Canola Agronomic Research Program (CARP)
FINAL REPORT

The Final Report should fully describe the work completed for the year and note the personnel involved. It should also note any deviations from the original plan and next and/or corrective steps as may be required if deviations are noted. A complete statement of expenses should be included. In the event of major changes within the budget, supporting notes should be included. The report should capture a complete summary of activity for the final year and an overview of the entire project.

Project Title: *Monitoring the canola flower midge with pheromone-baited traps*

Research Team Information

Lead Researcher:		
Name	***Institution***	***Project Role***
Boyd Mori, Assistant Professor	University of Alberta	PI
Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
Meghan Vankosky, Research Scientist	AAFC Saskatoon	Co-Investigator
Daniel Bray, Senior Research Fellow	University of Greenwich	Co-Investigator
Kyle Van Camp, PhD Student	University of Alberta	Collaborator

Project Start Date: **January 1, 2021** **Project Completion Date:** **December 31, 2022**

Reporting Period: **December, 2021** to **December 31, 2022**

CARP Project Number: **2020.10**

Instructions: This Final Project Report shall be completed and submitted on or about March 31st of the fiscal year that the agreement is in effect (upon completion of the project). The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a detailed account upon completion of the project.. Final project financial reporting should be provided at this time.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to the CCC contact listed below.

In addition, a Final Extension Report is due upon completion of the project, maximum 2-3 pages, to be used for publication on the Funders' websites and in the *Canola Digest*. Content will be used in extension material, for consumers and/or industry. Include an Executive Summary, brief project description, key findings and conclusions (with a summary graph/table or supporting image for the project), translation of key findings into best management practices and/or relevance to the canola sector and future research, and funding acknowledgment as determined in the grant award letter. The Final Extension Report is intended to support messaging to all audiences. Information needs to be clear, concise and in "grower-friendly" language.

Please include the funding acknowledgements outlined in your research agreement in all deliverables (publications, presentations, etc.) from this project.

1. Date of completion & status of activity (please check one)

Date of completion: 31 December 2022

☐ **Ahead of Schedule** ☐ **On Schedule** ☐ **Behind Schedule** ☐ **Completed**

Comments: Project is complete. (I can't check the box above, maybe compatibility issues with a Mac)

2. Abstract/Summary - Maximum of one page. This must include project objectives, results, and conclusions for use on the Funders' websites.

The canola flower midge, *Contarinia brassicola*, is a recently discovered insect pest of canola on the Prairies. Damage results when larvae feed on developing flower buds which prevents the flowers from opening and forming pods. The population density of canola flower midge is difficult to evaluate as densities are variable across the species range and damage is discreet and can be easily overlooked except under outbreak conditions. Midge control using traditional insecticides is complicated by the cryptic feeding nature of larvae. Larvae feed within developing buds and flowers, which minimizes the time and life stages that are susceptible to control.

Previous work identified the female-produced canola flower midge pheromone and optimized the pheromone blend and dose to create a potent male attractant. This project refined the pheromone trapping system and evaluated the relationship between adult midges captured in pheromone-baited traps and damage in the field. An optimized trapping system consisting of pheromone-baited Jackson trap, deployed 50 cm above the soil surface along the crop edge, was identified as the optimal combination of trap type and position to effectively monitor *C. brassicola*. Two daily peaks in male midge activity were observed, during the morning and late-afternoon, that could be used to increase the precision of monitoring strategies or potential treatment applications. Over the two years of this study (2021-2022), the number of male *C. brassicola* captured on pheromone-baited traps was not predictive of damage in the field, but in 1 of 2 years (2022) there was a significant relationship between the total number of midges captured and the total number of galls sampled over the season. Average air temperature and weekly cumulative rainfall, at the scale measured in this study, did not have an impact on the number of midges captured in either year.

To confirm the plant growth stages susceptible to midge egg-laying, plants were grown to the initial stem elongation (BBCH 31), yellow bud (59), full flower (65) and 30% of pods reach final size (73) stages and placed in the field. Plants in the later development stages (65-73) had more galls than plants at earlier growth stages, supporting evidence that buds need to be present on plants for egg laying. Through the course of this study, we identified *C. brassicola* larvae developing in canola pods. A field experiment with undamaged and mechanically damaged canola pods confirmed eggs can be laid and larvae develop in pods, but the exact mode of entry (e.g. if females can lay directly on undamaged or if pods need to have previous damage) remains uncertain. To determine if other brassicaceous plants can be hosts of *C. brassicola*, a field study was conducted with black mustard (*Brassica nigra*), wild mustard (*Sinapis arvensis*), stinkweed (*Thlaspi arvense*), and canola (*Brassica napus*). No evidence of *C. brassicola* infestation was observed on any of the alternate host plants deployed in the field. Finally, through laboratory studies, there was no difference in the sex ratio of emerging midges (males and females emerge in equal numbers) and on average live for 5 days or less.

Overall, this study has developed a reliable, pheromone-monitoring system that can be used to monitor populations of *C. brassicola* and determined that both buds/flowers and occasionally pods can be infested by *C. brassicola*. Although short-lived, the midge emergence is prolonged over the summer when canola plants are susceptible to infestations, and we urge continued monitoring and vigilance for this species as its full pest potential is yet to be determined.

3. Introduction – Brief project background, rationale, and objectives.

Initial studies on swede midge, *Contarinia nasturtii*, in northeastern Saskatchewan led to the discovery of the canola flower midge, *C. brassicola* (Soroka et al. 2019; Mori et al. 2019). This discovery spurred additional research into the life cycle and biology of *C. brassicola* on the Prairies (Vankosky and Mori, CARP Projects 2017-12 and 2017-13). Adult midges emerge in late-spring, mate and females lay eggs on flower buds; the resulting larvae feed within the flower and prevent pod formation. Midge damage was confirmed to be geographically widespread, but sporadic, from Portage-la-Prairie, MB in the south-east to Sangudo, AB in the north-west. Previously, damage was found on buds and flowers, indicating the timing of adult emergence must coincide with the susceptible crop stage, but the exact stage at which the crop becomes susceptible is not known. Many midges remain at low population levels for years before causing widespread economic damage (e.g. orange blossom wheat midge, *Sitodiplosis mosellana* (Olfert et al. 2008), saddle gall midge, *Haplodiplosis marginata* (Censier et al. 2015), and *C. brassicola* may be no different. To determine the pest status and develop successful monitoring tools for *C. brassicola* more research is needed.

Pheromone monitoring has the potential to improve our understanding of *C. brassicola* biology, phenology and population densities. Sex pheromone are species-specific volatile chemical signals used for mate finding between the sexes (Baker and Heath 2005). They have been successfully used as both monitoring and management tools (Witzgall et al. 2010, Samietz et al. 2012, Hallett and Sears 2013, Mori et al. 2014). Due to the potential threat of *C. brassicola* and its discreet feeding damage which makes it difficult to detect, we (Mori, Vankosky and Bray) identified the pheromone produced by *C. brassicola* females (CARP Project 2017-13). The pheromone blend, comprised of three components (R,S-2,7-diacetoxynonane, R,R-2,7-diacetozynonane, and R-2-acetoxynonane), was highly attractive to males in the field. The ratio and dose of the components were optimized in field studies (Bray et al. 2022), but further work is needed to determine the optimal trapping system and to determine the relationship between the number of males captured on pheromone-baited traps and larval density and damage. The relationship between the number of individuals captured in pheromone traps and the egg or larval stage and crop damage has been determined for several agricultural and forestry pests (e.g. Turnock 1987; Evenden et al. 1995; Hillier et al. 2004). These tools are especially useful when the adults are present before the damaging larval stages, as is the case for *C. brassicola*. However, these relationships have not been explored for *C. brassicola*.

Several factors can affect insect pest pressure. Abiotic conditions including temperature, moisture, and soil type can all effect emergence and population densities. This is especially true for gall midges, including the saddle gall midge (*Haplodiplosis marginata*) (Censier et al. 2015), swede midge (Readshaw 1966; Chen and Shelton 2007), orange blossom wheat midge (Wise and Lamb 2004; Doane and Olfert 2008) and *C. brassicola*, that overwinter in the soil. Without adequate moisture and temperature, emergence can delayed or inhibited altogether. Anecdotal evidence suggests that moisture level is a significant contributing factor to *C. brassicola* emergence, with higher midge populations at sites with higher growing season moisture (Soroka et al. 2019), but more research is needed. Midge synchrony with the crop is also an important factor. Emergence of saddle gall midge during the stem elongation stage of wheat cause more damage than those that emerge at later growth stages (Censier et al. 2015). Wheat is more susceptible to orange blossom wheat midge infestation during advanced heading compared to flowering (Elliot and Mann 1996). Additionally, the distribution of insects within a field can affect damage. The distribution of the saddle gall midge is not even throughout plots except under outbreak conditions. During periods of lower infestations an edge effect (e.g. higher populations at the edge of fields compared to the interior) has been noted (Censier et al. 2015). The distribution of *C. brassicola* throughout individual fields is not known, because sampling conducted thus far focused on field edges and the adjacent 5 m. Thorough knowledge of the distribution throughout fields is needed to predict damage levels and economic losses.

Accurate monitoring is a vital component of integrated pest management (Dent 1991). The project proposed here will address aspects of midge biology and the relationship of midge biology with abiotic factors to further refine the pheromone-based monitoring tool developed in CARP Project 2017-13.

Objective 1: Determine the factors affecting pheromone-baited trap capture of male canola flower midge.

Objectives 2: Elucidate the relationships between the number of midges captured on pheromone traps, larval density and crop damage.

Objective 3: Determine the abiotic conditions that affect midge emergence and population densities.

Objective 4: Investigate the emergence pattern, longevity and oviposition behavior of the canola flower midge.

4. Methods – Include approaches, experimental design, methodology, materials, sites, etc. Major changes from original plan should be cited and the reason(s) for the change should be specified.

Objective 1: Determine the factors effecting pheromone-baited trap capture of male canola flower midge.

Experiments were conducted to determine the effect of trap type, height and location in the field on the capture of male *C. brassicola* in canola fields in Central Alberta near the hamlet of Galahad. In all experiments, traps, baited with a standard *C. brassicola* pheromone lure (1 ug dose), were placed 25 m apart along linear transects at or parallel to the field edge. An additional experiment was added (compared to the original proposal) to investigate the time at which *C. brassicola* males are active and attracted to traps. This experiment was added as it will allow us to determine when (i.e.: time of day) adult midge are active in the field.

Experiment 1 tested the effect of three different trap types (wing, delta, and Jackson traps) which differ in shape and size, but all capture insects on a sticky liner. The experiment was conducted from June 15-July 21, 2021, at five canola fields. Traps were checked and their sticky cards replaced weekly throughout the duration of the experiment. There was a slight variance in the experiment compared to what was originally proposed as diamond traps were dropped from the experiment after consultation with other researchers and agronomists. The design of diamond traps makes them difficult to service and setup and thus would not be ideal for field use by agronomists, crop consultants, farmers, etc.

Experiment 2 tested the effects of height and location of Jackson traps (used based on results of Experiment 1) that were deployed at 3 different heights (0.1, 0.5 and 1 m above the soil surface) and at two different field locations (field edge and 25 m into the field). This experiment was conducted from June 29-July 13, 2021, at 10 canola fields. There was a slight variance in the experiment compared to what was originally proposed as originally traps were also to be deployed by a previous year canola field; however, this was dropped from the experiment after consultation with other researchers and agronomists. Identifying a previous canola field which was in a consistent vicinity to a current year field was difficult and appeared impractical from an applied standpoint as it would be difficult to draw conclusions based on these traps and what would be expected at nearby canola fields.

In 2022 a variation of the trap height experiment was conducted for the duration of the growing season (June 2 - September 8) to investigate the relationship between trap height and crop height on midge capture. Experimental procedures were conserved from the 2021 optimal height experiment. Variations to the previous experiment's procedure included an extended monitoring duration and the exclusion of traps placed 1 m above the soil surface, which were replaced by variable height traps set even with the height of the canola crop canopy each week.

Experiment 3 explored the daily patterns of *C. brassicola* activity by monitoring when adult males were attracted to pheromone baited traps. This information can be used as a proxy to indicate when males and females are actively searching for mates in the field. Two Jackson traps were deployed at each of two field locations and monitored hourly for a 24-hour period on July 27-28, 2021, and July 28-29, 2022.

Objective 2 & 3: Determine the relationship between the number of midges captured on pheromone traps, larval density, and damage in the field (2). Determine the abiotic conditions that effect midge emergence and population densities (3).

Experiment 4 was conducted to elucidate the relationships between the number of midges captured on pheromone traps to larval density and crop damage. A season-long field study was conducted at 12 canola fields from June 15-September 30, 2021. Two pheromone-baited Jackson traps were deployed along the field edge, 25 m apart, 50 cm above the soil surface. Each week the traps were checked and sticky cards replaced. Lures were replaced every 6 weeks. Additionally, 25 plants were collected each week and examined to determine the number galls. Weekly crop growth stage numbers were determined as an average of the growth stage represented by more than 50 percent of crop plants at each field location. Weather data from field locations was recorded by the Forestburg AGCM station and was accessed via the Alberta Climate Information Service website.

We examined the relationship between the number of midge captured in pheromone traps to the number of galls found at each site in several ways using linear regression. First each year, we examined the relationship between the total number of midges captured up to the end of the gall collection (after which little damage could still occur in the crop as midges would only be able to oviposition on tertiary racemes or volunteers which add little to overall yield). We also used a date shift method, by which we determined if the number of midges captured on pheromone traps 1 or 2 week(s) prior to gall collection could be predictive of damage. Due to large variations between years, relationships were examined on a yearly basis and results could not be combined across years. Finally, to determine if weather has an effect on the number of midges captured, we used Spearman's correlation to determine if weekly average temperature or weekly average rainfall impacted the number of midges captured in pheromone-baited traps.

Experiment 5 explored the distribution of *C. brassicola* damage throughout each field location by conducting yearly intra-field damage assessments. During each assessment 24 infield locations were sampled (six samples, 25 m apart, collected at each of four distances within the field's edge: 0 m, 25 m, 50 m and 100 m) by counting the number of flower buds damaged by *C. brassicola* on 25 racemes at each location (150 racemes per sample). Intra-field damage assessments were conducted at twelve canola fields on August 4, 2021 (growth stage 77 BBCH), and at ten canola fields on July 28, 2022 (growth stage 73 BBCH).

Objective 4: Investigate the emergence pattern, longevity, and oviposition behavior of the canola flower midge.

The pattern of emergence, longevity and oviposition behavior of the canola flower midge can all contribute to its pest status. A series of experiments designed to investigate key components of these influential ecological traits were conducted under laboratory and field conditions.

Experiment 6 investigated the characteristics of adult *C. brassicola* emergence from infested tissues. Samples of infested canola flower buds were gathered from multiple sites as soon as galls characteristic of *C. brassicola* were detected. Infested tissues gathered from the field were placed on containers of emergence medium and incubated within growth chambers to facilitate larval development and pupation. Emergence containers were monitored daily to record the rate and timing of adult midge emergence. Characteristics including sex ratio, development, and longevity were recorded in greater detail during periods of adult midge emergence too low for colony establishment.

Experiment 7 tested the effects of environmental conditions and host plant growth stages on female *C. brassicola* oviposition. Newly emerged adult midges were placed within large mesh colony cages containing multiple canola plants at different developmental stages (31, 59, 65, and 72 BBCH) to facilitate oviposition on flower buds. Environmental conditions within colony growth chambers followed schedules replicating daily patterns of humidity, air temperature, and light intensity observed at field sites. Adult midge remained within colony cages for a minimum of 24 hours to allow time for oviposition. After a period of 1-6 days all surviving adult midge within a cage were removed, the number and sex of surviving individuals was recorded, and cages were placed in a greenhouse to continue the development of infested canola plants.

Experiment 8 investigated the suitability of different canola growth stages for *C. brassicola* oviposition in field conditions. Twenty cohorts of four canola (*Brassica napus*) plants were grown within a greenhouse to different growth stages (31, 59, 65, and 72 BBCH) before being transported to field locations. From June 24 to July 13, 2022, cohorts including one plant of each growth stage were deployed 25 m apart along the edge of ten canola fields after being equipped with slow-release water reservoirs. After one week of field exposure plants were collected and placed into an isolated growth chamber where they were monitored for signs of *C. brassicola* infestation over the course of two weeks.

Experiment 9 investigated the suitability of three plant species closely related to canola as potential alternate hosts for *C. brassicola* oviposition in field conditions. Eighteen cohorts of black mustard (*Brassica nigra*), wild mustard (*Sinapis arvensis*), stinkweed (*Thlaspi arvense*), and canola (*Brassica napus*) plants were grown for three weeks within a greenhouse before being deployed at seven field locations from July 14 to August 10, 2022. Plants were transported, equipped, collected, and monitored using the same procedures as other field oviposition experiments.

Experiment 10 investigated the influence of mechanical damage on developing canola seed pods on *C. brassicola* oviposition in field conditions. Twelve cohorts of four canola (*Brassica napus*) plants were grown for seven weeks within a greenhouse before being deployed at six field locations from August 25 to September 7, 2022. During deployment at each site, two randomly selected plants were subjected to artificial damage conditions that simulated the damage caused by a pod-feeding insect pest: sterilized tweezers were used to tear a small (<1 mm) hole in developing pods, simulating damage from a cabbage seed pod weevil (*Ceutorhynchus obstrictus*), and sterilized pins were used to pierce developing pods, simulating damage from a tarnished plant bug (*Lygus sp*.). Flowers and buds were removed from both simulated-damage plants, as well as from a control-damaged specimen, while an additional control plant was left entirely undamaged. Plants were deployed, collected, and monitored using the same procedures as other field oviposition experiments.

5. Results – Present and discuss project results, including data, graphs, models, maps, design, and technology development.

Objective 1: Determine the factors effecting pheromone-baited trap capture of male canola flower midge.

Experiment 1: A generalized linear mixed effects model with a negative binomial distribution was used to determine if the number of midges captured per cm^2 differed with trap type. Site was used as a block (random effect) and week was included as an additional random effect. Trap type significantly affected the number of midges captured ($\chi^2 = 136.8$, df = 2, $p < 0.001$). Significantly more midges were captured per cm^2 with Jackson traps compared to other traps (Figure 1).

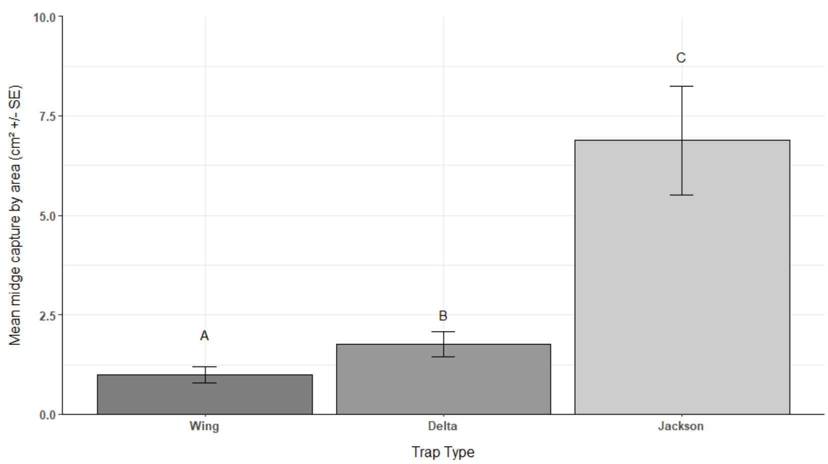

Figure 1: Mean (± SE) number of male *C. brassicola* captured per square centimeter of sticky card on three different trap types (wing, delta and Jackson). Letters indicate significant differences between the trap types (Tukey test, $p < 0.05$).

Experiment 2: A generalized linear mixed effects model with a negative binomial distribution was used to determine if the number of midges captured differed with trap height and location within the field. Site and week of data collection were both used as blocking factors (random effect). There was a significant interaction effect between trap height and trap location on trap capture ($\chi^2 = 7.7$, df = 2, $p < 0.05$). Traps placed along the edge of the field at 10 or 50 cm captured more midges than those at similar heights placed 25 m into the canola crop (Figure 2a). A generalized linear mixed effects model with a negative binomial distribution was also used to determine if the number of midges captured differed with trap height. Site and week of data collection were used as blocking factors (random effect). There was a significant effect of trap height on midge capture ($\chi^2 = 21.0$, df = 2, $p < 0.001$). Traps placed 10 cm above the soil captured significantly fewer midge than those placed at either 50 cm above the soil surface or even with the crop canopy. To determine if the number of midges captured was influenced by trap height and crop canopy height a generalized linear mixed effects model with a negative binomial distribution was used, once again applying site and week as blocking factors (random effects). There was a significant interaction effect between trap height and crop canopy height on trap capture ($\chi^2 = 8.2$, df = 2, $p < 0.02$). However, there was no significant difference between the midge capture of variable height traps set even with the crop canopy and constant height traps set at 50 cm (Figure 2b).

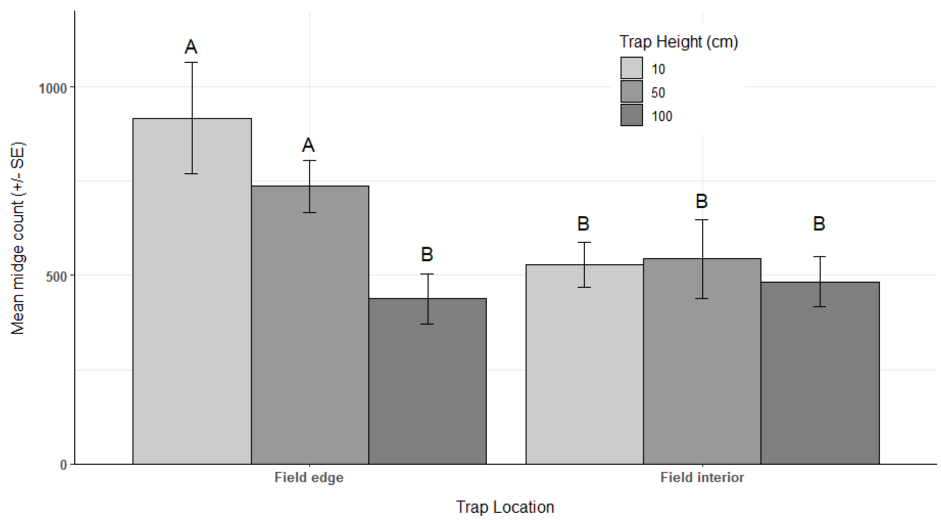

Figure 2a: Mean (± SE) number of male *C. brassicola* captured on Jackson traps deployed at three heights (10, 50 and 100 cm) at two locations (edge of the field and 25 m into the crop). Different letters indicate significant difference between traps (Tukey test, $p < 0.05$). There was a significant height by location interaction.

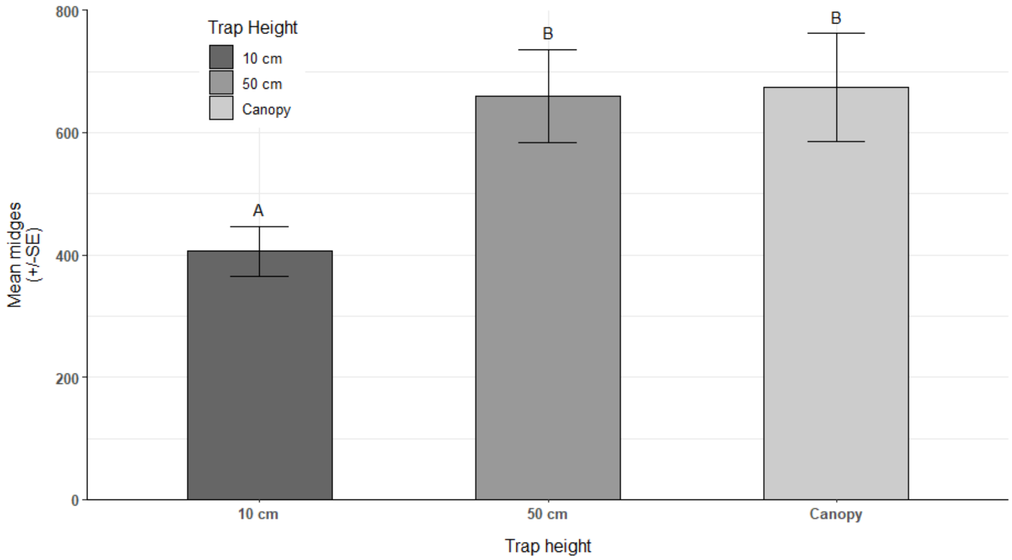

Figure 2b: Mean (± SE) number of male *C. brassicola* captured on Jackson traps deployed at three heights (10 cm, 50 cm, and even with the crop canopy). Different letters indicate significant difference between traps (Tukey test, $p < 0.05$).

Experiment 3: Hourly observations over a 24-hour period in 2021 and 2022 indicate similar patterns of daily male midge activity. Two periods of peak activity were recorded during approximately the same hours in samples from both years; a short period of activity occurred in the morning, lasting from 7 to 11 am and peaking at 9 am, followed by a prolonged period of activity in the evening that lasted from 3 to 10 pm. During the second period of activity two distinct peaks in male midge activity were recorded in 2021, at both 5 pm and 7 pm, a feature that was not observed during the evening interval in 2022 in which a single peak in activity was recorded at 7 pm. Little to no midge activity was recorded outside of these periods in either year (Figure 3).

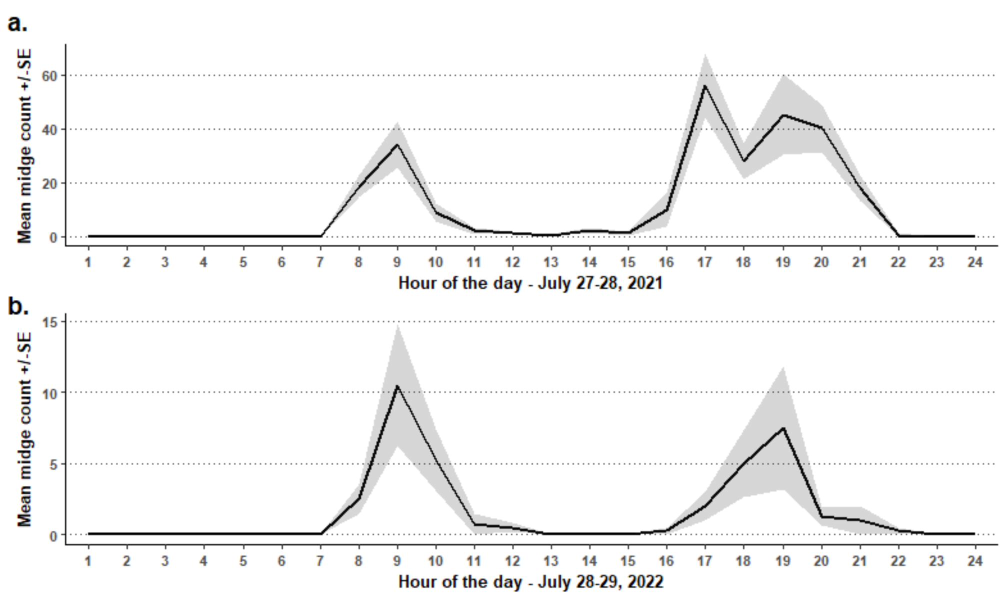

Figure 3: Mean number of male *C. brassicola* captured on Jackson traps each hour over 24-hour periods on July 27-28, 2021, and July 28-29, 2022.

Objective 2 & 3: Determine the relationship between the number of midges captured on pheromone traps, larval density, and damage in the field (2). Determine the abiotic conditions that effect midge emergence and population densities (3).

Experiment 4: Samples were obtained weekly from all sites throughout both growing seasons (June 15 – September 20, 2021; May 25 – September 8, 2022). The first emergence of adult *C. brassicola* males could not be determined in 2021, as adult midges were already present during the first week of sampling (June 15-21, 2020; GS 16) but was recorded in 2022 during the week of June 9-15 (GS 12). Fifty percent of the total cumulative midge capture each season was reached during July 13-19, 2021, (GS 64) and July 14-20, 2022 (GS 65). Peak flight occurred during July 21-27, 2021, (GS 65) and July 14-20, 2022, (GS 65). While the first infested canola flower buds observed in 2021 were identified on July 6, (4 galls; GS 61) it is relevant to note that a sharp increase in infested tissues occurred on July 21, 2021, (336 galls; GS 65). The first infested tissues observed in 2022 were identified on July 14 (431 galls; GS 65). Harvesting of mature canola at field locations began during August 30 – September 6, 2021, and August 25–31, 2022. The last adult *C. brassicola* males were captured during September 20-26, 2021, and September 8-14, 2022 (Figure 4 & 5).

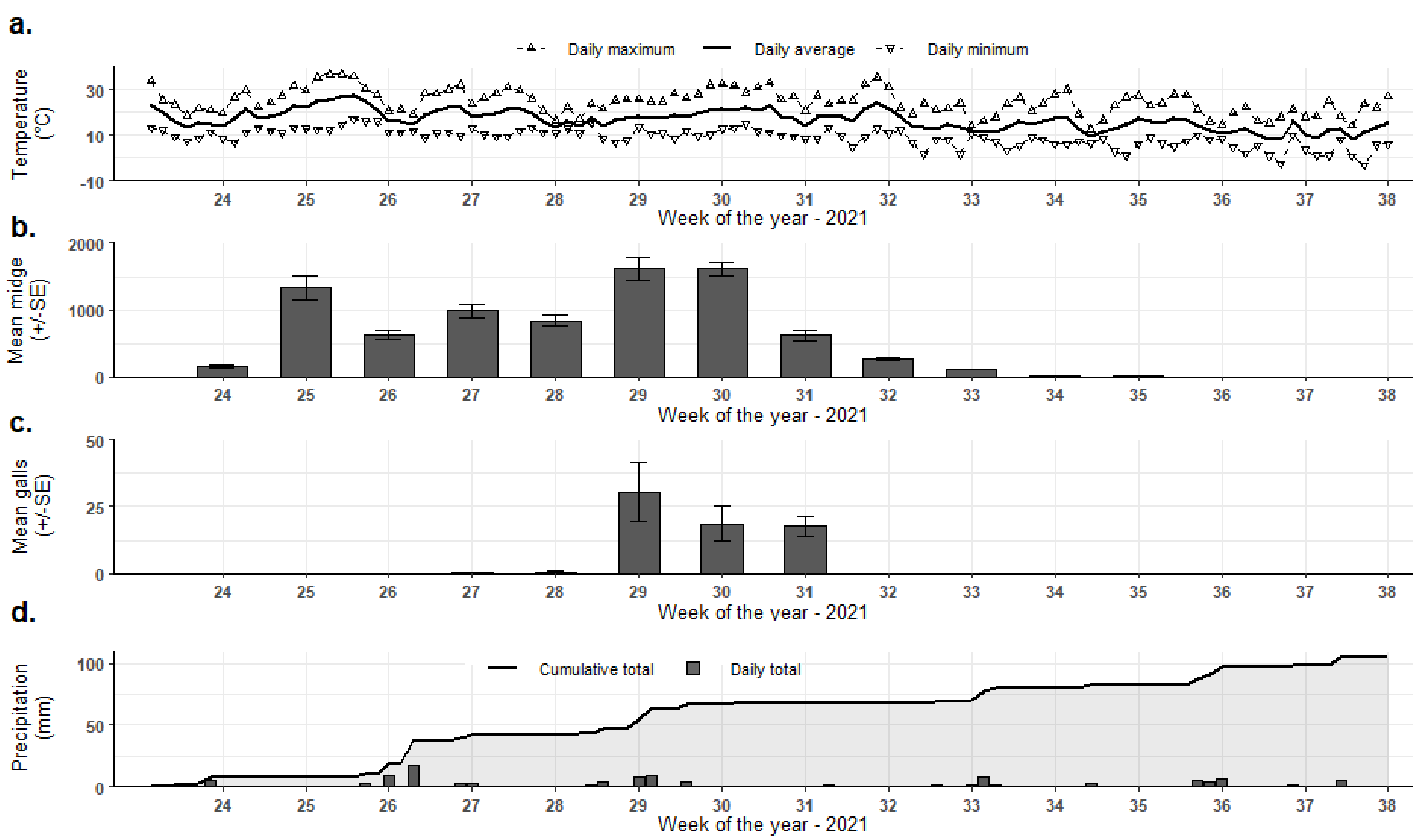

Figure 4: Comparison of environmental conditions and *C. brassicola* activity during the 2021 growing season. Measurements of daily air temperature and precipitation in figures (a.) and (d.) were collected from a monitoring station < 2 km from the nearest field location. Figures (b.) and (c.) respectively indicate the mean weekly counts of male *C. brassicola* (captured by two pheromone-baited Jackson traps) and infested canola flower buds (from 25 plant samples) between twelve sampling locations (± SE).

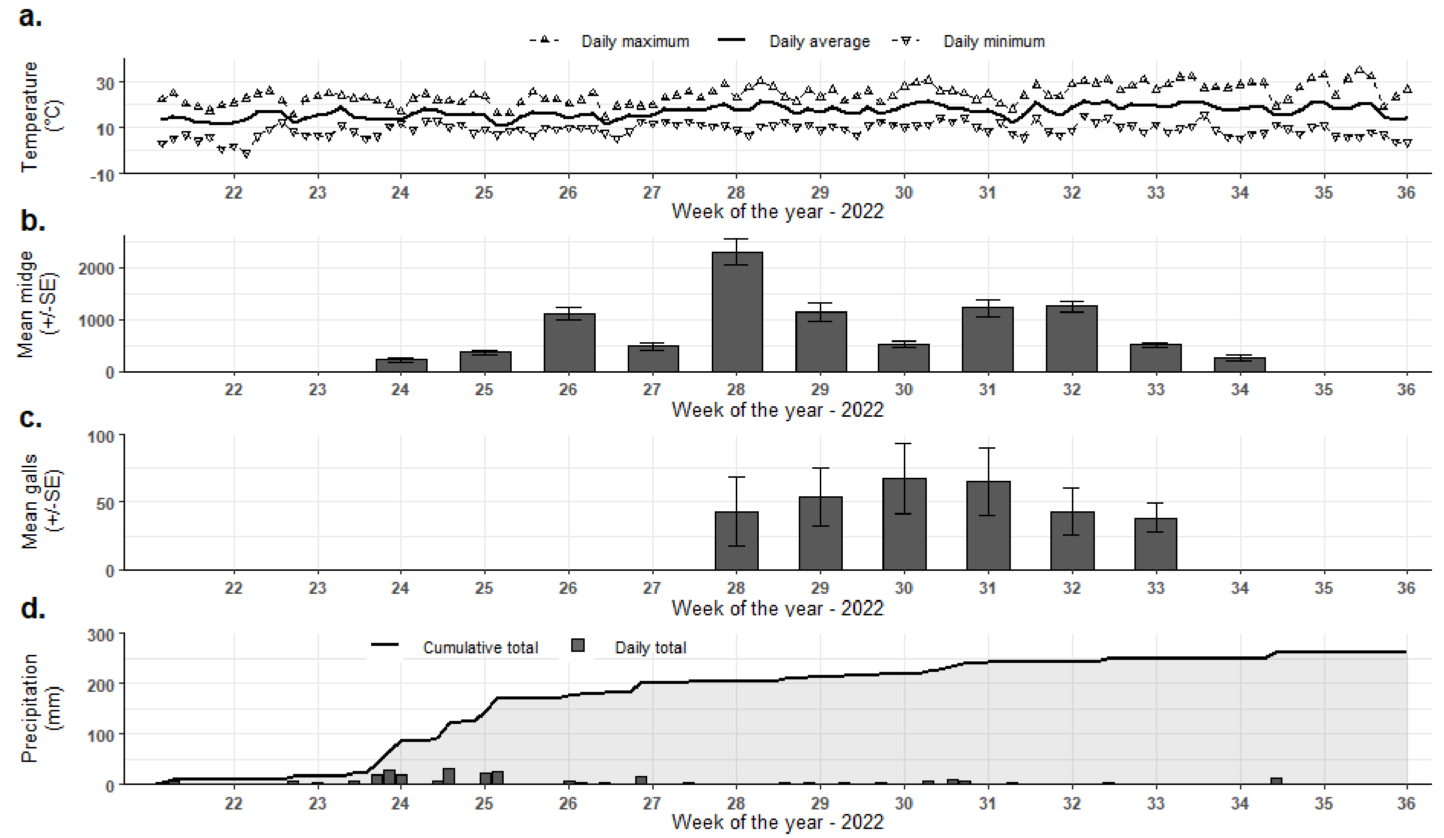

Figure 5: Comparison of environmental conditions and *C. brassicola* activity during the 2022 growing season. Measurements of daily air temperature and precipitation in figures (a.) and (d.) were collected from a monitoring station < 2 km from the nearest field location. Figures (b.) and (c.) respectively indicate the mean weekly counts of male *C. brassicola* (captured by two pheromone-baited Jackson traps) and infested canola flower buds (from 25 plant samples) between twelve sampling locations (± SE).

Due to significant year to year variation in trap capture and galls, no model was significant when years were combined. Several (2021, n = 16, 2022, n = 21) regression models were run on the data from 2021 and 2022. No models were significant in 2021, indicating the number of midge captured on pheromone-baited traps was not predictive of the weekly gall counts or the season total gall count ($F_{1,10}$ = 0.003-4.13, p = 0.07-0.98, across all models tested). In 2022, the only significant model compared the season-long total number of midges (up to the last gall collection date) to the total numbers of galls collected ($Galls = 0.036(midge) - 360.07$, $R^2 = 0.43$, $F_{1,8} = 6.02$, $p = 0.039$). Although this model based on season long totals was significant, biologically it is not helpful to predict damage throughout the season. In addition, when meteorological data (mean weekly temperature, and weekly cumulative precipitation) was examined to determine if there is a relationship between these variables and the number of moths captured, no significant models were found ($p > 0.05$, in all models tested). This was surprising as many cecidomyid midges are reliant on rainfall events and adequate soil moisture for emergence. However, with *C. brassicola*, even when Alberta was experiencing one of the most significant droughts in the previous two decades in 2021 significant numbers of midge emerged, and furthermore, significant populations were observed in 2022 indicating that the drought in 2021 did not have a large negative impact on the midge.

Experiment 5: A generalized linear mixed effects model with a negative binomial distribution was used to determine if the number of damaged flowers (per 150 racemes) differed with distance into the field. Site was used as a block (random effect). There was a significant difference in the number of damaged flowers based on distance into the field ($\chi^2 = 27.42$, df = 3, $p < 0.0001$). There is a clear edge effect in which plants located closer to the edge of a field have significantly higher numbers of flowers infested by *C. brassicola* (Figure 6).

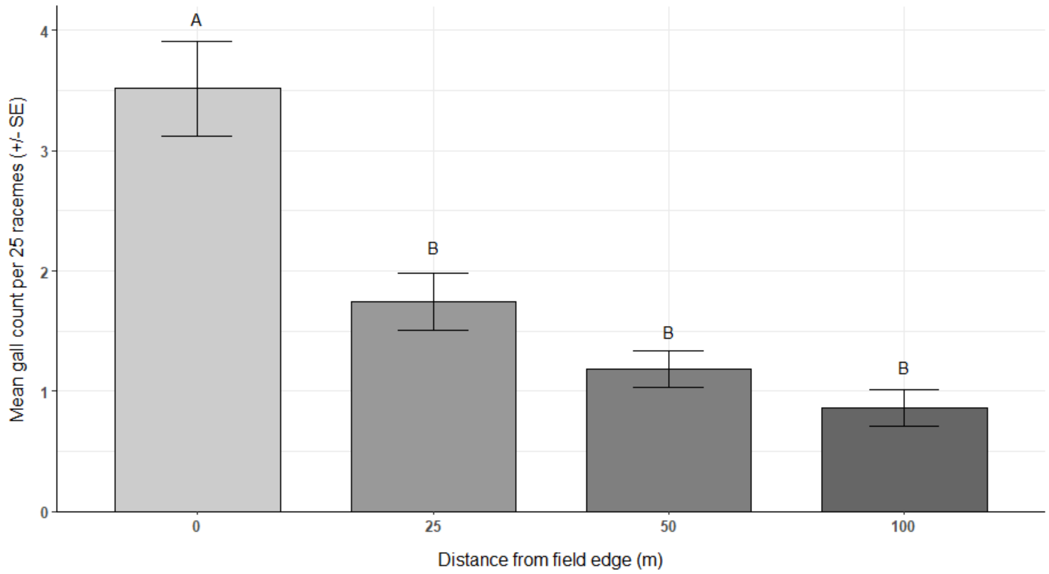

Figure 6: Mean number of damaged flowers (± SE) identified along four transects sampled at a distance of 0, 25, 50, and 100 m towards the field interior. Different letters indicate significant difference between traps (Tukey test, p < 0.05).

Objective 4: Investigate the emergence pattern, longevity, and oviposition behavior of the canola flower midge.

Experiment 6: Observations of adult *C. brassicola* emergence indicated a higher percentage of female midge emerging compared to males. There was no substantial difference between the total numbers of male and female midges that emerged from field collected samples (162 vs 179, average of 14.1 (+/- 5.3) and 16.3 (+/- 4.9), respectively).

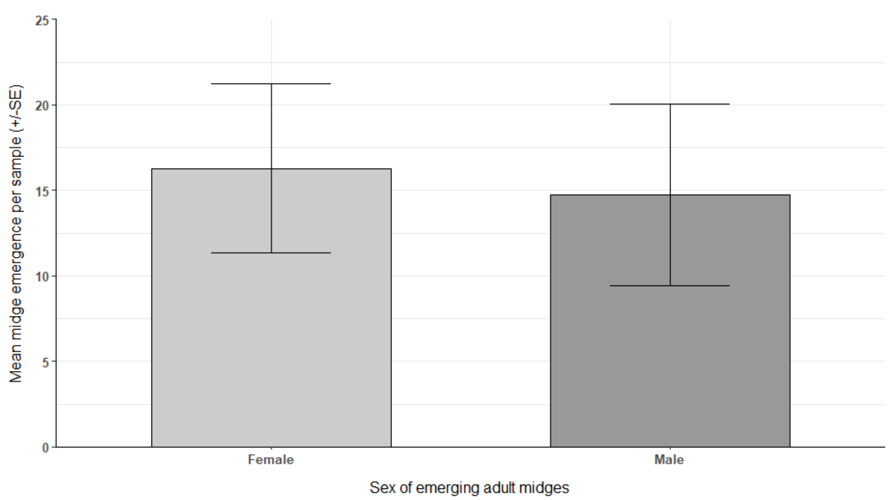

Figure 7: Mean number of male and female *C. brassicola* (± SE) emerging from infested canola flower buds gathered from field locations.

Experiment 7: Attempts to stimulate female oviposition on canola flower buds in vitro did not meet with success. In total, sixteen colonies were attempted containing 122 host plants stocked with 7420 adult *C. brassicola* (<48 hours post emergence from infested plant material). These colonies produced a total of fourteen (14) infested canola flower buds, together yielding 134 larvae. It is unclear what component(s) of *C. brassicola* reproduction are being inhibited within laboratory conditions. As a result of the inability to establish optimal environmental conditions for this species, the longevity of *C. brassicola* adults is not yet well defined.

Due to the continuous emergence of *C. brassicola* adults from field-collected plant samples, each colony cage was stocked several times with groups of midges that had emerged during the same period. Once a colony cage was stocked with its last group of newly emerged adult midges it was left for a period of 1-6 days. After this period all adult midge still alive were removed from the colony, sexed, and stored. Of the 7420 adult midges added to colony cages only 273 midges (3.68%) survived until collection.

Of the 273 adult *C. brassicola* that survived until their eventual collection, 250 individuals (91.3%) were alive for at least three days, 87 individuals (31.9%) were alive for four days, and only one individual (0.4%) survived for at least six days (Figure 8). No midges were observed to have survived for longer than six days within tested conditions. More female than male midges survived until collection in every colony sample taken. Overall, 202 (74%) of the surviving adult midges were female and 71 (26%) were male (Figure 8). This indicates that in the laboratory most midges only lived for 5 days or less.

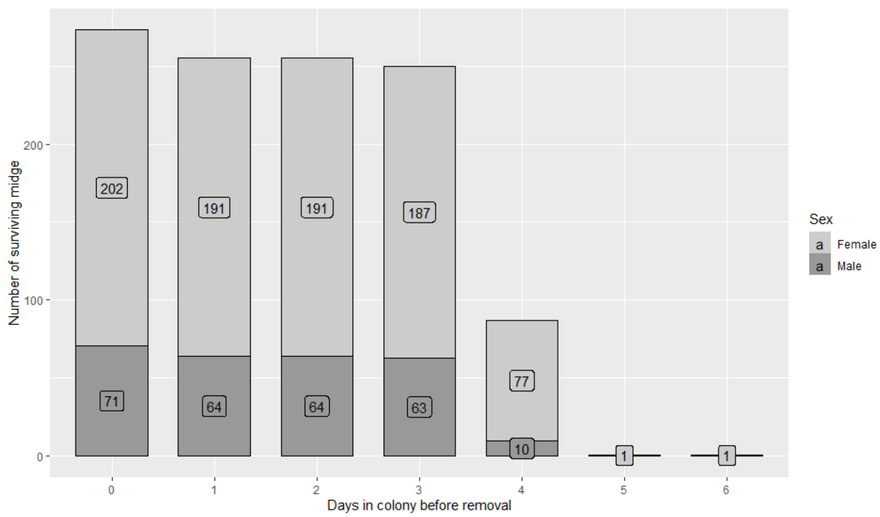

Figure 8: Number of adult *C. brassicola* of each sex that survived within colony cages until live collection, grouped by the number of days after the last midge was added to that colony. Day 0 represents all individuals collected alive.

Experiment 8: Canola plants at later growth stages (six and seven weeks of development, approximately GS 65 to GS 73) were observed to have more flower buds infested by *C. brassicola* than those at earlier growth stages (four and five weeks of development, approximately GS 31 to GS 59) when deployed at the edge of canola fields (Figure 9). Further experimentation and analysis will be necessary to determine whether these results suggest a preference in *C. brassicola* oviposition towards more mature plants.

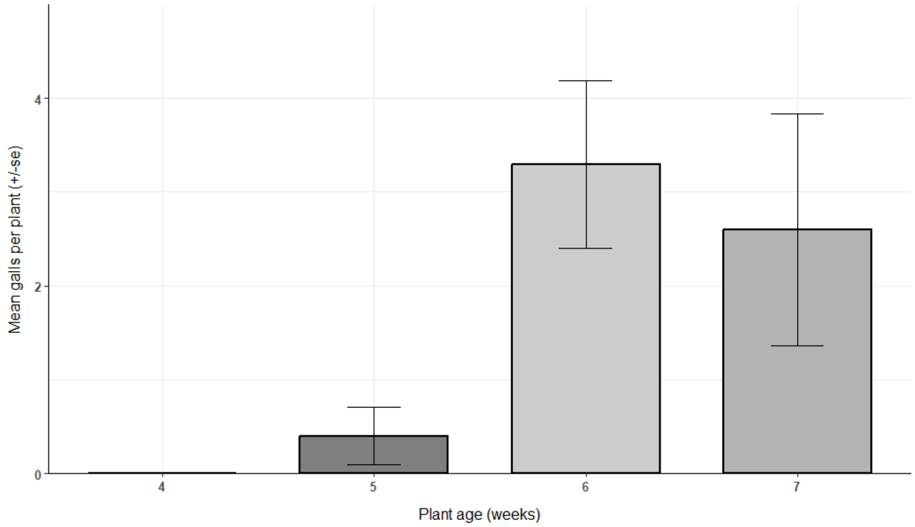

Figure 9: Mean number of *C. brassicola* galls (± SE) on canola plants at various stages of growth. Canola (*Brassica napus*) specimens four to seven weeks of age were deployed at field edges to approximate crop plants at specific growth stages (GS 31, 59, 65, and 73 BBCH, sequentially).

Experiment 9: No evidence of *C. brassicola* infestation was observed on any of the alternate host plants deployed at field locations. Oviposition by *C. brassicola* at the same time and location was confirmed for five out of 18 replicates by infested canola control specimens.

Experiment 10: Canola pod infestation by *C. brassicola* was observed in three of the 48 canola plants deployed at field edges. The three infested plants were all from different damage conditions (no damage, simulated Lygus damage, simulated weevil damage) and each had several infested pods (3, 6, and 15, respectively). This confirms other field observations by the current Researchers and collaborators who recently identified C. brassicola in pods in southern (near Strathmore) and central Alberta (near Forestburg), as well as in Saskatchewan (James Tansey, Personal Communication). Further studies will be necessary to understand the conditions that influence this novel infestation mechanism.

6. Conclusions and Recommendations – Highlight significant conclusions based on the discussion and analysis provided in the previous section with emphasis on the project objectives specified above; also provide recommendations for the application and adoption of the project results and identify any further research, development, and communication needs, if applicable.

Pheromone-baited Jackson traps, deployed 50 cm above the soil surface along the crop edge, were identified as the optimal combination of trap type and position to effectively monitor *C. brassicola*. Two daily peaks in male midge activity were observed, during the morning and late-afternoon, that could be used to increase the precision of monitoring strategies or potential treatment applications. This optimized trapping system can be used to develop monitoring protocols for *C. brassicola*. Pheromone lures are available through a commercial company, Chemtica International, for those interested in monitoring for *C. brassicola*.

Based on the data collected over the two years of this study the number of male *C. brassicola* captured on pheromone-baited traps was not predictive of damage in the field, but in 1 of 2 years there was a significant relationship between the total number of midges captured and the total number of galls sampled over the season. Meteorological conditions, at the scale measured in this study, did not have an impact on the number of midges captured in either year. We now know there is a significant damage edge effect, which will be useful information when designing future management strategies and, if warranted, management could potentially focus on the field edges. Daily patterns of male midge activity have been established, allowing for more precise timing of monitoring and potential treatment practices (it should be noted that no studies have determined the yield impact of this species in a controlled manner).

Emerging adult *C. brassicola* midges do not demonstrate a significant sex ratio towards either male or female emergence, but a higher proportion of those able to live for more than one day are female rather than male. It appears in the laboratory midges live for 5 days or less. The environmental conditions and plant host characteristics optimal for *C. brassicola* oviposition are still not well understood. No alternative hosts were infested over the course of our study; however, we were able to confirm that under certain conditions *C. brassicola* are capable of ovipositing and larvae develop in pods. Efforts to characterize the life history of *C. brassicola* under field and laboratory conditions have reaffirmed the distinctiveness of this cryptic species.

We urge continued monitoring and vigilance for this species as its full pest potential is yet to be determined. Dr. Mori is planning to collaborate with researchers in other parts of Canada and the world to determine if *C. brassicola* is a native or invasive species. Pheromone traps deployed in southern Sweden in summer 2022 did not capture *C. brassicola*, but further studies are needed. A controlled insecticide trial to determine the yield loss caused by *C. brassicola* is warranted. Overall, this study has developed a reliable pheromone-monitoring tool for C. brassicola and has furthered our understanding of this species' life history, which is critical as we continue to understand its potential as an agricultural pest.

7. Extension and communication activities: (e.g. extension meetings, extension publications, peer-reviewed publications, conference presentations, photos, etc).

Presentations:

1) Van Camp, K; Mori BA (2021) Characterizing midge emergence: An investigation of patterns within *Contarinia brassicola*. ALES Graduate Research Symposium. University of Alberta, Edmonton, AB.
2) Mori, B (2021) Insects: Past, Present, Future. Nutrien Manager of Agronomic Solutions Meeting, Edmonton, AB.
3) Mori BA; Vankosky M (2021) The canola flower midge, *Contarinia brassicola*. Saskatchewan Ministry of Agriculture Webinar.
4) Van Camp, K; Mori BA (2022) Scent-sational snares: Optimizing and evaluating pheromone-baited traps as tools for monitoring *Contarinia brassicola* on the Canadian prairies. Alberta Canola Research Update, Edmonton, AB.
5) Van Camp, K; Mori BA (2022) Scent-sational snares: Optimizing and evaluating pheromone-baited traps as tools for monitoring *Contarinia brassicola* on the Canadian prairies. ESA, ESC, and ESBC Joint Annual Meeting. Vancouver Convention Centre, Vancouver, BC.

8. Acknowledgements – Include actions taken to acknowledge support by the Funders.

All funders were acknowledged during each presentation. Canola Agronomic Research Program (CARP) provided financial assistance toward the Research Project including Alberta Canola, SaskCanola, Manitoba Canola Growers Association, and Western Grains Research Foundation.

9. Literature Cited

Baker, T. C., and J. J. Heath. 2005. Pheromones: Function and use in insect control, pp. 407-459. In L. I. Gilbert, K. Iatrou, and S. Gill (eds.), Comprehensive Molecular Insect Science, vol. 6. Elsevier Publishers, Amsterdam, The Netherlands.

Bray, D. P., Hall, D. R., Harte, S. J., Farman, D. I., Vankosky, M. A., & Mori, B. A. 2022. Components of the female sex pheromone of the newly-described canola flower midge, Contarinia brassicola. Journal of Chemical Ecology, 48(5-6), 479-490.

Censier, F., M. De Proft, and B. Bodson. 2015. The saddle gall midge, *Haplodiplosis marginata* (von Roser) (Diptera: Cecidomyiidae): Population dynamics and integrated management. Crop Protection 78: 137-145.

Chen, M., and A. M. Shelton. 2007. Impact of soil type, moisture, and depth on swede midge (Diptera: Cecidomyiidae) pupation and emergence. Environmental Entomology 36: 1349-1355.

Dent, D. 1991. Insect Pest Management. CAB International, Wallingford, UK.

Doane, J. F., and O. Olfert. 2008. Seasonal development of wheat midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae), in Saskatchewan, Canada. Crop Protection 27: 951-958.

Elliott, R. H., and L. W. Mann. 1996. Susceptibility of red spring wheat, *Triticum aestivum* L. Cv. Katepwa, during heading and anthesis to damage by wheat midge, *Sitodiplosis mosellana* (Gehin) (Diptera: Cecidomyiidae). The Canadian Entomologist 128: 367-375.

Evenden, M.L., J.H. Borden, and G.A. Van Sickle. 1995. Predictive capabilities of a pheromone-based monitoring system for western hemlock looper (Lepidoptera: Geometridae). Environmental Entomology 24: 933–943.

Hallett, R. H., and M. K. Sears. 2013. Pheromone-based action thresholds for control of the swede midge, Contarinia nasturtii (Diptera: Cecidomyiidae), and residual insecticide efficacy in cole crops. J Econ Entomol 106: 267-276.

Mori, B. A., C. Yoder, J. Otani, and M. L. Evenden. 2014. Relationships among male *Coleophora deauratella* (Lepidoptera: Coleophoridae) pheromone-baited trap capture, larval abundance, damage and flight phenology. Agricultural and Forest Entomology 16: 207-215.

Mori, B. A., L. Andreassen, J. D. Heal, J. R. Dupuis, J. J. Soroka, and B. J. Sinclair. 2019. A new species of *Contarinia* Rondani (Diptera: Cecidomyiidae) that induces flower galls on canola (Brassicaceae) in the Canadian prairies. The Canadian Entomologist 151: 131-148.

Olfert, O., R. H. Elliott, and S. Hartley. 2008. Non-native insects in agriculture: strategies to manage the economic and environmental impact of wheat midge, *Sitodiplosis mosellana*, in Saskatchewan. Biological Invasions 11: 127-133.

Readshaw, J. 1966. The ecology of the swede midge, *Contarinia nasturtii* (Kieff.)(Diptera, Cecidomyiidae). I.—Life-history and influence of temperature and moisture on development. Bulletin of Entomological Research 56: 685-700.

Samietz, J., R. Baur, and Y. Hillbur. 2012. Potential of synthetic sex pheromone blend for mating disruption of the swede midge, *Contarinia nasturtii*. Journal of Chemical Ecology 38: 1171-1177.

Soroka, J., L. Grenkow, B. Mori, and L. Andreassen. 2019. Injury by *Contarinia* (Diptera: Cecidomyiidae) to early-seeded and late-seeded canola in northeastern Saskatchewan, Canada, and assessment of seed treatments for midge control. The Canadian Entomologist 151: 219-235.

Turnock, W.J. 1987. Predicting larval abundance of the bertha armyworm, *Mamestra configurata* Wlk., in Manitoba from catches of the male moths in sex attractant traps. The Canadian Entomologist 119: 167–178.

Wise, I. L., and R. J. Lamb. 2004. Diapause and emergence of *Sitodiplosis mosellana* (Diptera: Cecidomyiidae) and its parasitoid *Macroglenes penetrans* (Hymenoptera: Pteromalidae). The Canadian Entomologist 136: 77-90.

Witzgall, P., P. Kirsch, and A. Cork. 2010. Sex pheromones and their impact on pest management. Journal of Chemical Ecology 36: 80-100.

10. Other Administrative Aspects: HQP personnel (PhD and/or MSc students) trained and involved; equipment bought; project materials developed

HQP: Kyle Van Camp (PhD student), as well as Nicholas Boyce, Rayven Landry-Doran, Carina Lopez, and Jose Correa Ramos (Undergraduate Summer Research Assistants).

Materials: Pheromone lures can be purchased through Chemtica International.

11. Appendices - If necessary, include any materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications.

12. Financial (to be provided to each Funding Agency (at the addresses indicated in 11.2)

a. Comprehensive Financial Statement that summarizes the total income and expenditures to date attributable to the Funders' Funding.
b. Explanation of variances from budget which are greater than 10%.
c. An invoice for each Funding Agency

13. Final Report Posting Do you consent to a version of this Final Report (with sensitive information removed) to be posted on the funder's website?	☐ Yes - this version can be posted THIS VERSION IS FINE TO BE POSTED ☐ Yes - a modified version will be sent ☐ No
14. Research Abstract Posting Do you consent to the 2-3 Research Abstract submitted with this Final Report to be posted on the funders and the Canola Council of Canada's website?	☐ Yes YES ☐ No

Please send an electronic copy of this completed document to:

Ellen McNabb
Research Administrator
Canola Council of Canada
400 – 167 Lombard Ave.
Winnipeg, MB R3B 0T6
Phone: (204) 982-2110
Fax: (204) 942-1841
E-Mail: mcnabbe@canolacouncil.org