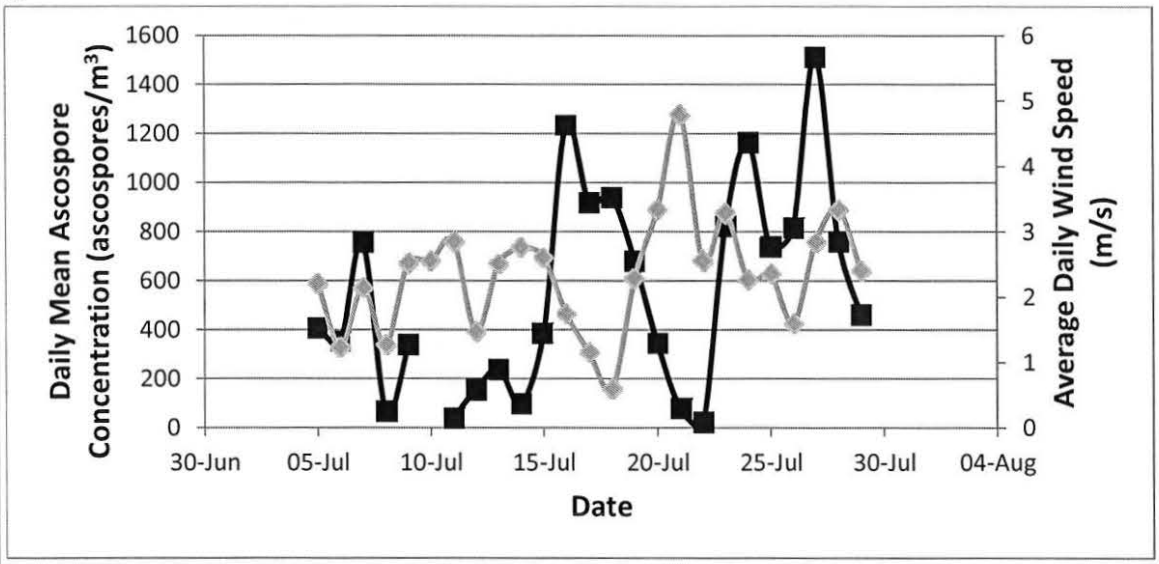


Figure 15. Daily mean ascospore concentrations (black) plotted against **A)** hours above 3 m/s **B)** hours above 4 m/s **C)** hours above 4.5 m/s and **D)** average wind speed (m/s)

Discussion

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, then warm dry conditions throughout the summer. Overall temperatures were slightly warmer and relative humidity percentages were lower in 2012 which affected canola flowering, seed set and size. Compared to Carman, Winnipeg experienced higher maximum temperatures and lower 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 the general weather pattern during both years.

The use of a misting system to promote disease development was successful in altering microclimates, however it did not influence disease incidence during the 2011 growing season when it was in use 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. Additional moisture increases relative humidity within the canopy during sclerotial germination and spore release; however relative humidity was not higher overall under the 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. In our study, petal tests did not show significant results, however in Winnipeg after misting began (July 14th, 2011) ascospores were only found on petals situated in the misted portion of the field compared to initial petal sampling on July 6th where few petals were found throughout the field. The process whereby ascospores land on the petals may be favoured in irrigated plots where petals become moist. However, very few ascospores were found in Carman in any of the plots, so there are other factors involved.

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 occured at similar times as the release in the adjacent 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 both Winnipeg and Carman. Thus, the general overall weather conditions clearly play a critical role in the timing of ascospore release in both host and non-host crops. 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 with larger peaks at 80 to 90% flowering which would likely influence neighbouring canola fields early in the flowering period. 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, 2011), however increased disease protection occurs when fungicides are applied during apothecia appearance 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 under non-host crops and releasing them into the surrounding atmosphere as proven in 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 have 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 wheat canopy in proximity as shown in this study. Adjacent field-born inoculum still however does influence neighbouring fields as proven 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. These findings were further validated within this study since ascospores were capable of travelling to 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 is also likely to impact spore concentration within bare soil from long distances. Qandah and Mendoza (2012) found that precipitation influences the steepness of ascopsore 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 in 2011 compared to 2012. The positive gradient occurring in Carman in 2012 may be explained

by strong prevailing winds increasing spore concentrations in the furthest 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; decreasing rates should be applied with distance from the inoculum source.

Climate is shown to be a major contributor to disease development as proven 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 the 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 exist due to weather and microclimatic conditions as well as canopy structures. In 2011 higher ascospore concentrations were observed 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 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 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 covered more of each site than wheat field portions, providing a larger inoculated area for spore dispersal. As a result of the ascospores being produced within an adjacent wheat field, 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 would have likely impacted the canola, and any external sources of inoculum did not result in additional disease incidence according to this study.

Another factor impacting ascospore dispersal is wind. Predominant wind directions changed often, therefore daily predominant winds were used to analyse 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. Wind direction does play a slight role in the movement of spores away from an inoculated 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). 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 host and non-host crop containing fields 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 sclerotia are present. Fungicides are also advised in susceptible crops located in proximity to fields with history of disease. Application timing 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.

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