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ADF Project Final Report Format

1. **Project title and ADF file number.**

   Ecology of swede midge host plant interactions, Project #20140159, Reporting Period: 2019-2020 (Year 4)

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4. **Abstract/ Summary**: *An outline on overall project objectives, methods, key findings and conclusions for use in publications and in the Ministry database (Maximum of 500 words or one page in lay language).*

The swede midge, *Contarinia nasturtii* (Diptera: Cecidomyiidae), is an invasive insect pest of canola (*Brassica napus* L. and *B. rapa* L.) and other brassicaceous vegetable crops. Swede midge causes significant damage to canola in eastern Canada. Swede midge larval feeding can cause significant plant distortion including crumpled leaves, swelling of buds or petioles, corky scarring and death of the meristem. However, little is known about the chemical ecology of swede midge and its associated susceptible and resistant host and non-host flora. Here, we examined 1) the host range of swede midge with emphasis on common brassicaceous weeds found on the Prairies; 2) host plant resistance in these weed species; 3) the potential biochemical basis of resistance; and 4) examined the factors that make plants susceptible to swede midge damage. We found swede midge could infest all plant species tested except for *Descurainia sophia* (flixweed). Swede midge had lower preference for *Camelina microcarpa*, *Lepidium densiflorum*, and *Arabidopsis thaliana* compared to other plant species tested indicating possible host plant resistance (nonpreference). All crop cultivars tested were highly susceptible to swede midge infestation. Glucosinolates do not appear to play a role in swede midge host plant choice and other factors should be investigated for mechanisms of resistance (e.g. plant morphology, or other chemical factors). Investigation of infested *B. napus* (AC Excel) at the gene-level revealed that although swede midge triggered a plant defense response, swede midge larvae can still survive on the plant. Several genes involved in plant cell structure were highly expressed in infested plants, indicating they may be involved in swede midge gall formation, which can result in severe plant deformation and yield loss. Further investigations of these genes and their involvement in gall formation is warranted to potentially develop resistance to swede midge.

5. **Extension Messages:** *key outcomes and their importance for producers/industry (3-5 bullet points in lay language).*

- Swede midge can infest most common brassicaceous weeds on the Prairies. Therefore, if swede midge does establish on the Prairies, control of these weeds will be vital to remove alternative hosts and reduce insect population densities.

6. **Introduction:** *Brief project background and rationale.*

The swede midge, *Contarinia nasturtii* (Diptera: Cecidomyiidae), is an invasive insect pest of canola (*Brassica napus* L. and *B. rapa* L.) and other brassicaceous vegetable crops (Chen et al. 2011). Swede midge causes significant damage to canola in eastern Canada (e.g. Quebec and Ontario) and, although it has yet to establish, it threatens canola production on the Prairies (Williams and Hallett 2017; CFIA 2009). Swede midge larval feeding can cause significant plant distortion including crumpled leaves, swelling of buds or petioles, corky scarring and death of the meristem (Barnes 1946; Hallett and Heal 2001). However, little is known about

the chemical ecology of swede midge and its associated susceptible and resistant host and non-host flora. Because chemical control of a multi-generational cryptic pest such as swede midge is difficult, because cultural control results may be variable even if they were known, and because no effective biological control agents have been found in North America, it is paramount that host plant resistance be investigated as a source of durable, potentially long-term control of swede midge, as has been found for wheat midge on the prairies.

7. Objectives and the progress towards meeting each objective.

Objectives (*Please list the original objectives and/or revised objectives if Ministry-approved revisions have been made to original objective. A justification is needed for any deviation from original objectives)*	**Progress (e.g. completed/in progress)**
a) Examine host range of swede midge	A list of potential Brassica reservoir hosts on the prairies has been established. A subset of 12 species have been tested in a series of no-choice oviposition and larval development bioassays and choice oviposition assays. ***Complete***
b) Investigate host plant resistance of non-hosts	Results indicate all plants tested are susceptible to swede midge, but the number of eggs laid and larvae developing differ between species indicating some host plant resistance may be present (antixenosis, antibiosis, tolerance). Laboratory studies on seed yield of select plant lines found no differences between infested and uninfested plants. ***Complete***
c) Investigate biochemical basis of resistance	There appears to be some resistance to swede midge in *Descurania sophia* (Flixweed). The glucosinolate profiles of select plants is currently being measured. ***Partially complete***
d) Examine plant susceptibility factors to swede midge feeding	Investigation into the plant recognition pathway for swede midge larvae has begun with a comparative transcriptome (e.g. gene expression) study. Sequencing has been performed on plants 0, 3, and 10 days post-swede midge infestation, and plant hormones analyzed. ***Complete***

Please add additional lines as required.

8. Methodology: *Specify project activities undertaken during entire project period. Include approaches, experimental design, methodology, materials, sites, etc.*

The preference of adult female swede midge towards a variety of host plants was tested in the laboratory during the lifespan of this project (Table 1). All of the species tested belong to the family Brassicaceae; some are crop plants, but most are annual weeds that could serve as alternative developmental hosts for swede midge larvae across the Canadian Prairies.

No choice oviposition and larval development bioassays

No-choice oviposition bioassays were conducted on 12 plant species (Table 1) at the early bud stage. Two separate trials of these 12 plants were tested prior to and after a lighting retrofit at AAFC. Planting was staggered in order to have plants at a similar growth stage (i.e. bud) for oviposition, which included a 6-week vernalisation period for *Camelina microcarpa*, *Descurania sophia*, and *Lepidium densiflorum*. Single plants were placed in square cages and 8 female and 4 male swede midge (<24 hours old) were added to the cage. After 48 hours the midge adults were removed. In the oviposition experiments, plants were then dissected and examined for the presence of eggs. For the larval development experiment, plants were maintained for an

additional 10 days, after which the plants were dissected to determine the presence and number of larvae. To determine the relative developmental stage of the larvae, all larvae were examined for the presence of a spatula, a morphological feature which is found in third instar larvae. Analysis-of-variance (ANOVA) was used to compare the number eggs laid and number of larvae developing on the various plant species and was performed in SAS v. 9.1 (SAS Institute, Cary, NC).

Oviposition choice bioassays

Choice oviposition bioassays were conducted on 11 plant species, as above (except for *Arabidopsis thaliana*), at the early bud stage. *Arabidopsis thaliana* was not tested as it is a model organism and swede midge on the Prairies would not come in contact with it in the wild. Plants were grown, as described for the no-choice experiment, and placed in a circle in a large insect cage (Bugdorm). Eighty-eight females and 44 male swede midge (<24 hours old) were added to the cage (8 females and 4 males per plant). After 48 hours the midges were removed, and the plants dissected and examined for the presence of eggs. Plants were randomized around the circle in each replicate (n=7). A second choice experiment was conducted with the same 11 plant species, plus the addition of *Brassica oleraceae* (cauliflower).

Choice experiments should be analyzed using multivariate statistics because choice experiments do not meet the assumption of independent treatments required for univariate analysis of variance (ANOVA; Chesson 1983; Roa 1992). However, due to the number of choices presented to the swede midge (11) and low replication (n < 8) compared to the number of treatments, we did not have enough degrees of freedom to conduct a powerful multivariate analysis. There was also considerable variation between replicates and host plants that complicated the analysis. Here we presente univariate ANOVA results to compare female preference for the host plants. Experience gained during this experiment will guide the design of future choice experiments to better understand swede midge host preference for brassica crops and alternative hosts. Analysis-of-variance (ANOVA) was performed in SAS v. 9.1 (SAS Institute, Cary, NC).

Mechanisms of swede midge resistance

To determine if tolerance is a possible mechanisms of swede midge resistance, selected plants (*B. napus*, *C. sativa*, *E. cheiranthoides*, *N. paniculata*, and *T. arvense* were grown as above, and in a paired design, were either exposed to 8 ovipositing swede midge females or left as untreated controls. Females were allowed to oviposit for 24 hours after which they were removed and the plants allowed to complete their development. Once development was complete, plants were removed from cages and dried at room temperature for 3 weeks. Seed was harvested by hand from each plant, and 1000 seed weight was determined. The effect of host plant species and infestation status on yield was determined using a two-factor ANOVA design using SAS 9.1 (SAS Institute, Cary, NC).

In addition, to determination of tolerance via seed yield, we also wanted to determine if tolerance to swede midge damage could be assessed via an indirect method, application of 2-4-D to mimic damage symptoms. *Brassica napus*, *B. oleracea*, *A. thaliana*, *D. muralis*, *N. paniculata*, and *S. arvensis* were grown as above, and after 6 weeks of growth, 2-4-D was injected onto each growing point, untreated controls were injected with water following the methods of Brewer et al. 1994. The plants were allowed to continue to grow, and after one week their damage symptoms were visually assessed to compare with known swede midge damage.

Investigation of the biochemical basis of resistance

In another choice bioassay, *S. alba* lines with various levels of seed glucosinolate (GLS) and erucic acid (EA) content (J. Soroka, unpublished data) were tested to determine if seed glucosinolate content influences the biochemical basis of resistance. Four varieties of *S. alba* (AC Pennant (high GLS, high EA), JS99-503 (high GLS, low EA), JS00-531 (low GLS, low EA) and JS99-504 (low GLS, high EA)) and *B. napus* (AC Excel) where tested

following the same experimental procedure as above; however, the number of midges were reduced to 40 females and 20 males (8 females and 4 males per plant).

Finally, to determine if tissue glucosinolates play a role in potential resistance to swede midge, 10 plant species (*A. thaliana*, *B. napus*, *B. oleracea*, *C. sativa*, *D. muralis*, *E. cheiranthorids*, *N. paniculata*, *S. alba*, *S. arvensis*, and *T. arvense*) (n = 9 of each) were grown to the bud stage. The growing points of each plant were then removed and flash frozen in liquid nitrogen, prior to freeze drying. Samples were then provided to the Oilseed Chemistry Laboratory at AAFC-Saskatoon for glucosinolate analyses following the protocols of Raney and McGregor (1990) and Soroka and Grenkow (2013).

Examining plant susceptibility factors to swede midge

In order to investigate the recognition system used by plants to perceive insect attack, we conducted an experiment to determine the gene expression levels within the plants post swede midge infestation. A moderately susceptible line, *B. napus* (AC Excel), was grown in the greenhouse until the 6-leaf stage, after which plants were infested with swede midge larvae or left as untreated controls. Plant meristematic tissue was harvested from 5 plants on day 0 (control only), day 3, and day 10 (control and infested). The tissue was cut in half and immediately flash frozen in liquid nitrogen. RNA was extracted from the samples and 3 replicates from each treatment (control or infested) at each time point were sent to the NRC Saskatoon Laboratory for high-throughput Illumina sequencing (RNA-Seq). Additionally, the other half of the plant sample was sent to Dr. Eiji Nambara (University of Toronto) for plant hormone analysis.

RNA-Seq analysis:

Libraries were prepared at NRC with an Illumina TruSeq stranded mRNA kit according to the manufacturer's protocol. Paired-end, 125 bp cDNA libraries were sequenced on an Illumina HiSeq 2500 over three lanes, resulting in ~35 million reads per library. After sequencing, the data was downloaded from NRC and the quality of sequenced reads was examined with FastQC v0.11.5 (Andrews 2013), and the reads were cleaned to remove adaptors and low-quality sequences with Trimmomatic v0.36 (Bolger et al. 2014). After clean up, there were ~30 million reads per library (n = 15 libraries). Reads were then mapped to the *B. napus* genome (va. DH12075 provided by Dr. Isobel Parkin) in CLC Genomics Workbench v.8.1. The resulting read counts from CLC were used for differential expression analyses using 'edgeR' v.3.16.5 (Robinson et al. 2010) in R v.3.3.2 (R Core Team 2016). Only those transcripts with at least 1 count per million reads (CPM) in at least three of the libraries, which would correspond with a gene being differentially expressed (DE) in at least one library, were tested statistically. All DE genes with at least $\log_2$ fold change of 2 (4-fold increase) in each tissue type were examined for significant differences (i.e. control vs. 3 or 10-day post infestation). A correction for multiple comparisons was made using the false discovery rate (p <0.05) (Benjamini and Hochberg 1995). To further evaluate the pattern of gene expression in the infested tissue, a gene ontogeny (GO) enrichment analysis was performed with the 'topGO' package (Alexa and Rahnenfuhrer 2019) in R v.3.3.2.

Plant hormone analysis:

Plant hormones are known to aid in plant defense responses to insect attack. By measuring hormone levels we will gain knowledge on plant receptors involved in plant perception of swede midge. In order to determine how the plant responds to swede midge infestation the concentration (ng hormone/g dry plant weight) for 5 plant hormones was measured including: salicylic acid (SA), indole-3-acetic acid (IAA), jasmonic acid-isoleunce (JA-Il), trans-zeatin (tZ), and 2-isopentyl adenine (2iP). Briefly, samples of infested tissue (collected as above) were freeze dried and then homogenized. Internal standards were added, and the samples extracted with methanol containing 1% acetic acid. Samples were subjected to three cartridge columns, HLB, MCX, and WAX (Waters) and subjected to LC-ESI-MS/MS analysis (Agilent LC-MSMS 6410). LC conditions and MS followed those in Preston et al. 2009. For the full protocol, please see Preston et al. 2009. The concentration of each plant hormone was compared between the control and infested tissue at each time point with either a Welch's two-sample t-test when the data were normally distributed or a Wilcoxon rank sum test when data were not normally distributed. Treatment effects were considered significant at $p < 0.05$.

All statistical tests in all experiments were conducted in R v.3.3.2 (R core Team 2016), unless otherwise noted.

9. **Results and discussion:** *Describe results accomplished during the entire project period under each objective listed under section 6. The results need to be accompanied with tables, graphs and/or other illustrations. Provide discussion necessary to the full understanding of the results. Where applicable, results should be discussed in the context of existing knowledge and relevant literature. Detail any major concerns or project setbacks.*

Objective A: Examine host range of swede midge; and **B:** Investigate host plant resistance of non-hosts

Two series of no-choice tests were conducted inside growth cabinets at AAFC-Saskatoon, the first series occurred before the lights were retro-fit in the growth cabinet and the second was conducted after the light retro-fit. In the first series, the identity of the host plant affected the number of eggs laid by female swede midge ($F_{11, 101} = 2.80$, $p = 0.0032$), indicating host preference. Of the 12 brassica species tested, one was rejected as an oviposition host (*D. sophia*) and all others were accepted, although the mean (±SE) number of eggs laid on the acceptable hosts ranged from 5.00 ± 2.37 to 76.27 ± 29.37 (Figure 1). Some individual *B. napus* and *A. thaliana* plants had over 90 eggs laid on them, while some individual *C. sativa*, *S. alba*, *T. arvense*, and *D. muralis* plants received over 100 eggs. Unlike, Boddum et al. (2018) who found no eggs were laid on *A. thaliana*, we found both eggs were laid and larvae could develop on *A. thaliana*. If swede midge can consistently lay eggs and larvae develop on *A. thaliana* there may be potential to create a model system to investigate gall formation on a natural host (e.g. swede midge – *A. thaliana* interactions) which is not currently available. A model system would allow the dissection of plant responses to swede midge, and may aid in the identification of susceptibility or resistant factors. Further investigation into this system is warranted as *A. thaliana* is already a model plant species used to investigate plant physiology, morphology, defenses, etc. (Meinke et al. 1998, Koornneef and Meinke 2010).

In the second series of no-choice tests, host plant identity also affected the number of eggs laid by female swede midge ($F_{6, 35} = 3.99$, $p = 0.0038$). In this experimental series, four host plants were rejected by ovipositing females: *C. microcarpa*, *C. sativa*, *D. sophia*, and *N. paniculata*. Only *D. sophia* was rejected before and after the light retrofit in the experimental chamber. During this experimental series, the mean (±SE) number of eggs on the acceptable hosts ranged from 1.33 ± 0.95 to 106.33 ± 35.95 (Figure 2). The most eggs laid on an individual plant was 245 laid on *D. muralis*. In both experimental series, females laid more eggs on *B. napus*, *S. alba*, *D. muralis*, and *T. arvense* than on the other potential hosts, although the differences were not always significant. The no-choice oviposition experiment confirms that swede midge will oviposit on all plant species tested at the bud stage except for *D. sophia* (flixweed). Hallett (2007) also found that swede midge did not infest *D. sophia* in the field in Ontario. The number of eggs laid by female swede midge varied considerably between host plants of the same species in both experiments. Variation may have resulted from slight differences in the appearance, nutrient content, or developmental stage of the individual plants, or individual variation between the female swede midge used in the experiments (i.e., age, egg load, etc.). However, results from the no-choice experiments clearly demonstrate that swede midge can and will lay eggs on a wide variety of brassica host plants including most of the common weed species on the Prairies. This study confirms that swede midge will oviposit on *C. sativa*, *D. muralis*, *N. paniculata*, and *L. densiflorum*, none of which had been tested previously.

The first choice experiment, conducted in 2018, gave female swede midge the choice of 11 host plants for oviposition (Table 2). Female swede midge exhibited preferences for some of the host plants compared to others ($F_{10, 40} = 34.83$, $p < 0.0001$). Three host plants were rejected by swede midge: *E. cheiranthoides*, *L. densiflorum*, and *D. sophia*. The most preferred hosts were *B. napus* and *S. alba* (Table 2). These results agree with results of the no-choice experiments in which no eggs were laid on *D. sophia*, and lower numbers of eggs were laid on *E. cheiranthoides* and *L. densiflorum* (Figure 1 & 2).

The second choice experiment was conducted in 2019. It included the same 11 plants as in the 2018 experiment, with the addition of *Brassica oleracea*. In the choice arenas, female swede midge laid more eggs on

some plants compared to others ($F_{11, 66} = 24.91$, $p < 0.0001$). Females rejected *L. densiflorum*, *D. sophia*, and *C. microcarpa* for oviposition and strongly preferred *S. alba* and *B. napus* (Table 3). In addition, *B. oleracea* was an acceptable host, but received significantly fewer eggs than the most preferred hosts (Table 3). *Brassica napus* appears to be the most preferred host out of the species tested, which appears to rule out trap cropping for swede midge on the prairies as we did not find a species that swede midge preferred more than *B. napus*.

Swede midge larvae are restricted to developing on the host where their egg is deposited. Thus, swede midge populations should increase when female preference for host plants results in improved larval performance (optimal oviposition theory; Jaenike 1978). To address the impact of host plant on larval performance, we used a no-choice experimental design but instead of counting the eggs that were laid, we counted the larvae that hatched from the eggs one week after oviposition and classified them based on their stage of development. To determine the effect of host plant species on larval performance, we compared: (a) the number of larvae found on the plants after one week, and (b) the proportion of total larvae that reached the third instar of larval development after one week. The number of swede midge larvae present one week after oviposition differed between host plant species ($F_{11, 112} = 5.07$, $p < 0.0001$). The most larvae were found on *B. napus* and no larvae were found on *C. microcarpa* or *D. sophia* (Figure 3). Less than five larvae per plant were found on *A. thaliana* and *L. densiflorum* (Figure 3). Host plant species also affected the rate of larval development, based on the number of third instar larvae counted on the plants after one week ($F_{8, 61} = 3.48$, $p = 0.0023$). After one week, larval development by cohort was most advanced on *B. napus* (64.6%), *D. muralis* (65.7%), and *N. paniculata* (78.1%) (Table 4). Larval development by cohort was least advanced for larvae developing on *L. densiflorum* (15.9%) and *A. thaliana* (18.8%) (Table 4).

Altogether, these results indicate the swede midge females have a preference for host plants, but when preferred hosts are unavailable (as indicated in no-choice experiments) most weeds within the Brassicaceae found on the Prairies can be suitable hosts. This is similar to other studies that found swede midge females had flexibility in the plants on which to oviposit depending on which host plant species were available (Boddum et al. 2018). The complete lack of oviposition on *D. sophia* indicates that either it is not a suitable host or that the plant stage tested was not suitable for swede midge oviposition and development. Anecdotal observations of *D. sophia* indicate that it is not a host at any plant stage, thus the mechanism of host plant resistance would appear to be non-preference (i.e., antixenosis). The morphology of *D. sophia* is quite different than the crop species tested, and this cannot be ruled out as a factor contributing to resistance. A low number of eggs were laid on *C. microcarpa* in the first no-choice experiment and in both choice experiments, and no eggs were laid on this species in the second no-choice experiment and no larvae were found developing on *C. microcarpa*. However, it is not known if the lack of developing larvae was due to a lack of eggs laid, or if the plant created a defensive compound that killed any potential eggs. Further investigation is warranted on *D. sophia*, *C. microcarpa*, *L. densiflorum*, and *A. thaliana* to determine what makes these plants non-hosts for swede midge.

A swede midge outbreak on the prairies could be facilitated by the diversity of cruciferous weeds that grow along field margins, in un-managed areas, and in crops. However, many of these plants provide important ecosystem services by providing alternative food sources for beneficial insects such as pollinators and parasitoids. Thus, we were curious about the potential for population decline of alternative hosts and crop plant hosts (e.g., *B. napus*) of swede midge on the prairies. In a laboratory experiment, we compared the yield (1000 seed weight in grams) of plants exposed to swede midge oviposition and larval infestation to the yield of plants grown without exposure to swede midge. Seed weight varied between host plant species ($F_{4, 30} = 35.20$, $p < 0.0001$), which we expected due to differences in seed characteristics between the five plant species tested in this experiment (Figure 4). There was no effect of swede midge infestation on seed weight ($F_{1, 30} = 0.41$, $p = 0.5279$), and no interaction of host plant and midge infestation ($F_{4,30} = 0.47$, $p = 0.7538$). These results suggest that plants attacked by swede midge should yield comparably to non-attacked plants. However, a similar experiment should be conducted in field conditions to determine the effects of swede midge on crop and host plants in uncontrolled conditions and with varying levels of pest pressure, as observed in field crops.

Understanding yield losses associated with insect pests helps to estimate the impact of pests and determine appropriate economic thresholds to aid in the implementation of ecologically and agriculturally sound integrated insect pest management. Conducting research to estimate the yield impacts of insect pests is often difficult. For example, population densities may be difficult to manage in field conditions or mechanical

damage used to mimic insect damage might not accurately represent damage caused by insects. Mimicking insect feeding damage in laboratory and field studies can allow for experiments to be conducted without live insects, so this method should be tested as an option and not immediately discounted. Previously, research conducted on sunflower midge, *Contarinia schulzi*, indicated that injection of the herbicide 2-4D into the growing points of the plants could be used to mimic damage caused by the pest (Brewer et al. 1994). We tested the same method to determine if 2-4D could be used to mimic swede midge damage. Unfortunately, plant response to herbicide injection was not similar to swede midge infestation for any of the species that were tested (between midge infested and herbicide-injected plants (*B. napus*, *B. oleracea*, *A. thaliana*, *D. muralis*, *N. paniculata*, and *S. arvensis*). Injection with herbicide caused the plants to wilt, instead of affecting the growing points of the plants, as swede midge do. Control (injected with water) and herbicide-injected plants are shown in Figures 5 and 6.

Objective C: Investigate biochemical basis of resistance

In the third choice experiment, swede midge were given a choice of hosts consisting of *B. napus* and four lines of *S. alba* that vary in seed glucosinolate content. Female swede midge did not exhibit a preference for any host species or variety in this experiment (Wilk's $\lambda = 0.2494$, $F_{4, 5} = 3.76$, $p = 0.0894$; Table 5). Thus, seed glucosinolate content can probably not be used as a predictor of female choice for oviposition hosts and raises questions about the role of glucosinolates in host plant selection overall. Recent evidence indicates that glucosinolates may not be a factor in host plant choice of swede midge based on olfactory cues (Boddum et al. 2018); however, glucosinolates are known to be attractive oviposition cues for other species, especially, when contact with the plant is allowed (Sun et al. 2009). Thus, exploration of the glucosinolate content of leaf tissue was also proposed. Unfortunately, analysis of tissue glucosinolates is currently on hold. Samples were provided to the Oilseed Chemistry Laboratory at AAFC-Saskatoon in January 2020, but since this time period a network server crash followed by the recent COVID-19 pandemic and subsequent remote-work policy leaves the processing of these samples suspended indefinitely. However, given that swede midge can oviposit and larvae develop on the wide variety of species tested with various levels (low to high) of glucosinolates (e.g. Daxenbichler et al. 1991, Soroka and Grenkow 2013) may not be a contributing factor to host plant resistance. In addition, transcriptomic data obtained in another study found swede midge contain detoxification genes (e.g. myrosinase, glutathione-S-transferases, sulfatases, etc.) (Mori – unpublished) that have been shown in other insects to modify or metabolize glucosinolates and prevent them from harming the insect (Winde and Wittstock 2011).

Objective D: Examine plant susceptibility factors to swede midge feeding

Plant transcriptome experiments allow for the identification of all actively expressed genes at the specific stage harvested. There are over 100,000 genes in *B. napus* (Chalhoub et al. 2014) with varying genes expressed at different life stages. At 3 days post-swede midge infestation there were no differentially expressed (DE) genes between the control and the infested plant (results not shown). The lack of DE genes at this time point may be due to variability in the samples. Plant hormone analysis also revealed little change between hormone levels 3 days post-infestation (Figure 7). The only significant difference in plant hormone concentration was an increase in SA ($t = -2.67$, $df = 5.97$, $p = 0.037$). This confirms the SA pathway is activated upon swede midge feeding as is known to occur with many other insect pests including aphids (Thompson and Goggin 2006), cotton boll weevil (Artico et al. 2014) and other gall midges (Ollerstam and Larsson 2003). Salicylic acid is an important phytohormone involved in regulation of plant defenses against insects and is involved with both local defense and induction of systemic resistance (War et al. 2012).

At 10 days post swede midge infestation 6,437 genes were differentially expressed; 4,624 (71.8%) genes were upregulated, while 1,813 (28.2%) were downregulated. The GO enrichment analysis of GO biological terms (i.e. genes involved in biological processes) reveals many genes involved with response to plant stress, immunity and response to various stimuli are overrepresented (Table 6). This data provides the first insight into the response of *B. napus* to swede midge infestation and will provide valuable information on how the plant

responds to swede midge which may aid in identification of potential resistance mechanisms. Annotation of these differentially expressed genes revealed many different genes involved in cell signalling and response to stresses.

In order to respond to an insect threat, a plant must recognize it and trigger an appropriate response. Plants have pattern recognition receptors (PRRs) that can recognize conserved patterns in microbes, herbivores (including insects), and nematode-associated molecular patterns (MAMPs, HAMPs, and NAMPs, respectively) (Choi and Klessig 2016). Chitin, a large component of the insect exoskeleton, is a HAMP. Over 150 genes involved in response to chitin were enriched in infested plants (Table 6). These genes would enable *B. napus* to recognize the insect and start to mediate a defense response. Well known PRRs in plants are leucine-rich repeat (LRR) receptor-like kinases (RLK) (Panstruga et al. 2009), 53 LRR-RLKs were upregulated in infested plants compared to uninfested controls (S. Table 1). After recognition signal transduction occurs via mitogen-activated protein kinase (MAPK) pathways followed by activation of transcription factors (He et al. 2006). Twelve MAPK genes were upregulated following infestation (S. Table 1). MAPK genes triggers a signal cascade which ultimately results in defense gene activation via transcription regulators including WRKY transcription factors (Panstruga et al. 2009); 78 WRKY transcription factors were upregulated in infested plants (S. Table 1). Taken together, this information reveals one potential way swede midge is recognized by the plant (e.g. PRR, most likely LRR-RLK, may recognize chitin, and then trigger a plant defense via the MAPK-WRKY pathway). Even though this well recognized pathway is activated it fails to reduce swede midge damage, which emphasizes the need for further exploration of these genes to understand their exact role in swede midge infestations. Ultimately, swede midge may be using some of these factors to its benefit, and the "susceptibility" factors (Harris et al. 2015), those that the midge are using to successfully infest the plant, should be investigated further.

At 10-days post-infestation plant hormone levels are significantly different between the control and infested plants. Although there is a large increase in SA, there was no significant difference between control and infested plants, most likely due to the large amount of variation present ($W = 7$, $p = 0.31$). The levels of IAA and ABA significantly decreased following infestation at 10-days post-infestation (IAA: $t= 2.76$, $df = 7.93$, $p = 0.025$; ABA: $t = 3.53$, $df = 8.00$, $p = 0.008$) and there was a significant increase in the concentration of JA-Il ($W = 0$, $p = 0.007937$). Finally, there was no significant difference in the levels of tZ ($W = 11$, $p = 0.84$) or 2iP ($t = 0.66$, $df = 7.98$, $p = 0.53$) 10-days most midge infestation. JA-Il is considered to be the main regulator of plant resistance to insect herbivores and, thus the jasmonic acid (JA) signalling pathway is at the heart of plant response to insect pests (Erb et al. 2012). Transcriptomic analyses identified upregulation of several genes involved in the jasmonic acid pathway including allene oxidases, lipoxygenases and phosopholipases (Aljbory and Chen 2017) (S. Table 1). Both ABA and IAA can actively regulate steps within the JA signalling process and are thus integral to the plants response to insects. Ultimately, the jasmonic acid pathway can activate defense responses including upregulation of protease inhibitors (3 highly upregulated in infested plants (S. Table 1) which may reduce the growth rate of insects (Zavala et al. 2004). Determining the exact roles these hormones play in terms of *B. napus* response to swede midge will need to be dissected further.

Swede midge infestation severely alters plant morphology leading to formation of a pseudo-gall like structure from which the larvae can feed (Chen et al. 2011). In order to create this structure, the midge most likely has proteins in its saliva, known as effectors, that can interact with the host plant and co-opt host plant machinery to its benefit. To create a gall, plant cell structure may be modified by pectate lyases, expanses, extensins pectinesterase inhibitors, and beta-galactosidases which all play a role in plant cell modification (Showmaker et al. 2016), many of which were upregulated in infested plant tissue, while some were also downregulated (S. Table 1 and S. Table 2). Interestingly, a large number (22) of growth regulating factors were downregulated in infested plants (S. Table 2). Arabidopsis expressing growth regulating factor-1 (GRF-1) had reduced stem elongation and flowers forming within the rosette, and it is hypothesized that GRF-1 may regulate meristem function through control of cell proliferation (Van der Knapp et al. 2000). These growth regulating factors warrant further exploration as they may allow swede midge to form galls on host plants.

10. **Conclusions and Recommendations:** Highlight significant conclusions based on the findings of this project, with emphasis on the project objectives specified above. Provide recommendations for the application and adoption of the project findings.

    Swede midge has the potential to infest the major brassicaceous weed species present on the Prairies, thus any swede midge management program must consider the alternative hosts present in the agroecosystem.

    Putative resistance (i.e. nonpreference) has been identified in *C. microcarpa*, *L. densiflorum*, *A thaliana*, and *D. sophia*.

    Glucosinolates may not play a major role in host selection by the swede midge.

    The plant response to swede midge infestation is a complicated process with the concentration of several plant hormones increasing and others decreasing over the course of the infestation. Several thousand genes appear to be involved with infestation. Plants appear to elicit a defense response, but swede midge is still capable of infesting the plant.

    Transcriptomic analyses revealed several potential genes that the may be co-opted by the swede midge to form galls including pectate lyases, expanses, extensins pectinesterase inhibitors, and beta-galactosidases.

    Growth regulating factors were strongly downregulated in infested plants and should be further explored for their role in swede midge gall formation.

11. **Is there a need to conduct follow up research?** Detail any further research, development and/or communication needs arising from this project.

    There is a need to continue to explore swede midge plant interactions in order to further explore the potential plant genes which swede midge may co-opt for gall formation. If it is possible to identify the role of these genes, and how swede midge interacts with them, in the future it may be possible to modify these genes so swede midge can no longer interact with them.

12. **Patents/ IP generated/ commercialized products:** List any products developed from this research.

    None

13. **List technology transfer activities:** Include presentations to conferences, producer groups or articles published in science journals or other magazines.

    Talks:
    Mori BA. 2018. Insect scouting and identification. Crop Diagnostic School, Government of Saskatchewan Ministry of Agriculture, SaskCanola, Saskatchewan Pulse Growers, SaskFlax, and SaskWheat, Melfort, SK.

    Mori BA. 2018. Canola insect damage assessment and diagnostics. CanoLAB Diagnostic Workshop, SaskCanola and Canola Council of Canada, Saskatoon, SK

    Reports:
    Mori BA, Soroka J & Olfert O. 2018. Ecology of swede midge-host plant interactions. Western Committee on Crop Pests – Saskatchewan Entomology Research Summary

    Media:
    King C. “Assessing the swede midge threat.” Top Crop Manager – Western Edition. October Issue

14. List any industry contributions or support received.

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15. Acknowledgements. Include actions taken to acknowledge support by the Ministry of Agriculture and the Canada-Saskatchewan Growing Forward 2 bilateral agreement (for projects approved during 2013-2017) or Canadian Agriculture Partnership (For projects approved beyond 2017).

Funders (including Ministry of Agriculture) were acknowledge in all talks and reports.

16. Appendices: Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited.

Table 1. List of 12 brassica plants species to be tested for swede midge resistance/susceptibility.

Plant Species	Common Name	Swede midge host	Reference
Brassica napus	Canola	Yes	Hallett 2007
Camelina sativa	Camelina, false flax	?	
Camelina microcarpa	Small seed false flax	Yes	Hallett 2007
Diplotaxis muralis	Annual wall-rocket	?	
Erysimum cheiranthoides	Wormseed wallflower	No/?	Hallett 2007
Neslia paniculata	Ball mustard	?	
Sinapis arvensis	Wild mustard	Yes	Hallett 2007
Thlaspi arvense	Stinkweed	Yes	Hallett 2007
Arabidopsis thaliana	Thale cress	No	Boddum 2013
Descurainia sophia	Flixweed	No	Hallett 2007
Lepidium densiflorum	Peppergrass	?	
Sinapis alba	White mustard	Yes	Stokes 1953

Table 2. Mean (±SE) number of eggs laid on potential host plants by swede midge in a choice experiment when adult females were given a choice between 11 plant species. Means followed by the same letters are not significantly different ($p > 0.05$).

Host Plant Species	Mean (±SE) Eggs
Erysimum cheiranthoides	0
Lepidium densiflorum	0
Descurainia sophia	0
Camelina microcarpa	3.67 ± 2.44 A
Camelina sativa	3.67 ± 2.79 A
Neslia paniculata	7.00 ± 4.43 A
Thlaspi arvense	9.50 ± 5.12 A
Sinapis arvensis	13.83 ± 4.97 A
Diplotaxis muralis	53.67 ± 22.87 B
Brassica napus	124.33 ± 23.68 C
Sinapis alba	523.50 ± 96.75 D

Table 3. Mean (±SE) number of eggs laid on potential host plants by swede midge in a choice experiment when adult females were given a choice between 12 plant species, including *Brassica oleracea*. Means followed by the same letters are not significantly different ($p > 0.05$).

Host Plant Species	Mean (±SE) Eggs
Lepidium densiflorum	0
Descurainia sophia	0
Camelina microcarpa	0
Thlaspi arvense	2.14 ± 2.14 A
Sinapis arvensis	7.43 ± 5.55 A
Diplotaxis muralis	12.86 ± 4.90 A
Neslia paniculata	13.00 ± 6.82 A
Brassica oleracea	20.71 ± 6.76 A
Camelina sativa	21.14 ± 6.73 A
Erysimum cheiranthoides	52.86 ± 23.98 A
Sinapis alba	275.71 ± 82.15 B
Brassica napus	495.86 ± 64.04 B

Table 4. Mean (±SE) percent of swede midge larvae that reached the third instar of larval development one week after oviposition on 12 potential host plants as an estimate of larval performance.

Host Plant Species	Mean (±SE) Percent of Larvae at the 3rd Instar Stage
Arabidopis thaliana	18.8%
Brassica napus	64.6 ± 7.0%
Camelina microcarpa	No Larvae
Camelina sativa	58.6 ± 11.1%
Descurainia sophia	No Larvae
Diplotaxis muralis	65.7 ± 8.6%
Erysimum cheiranthoides	34.0 ± 6.6%
Lepidium densiflorum	15.9 ± 9.3%
Neslia paniculata	78.1 ± 3.1%
Sinapis alba	34.7 ± 10.7%
Sinapis arvensis	32.5 ± 12.2%
Thlaspi arvense	44.3 ± 7.1%

Table 5. Mean (±SE) eggs laid by female swede midge in a choice experiment with *Brassica napus* and four varieties of *Sinapus alba* that vary in seed glucosinolate content.

Host Plant Species/Variety	Mean (±SE) Eggs
Brassica napus	100.56 ± 28.95
Sinapis alba (JS99-503) (High GLS, Low EA)	43.67 ± 18.55
Sinapis alba (JS00-531) (Low GLS, Low EA)	36.89 ± 8.63
Sinapis alba (JS99-504) (Low GLS, High EA)	149.89 ± 45.37
Sinapis alba AC Pen. (High GLS, High EA)	96.89 ± 18.00

Table 6. Overrepresented GO terms (biological process) in the upregulated *B. napus* genes 10-days post *swede midge*. GO enrichment analysis was conducted with TopGO (Fisher Classic method, $p < 0.05$).

GO.ID	GO Term	Number of Annotated Genes
GO:0050896	response to stimulus	1981
GO:0006950	response to stress	1226
GO:0042221	response to chemical	1159
GO:0006952	defense response	534
GO:1901700	response to oxygen-containing compound	816
GO:0098542	defense response to other organism	379
GO:0009605	response to external stimulus	635
GO:0043207	response to external biotic stimulus	526
GO:0051707	response to other organism	526
GO:0009607	response to biotic stimulus	537
GO:0009617	response to bacterium	309
GO:0042742	defense response to bacterium	267
GO:0045087	innate immune response	216
GO:0006979	response to oxidative stress	221
GO:0010033	response to organic substance	869
GO:0010200	response to chitin	161
GO:0002376	immune system process	224
GO:0010243	response to organonitrogen compound	170
GO:0006955	immune response	219
GO:0051704	multi-organism process	587

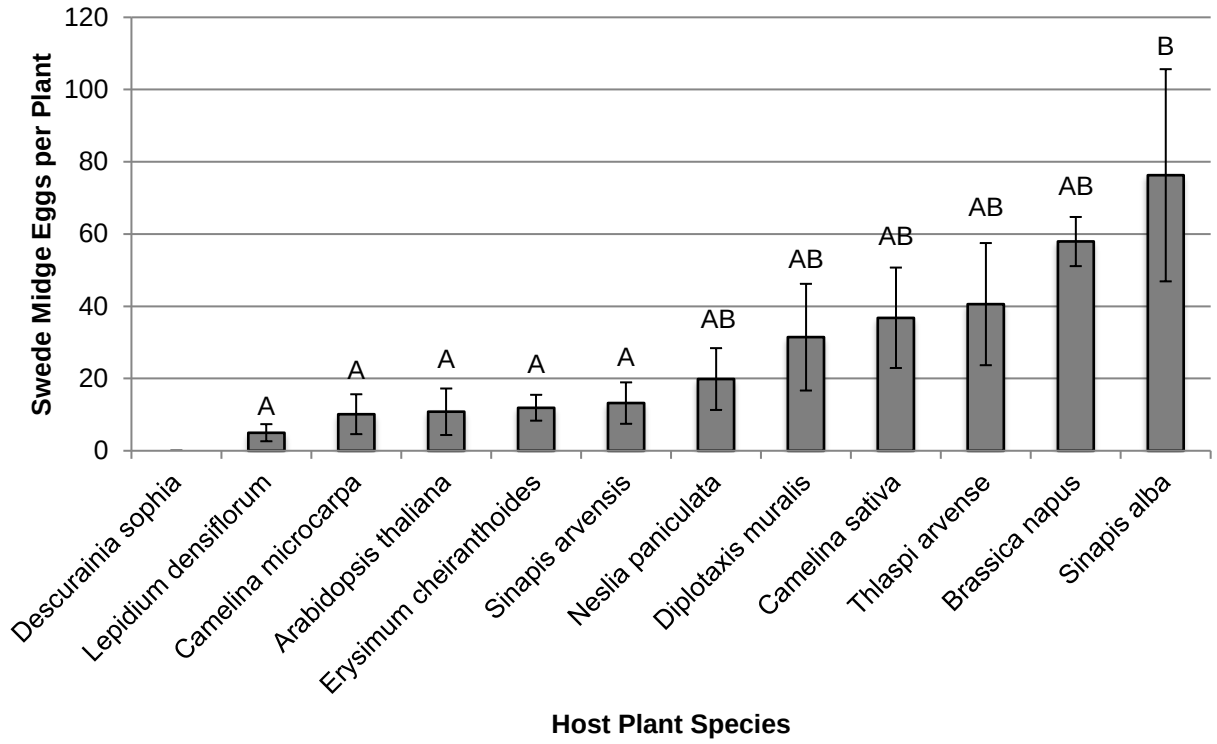

Figure 1. Mean (±SE) eggs laid per plant on 12 species of potential larval hosts of swede midge in a no-choice laboratory bioassay. Means with the same letters are not significantly different ($p > 0.05$).

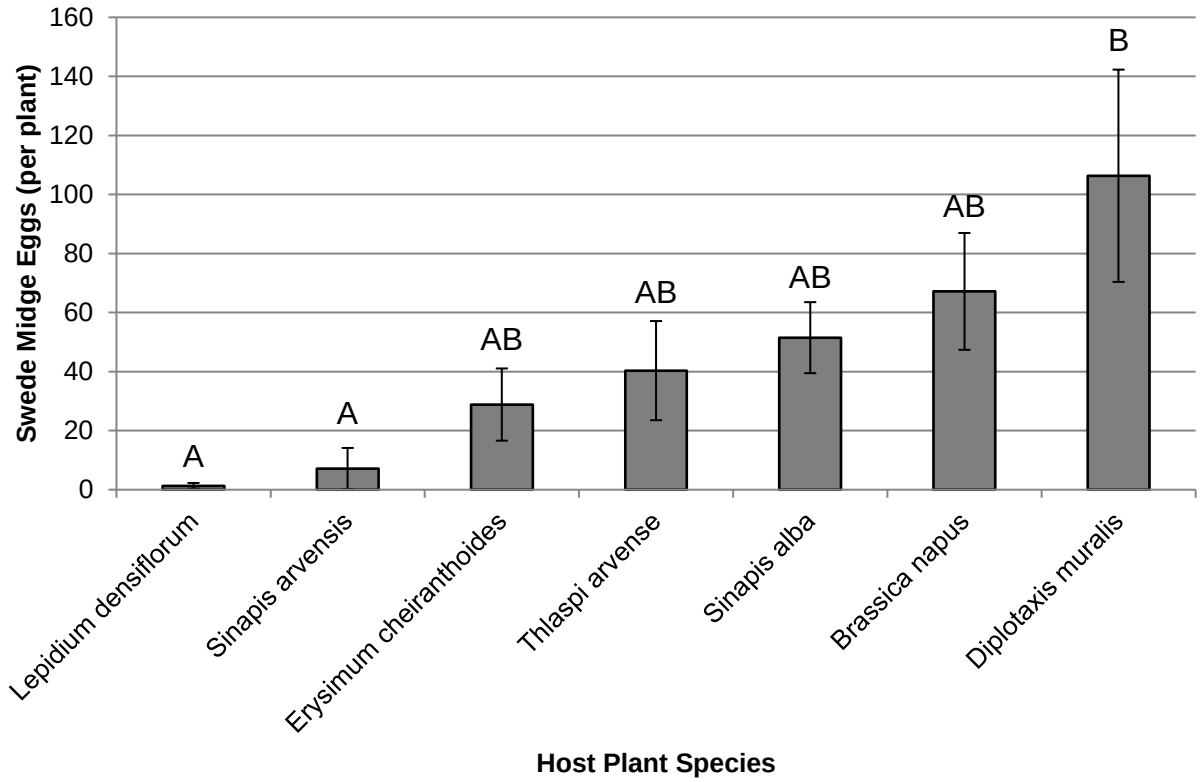

Figure 2. Mean (±SE) eggs laid per plant on in an experiment with 11 species of potential larval hosts of swede midge in a no-choice laboratory bioassay; the four plant species (*C. microcarpa*, *C. sativa*, *D. sophia*, and *N. paniculata*) that were rejected by swede midge are not shown. Means with the same letters are not significantly different ($p > 0.05$).

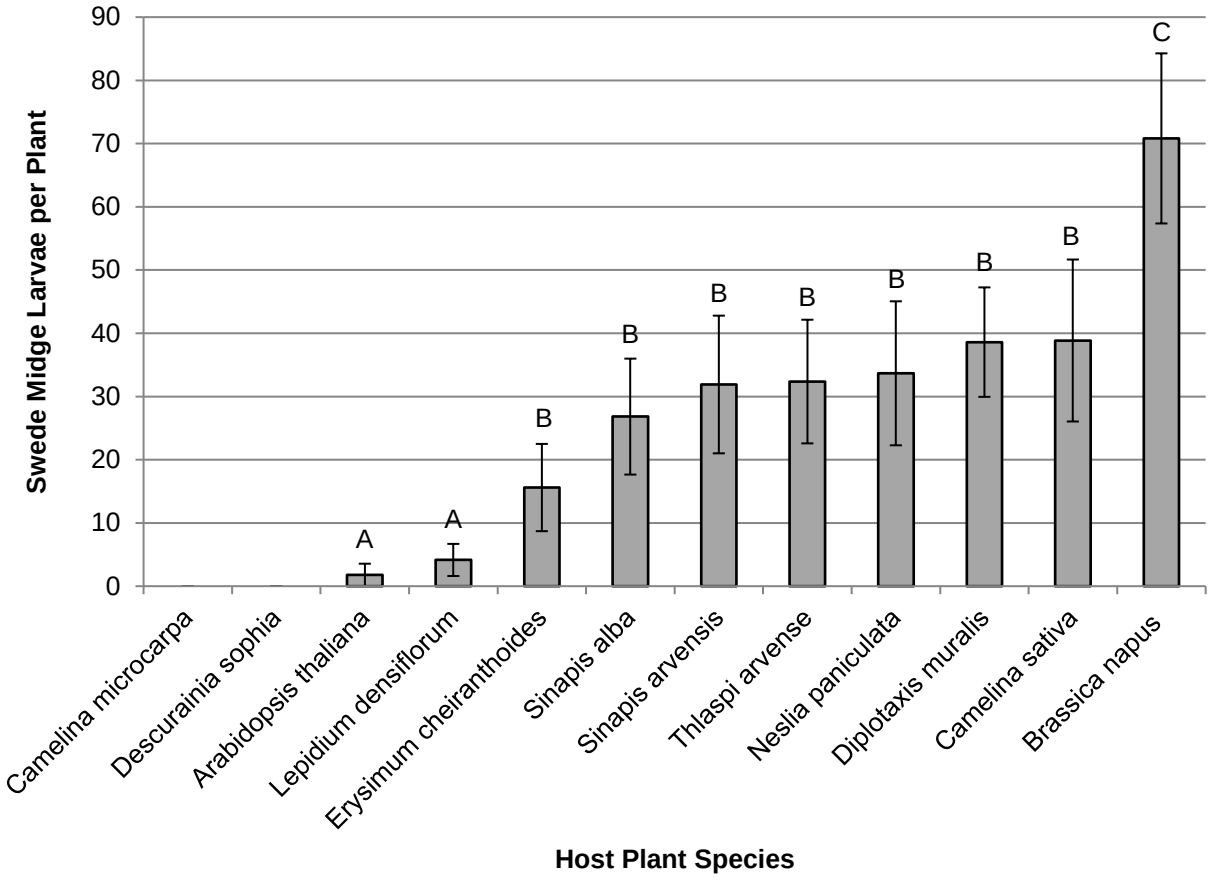

Figure 3. Mean (±SE) swede midge larvae per plant counted on host plants one week after individual plants were exposed to adult female swede midge in a laboratory bioassay. Means with the same letters are not significantly different ($p > 0.05$).

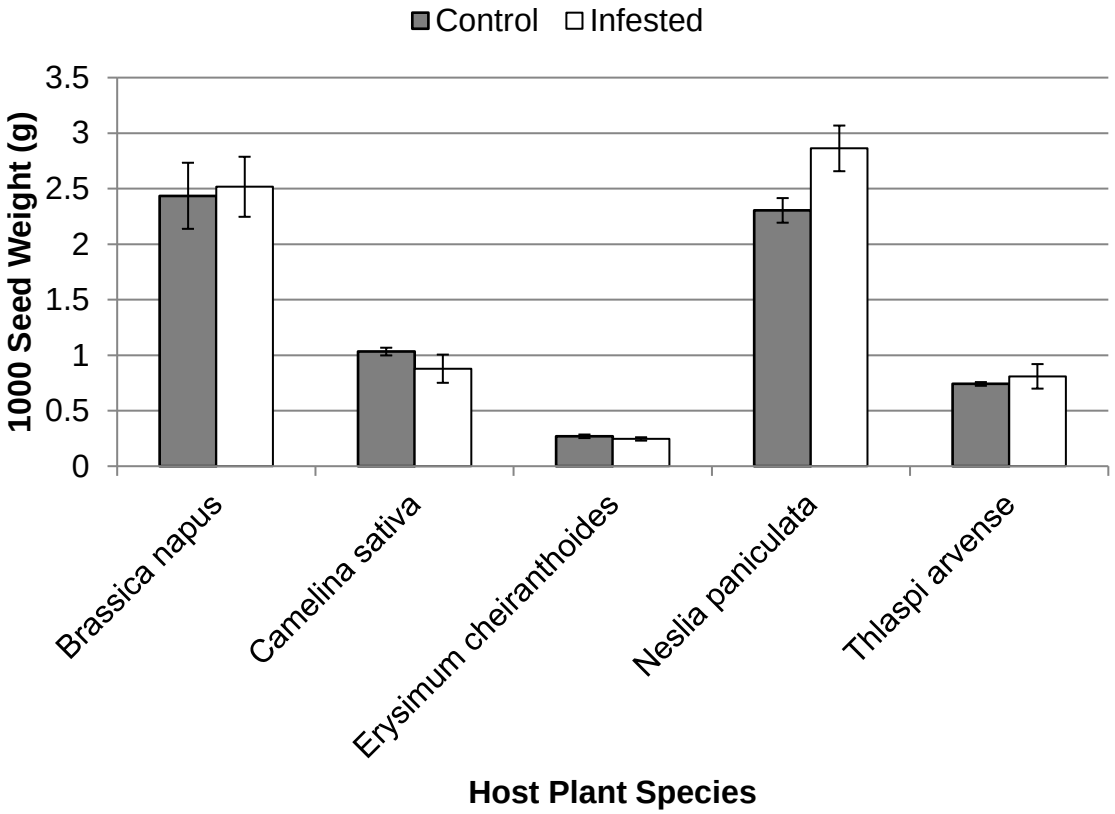

Figure 4. Mean (±SE) 1000 seed weight of five host plants of swede midge that were infested with swede midge eggs at a susceptible stage compared to the control. For each plant species, there was no effect of swede midge infestation on seed weight ($F_{1, 30} = 0.41$, $p = 0.5279$).

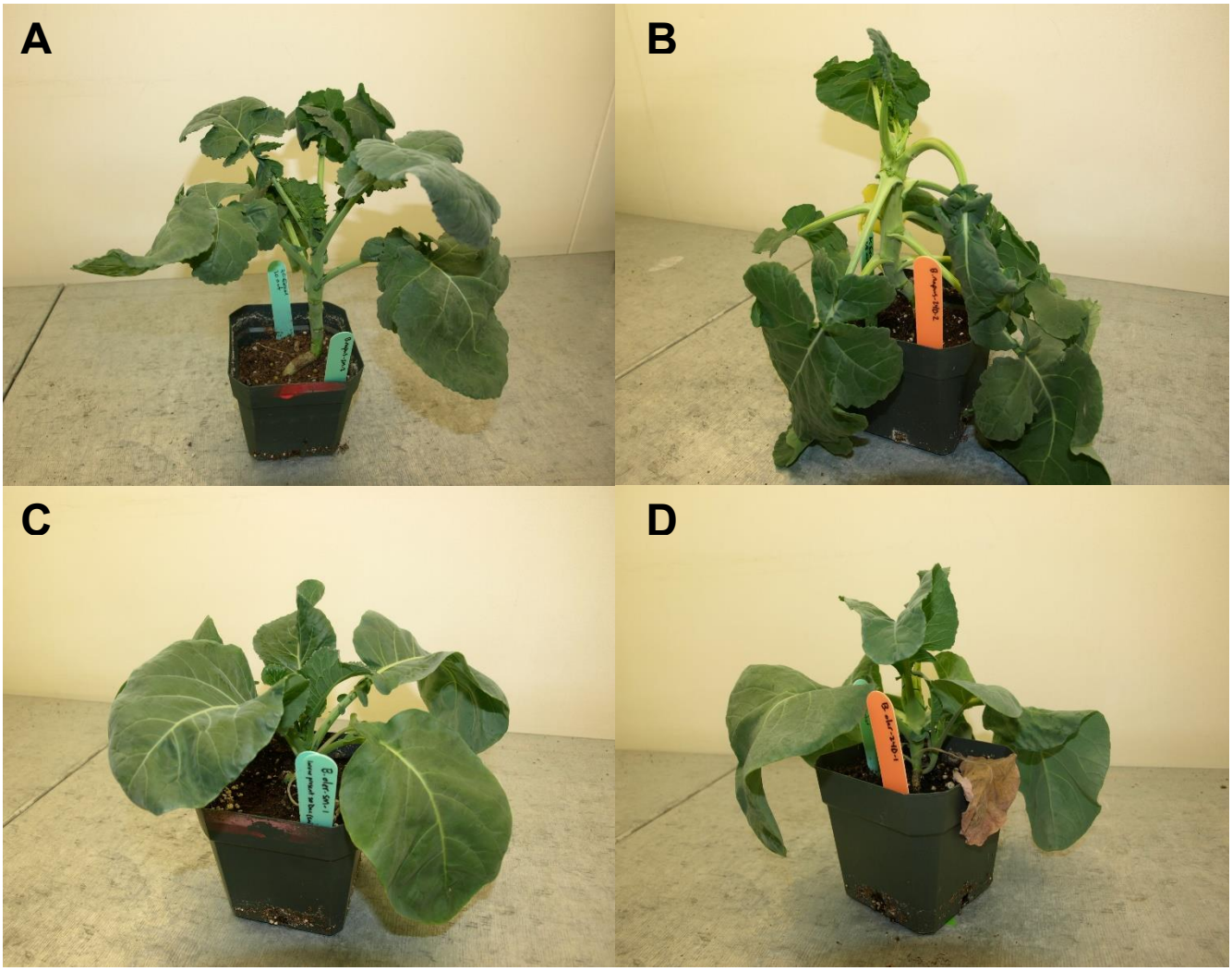

Figure 5. (A) *Brassica napus* injected with water (control) at the growing points; (B) *B. napus* injected with herbicide at the growing points; (C) *Brassica oleracea* injected with water (control) at the growing points; and (D) *B. oleracea* injected with herbicide at the growing points.

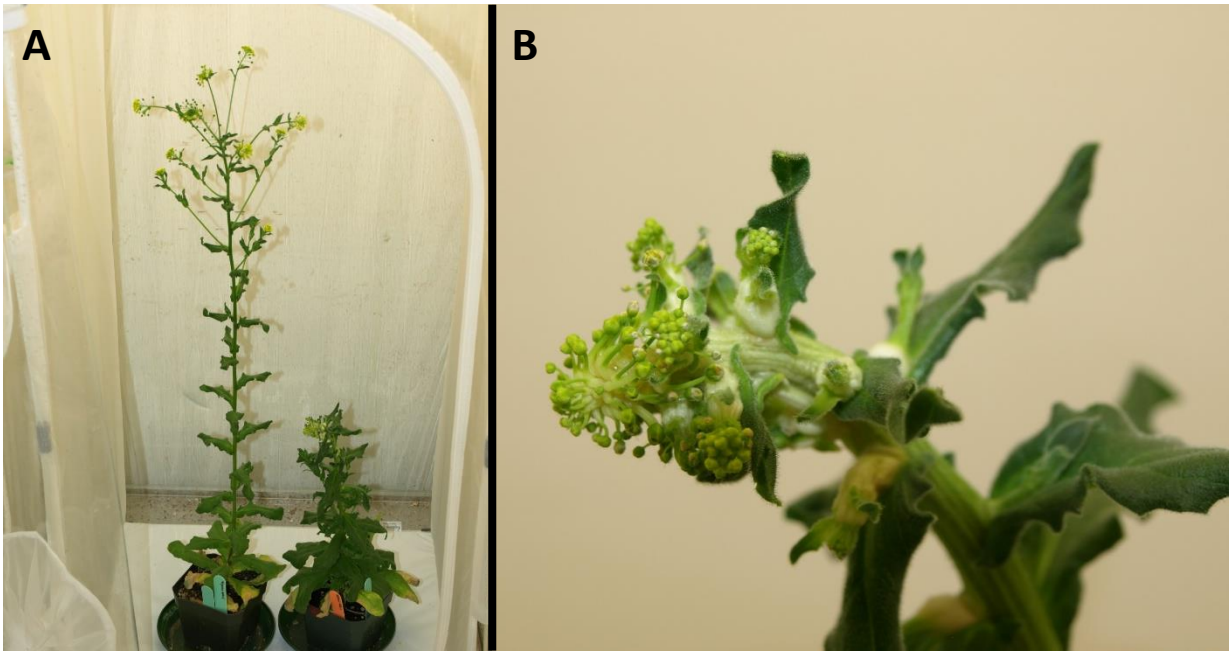

Figure 6. (A) Side-by-side comparison of the effect of herbicide (right) and water (control; right) injection on the growth of *Neslia paniculata*; (B) the wilting and malformation of a growing point of *N. paniculata* injected with herbicide.

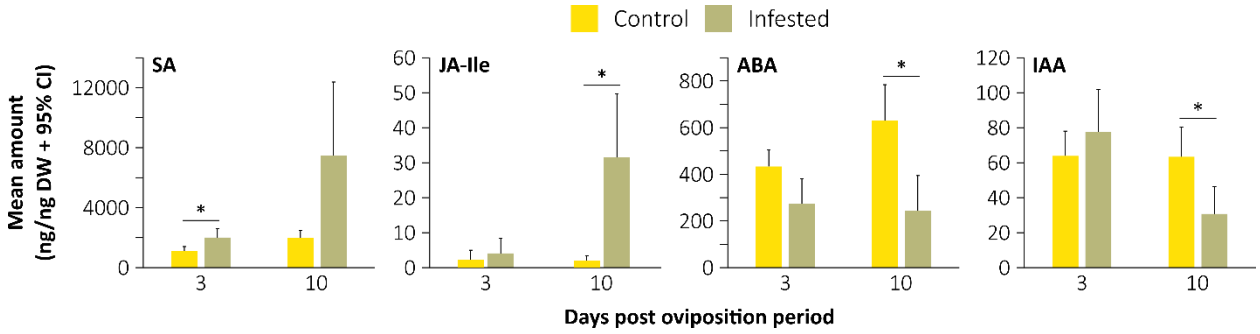

Figure 7: Mean amount of plant hormones (SA, JA-Il, ABA and IAA) in *B. napus* tissue 3 and 10-days post-swede midge infestation. * indicates significant differences between the control and infested plants ($p < 0.05$).

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