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**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:** Effect of hairiness in Brassica lines on the abundance, feeding and oviposition behavior of flea beetles, diamondback moths and Aster leafhoppers.

**Research Team Information**

| **Lead Researcher:** | | |
|---|---|---|
| ***Name*** | ***Institution*** | ***Project Role*** |
| Chrystel Olivier | AAFC - Saskatoon | PL |
| **Research Team Members (add rows as required)** | | |
| ***Name*** | ***Institution*** | ***Project Role*** |
| Dwayne Hegedus | AAFC-Saskatoon | Co-investigator |
| Tyler Wist | AAFC-Saskatoon | Co-investigator |
| Meghan Vankosky | AAFC-Saskatoon | Co-investigator |
| | | |

**Project Start Date:** April 1, 2018 **Project Completion Date:** Dec 31, 2021

**Reporting Period:** April 1, 2018 to December 31, 2021

**CARP Project Number:** 2018.7

**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 set out in the 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: _____**

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

**Comments:**

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


Flea beetles, (Crucifer flea beetles, *Phyllotreta cruciferae* (Goeze) (CFB) and striped flea beetles, *Phyllotreta striolata* (Fabricius) (SFB) (Coleoptera: Chrysomelidae)), diamondback moths (DBM) and aster leafhoppers are major insect pests that threaten canola production each year. The objectives of the project were to conduct lab bioassays and field trials with naturally-hairy *B. napus* lines and *B. villosa* to assess: 1) feeding damages of flea beetles and DBM, 2) feeding and oviposition behavior of flea beetles, DBM and aster leafhoppers and 3) AY transmission by aster leafhoppers

In laboratory assays, striped flea beetles were used because they were plentiful to catch in the field early season and the crucifer flea beetles were not. Also, striped flea beetles emerge from overwintering two weeks before crucifer so they are the first flea beetles to challenge young canola seedlings on the farm. Hairy leaves of the DOS2 brassica were less fed-upon by SFB than glabrous AC Excel which the very hairy *B. villosa* was not preferred by SFB at all. More stem clipping by flea beetles however, occurred on the DOS-02 plants whose petioles and stems are not hairy, which suggests the need for breeding for trichome-covered petioles and stems as well as leaves. Cotyledons of DOS2 and *B. villosa* were also less preferred for feeding than those of AC Excel even though they are as hairless as AC Excel canola cotyledons and this phenomenon warrants further attention. In lab bioassays, DBM larvae overwhelmingly avoided the hairy leaves, with the first instars having difficulty mining the hairy canola leaves. Trichome-covered leaves bode well as a DBM deterrent as this trait is expressed on leaves throughout the life of the plant, especially under drier environmental conditions.

In the lab bioassays, aster leafhopper did not exhibit feeding deterrence or preference for hairy or non-hairy canola plants (similar average number of aster leafhoppers in all plants), but laid very few eggs on all crucifer plants. In the 2020 field plots, there were very few aster leafhoppers. All plants tested negative for the presence of aster yellows phytoplasma, AYp, the cause of aster yellows disease.

In 2020, a small scale field trial was conducted at the Saskatoon RDC Lowe Road site and showed that leaf damages (% of fed leaf areas) at the cotyledon stage were very similar between hairy (DOS2) / non-hairy canola cultivars (AC Excel), but DOS2 cotyledons are not hairy. However, at later stage (4 leaf stage), leaf damages were higher on AC Excel compared to DOS2, and at this time, DOS2 leaves expressed the hairy trait on all leaves. In 2021, heavy flea beetle pressure coupled with hot and dry conditions overcame all of the experimental seedlings that were planted at the experimental farm. In the 2020 field plots, there were very few diamond back moths (larvae and adults), and no DBM damage could be estimated.

Development of hairy canola lines is still a work in progress. Hairy Brassica lines that have been developed are not Canola quality and so field testing of plants should be considered as only very preliminary. Deterrence of DBM larvae is promising, while the destruction of early-season seedlings by flea beetles in 2021 is not. The cotyledons are embryonic and not plant tissue, and this situation poses a problem with no trichome expression on cotyledons. Cotyledons therefore, cannot be protected by defensive trichomes, but another potential form of deterrence seems to be in action on the transgenic, DOS-02 and *B. villosa* lines that warrants further study.


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

Flea beetles, (Crucifer flea beetles, *Phyllotreta cruciferae* (Goeze) (CFB) and striped flea beetles, *Phyllotreta striolata* (Fabricius) (SFB) (Coleoptera: Chrysomelidae)), diamondback moths (DBM) and aster leafhoppers are major insect pests that threaten canola production each year. Flea beetles feed on canola seedlings and later on pods, while DBM larvae feed on green tissue and the aster leafhopper is a sap feeder vectoring Aster Yellow disease. The local flea beetle, DBM and aster leafhopper populations are difficult to control, and there is no model that can predict the population of these pests from one year to the next and no resistant canola varieties exist. Therefore, insecticide application is the only control option for all three of these pests. Recently, AAFC scientists identified natural lines of *Brassica napus*, and the related *Brassica villosa* species, that exhibited high levels of hairs (trichomes) on their leaves and stems. Seedling-stage "Hairy" canola, (a transgenic line) has demonstrated resistance to flea beetle feeding in previous work conducted at AAFC Saskatoon. This project was conducted in coordination with another proposal submitted to the Canola cluster (D. Hegedus et al: Genetic resources for flea beetle resistance in canola), which will focus on the genetics of the trichome abundance (hairy) trait in Brassica plants.

This project aimed to study the effect of the hairiness of the hairy *Brassica* lines (non-transgenic hairy canola lines acquired from PGRC accessions, doubled-haploid hairy derivatives of these accessions and *B. villosa*) on the feeding and oviposition behaviour of flea beetles, DBM and aster leafhoppers in field trials and lab bioassays.
The objectives of the project were to conduct lab bioassays and field trials with naturally-hairy *B. napus* lines and *B. villosa* to assess: 1) feeding damages of flea beetles and DBM, 2) feeding and oviposition behavior of flea beetles, DBM and aster leafhoppers and 3) AY transmission by aster leafhoppers and 4) to gather information on the interactions between flea beetles, DBM and aster leafhopper with *B. villosa* and hairy lines on *B. napus*.

**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.

**1. Naturally hairy *B. napus* seed increase (Olivier, Hegedus)**

1.1.Brassica species and lines (Fig.1.1; 1.2 & 1,3) used in the project

In order to have sufficient seeds to conduct field trials and bioassays, seed increase of a double haploid line (DOS-2) derived from a naturally hairy *B. napus* line and a *B. villosa* line were conducted in the greenhouse.

*Brassica villosa* (GCN14116) was obtained from the Centre for Genetic Resources (Wageningen, The Netherlands). Plants were grown in 15 cm pots in a controlled environment greenhouse [16h/8h (day/night) supplemented with halogen lighting, 22/20°±2C, and 75±5% humidity] for 8 months and then plants were transferred to 4° for 12 weeks for vernalization. *Brassica villosa* has semi-self-incompatibility and produces only a few seeds, between 10-50 seeds per plant after several successive vernalization treatments.

*Brassica napus* DOS-2 is a double haploid line developed at the AAFC (Saskatoon Research Center) with the ability to produce 300±50 trichomes leaf up to the 4-5 leaf stage. DOS-2 was grown in 15 cm pots in a controlled environment greenhouse [16h/8h (day/night) supplemented with halogen lighting, 22/20°±2C, and 75±5% humidity] for 3 months and then transferred to the vernalization room for 8 weeks to initiate flowering.

AAFC (Drs. Margaret Gruber and Julie Soroka) and University of Saskatchewan (Dr. Peta Bonham-Smith) researchers developed the *B. napus* line "Hairy canola" that produces hairs (trichomes) on its leaves, petioles and stems, and exhibits a good level of resistance to flea beetles. This line was accomplished by introducing one gene (GL3: glabrous 3) from a hairy, distant relative of canola, *Arabidopsis thaliana*, to induce hair formation and a second transgene (dsRNA TTG1) to restore normal plant growth (Gruber et al., 2006; Alahakoon et al., 2016). The non-hairy cotyledons in this line also exhibited increased flea beetle tolerance due the presence of anthocyanin pigments, a trait that is also regulated by GL3 (Gruber et al., 2018). AC Excel (*B. napus*) is a commercially available cultivar registered to AAFC developed by Dr. Gerhardt Rakow and is the progenitor line for the hybrid canola produced in Canada.

*Alahakoon UI, Taheri A, Nayidu NK, Epp D, Yu M, Parkin I, Hegedus DD, Bonham-Smith P, and Gruber MY (2016) Hairy Canola (Brasssica napus) re-visited: Down-regulating TTG1 in an AtGL3-enhanced hairy leaf background improves growth, leaf trichome coverage, and metabolite gene expression diversity. BMC Plant Biology 16: 12.*

*Gruber MY, Wang S, Ethier S, Holowachuk J, Bonham-Smith PC, Soroka J, Lloyd A (2006)''HAIRY CANOLA''– Arabidopsis GL3 induces a dense covering of trichomes on Brassica napus seedlings. Plant Mol Biol 60: 679–698.*

*Gruber MY, Alahakoon U, Taheri A, Nagubushana N, Zhou R, Aung B, Sharpe A, Hannoufa A, Bonham-Smith P and Hegedus DD (2018) The biochemical composition and transcriptome of cotyledons from Brassica napus lines expressing the AtGL3 transcription factor and exhibiting reduced flea beetle feeding. BMC Plant Biology 18: 64.*

1.2. Number of trichomes on *B. napus* leaves

DOS-2 and AC Excel (AC Excel (*B. napus*) is a commercially available cultivar that causes no copyright issues when used in experiments) plants were grown at cold (12°C night/18°C day) and warm (22°C night/25°C day) temperatures with 16L/8D, 50-60% relative humidity, and 400-500 µmol/$m^2$/s in both growth chambers with 3 different soil moisture levels: dry (20-30% soil moisture), wet (40-50% soil moisture) and very wet (60-70% moisture). 5 plants were grown per treatment until the 8 leaf stage, and all leaves were observed under a binocular microscope and trichomes were counted.

1.3. Trichome observation using the light source of the Synchrotron

The objective of this study was to examine the structural differences among various trichomes at the molecular level using synchrotron techniques. The chemistry of trichomes was examined using Mid-IR beamline and BioXAS beamline at the Canadian Light Source (Synchrotron) on the USask campus. Four week old *Brassica napus, B.juncea, B.villosa, Sinapis alba* and mature plant of *S. alba*, which showed varying degree of susceptibility to insects, were included in the study.

For Mid-IR experiment, trichome samples were mounted on CaF2 windows and fourier transformed Mid-IR spectra were collected at 900-2000 cm-1 energy range. Orange/quasar data mining software and workflow obtained and installed from the beamline were used for data analysis. We collaborated with Mid-IR scientist for data analysis and interpretation of results.

Experiments were conducted on the BioXAS beamline using spectral energy range 5-21KeV. Trichomes of 4- week old *Brassica napus, B. juncea, B. villosa* and *Sinapis alba* were examined. X-ray fluorescence imaging spectra were collected on trichomes of freshly harvested leaves, mounted on tape attached to a disk. Occurrence and distribution of metals and metalloids in the trichomes and their co-localization patterns were mapped. Spectra from various standards were also collected under the same experimental conditions.

**2. Conduct field trials with naturally-hairy *B. napus* lines and *B. villosa* to assess feeding damage of flea beetles, DBMs and aster leafhoppers (Olivier, Hegedus, Wist)**

2.1. Field trials

No field trials were conducted in 2018 and 2019 due to the low number of DOS2 and *B. villosa* seeds available. Enough seed was available for small field trials to be conducted in 2020 and 2021.

A small field trial, consisting of 8 rows of 100 seeds/row (4 rows with DOS2 and 4 rows with AC Excel, alternate 2 rows) was seeded by hand in May 2020 and 2021 at the Saskatoon Research Center farm, Lowe road site. FB damages from spring and fall population were assessed as soon as germination started and until the end of the growing season. Assessment was done by counting the % of eaten surface on 100 plants per row.

2.2. FB, DBM and leafhopper population

In all project years, cereal crops (barley, oat, wheat) and canola were planted, each in 1.012 HA fields to track the populations of aster leafhoppers, flea beetles and diamondback moth (DBM) at the AAFC Lowe Road farm. Flea beetle and leafhopper populations were monitored with sweep nets and yellow sticky cards. No-mess adhesive yellow sticky cards and card holders were purchased from Alphascents (Size 18 x 14cm). In 2020 and 2021, diamondback moth (DBM), pheromone traps were deployed to assess the migration of DBM adults and compare populations across years.

**3. Conduct lab-based bioassays with naturally hairy *B. napus* lines and *B. villosa* to assess feeding and oviposition behavior of flea beetles, DBM and aster leafhoppers**

Bioassays protocols were set up for the feeding/oviposition and AY transmission experiments as per Olivier et al., 2014 (Canadian Plant Disease Survey, 94: 162-175), except for the flea beetle oviposition bioassays described below. Briefly, one row of seedlings, each seedling belonging to a different line, was set up in a cage. Care was be taken to place seedlings in cages (Fig.1.4) that were at very similar growth stages and height. Depending on the growth stage, rows contained five (cotyledons, first/second leaf, third/fourth leaf) or three plants (fifth/sixth leaf and bolting). Trays with the 10 cages were placed in a controlled environment chamber (Conviron, model PGV35) set at 20°C, 50-60% relative humidity, 16h light/8h dark photoperiod and 400 µmol/m2/s1 light intensity. The experiment was repeated three times and at least 3 growth stages were tested for each bioassay. In the bioassay involving aster leafhopper, plants were sampled at the end of the bioassay and tested by PCR for the presence of phytoplasma as per Dumonceaux et al., 2014 (Plos One. Doi:10.1371/journal.pone.0116039). The experimental design was a randomized complete bloc choice (various lines/cage) experiment for all bioassays. A no-choice experiment (same lines/cage) was also be conducted. Statistical analysis was conducted using Anova; however, if data were missing or treatments are added, GLM or Proc Mixed were used.

3.1.Flea beetle bioassays:

Choice and no-choice striped flea beetle (SFB) feeding bioassays were conducted in small cages (Fig. 1.4) at cold temperatures (12°C night/18°C day) and warm temperatures (22°C night/25°C day) with 16L/8D, 50-60% relative humidity, and 400-500 µmol/$m^2$/s in both growth chambers with 4 different soil moisture levels: dry (20-30% soil moisture), wet (40-50% soil moisture), very wet (60-70% moisture) and saturated (100% soil moisture). Striped flea beetles were collected in the field in the spring and in the fall, and maintained on cabbage and canola plants in a warm growth chamber. Ten flea beetles were starved overnight before being placed in cages containing Brassica seedlings (10 striped flea beetles/cage). Flea beetle location on the plants was recorded every hour for 7 hrs and damage were recorded 72 hrs after flea beetle introduction. Bioassays were repeated at least 3 times. All bioassays planned in this project were conducted.

*B. napus* Hairy Canola (hairy, 800 trichomes leaf), DOS 2 (moderately hairy, 300 trichomes per leaf), AC Excel (mildly hairy, 75-100 trichomes per leaf), and Round-up ready (RR) (mildly hairy, 100-125 trichomes per leaf) as well as *Brassica villosa* (extremely hairy, > 3000 trichomes per leaf) were evaluated. There were enough seeds of DOS-2, AC Excel and RR, but few seeds of *B. villosa* and very few seeds of the transgenic Hairy Canola (no more production of those seeds). Therefore, seeds of *B. villosa* and Hairy Canola were used only sporadically.

3.2 Flea beetle colonies

The high standard deviations observed in many experiments and the differences in feeding behavior between the spring and fall population lead us to attempt rearing striped flea beetle (SFB) and generated a self-sustaining colony.

The first attempt was started in the fall 2019. Fall SFB (n=500) were placed in moist soil at 5°C for 2 months (October 2019, and a total of 50 adults emerged when the soil was placed back at room temperature (January 2020). The low survival was believed to be caused by low soil moisture during overwintering. The 50 survivors were placed in cages containing canola seedlings and successfully laid eggs.

In August of 2021, another colony was started with 160 mixed sex beetles following a protocol adapted from Nagalingam and Costamagna, 2019. SFBs were collected on August 30, 2021 from the shelterbelt area south of Block 16 at the AAFC Lowe Road farm. Since this was the fall population, 'true' hibernation is required for the beetles to lay eggs successfully. The growth cabinet conditions for pre-hibernation were 19°C for 8 hours of light and 7°C for 16 hours of dark. This step is to expose the beetles to the fall temperature for 1 month. For true hibernation, the jar is covered with black cloth and kept in a fridge at 5°C for 6 weeks (Fig. 1.5). After 6 weeks, the beetles were ready to start a new colony.

3.3. Diamondback moth (DBM) and aster leafhopper bioassays.

3.3.1. DBM oviposition bioassays were conducted following the protocol described by Alahakoon et al., 2016 (Can. Entomol., 148: 603-615). Briefly, after mating, 20 females and 5 males were deposited in cages containing plants at the

4/5, 7/8 leaf and bolting growth stages and left for 7 days. Number and localization of eggs was recorded every 48 hours. Each experiment consisted of 5 replicates and was repeated three times..

3.3.2. DBM larval movement and feeding bioassays: Late instar diamondback moth larvae were selected from the AAFC colonies maintained by Dr. Wist. In each cage, 1-5 larva were placed per plant depending on the growth stages (1 larva/plant at the 4/5 leaf and 7/8 leaf stage and 5 larvae per plant at the bolting stage). Observations of larval movement and location were recorded after 12, 24, 48 and 72 hours, and plant damage was recorded after 12, 24, 48 and 72 hours, 1 and 2 weeks as per Ulmer et al., 2001 (Can. Entomologist: 133: 509-519). Two weeks after insect introduction, the number of larvae and pupae were recorded. Each experiment consisted of 5 replicates and was repeated 5 times.

3.3.3. Aster leafhopper feeding, oviposition and transmission of AY bioassays
Bioassays protocols were set up for feeding/oviposition and AY transmission experiments as per Olivier et al., 2014 (Canadian Plant Disease Survey, 94: 162-175), Depending on the growth stage, rows contained five (cotyledons, first/second leaf, third/fourth leaf) or three plants (fifth/sixth leaf and bolting). The experiment was repeated three times and at least 3 growth stages were tested for each bioassay over the course of the project. Plants were sampled at the end of the bioassay and tested by PCR for the presence of phytoplasma as per Dumonceaux et al., 2014 (Plos One. Doi:10.1371/journal.pone.0116039).

**4. Gather information on the interactions between flea beetles, DBM and aster leafhopper with *B. villosa* and hairy lines on *B. napus* (Olivier, Wist, Vankosky).**

In all field trials, sticky cards were set up in every plot from two repetitions and changed weekly. Sweeps were conducted weekly around the field trails in the surrounding canola to assess populations of target insects as a comparison of the pressure on the small plots. Flea beetles, leafhoppers and DBM were collected, identified, and counted from sweeps and yellow sticky cards. The aster leafhoppers were tested for the presence of AY phytoplasma using the 16Sr and Cpn60 gene as per Dumonceaux et al., 2014 (Plos One, Doi:10.1371/journal.pone.0116039). The insect by catch was stored in the freezer for further identification (with focus on other canola pests and parasitoids) and counts.

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

**1. Naturally hairy *B. napus* seed increase (Olivier, Hegedus)**

1.2. Number of trichomes on *B. napus* leaves

The average number of trichomes was counted on leaves until canola growth stage 5. The average number of trichomes on leaves of DOS-2 at the 2-3 leaf stage was significantly higher (250-300 per leaf) as compared to AC Excel (75-100 per leaf), but was significantly higher in drier soil, especially in warm temperatures (Fig. 1.6).

1.3. Trichome observation using light source.
Results of the Mid-IR spectroscopy among species are shown in Fig. 1.7.A & B (graph & table). Spectral differences along the length of the trichomes were also observed indicating structural differences within trichomes. There were protein-like compounds as well as compounds with C-C, C-O and CH2 groups as indicated by stretching and vibrational characteristics of various chemical bonds. Some of these compounds indicate the cell wall components while others indicate the components within trichomes.
Preliminary observations on BioXAS experiments revealed the presence of various metals/metalloids in trichomes and their distribution in different parts of the trichomes. When combined with elemental profiles, organic molecules observed in these trichomes will enhance our knowledge on plant trichomes and their possible role in defense against insects.

**2. Conduct field trials with naturally-hairy *B. napus* lines and *B. villosa* to assess feeding damage of flea beetles, DBMs and aster leafhoppers (Olivier, Hegedus, Wist)**

2.1. Small field trials

Seeds were sown on May 20 2020 and weekly assessment of FB damages started June 5 (Fig 2.1). FB assessment showed that damage (% of fed upon area) at the cotyledon stage were similar between hairy (DOS-2) and non-hairy canola cultivars (AC Excel), although it should be noted that *B. napus*, even hairy B. *napus*, does not produce hairs on the

cotyledons because it is embryonic tissue. At the cotyledon stage, FB damage was on average 75% (Fig 2.2); However, at the fourth leaf stage, damage was higher on AC Excel compared to the hairy-leaved DOS-2. At the end of the season, FB were feeding on DOS-2 plants, due to its late maturity (Fig.2.3), lack of bolting and because it was greener than the AC Excel. However, feeding on canola plants by second generation flea beetles is common each year. In 2020, no DBM larvae or typical DBM damage was observed on any of the plants in either AC Excel or DOS-2 plots and none of the canola plants of either variety in the small plots showed symptoms of aster yellows infection.

Field trials in 2021 were compromised due to the severe drought resulting in poor seed germination and poor seedling establishment and total destruction of any plants at the cotyledon stage that actually grew in the trial by flea beetles. No further assessments could be made beyond the 100% yield loss of all experimental plants due to flea beetle feeding damage and drought.

2.2. Population of FB, aster leafh and DBM at the experimental farm in Lowe Road

In the 2020 test year for the hairy canola lines, an adjacent canola field was planted and followed for populations of insects. We did not have access to our farm in spring of 2020 except to plant the canola so DBM pheromone trapping started late that year. No DBM adults were caught on traps in 2020 or in 2021. However, in the canola field, we did have a small population of DBM larvae (Fig. 2.4) that started in the last week of July 2020 so there must have been DBM adults that migrated into Saskatchewan that year. 2020 was the only project year where DBM migrated to Saskatoon (Fig. 2.4)

In 2020, the second generation of flea beetles was dominated by striped flea beetles (*P. striolata*) in August (Fig. 2.5) where they were caught feeding in the late-stage canola adjacent to the hairy canola plots. In 2021 the drought negatively impacted the striped flea beetle population. The second (or overwintering) generation of flea beetles was dominated by crucifer flea beetles (*P. cruciferae*) in 2021 (Figure. 2.5) and the hot dry conditions increased their populations in the late-stage canola crop in August.

Sweep samples in canola are only possible after the spring generation of flea beetles has fed on the crop so yellow sticky cards are used to monitor the early season flea beetle populations. In 2020, we did not have access to our farm and buildings to place yellow sticky traps. In 2018, the overall flea beetle population was low, with a large population of striped and crucifer flea beetles that overwintered and entered canola fields and plots in 2019 (Fig. 2.6). In 2019, it's evident that the striped flea beetles emerged from their overwintering sites earlier than the crucifer but they were active and feeding on plants by the second week in June (Fig. 2.6). The early season population of both flea beetle species in 2021 was lower than in 2019 but from the sweep samples, that first generation of crucifer flea beetles was very fecund and their offspring survived to form an enormous second generation of crucifer flea beetles while the striped flea beetle population crashed in the drought conditions (Fig. 2.5).

Aster leafhopper populations were followed over the project years with 50 sweeps of a standard sweep net when crops were large enough to sample. Aster leafhopper populations increased in the cereal crops over the course of the growing season, especially in barley in 2018 and in oat in 2019 (Fig. 2.7). These results indicate that the aster leafhoppers were reproducing in these cereal crops and mostly stayed in the cereals through August except for a few that moved into canola in both years (Fig. 2.7).

In 2020 and 2021, aster leafhoppers were present in cereal fields when fields were large enough to sweep. In 2020, the population progressed and reproduced in the cereal crops, especially wheat at the Lowe Road farm, and a few aster leafhopper adults ventured into the canola by the first week of August in 2020 and were found in the canola field throughout August (Fig. 2.8). In 2021, the wheat field attracted the first migrant aster leafhoppers but their population did not increase over the growing season (Fig. 2.8), likely due to the extreme heat and drought that negatively impacted all of the insect populations in all of the crops at the AAFC Lowe Road farm. Interestingly, in the first week of August 2021 (3$^{rd}$), aster leafhoppers were found in low numbers in the canola field at approximately the same time as in 2020 which indicates that some within crop movement is occurring from cereals to canola in this F1 generation of leafhoppers.

As an indicator of the aster yellows pressure each year, we check with the Saskatchewan Provincial Ministry of Agriculture's disease survey. In 2020, from the Saskatchewan Provincial Ministry aster yellows survey, only three areas in Saskatchewan had fields with any incidence of aster yellows. In 2020, 23% of the 261 fields surveyed had at least 1 plant with AY symptoms and those fields are clustered. The overall incidence across the survey was that 0.72% of all plants surveyed had AY symptoms so 2020 was a low year for aster yellows infection in Saskatchewan even though there was a

migration of aster leafhoppers into the province (Fig. 2.8). Saskatoon and area had no fields with aster yellows incidence which correlates with the lack of aster yellows infected plants that we detected in our Saskatoon fields. Of the subsample of the collected aster leafhoppers from 2020, none of the samples from the Saskatoon Farm were infected with aster yellows.

A subsample of leaves were taken from DOS-2 (2 leaves/plant, 50 plants) and AC Excel plants (2 leaves/plant and 50 plants) grown next to the Canola field at the AAFC Saskatoon Lowe Road farm all tested negative for the presence of the aster yellows phytoplasma indicating that no transmission of AYp had occurred to these plants through aster leafhopper feeding.

In 2021, from the first arriving aster leafhoppers (May-June 9 2021), we tested 32 leafhoppers caught in ditches with sweep nets and only one was positive for aster yellows phytoplasma (AYp). None of the early season migrants at the AAFC Lowe Road farm were infected with AYp and only one Aster yellows infected plant was found in the Lowe Road canola field. So, aster leafhoppers likely did arrive to Saskatchewan with some active AYp infections but the number was low. The provincial aster yellows survey recorded more incidences and trace amounts of aster yellows infected plants in canola fields than in 2020. In 2021, 13/212 = 6.1% of fields in Saskatchewan had an incidence of Aster yellows (detected on the 100 plant/field survey) (range 1-16% of plants infected, with heaviest infection in the Meadow Lake region). There were 27 of 212 fields (12.23%) with trace amounts of AY infected plants where surveyors notice AY infected plants but they were not captured in the 100 plant survey giving 18.9% of all sampled fields with aster yellows infected plants (40/212). Trace numbers of AY infected plants were found in RMs around Saskatoon.

**3. Conduct lab-based bioassays with naturally hairy *B. napus* lines and *B. villosa* to assess feeding and oviposition behavior of flea beetles, DBM and aster leafhoppers**

During the growing season 2018, the vast majority of the collected flea beetles were *Phyllotetra striolata* (striped flea beetles) (Fig. 2.2.3), so all bioassays were done with *P. striolata*.

3.1. Flea beetle bioassays

In choice and no-choice bioassays, SFB damage was higher in warm temperatures as compared to colder temperatures and when the soil was wet (Fig. 3.1 & 3.2).

In choice and no-choice bioassays, SFB tended to avoid areas with trichomes and fed more readily on trichome-free areas, such as the stems and petioles. SFBs tended to avoid and not feed on *B. villosa*. In choice and no-choice experiments, SFB tended to be located more so on stems and petioles as compared to cotyledons and leaves of DOS-2 (Fig.3.3). There was no significant difference in SFB location between stem, petiole and cotyledon when SFB were placed on AC Excel plants. More specifically, in choice experiments, SFB preferred to feed on DOS-2 stems (no trichomes) and AC Excel leaves (mildly hairy) as compared to DOS-2 leaves (moderately hairy) and AC Excel stems (Fig. 3.4 & 3.5). This altered feeding behavior led to stem clipping of the DOS-2 seedlings causing increased seedling mortality as compared to the less hairy AC Excel seedlings. This observation was very useful as it illustrates the importance of breeding for the presence of trichomes on the stems, as well as on the leaves and petioles.

In choice and no-choice experiments, SFB tended to avoid non-hairy cotyledons of *B. villosa* and DOS-2, suggesting the presence of repellents or anti-xenotic compounds (other than trichomes) in the cotyledons. Previously, it was demonstrated the resistance of non-hairy cotyledons in the Hairy Canola developed by M. Gruber was due to elevated levels of anthocyanin pigments. Further, natural product chemistry investigation of the cotyledons of the DOS-2 and *B. villosa* lines is warranted.

Spring population of SFB can be seen in canola crops on seedlings (and caught on yellow sticky cards), while fall populations of SFB are rarely seen in canola crops, as opposed to Crucifer flea beetle (CFB) that can be seen in spring and in the fall in canola crops. Spring SFB are voracious, but fall SFB seem to eat less (Fig. 3.6 & 3.7). One of the challenge we faced was to know when the spring SFB population ends and when the fall SFB population starts. Very little is known about the SFB bionomics, and activities after the spring generation lays eggs. As well, very little is known about the SFB fall feeding (which plants do they feed on, where?), overwintering sites and survival.

3.2. Flea beetle Colonies

Three successive generations of SFBs were produced since August 2021 in our lab colony (Fig. 3.9). The emergence of the third lab colony generation coincided with the emergence of the spring generation so efforts were focused on using higher numbers of the spring field-collected SFB to increase the lab colony.

3.3. DBM bioassays

Bioassays regarding oviposition and feeding behavior indicate that DBM can lay eggs on *B. napus* AC Excel, DOS-2 and *B. villosa*, with a preference for *B. villosa* stems and DOS-2 leaves compared to AC Excel (Fig 3.10). First instar larvae had difficulties mining in the leaves of *B. villosa*, as their tunnels were very short. Larvae crawled between trichomes or tried to walk across the trichomes by producing silk mats (Fig 3.12), indicating that trichomes reduces feeding efficacy. In bioassays using detached leaves, DBM larvae overwhelmingly avoided the hairy leaves (Fig 3.13).

3.4.Aster leafhopper feeding, oviposition and transmission of AY bioassays

Bioassays with aster leafhoppers were started in January 2020. A total of 5 AY-infected leafhoppers were placed in each cage and left for 7 days. Localisation of the leafhopper were observed hourly on the first day after placing the leafhoppers in the cage. Leafhoppers feed and survived on hairy and glabrous *B. napus*, but aster leafhopper mortality was high (12.9%) regardless of the plant growth stage. No significant difference was found in mortality between the moderately hairy and less hairy plants. In our bioassays, leafhoppers did not reproduce on Brassica plants as no eggs were observed on leaves of *B. villosa*, *B. napus* DOS-2 or AC Excel. In the leafhopper colonies, a few eggs were occasionally found in leaves of these Brassica plants.

Transmission bioassays indicate that aster leafhoppers can transmit AYp to the DOS-2 and AC Excel at low frequency, with 1/54 plants testing positive for phytoplasma. AY leafhoppers did not show any preference in feeding on stems or petioles, regardless of the lines and there was no statistical difference in the number of leafhoppers feeding on DOS-2 compared to AC Excel.

**4) Gather information on the interactions between flea beetles, DBM and aster leafhopper with *B. villosa* and hairy lines on *B. napus*.**

This objective was not achieved, as statistical analyses and more field observations remain to be conducted.

**Figures, graphs and tables**

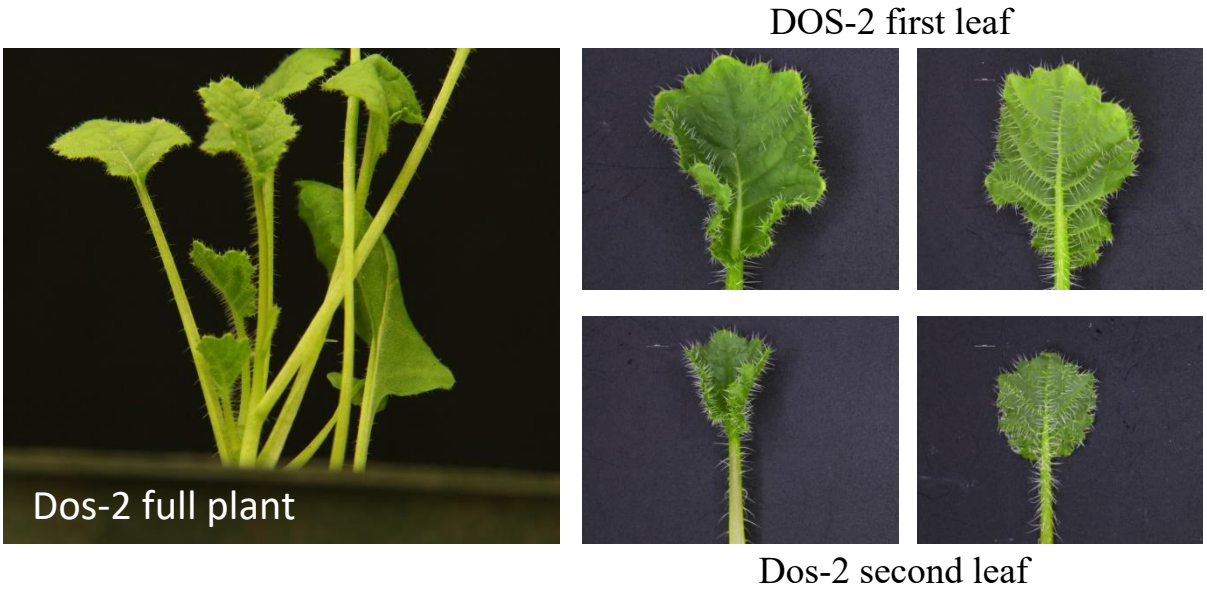


**Fig. 1.1:** Dos-2 full plants and first and second leaf of DOS-2 with trichomes


**Fig.1.2**: *B. villosa* full plant and leaf and petiole with trichomes

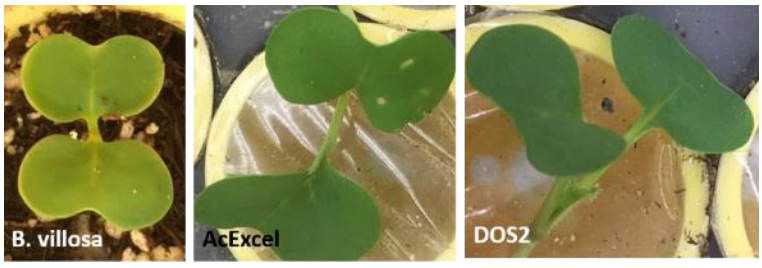


**Fig1.3**: Glabrous cotyledons of *B. villosa*, AC Excel and DOS-2.

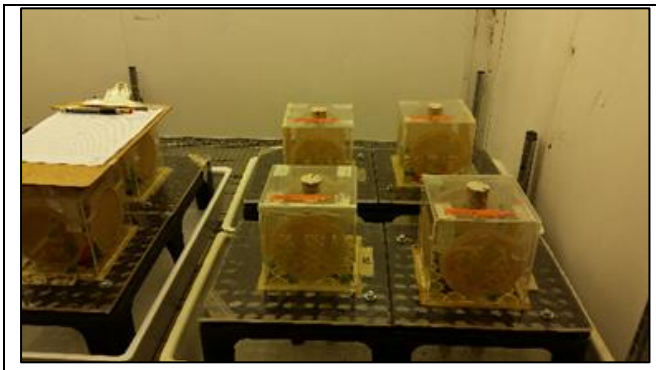

**Fig. 1.4**: Flea beetle bioassay set up.

**Fig. 1.5:** Jars with SFB in growth cabinet conditions for pre-hibernation (19 °C for 8 hours of light and 7°C for 16 hours of dark), to expose the beetles to the fall temperature for 1 month. For true hibernation, the jar is covered with black cloth and kept in a fridge at 5°C for a 6 weeks. After 6 weeks, SFB are ready to start a new colony and set up in a regular growth cabinet (22°C night/25°C day) with pots of canola seedlings.

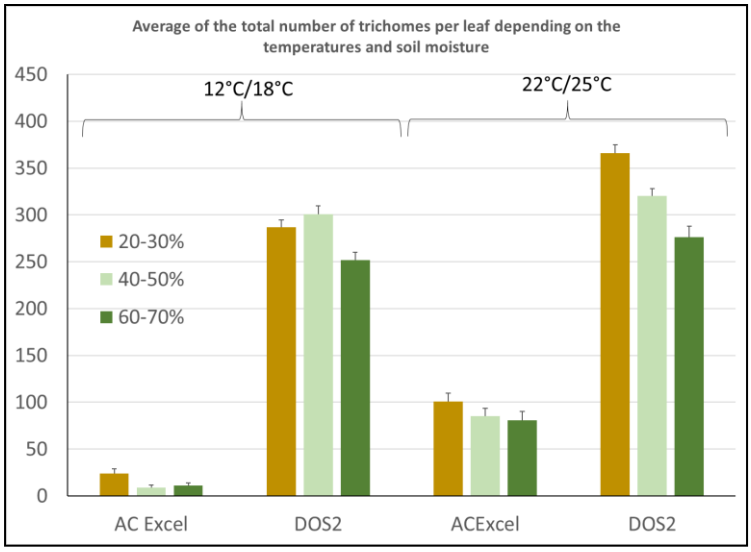


**Fig1.6:** Average total number of trichomes per leaf of DOS2 and AC Excel, at the 2-3 leaf stage, depending on the temperature and soil moisture used to grow the plants.

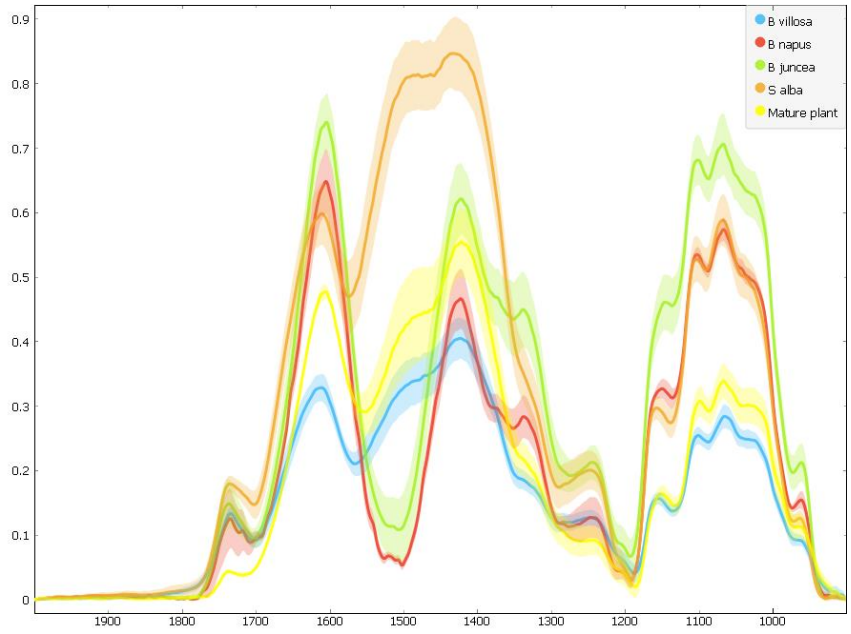


**Fig.1.7.A:** Mid-IR spectra of trichomes of B. villosa, B. napus, B. juncea and S. alba (2 leaves stage and mature)

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ***B. Villosa*** | 1734 | | 1612 | 1517 | | 1423 | 1338 | 1242 | 1159 | 1103 | 1064 | 1020 | 960 |
| **Protein** | 1739 | 1652 | | 1541 | 1448 | 1429 | 1338 | 1243 | 1160 | 1112 | 1080 | 1038 | |
| ***B. napus*** | 1735 | | 1606 | | | 1423 | 1336 | 1238 | 1150 | 1102 | 1066 | 1015 | 960 |
| ***B. juncea*** | 1737 | | 1604 | | | 1421 | 1336 | 1242 | 1147 | 1103 | 1067 | 1026 | 962 |
| ***S. alba*** | 1738 | 1651 | 1610 | 1535 | | 1421 | 1326 | 1247 | 1157 | 1103 | 1066 | 1019 | 960 |
| **Mature *S.alba*** | 1736 | | 1606 | 1513 | 1450 | 1421 | 1331 | 1237 | 1149 | 1100 | 1065 | 1024 | 960 |
| | | | C-C aromatic | | | CH2 deformation | | C-O stretching | C-O-C | C-O stretching | | | C-O bending |
| | | | Phenolics? | | | Cellulose | | | Hemicellulose | Pectin? | Cellulose | Galactomannan | Pectins |
| | | | Three v (C-C) aromatic conjugated with C=C (1551, 1515 & 1440). | | | | | | Lignin | | Hemicellulose | | |

**Fig.1.7.B:** Table with preliminary Peak Assignment at various energy levels observed for trichomes in Fig.1.7.a.

FB damages on DOS 2 (left) and AcExcel (right) on June 5, 2020.
Small field trial, AAFC experimental farm Lowe road

**Fig. 2.1:** FB damages on DOS2 (left) and AC Excel (right), June 5, 2020. Small field trial, AAFC experimental Farm, Lowe Road Saskatoon.

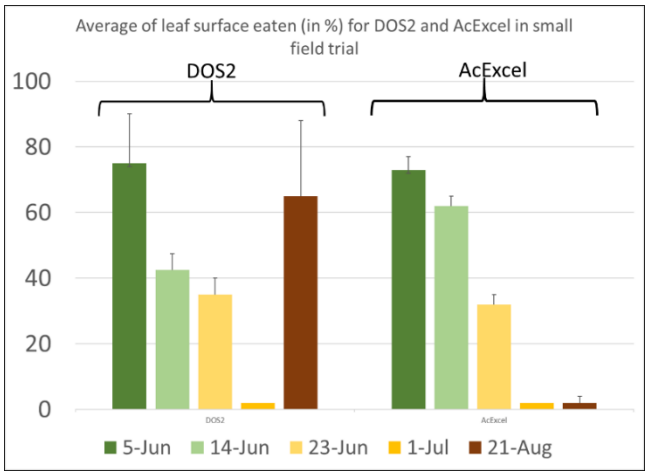


**Fig2.2**: Average of leaf surface damaged by FB in the small field trials

**Fig.2.3**: Rows of DOS2 (left) and AC Excel (right), August 23, 2020. Due to the late maturity of DOS2, FB damages is very high on DOS2 leaves (35-50%).

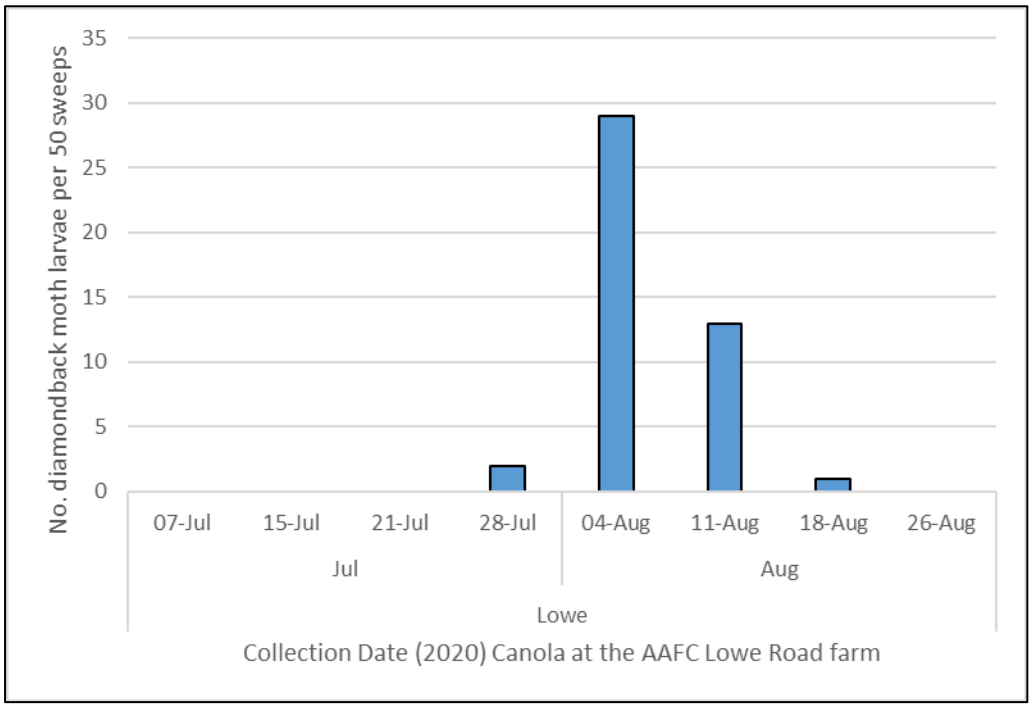


**Fig. 2.4:** Total number of diamondback moth larvae per 50 sweeps through July and August 2020 in the Canola field at the AAFC Lowe Road farm.

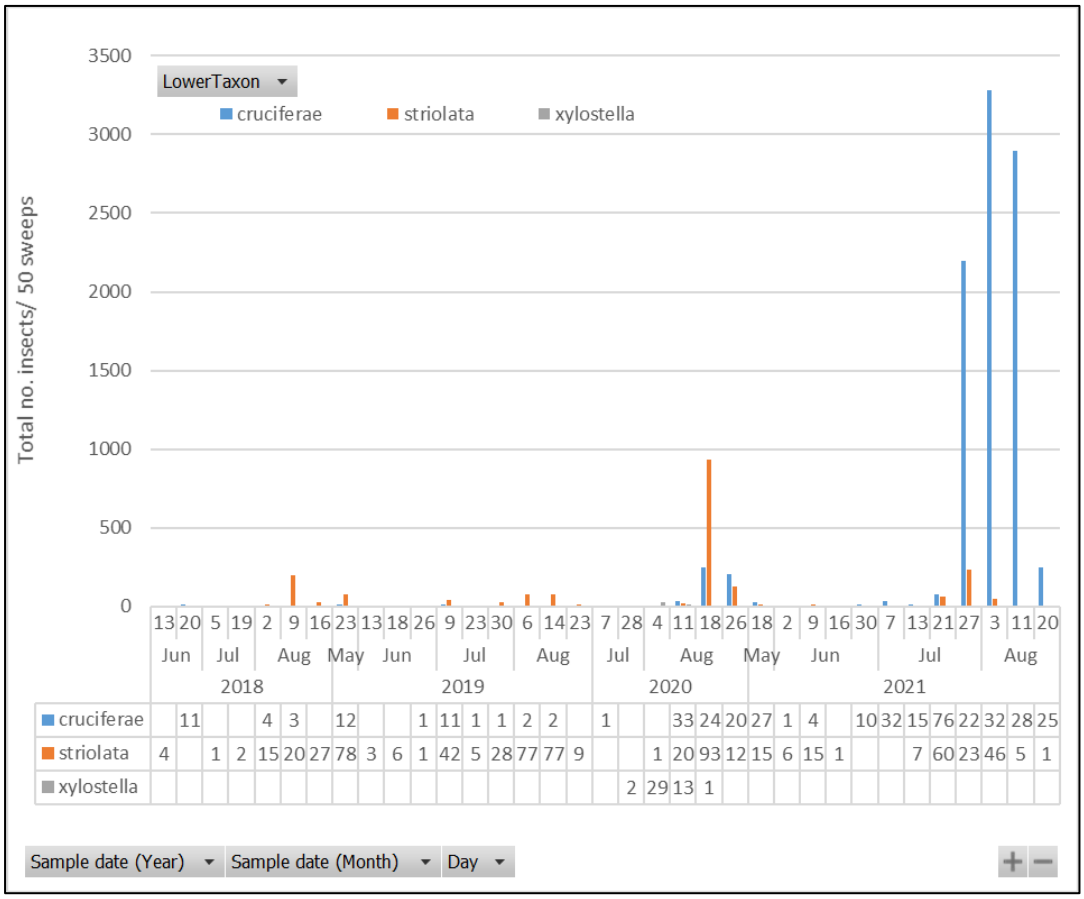


**Fig. 2.5:** Total numbers of flea beetles (*Phyllotreta crucifera* and *P. striolata*) and diamondback moth larvae (*Plutella xylostella*) collected in 50 sweep samples in Canola sampled at the AAFC Lowe Rd Farm 2018-2021.

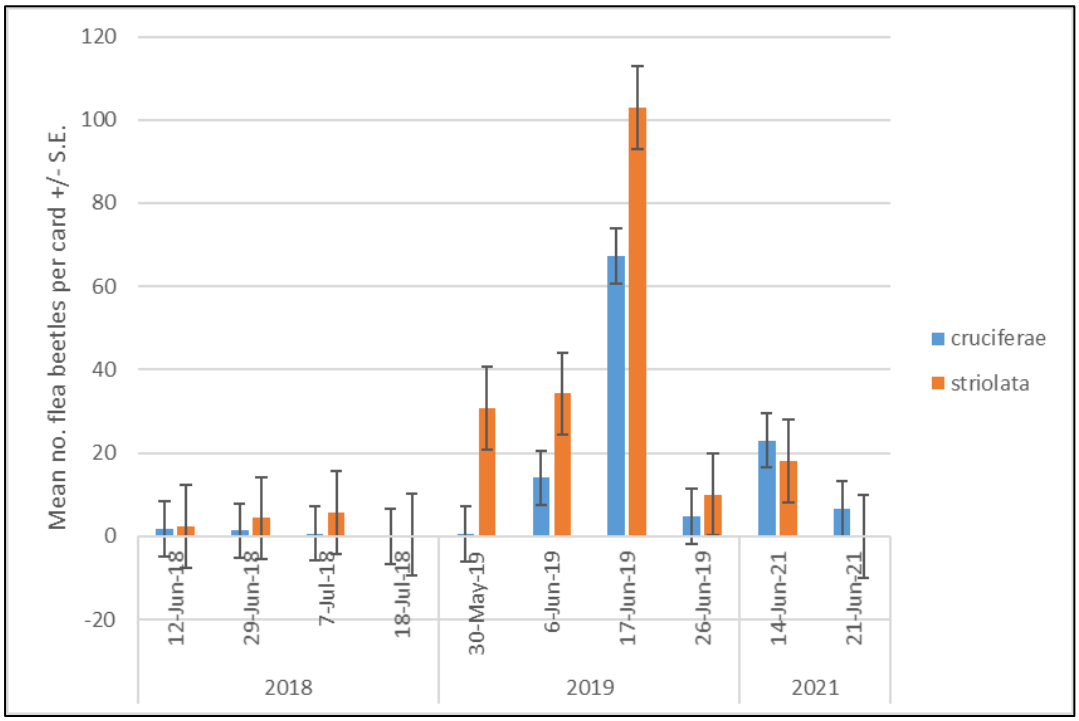


**Fig. 2.6:** Spring/overwintered populations of flea beetles (*Phyllotreta crucifera* and *P. striolata*) presented in average number per yellow sticky card (n=3 2018, n=4 2019, 2021) ±S.E. in non-insecticide treated (seed or spray) canola plots at the AAFC Lowe Road Farm over the project years minus 2020.

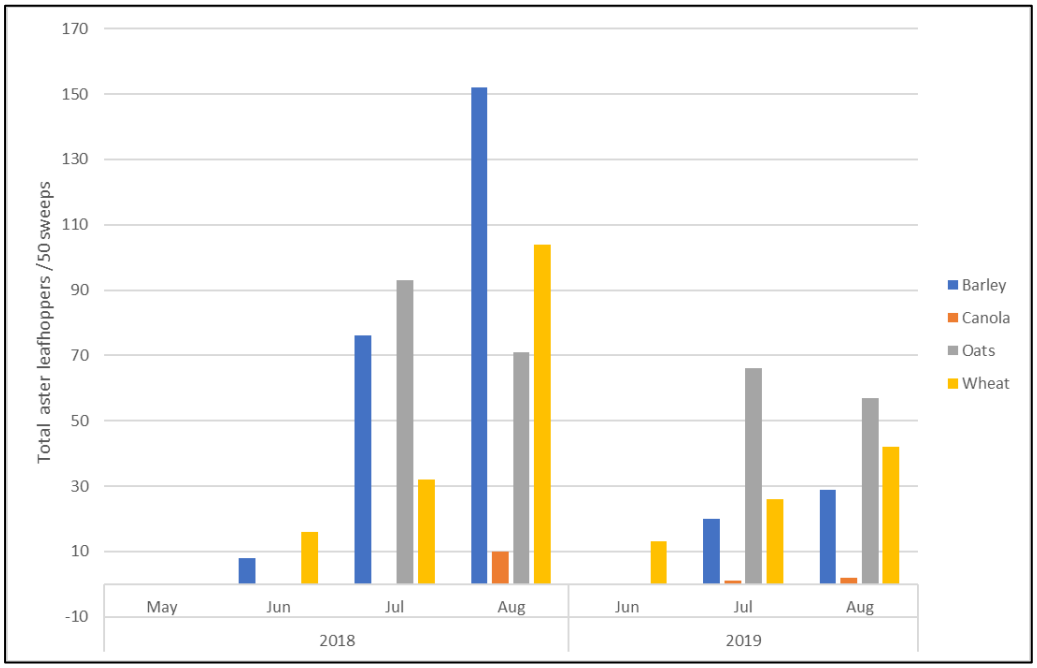


**Fig. 2.7:** Total number of aster leafhoppers (adults and nymphs combined) in barley, oat, wheat and canola fields at the AAFC Lowe Road farm in 2018 and 2019.

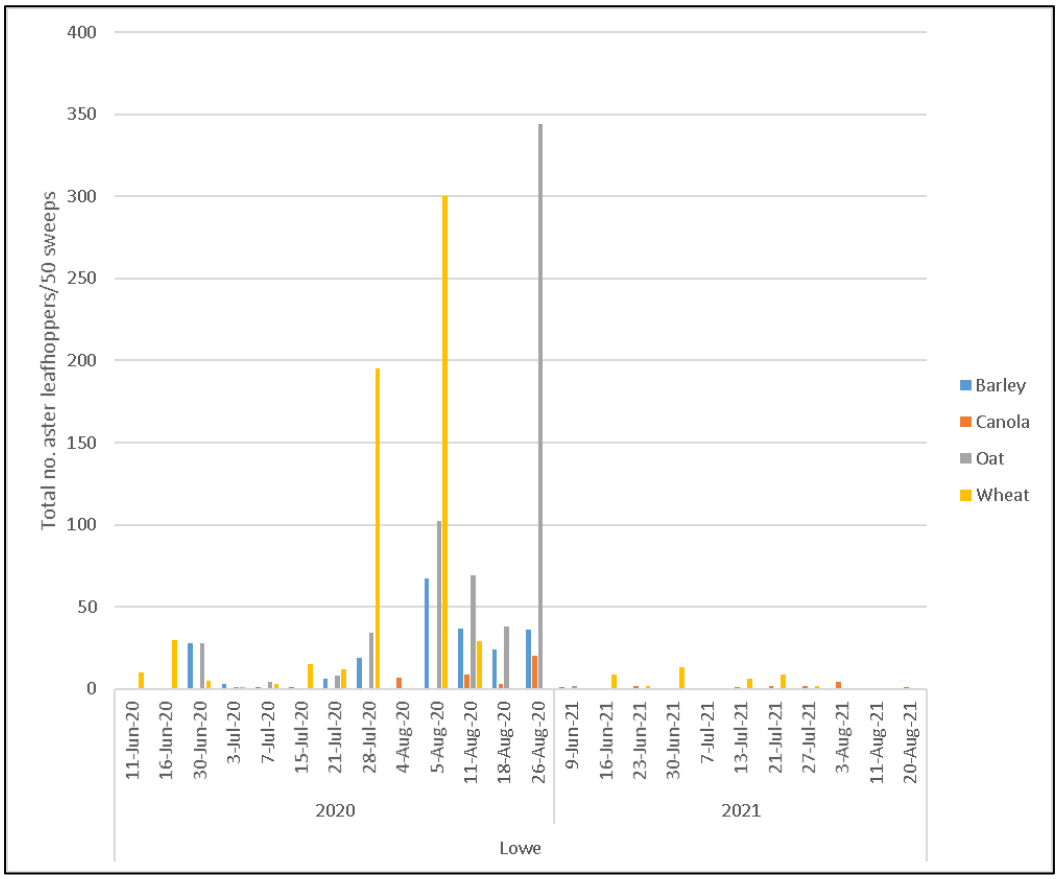


**Fig. 2.8:** Total number of aster leafhoppers (adults and nymphs combined) in barley, oat, wheat and canola fields at the AAFC Lowe Road farm in 2020 and 2021.

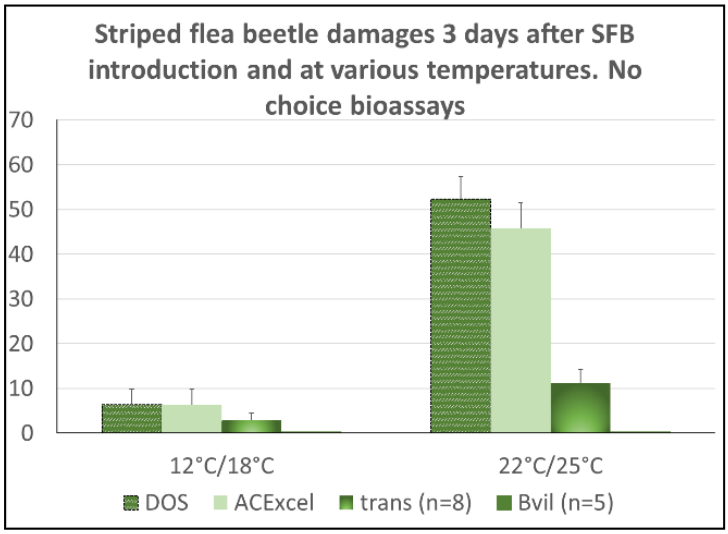


**Fig.3.1.** Feeding damages on DOS2, AC Excel, transgenic canola and *B. villosa* caused by SFB 3 days after SFB introduction and depending on the temperatures.

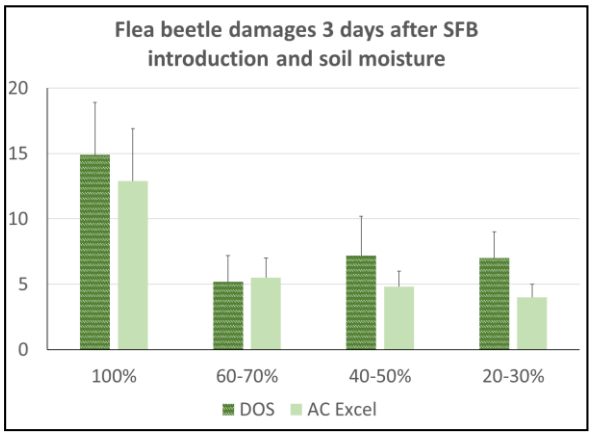


**Fig.3.2** : Feeding damages on DOS2 and AC Excel caused by SFB 3 days after SFB introduction and depending on soil moisture.

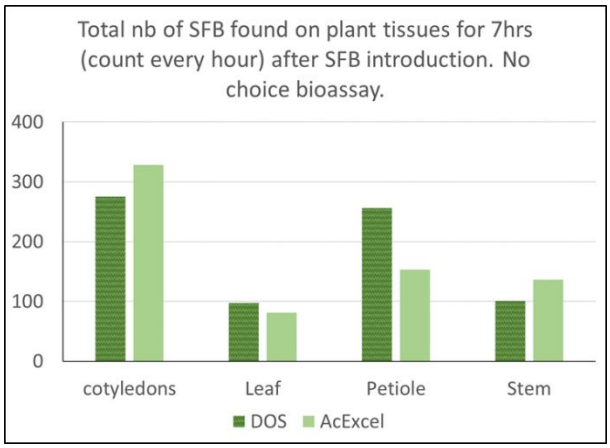


**Fig.3.3.** Total number of SFB found on cotyledons, leaves, petioles and stems of DOS2 and AC Excel in choice and no choice bioassays during the first 7hrs of their introduction on plants.

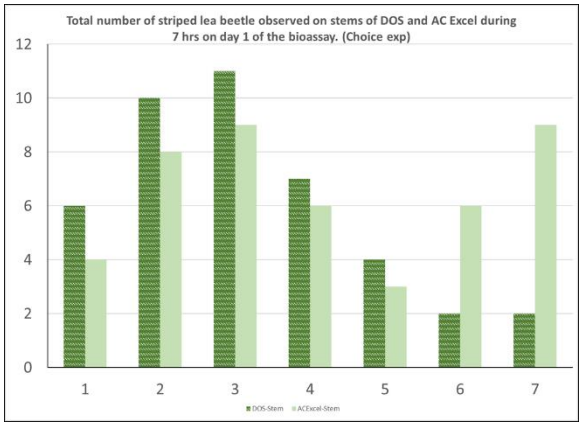


**Fig. 3.4:** Total number of SFB found on stem of DOS2 and AC Excel during the first 7hrs after SFB introduction in choice bioassays.

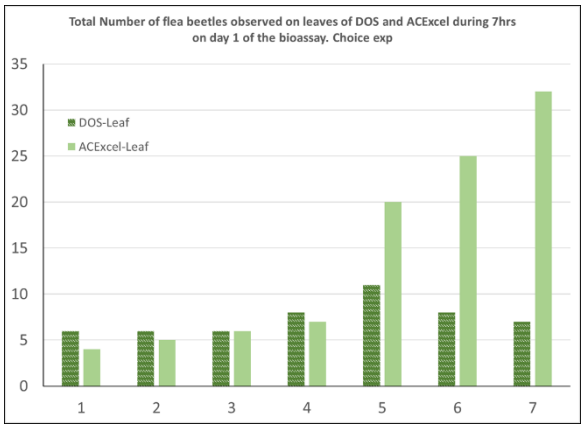


**Fig.3.5:** Total number of SFB found on leaves of DOS2 and AC Excel during the first 7hrs after SFB introduction in choice bioassays.

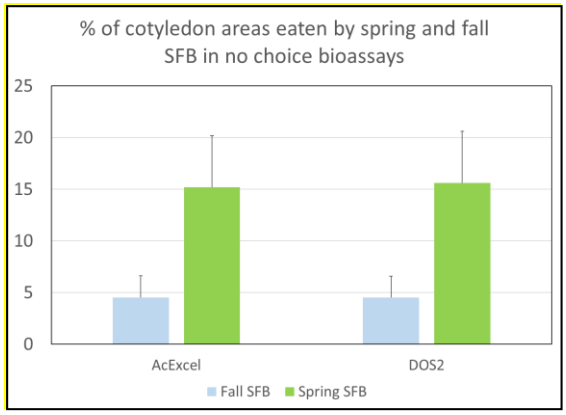


**Fig 3.6.** Feeding damages on DOS2 and AC Excel in no-choice bioassays caused by spring (May & June collections) and fall (August collections) SFB populations .

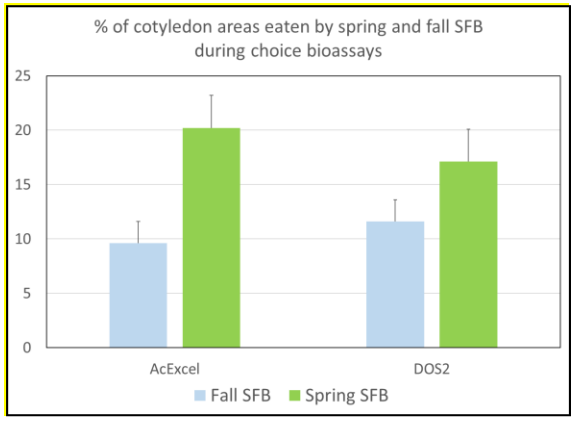


**Fig 3.7**.Feeding damages on DOS2 and AC Excel in choice bioassays caused by spring (May & June collections) and fall (August collections) SFB populations .

**Fig 3.9:** SFB lab colony larva observed in the soil .

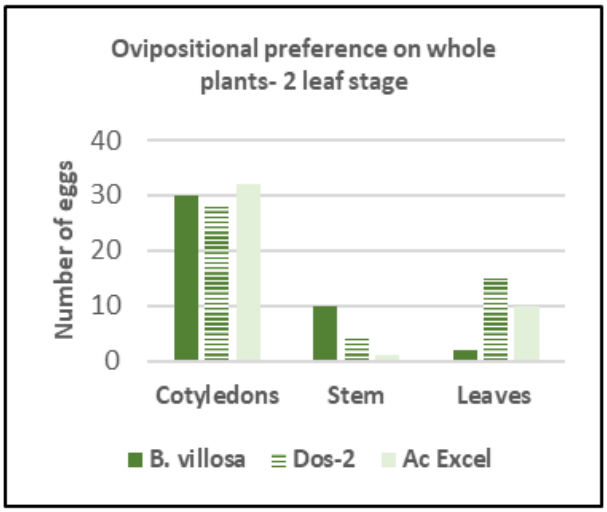


**Fig 3.10:** Ovipositional preference of DBM on DOS2, AC Excel and *B. villosa* (Total number of eggs/tissues)

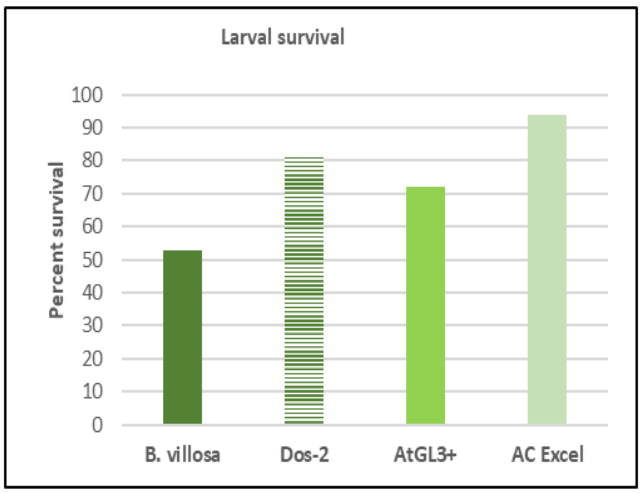


**Fig.3.11**: % of 1st instar survival on leaves of *B. villosa*, DOS2, Transgenic plant (AtGl3+) and AC Excel.

**Fig.3.12:** First instar larvae of DBM having difficulty mining and feeding on *B. villosa* leaves.

**Fig. 3.13**: Newly emerged larvae (2 larvae/ leaf) on leaves of *B. villosa* (top and left) & AC Excel (right and bottom) moved from *B. villosa* to AC Excel leaf within 24hrs and actively feed on AC Excel leaves

**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.

During the project, field trials could not be conducted every year, due to the small number of seeds and Covid restrictions. In 2020, a small scale field trials was conducted at the Saskatoon RDC Lowe Road site and showed that leaf damages (% of fed leaf areas) at the cotyledon stage were very similar between hairy (DOS2) / non-hairy canola cultivars (AC Excel). However, at later stage (4 leaf stage), leaf damages were higher on AC Excel compared to DOS2. In the 2020 field plots, there were very few diamond back moths (larvae and adults), and no DBM damage could be estimated. Therefore, we could not confirm the bioassay results in the field.

In lab bioassays, DBM larvae overwhelmingly avoided the hairy leaves, with the first instars having difficulty mining the hairy canola leaves. Trichomatous leaves bode well as a DBM deterrent as this trait is expressed on leaves throughout the life of the plant.

In the lab bioassays, aster leafhopper did not exhibit feeding deterrence or preference for hairy or non-hairy canola plants (similar average number of aster leafhoppers in all plants), but layed very few eggs in all crucifer plants. In the 2020 field plots, there were very few aster leafhoppers. Leaf samples were taken from the plot and have all been tested negative for the presence of AY.

Development of hairy canola lines is still a work in progress. Hairy Brassica lines that have been developed are not Canola quality and so field testing of plants should be considered as only very preliminary. Deterrence of DBM larvae is promising, while the destruction of early-season seedlings by flea beetles in 2021 is not. The cotyledons are embryonic and not plant tissue, and this situation poses a problem with no trichome expression on cotyledons. Cotyledons therefore, cannot be protected by defensive trichomes, but another potential form of deterrence seems to be in action on the transgenic, DOS-02 and *B. villosa* lines that warrants further study.

**7. Extension and communication activities**: (e.g. extension meetings, papers produced, conference presentations made, photos).

**Fredericton & Berlin 2019, article on synchrotron work**

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

**-acknowledgments at the end of the presentation.**

**9. Literature Cited**.

**10. Other Administrative Aspects:** personnel involved; equipment bought; project materials developed

**Jennifer Holowachuck, EG-04 Technician**

**Ruwandi Andrehenadi, Biologist**

**No major equipment was purchased for this project.**

**DOS-02 Canola and Brassica villosa lines were developed to the point where bioassays and limited field tests were able to be conducted.**

**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

**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