FINAL PROJECT REPORT
Canola Agronomic Research Program (CARP)

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. The report should also provide an update on the status of the Project including forecasted date of completion. A complete statement of expenses should be included. In the event major changes are anticipated within the budget supporting notes along with a proposed budget should also be included. The report should also capture a complete summary of activity for the year.

Project Title: Assessing the impact of *Contarinia* sp. on canola production across the Prairies

Research Team Information

Lead Researcher:		
Name	***Institution***	***Project Role***
Meghan Vankosky	Agriculture and Agri-Food Canada, Saskatoon RDC	Co-PI
Boyd Mori	University of Alberta, formerly AAFC-Saskatoon RDC	Co-PI
Research Team Members (add rows as required)		
Name	***Institution***	***Project Role***
Owen Olfert	AAFC-Saskatoon RDC	Survey design expert; insect ecology and population dynamics
Martin Erlandson	AAFC-Saskatoon RDC	Insect population genetics
Dwayne Hegedus	AAFC-Saskatoon RDC	Insect molecular biology
Scott Meers	Alberta Agriculture and Forestry	Field crop entomology
Scott Hartley/James Tansey	Sask. Ministry of Agriculture	Field crop entomology
John Gavloski	Manitoba Agriculture	Field crop entomology
Tim Haye	CABI-Switzerland	Insect biological control

Project Start Date: 01 April 2017 **Project Completion Date:** 31 March 2020

Reporting Period: 01 April 2019 to 31 March 2020

CARP Project Number: 2017.12

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 to the Final Project Report, a one page Research Abstract including rationale, objective, methodology, summary and conclusions (with a summary graph/table or supporting image for the project), acknowledgement and references is due upon completion. The Research Abstract is intended for use in publications such as the *Canola Digest* and the CCC Research Hub and is intended to support messaging to all audiences.

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

1. Date of Completion:

31 March 2020

2. Status of Activity: (please check one)

_____ Ahead of Schedule _____ On Schedule _____ Behind Schedule __x___ Completed

Comment: Work to meet all project objectives progressed as expected and information regarding all objectives is provided below. Due to the timing of this Final Report, some analyses for the project are ongoing, however, all of the data required to conduct these analyses has been collected (i.e., no further field work is required).

3. Completed actions, deliverables and results; any major issues or variance between planned and actual activities.

In 2016, a new species of midge was reported in Saskatchewan for the first time. The new species was formally described by Mori *et al.* (2019) and is named *Contarinia brassicola*. It is informally known as 'canola flower midge' as the adult midge lay eggs on canola flower buds and the larvae develop inside the developing flowers. The overarching objective of this project was to learn more about the biology, distribution, and potential economic impact of canola flower midge.

Objective 1: To determine the distribution of canola flower midge and swede midge on the Prairies and produce distribution maps that can be used by producers to evaluate risks in their area.
In 2017, 2018, and 2019, a network of pheromone traps for swede midge (*Contarinia nasturtii*) was used to determine if swede midge have invaded Manitoba, Saskatchewan, and Alberta. Between 30 and 60 traps were deployed by scientists, agronomists, and growers across the three provinces in all three years of the project, following the standardized protocol developed in Ontario. No swede midge were detected in any year of the project, and no symptoms of swede midge damage were detected during the driving survey for canola flower midge. Thus there is no indication that swede midge are present in Alberta, Saskatchewan, or Manitoba. The Prairie Pest Monitoring Network will continue to deploy pheromone traps for swede midge for the next few years as an early warning system to detect incursions of this serious pest of canola and other brassica crops and vegetables.

To determine the distribution of canola flower midge, a driving survey was conducted in all three years of the project. Canola fields were randomly selected along transects running north to south, with two fields per rural municipality (RM) in Saskatchewan and the equivalent number of fields selected in Alberta and Manitoba. At each field, 10 racemes at 10 locations along the field edge were randomly selected and examined for canola flower midge damage (n=100 racemes per field). In Alberta in 2019, 25 racemes were examined for damage at 10 locations in each field (n=250 racemes). The area surveyed in each year varied slightly, based on available personnel and results from the previous year's survey. In 2019, for example, the survey effort in Saskatchewan focused on the north eastern part of the province, where population densities were greatest in 2017 and 2018, in order to determine if changes in population density occurred between years. In 2019, we also dedicated more time to sampling in southern Manitoba. Between 2017 and 2019, 703 fields were surveyed for canola flower midge. Figure 1 illustrates the distribution of canola flower midge based on sampling in all three years. Figure 2 illustrates the sampling effort in 2019. The canola flower midge is distributed across the prairies, although it occurs in quite low densities across the majority of its range. It has been detected as far north as the Alberta Peace River Region. In Alberta and Saskatchewan, the southern limits of its distribution occur around Red Deer and Rosetown/Yorkton. In Manitoba, canola flower midge were detected in Portage la Prairie in 2018 and south of Brandon in 2019. One factor that may affect midge distribution is soil type. Canola flower midge distribution appears to closely align with the dark grey, black, and dark brown soil zones in western Canada.

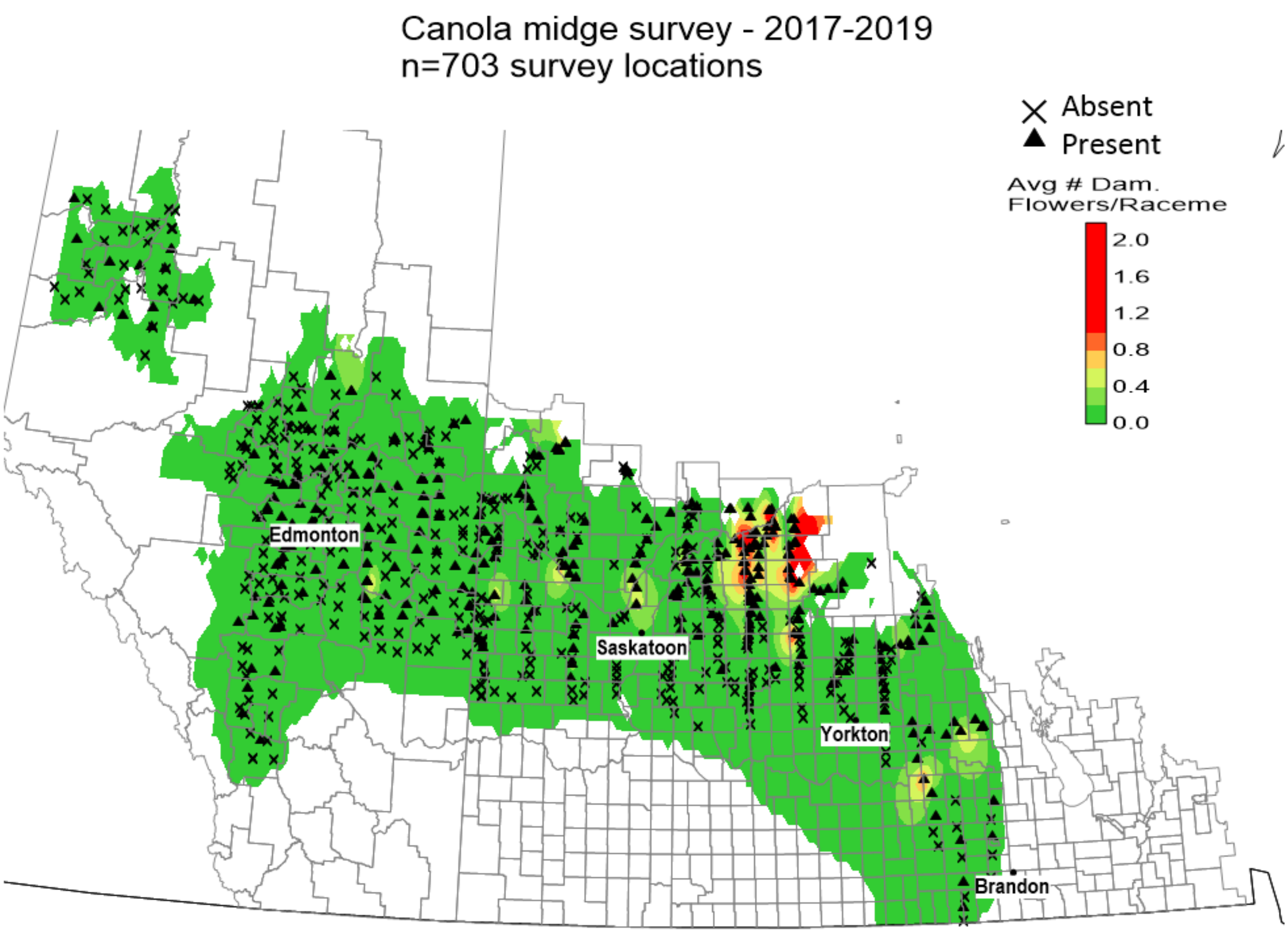

Figure 1. The distribution of canola flower midge across western Canada based on data collected in 2017, 2018, and 2019.

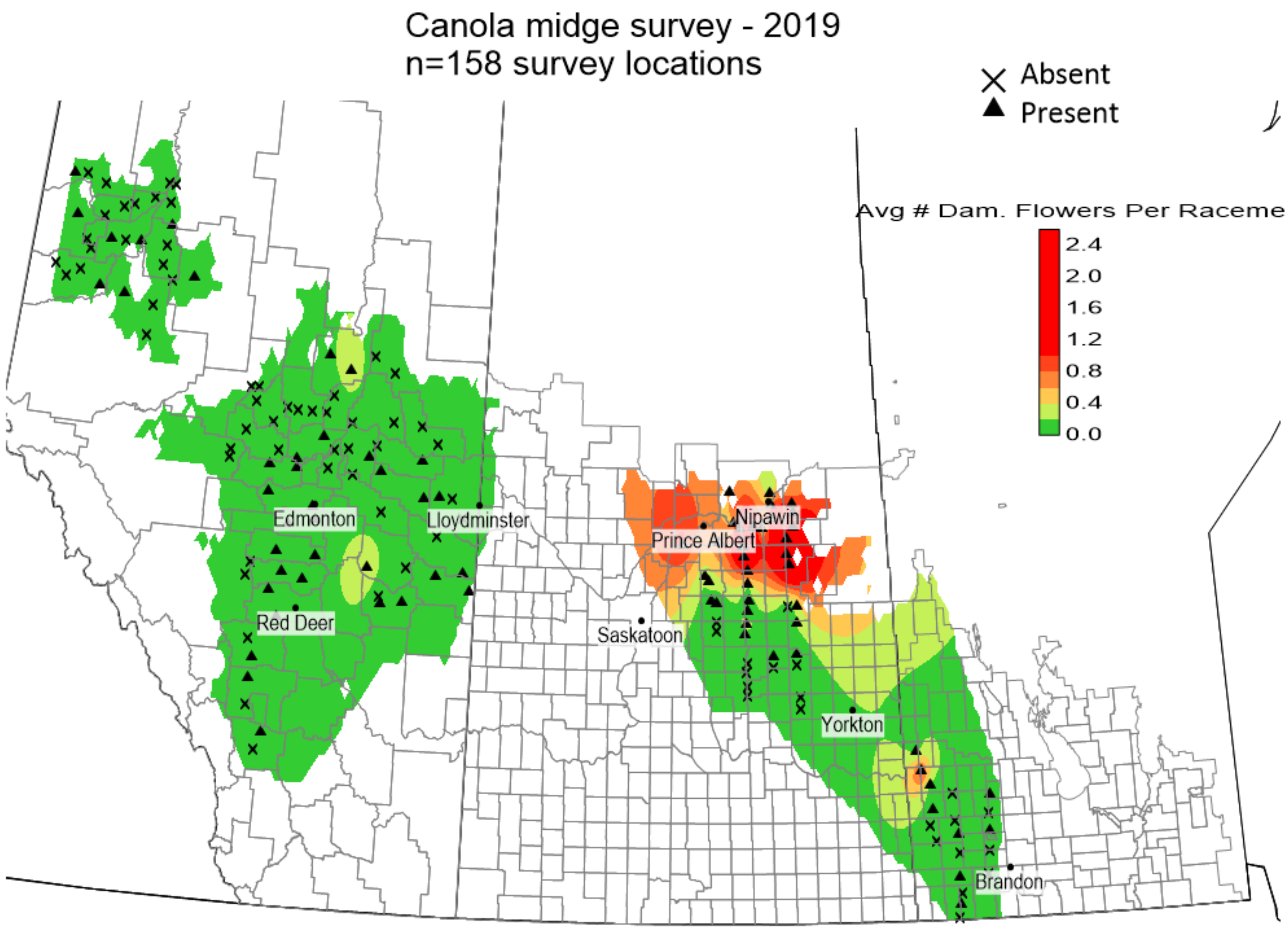

Figure 2. The distribution of canola flower midge based on sampling effort in 2019.

Objective 2. To understand the life history and phenology of canola flower midge and its impact on canola yield.
Plant samples were collected from five locations in northeastern Saskatchewan in all three years of the project during the growing season. Each week, 25 plants were collected from each site and returned to the lab, where they were carefully examined for eggs and larvae of canola flower midge. The location of eggs and larvae was recorded, as was the location where eggs and larvae were found on each plant.

In 2019, the first eggs were detected on canola plants the week of 04 July. The presence of eggs on the plants was quite variable between plants at each site, and between sites. However, there appears to have been two peaks of egg production, with the first occurring in the middle two weeks of July (Table 1) and a second peak in early August (week of 07 August) (Table 1). On the week of 11 July we counted 674 eggs on 38 of the 117 plants sampled at five sites. The number of larvae detected on individual plants during the 2019 growing season ranged from 0 to 234. During the week of 11 July and 24 July, we counted 2475 and 2999 larvae, respectively, on approximately 120 plants collected from five sites. The number of larvae per plant (calculated based on the number of plants with larvae detected; Table 1) was greater than 15 for four consecutive weeks in July, starting the week of 11 July. In August, larval numbers decreased.

We used cages, open to the soil surface, installed early in the growing season to detect periods of adult emergence. These cages were located in current year canola fields and in fields where canola was grown the previous year. In 2019, some emergence cages were located in fields where canola had been grown in two consecutive years. In all years of the project, two peaks of flight activity were detected inside emergence cages (Figure 3). This suggests that there are two generations of canola flower midge adults per year. Interestingly, more adult midge emerged from cages where canola was growing compared to cages where canola was grown the previous year. It is possible that canola flower midge pupae in the soil detect cues from their host plant to stimulate adult emergence in order to ensure that midge emergence coincides with the presence of a suitable host. Additional research is required to test this hypothesis.

All eggs found on canola plants at all field sites in all years of the project were found on canola buds, very close to canola buds, or tucked inside opening canola flowers (Figure 4). All larvae found on canola plants were also inside developing canola flowers (Figure 5) and their feeding resulted in galled flowers that did not produce pods. Estimating the impact of canola flower midge on canola yield in the field proved to be more difficult than anticipated. In 2019, we collected 3000 canola racemes in mid to late August from five sites to estimate the number of pods per raceme that would produce harvestable yield and then dissected 2800 pods from those racemes to determine the mean number of seeds per pod. On average, there were 11 pods with harvestable seeds per canola raceme that produced approximately 26 seeds each. The most damage observed in canola fields during the driving survey was 2.38 flowers per raceme, which would equate to a loss of approximately 2 pods per raceme (18.2% of yield per raceme) in the field, assuming equal distribution of midge larvae across the entire field. In the majority of fields surveyed in 2017, 2018, and 2019, canola flower midge damage likely had negligible effects on yield. Two fields were identified in 2019 with significant canola flower midge damage. At the field in Saskatchewan, nearly seven flowers per raceme had symptoms of canola flower midge damage. Based on our estimate of 11 harvestable pods per raceme, an infestation level of seven flowers would result in a loss of seven pods, or 63.6% of yield per raceme.

Table 1. Summary of eggs and larvae found on canola plants during the growing season across 5 sites. Eggs/plant, larvae/plant, and damage/plant were calculated based on the number of plants with eggs, larvae, or damage present, not on the total number of plants sampled during each week. Damage refers to flowers and buds used by midge larvae for development.

Week	*Plants Sampled*	*Plants w. Eggs*	*Total Eggs*	*Eggs per Plant*	*Plants w. Larvae*	*Total Larvae*	*Larvae per Plant*	*Damage per Plant*
4 July	125	8	40	5.00	2	3	1.50	1.00
11 July	117	38	674	17.74	54	2475	45.83	4.11
17 July	96	19	176	9.26	37	670	18.11	3.33
24 July	122	18	74	3.89	77	2999	38.95	5.04
31 July	122	2	4	2.00	35	692	19.77	5.36
7 Aug	120	3	55	18.33	11	54	4.91	7.15
14 Aug	125	2	15	7.50	12	47	3.92	3.97
21 Aug	125	1	2	2.00	5	44	8.80	6.14
28 Aug	125	0	0	0	0	0	0	11.93

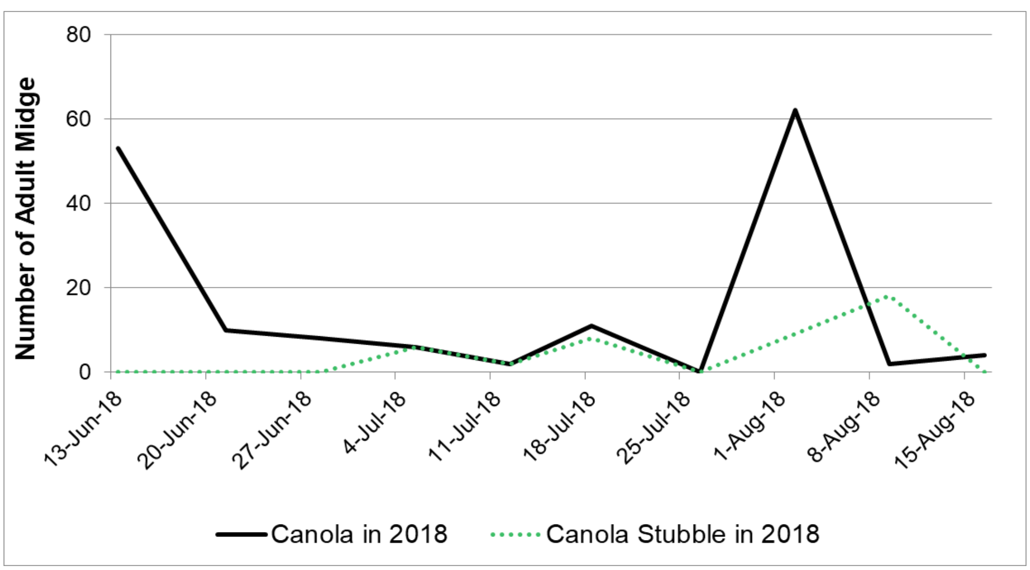

Figure 3. Emergence of adult canola flower midge inside cages placed on current year canola in 2018 or canola stubble (where canola grew in 2017), indicating two peaks of adult emergence.

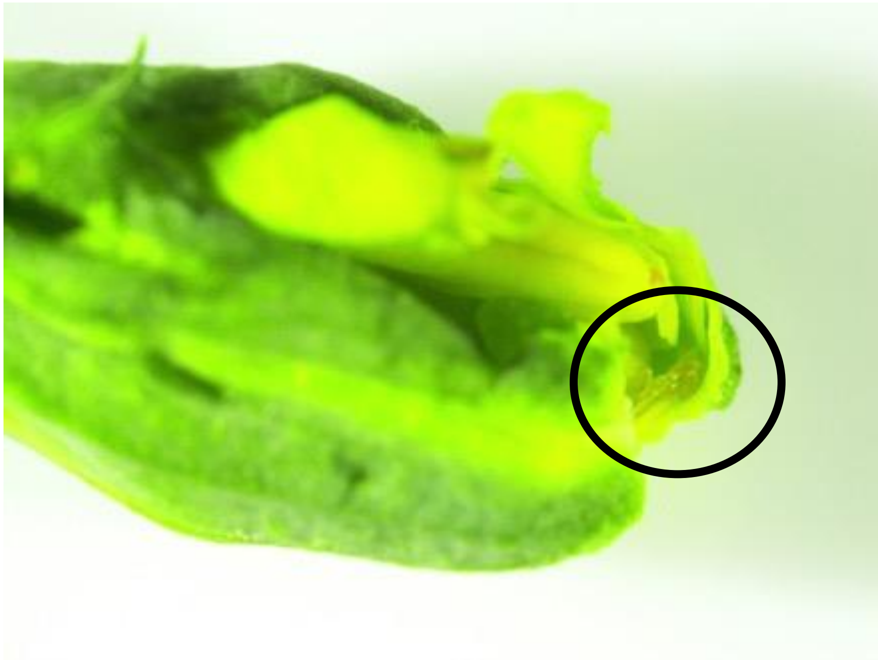

Figure 4. Canola flower midge eggs (inside black circle) inside a canola flower. Image by Shane Hladun and Jennifer Holowachuk.

Figure 5. Larvae of canola flower midge developing inside a galled canola flower. Image by Shane Hladun and Jennifer Holowachuk.

Objective 3: To use population genetics analyses to determine the source of swede midge and determine distribution patterns of the undescribed midge in the Prairies.

No swede midge were found on the Prairies during the duration of this study; however, population genetic analyses were conducted on the canola flower midge to help determine its distribution pattern and potential area of origin. Using samples collected from 16 locations (Table 2) spanning the known geographic distribution of the canola flower midge, DNA was extracted and a portion of the mitochondrial CO1 gene (439 bp of 'the barcoding region') and double-digest restriction site associated DNA (ddRAD) libraries were sequenced for 120 individuals. The CO1 sequence data was compared with available sequence data available in GenBank (NCBI) and all individuals were positively identified as canola flower midge, *C. brassicola*. There were 17 CO1 haplotypes (i.e. unique DNA sequences) identified from all the individuals sampled across the 16 locations (Figure 6). Haplotype diversity was highest in Saskatchewan and Alberta, followed by Manitoba. The high number of haplotypes and their diversity across the range of the canola flower midge provides some evidence that it may be native to the region. Generally, invasive species have low haplotype diversity in their invaded range compared to their native range. For instance, Laffin et al. (2005) observed only 5 cabbage seedpod weevil (*Ceutorhynchus obstrictus*) haplotypes in the invaded North American range, whereas 11 haplotypes were observed in the native range. Additionally, Mori et al. (2016) observed only 4 haplotypes of the red clover casebearer moth (*Coleophora deauratella*) in the invaded North American range, whereas 13 were observed in the native range.

Table 2. Site number, location, GPS coordinates, sample sizes and haplotypes (number of individuals) for the 16 sites where the canola flower midge was sampled for the population genetics analyses.

Site. No.	***Site***	***Abbreviated Name***	***Province***	***Latitude***	***Longitude***	***Sample Size***	***Haplotype (n)***
1	Sangudo	San	AB	53.90	-114.94	6	A (5), F
2	Athabasca	Ath	AB	54.73	-113.35	8	A (2), B (6)
3	Lamont	Lam	AB	53.72	-112.35	6	A (4), E, J
4	Forestburg	For	AB	52.57	-112.19	8	A (7), E
5	Vermilion	Ver	AB	53.33	-111.18	8	A (7), M
6	Major	Maj	SK	51.94	-109.80	8	A
7	Meadow Lake	Mea	SK	54.15	-108.32	7	A (4), B, I (2)
8	North Battleford	Nor	SK	52.84	-108.06	6	A (5), E
9	Fairy Glen	Fai	SK	53.05	-104.52	8	A, C (6), F
10	Steen	Ste	SK	52.67	-103.52	5	A (4), B
11	Porcupine Plain	Porc	SK	52.60	-102.70	7	A (4), D (3)
12	Preeceville	Pre	SK	52.06	-102.47	8	A (6), B, L
13	Thunder Hill	Thu	MB	51.99	-101.54	7	A (3), D (2), F (2)
14	Swan River	Swa	MB	52.29	-100.92	8	A
15	Dauphin	Dau	MB	51.07	-100.02	9	A (8), C
16	Portage la Prairie	Port	MB	49.96	-98.28	7	A

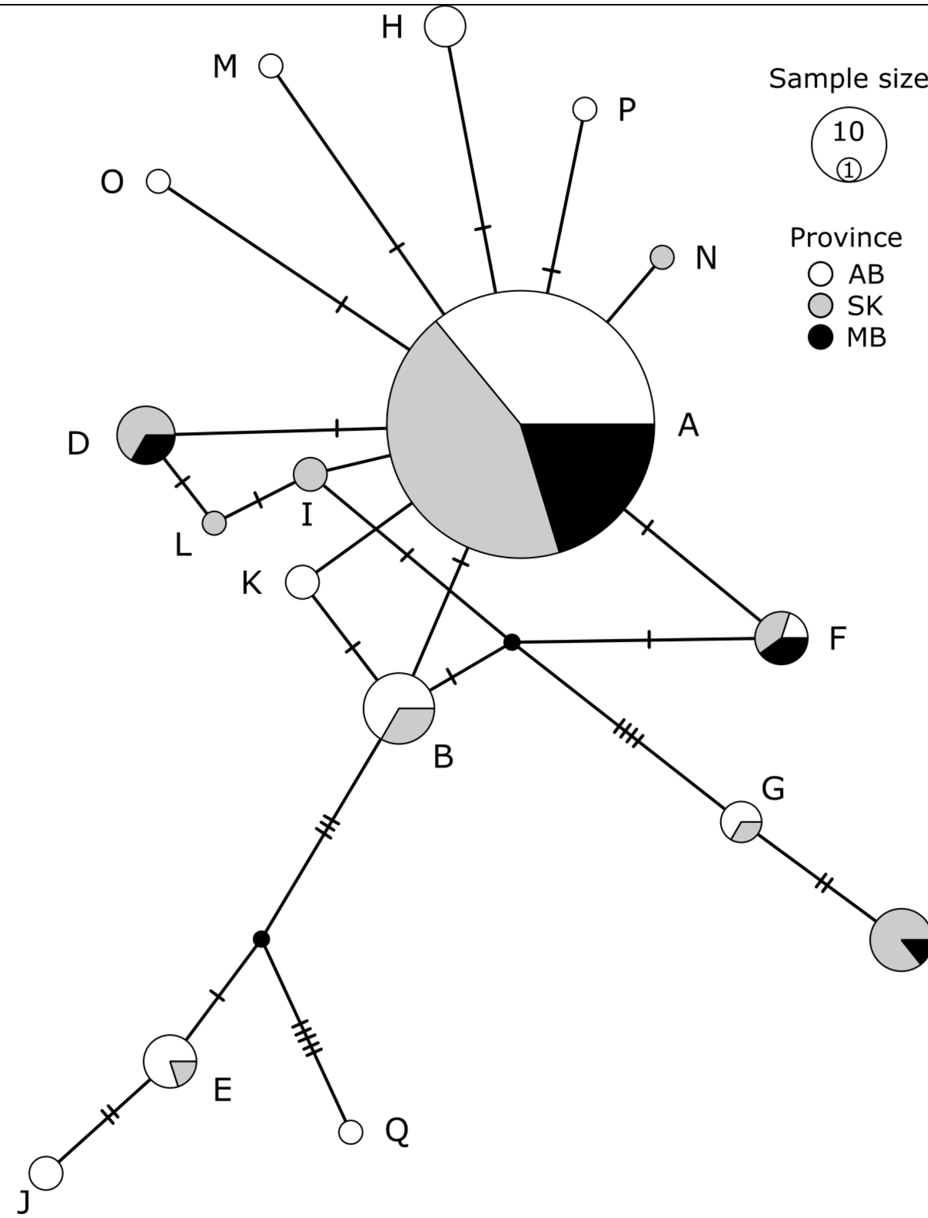

Figure 6. Mitochondrial DNA CO1 haplotype network for the 17 known canola flower midge haplotypes with corresponding letter. Haplotypes G, H, K, N, O, P, and Q were identified previously (Mori et al. 2019), but were not in the current study. Size of the nodes is proportional to the number of individuals with each haplotype. Colour of each node represents the proportion of individuals from each province with the corresponding haplotype. Small black nodes and dashes on the network represent intermediate haplotypes that were not identified.

Stacks v. 2.0 was used to analyze the ddRAD libraries and call single nucleotide polymorphisms (SNPs) markers. The output of Stacks was further filtered to remove incomplete data and analyzed with the program STRUCTURE. STRUCTURE was used to infer the most probable number of genetic clusters (i.e. groups = K) within the dataset and can help determine if populations are isolated or intermixed. The STRUCTURE analyses predicted the most likely number of clusters was K = 3 (Figure 7). Those populations at the edge of the range (e.g. Athabasca, Sangudo, Swan River and Portage la Prairie) are more distinct than those populations in the middle of the range (Figure 7). Although, Swan River and Portage la Prairie appear quite similar to each other, further analysis of their Fst-values (fixation index) reveals they are also fairly distinct. One possible explanation for these two sites appearing so different from the other sites could be that the Portage la Prairie sample was obtained from a research farm within the City which may have isolated these individuals from other populations. Similarly, the population in Swan River is on the edge of the agricultural extent and might be isolated from other populations due it is remoteness. Across the remaining populations mixing of individual appears to be occurring and no isolation exists (Figure 7).

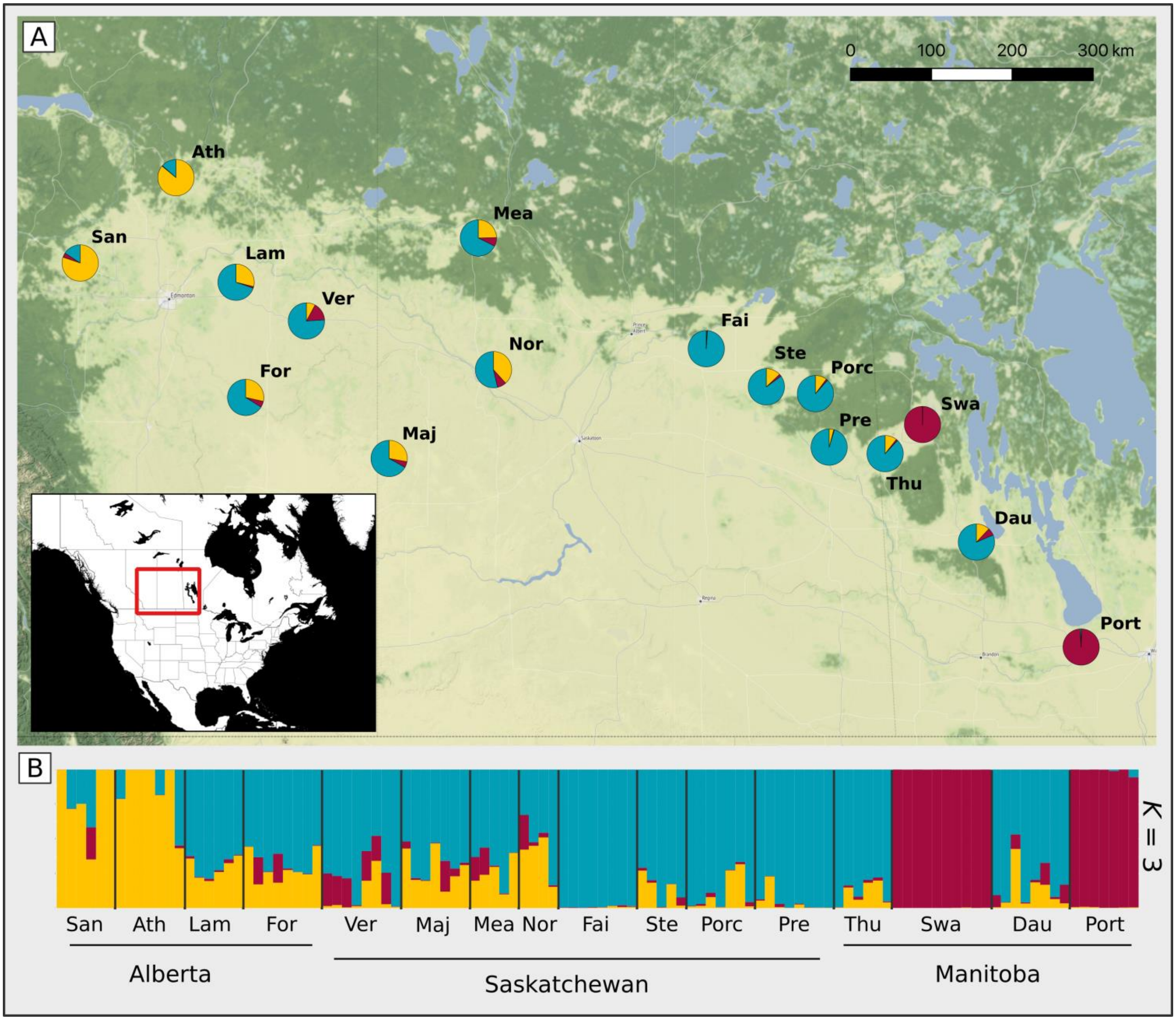

Figure 7. Population genetic clustering of *C. brassicola* in the Canadian Prairies using genomic SNPs. The pie charts on the map in (A) depict both the sampling location and average genetic assignment of each population according to STRUCTURE analysis. The individual population genetic assignments output by STRUCTURE are shown in (B).

Objective 4: To survey canola fields infested with midges for potential biological control agents (i.e. natural enemies such as parasitoids).

Previous work identified two parasitoids species, *Inostemma* sp., and a *Gastrancistrus* sp., attacking midge in canola in Saskatchewan (Mori et al. 2019). In order to determine the distribution of these parasitoids, and to determine the relative parasitism levels across Saskatchewan larval samples obtained during the driving survey were brought back to the laboratory and reared for midge and parasitoids. In 2018, of the 41 sites from which midge larvae were collected in Saskatchewan, parasitoids were identified from 3 sites. Only a single parasitoid emerged from each site in RM 487 (RM of Nipawin), 488 (RM of Torch River), and 588 (RM of Meadow Lake). In 2019, a single parasitoid emerged from a larval sample collected in RM 458 (RM of Willow Creek), accounting for 5% of larvae parasitized at that site. The greatest levels of parasitism were observed in 2017, when parasitoids were reared from larvae collected at 9 sites and parasitism levels ranged from 0 to 33%. Parasitoid specimens have been sent to the Canadian National Collection for morphological identification by parasitoid experts, but have yet to be identified.

References:

Laffin, R. D., Dosdall, L. M., & Sperling, F. A. H. (2005). Population structure of the cabbage seedpod weevil, Ceutorhynchus obstrictus (Marsham)(Coleoptera Curculionidae): origins of North American introductions. *Environmental entomology*, *34*(2), 504-510.

Mori, B. A., Andreassen, L., Heal, J. D., Dupuis, J. R., Soroka, J. J., & Sinclair, B. J. (2019). A new species of Contarinia Rondani (Diptera: Cecidomyiidae) that induces flower galls on canola (Brassicaceae) in the Canadian prairies. *The Canadian Entomologist*, *151*(2), 131-148.

Mori, B. A., Davis, C. S., & Evenden, M. L. (2016). Genetic diversity and population structure identify the potential source of the invasive red clover casebearer moth, Coleophora deauratella, in North America. *Biological invasions*, *18*(12), 3595-3609.

4. Significant Accomplishments

The distribution of *Contarinia brassicola* across the prairie provinces was determined during this project. The midge occurs in the Peace River region of Alberta and across central Alberta and Saskatchewan. Presence of *C. brassicola* was confirmed in western and southern Manitoba. Although generally low across its range, the population density of *C. brassicola* is highest in north eastern Saskatchewan and two fields were identified in 2019 where damage levels due to *C. brassicola* infestation may have had an impact on crop yield. The project demonstrated that adult midge only lay eggs on or near canola buds and that larvae only develop inside the buds. There are at least two generations of *C. brassicola* per year. Populations of *C. brassicola* are used as hosts by at least two species of parasitoid. Population genetic analyses revealed high genetic diversity across the known range of *C. brassicola* and provide support that it may be a native species.

Acknowledgements: This research is part of the Canola Agronomic Research Program (CARP Grant 2017.12) with project funding from the Alberta Canola Producers Commission (Alberta Canola) and the Saskatchewan Canola Development Commission (SaskCanola). We are grateful for the field assistance provided by summer students (J. Drury, A. Jahnke, J. Nokusis, A. Hamilton, J. Smith, J. Kim, and K. Saita) during this project. Mr. Ross Weiss produced the distribution maps for canola flower midge. The driving survey was conducted with the assistance of J. Soroka, J. Holowachuk, S. Harris, S. Hladun, and J. Williams.

5. Research and Action Plans

A new proposal (led by Boyd Mori) has been submitted to CARP to build upon the knowledge gained from this project and from CARP 2017.13 (Identification of canola flower midge pheromones and development of a monitoring tool). The emphasis of this project will be to optimize a monitoring system and to study the relationship between trap capture and midge population density and yield loss in canola fields.

As analyses for this project are completed, at least one scientific manuscript detailing our results will be submitted for publication in peer-reviewed journals. Results from this project will continue to be shared at outreach and extension events.

6. Final Project Budget and Financial Reporting

Please see financial report, to be submitted in March 2020.

Please forward an electronic copy of this completed document to:

Gail M. Hoskins
Crop Production Administrator and CARP Coordinator
Canola Council of Canada
400 – 167 Lombard Ave.
Winnipeg, MB R3B 0T6
Phone: (204) 982-2102
Fax: (204) 942-1841
E-Mail: hoskinsg@canolacouncil.org