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Full Research Project Final Report Form

- All sections must be completed.
- One electronic copy and one signed original copy are to be forwarded to the lead funding agency as per the investment agreement.
- A detailed statement of expenses incurred during the course of the project must be submitted along with this report.
- For any questions regarding the preparation and submission of this report, please contact the representative of the lead funding agency.

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Section A: Project overview

1. Project number: **2009F074R**
2. Project title: **Developing Root Maggot-Resistant Canola**
3. Research team leader: **Dr. Lloyd Dosdall**
4. Research team leader's organization: **Dept. AFNS, University of Alberta, Edmonton, AB**
5. Project start date (MM/DD/YYYY): **11/01/2009**
6. Project completion date (MM/DD/YYYY): **06/30/2013**
7. Project final report date (MM/DD/YYYY): **08/10/2013**

Section B: Non-technical summary (max 1 page)

A research project was undertaken to develop canola germplasm resistant to infestation by root maggots. Root maggots are serious insect pests of canola across western Canada, and especially throughout central and northern Alberta. Root maggots cause damage when their larvae feed on canola root tissues. Research was initiated using intergeneric hybrid genotypes developed from crosses of *Sinapis alba* with *Brassica napus*; *S. alba* is the parental genotype resistant to root maggot attack and *B. napus* is susceptible.

Resistance to root maggots was confirmed in some of the intergeneric hybrid accessions (Objective 1). Doubled haploids with putative root maggot resistance were crossed with elite canola cultivars to produce F_1's ; these hybrids were used to generate a new population of resistant and susceptible plants that were subjected to attack by field populations of root maggots at Guelph, ON and Edmonton, AB. Segregation of resistant and susceptible genotypes occurred in this new population.

Biochemical markers associated with root maggot resistance were developed and validated (Objective 2). Three markers were identified that were constant and linked with root maggot resistance and susceptibility. Two of the markers were associated with resistant genotypes and one had consistent association with the susceptible lines.

Determination of constant markers associated with root maggot resistance enabled identification of the biochemical pathways associated with these traits (Objective 3). The compounds associated with the markers were determined. Resistance markers represent flavonoid compounds, and their presence appears to relate to the mechanism by which crucifer specialists (like root maggots) detoxify various glucosinolate by-products that can otherwise be toxic to them. The high concentration of flavonoids in resistant lines would diminish concentrations of isothiocyanates, which are compounds highly attractive to root maggots. Flavonoids can also modify leaf reflectance properties, and so reduce the attractiveness of resistant canola to ovipositing females.

Behavioral studies to determine the mechanisms of resistance to root maggot attack (Objective 4) focused on visual and olfactory cues, and associational resistance. Visual assessments of the resistant and susceptible germplasm indicated variations in the green and ultraviolet light reflected from resistant plant foliage, and as a consequence, female flies were less attracted to resistant genotypes. When flies were exposed to odors elicited by the various genotypes (in the absence of visual cues), some of the resistant genotypes were as resistant as the resistant parental genotype (*S. alba*). In field plantings of monocultures of *S. alba* and *B. napus*, versus mixed plantings of the two parental lines along with the resistant hybrids, damage to some resistant genotypes increased as a greater proportion of susceptible *B. napus* plants were included in the mixture. One resistant genotype demonstrated promise for conferring root maggot resistance in susceptible plants in the field trials.

In summary, all of the research objectives of this study were met. Our research team developed and confirmed resistance to root maggots in new *B. napus* hybrids, with the resistance traits derived initially from *S. alba*, and the resistance has been associated with biochemical markers. The key biochemical compounds associated with resistance were identified, and the mechanisms of resistance were confirmed in behavioral laboratory and field studies.

Section C: Project details

1. Project team (max ½ page)

Describe the contribution of each member of the R&D team to the functioning of the project. Also describe any changes to the team which occurred over the course of the project.

a) Research Team Leader: (requires personal data sheet only if leader has changed since last report)		
Name	***Institution***	***Expertise Added***
Dr. Lloyd Dosdall	University of Alberta, AB	Insect Pest Management, Insect Ecology

b) Research Team Members (each member requires a personal data sheet only if changed since last report.) *Additional rows may be added if necessary.*		
Name	***Institution***	***Expertise Added***
Dr. Laima Kott	University of Guelph, ON	Canola Breeding, Plant Genetics

No changes were made to the team during the course of the project.

Background (max 1 page)

Root maggots are severe and chronic pests of canola throughout western Canada, particularly in central and northern Alberta and Saskatchewan. In years of severe root maggot infestations, crop losses have been estimated at $100 million annually in Alberta alone (Soroka et al. 2004). Root maggot infestations were high in western Canada throughout the 1990s (Soroka et al. 2004), but declined in the early 2000s. These reductions in infestation levels can be attributed to: 1) the reduction in acreage of *Brassica rapa* and increases in production of *Brassica napus* because *B. napus* is less susceptible to attack by these pests (Dosdall et al. 1994); 2) a trend toward producer implementation of agronomic practices that favor development of healthy plant stands like adequate seeding rates, early seeding, and reduced tillage, all of which enable plants to better compensate for root maggot attack (Dosdall et al. 1996, 1998, 2006; Clayton et al. 2004); and 3) drought in 2002 and 2003. Root maggots thrive in moist conditions (Griffiths 1986); consequently, the return of normal precipitation in recent years (following the widespread droughts of 2002 and 2003) resulted in increases in root maggot populations. In Alberta and Saskatchewan, serious crop losses to canola were inflicted by root maggots in 2006, 2007, 2008, 2010, and 2012.

The recent increases in canola crop damage by root maggots can also be attributed to the practice of shortened canola rotations by some western Canadian farmers. Long-term research has shown that root maggot infestations gradually increase over time when canola is grown continuously on a particular field rather than rotated with a crop that is a non-host for root maggots like wheat or field pea (Dosdall et al. 2012).

Some cultural practices have been developed for reducing the impact of root maggots in canola, including seeding the crop at recommended or higher densities (Dosdall et al. 1996), planting at wider row spacings (Dosdall et al. 1998), planting less susceptible cultivars (Dosdall et al. 1994; Dosdall et al. 2000), and maintaining well fertilized plant stands (Dosdall et al. 2002, 2004). In addition, studies were recently completed to identify agronomic practices that enhance populations and activities of root maggot natural enemies, especially the beetle species *Aleochara bilineata* that is both a predator and parasitoid of root maggots (Broatch et al. 2010; Hummel et al. 2010). However, the development of commercial canola varieties resistant to root maggots has not been attempted previously, and would provide a crucial component in the integrated management of these pests.

In research we conducted to develop canola germplasm resistant to attack by the cabbage seedpod weevil, we developed a large number of accessions from crosses between *B. napus* and *Sinapis alba*, which are species susceptible (*B. napus*) and resistant (*S. alba*) to both the weevil and to root maggots (Dosdall et al. 1994; Dosdall and Kott 2006). We have screened some of these intergeneric hybrid lines and have found that weevil-resistant material is not also resistant to root maggots. However, some of the lines susceptible to weevils do have root maggot resistance (Ekuere et al. 2005; Kott and Dosdall 2005). To date, the two pests tend not to occur in similar ecoregions, with weevils found in southern Alberta and Saskatchewan and root maggots in central and northern regions, so the two strategies should be compatible.

Our goal was to develop and validate canola germplasm with resistance to root maggots.

2. Objectives and deliverables (max 1 page)

The primary goal of this research is to deliver root maggot-resistant canola germplasm to canola breeders in the commercial and public sectors. This requires introgression of biologically sourced resistance into elite canola germplasm, coordinated with field screening and marker development for rapid incorporation of target genes.

The major objectives of our proposal were:

1) to confirm resistance to root maggots in intergeneric hybrid genotypes developed from crosses of *Sinapis alba* with *Brassica napus*, and then backcrossed with the *B. napus* parent for several generations;
2) to develop biochemical markers in canola germplasm associated with root maggot resistance and validated through field and laboratory assessments;
3) to identify the major biochemical pathway(s) associated with root maggot resistance or susceptibility; and
4) to determine the mechanism(s) of resistance to root maggot attack through evaluations of behavioral differences preceding egg laying in resistant and susceptible germplasm by mated, gravid females.

3. Research design and methodology (max 4 pages)

Canola-quality germplasm resistant to attack by root maggots was already developed and reported in previous research (Kott and Dosdall 2005). This germplasm was used to generate populations of doubled haploids (DHs) for field screening and other research (described below). From pre-screened doubled haploid lines from 2005, several pairs of resistant and susceptible isolines were selected and utilized for the different research components which included:

a) selection, field and laboratory screening to identify the most resistant lines;
b) determination of resistance mechanisms through behavioral studies of adult root maggots;
c) biochemical marker identification; and
d) determination of specific biochemical pathways.

Lines with superior root maggot resistance were crossed with elite canola germplasm from the University of Guelph breeding program, and combined with traits for resistance to blackleg, resistance to Sclerotinia, high protein and oil contents, and favorable yields. Standard microspore extraction was used to produce several new DH lines each year for field screening and laboratory assessments.

After hand crossing, DH extraction was done by the microspore extraction method. Between 100 and 200 new DHs were produced each year for screening in laboratory assays and research plots. Screening involved both field and laboratory assessments proven to be effective from earlier work (Kott and Dosdall 2005).

Field assessments subjected plants to root maggot infestations in replicated plots in central Alberta and near Elora, Ontario. Root maggot susceptibility was assessed according to the method of Dosdall et al. (1994), requiring assessments of feeding damage by root maggot larvae on canola taproots at the end of the season.

Studies to determine mechanisms of root maggot resistance focused on 1) determining the near-ultraviolet light reflectance of foliage in resistant and susceptible lines because alightment behaviors of root maggot adults has been found to vary with the ratio of stimulatory and inhibitory reflected wavelengths (Koštál 1993); 2) visual assays using a method developed at the University of Alberta (Tansey et al. 2010a) to determine the behavioral responses of male and female flies to various canola genotypes; 3) studies on behavioral differences in male and female root maggots to the odors emitted by resistant and susceptible canola plants; and 4) determination of associational resistance, or the potential for canola plants susceptible to attack by root maggots to be less infested when grown in close proximity with resistant plants.

The laboratory experiments relied upon the development of root maggot caged colonies. Adult root maggots for various assays were obtained from a laboratory colony of *Delia radicum* held at the University of Alberta, Edmonton. This colony originated from specimens at the Saskatoon Research Centre of Agriculture and Agri-Food Canada and the Agriculture and Agri-Food Canada research centre at St. Jean-sur-Richelieu, PQ. The colony was maintained in mesh cages under laboratory conditions (21°C, 12 L:12 D). The adults were provisioned with 10% honey

solution and a diet consisting of 70% full fat soy flour, 30% food grade brewer's yeast, and 1% yeast hydrolysate. Females were allowed to oviposit into approximately 500 g, cut portions of rutabaga taproots (*B. napus* L. ssp. *rapifera*) placed into plastic containers (11 cm in diameter) filled with sterilized sand dampened with distilled water. The moisture content of the sand was maintained with the addition of distilled water over the course of larval development. Adults were utilized in experiments 7-14 days after they eclosed from puparia. The sex of flies was determined through visual inspection.

We assessed the reflectance of each replicate plant using a Jaz spectrometer (Ocean Optics Inc., Dunedin, FL) with an attached reflection probe (QR400-7-SR-BX, Ocean Optics Inc., Dunedin, FL) positioned within a rigid opaque sleeve that standardized the distance to the sample at 9 mm. We assessed reflectance from 200-1000 nm in 0.36-0.46 nm increments. Measurements were corrected to absolute reflectance by a 99% Spectralon reflectance standard (SRS-99-010, Labsphere, North Sutton, NH). We measured and averaged the reflectance of all fully expanded non-senescing leaves to give one foliar spectrum for each replicate plant. Our analysis focused on the 350 nm (UV) and 550 nm (G) areas of the spectrum as these showed the greatest variation and these regions are also important for *D. radicum* vision.

Each replicate plant was photographed using a Nikon D70 (Nikon Canada, Mississauga, ON). The images were then processed and measured using ImageJ software. The photographs were taken against a red background to better allow the removal of the background using a series of threshold filters. Each of the remaining pixels was represented by a red, green and blue percentage. We then calculated the mean of these values for the remaining pixels. Red values were discarded from the analysis as *D. radicum* is less sensitive to these wavelengths. We also placed a ruler in each photograph to determine pixel size to allow us to calculate foliar area.

Visual responses of root maggot females to resistant and susceptible lines were assessed using a six-way choice arena constructed of acrylonitrile butadiene styrene pipefittings and modified from that described by Tansey et al. (2010a). To minimize the potential influence of olfactory cues confounding the visual data, we capped each end tube of the arena with a clear cellophane film. Light was provided with a Hortilux - Blue™ daylight metal halide lamp positioned 1.2 m above the arena, as this lamp provides a more balanced light spectrum that more closely approximates natural sunlight than conventional florescent lighting. We first placed plants of the different genotypes (at the 5-6 true leaf stage) in front of the chambers, then we added a root maggot adult. After 20 mins we recorded the position of the fly within the chamber and considered that the end tube in which it was located represented a preference for that particular genotype. Many replicate flies were assessed.

Responses of *D. radicum* to the olfactory cues associated with *S. alba*, *B. napus*, and the intergeneric hybrids were evaluated in a Y-tube olfactometer as described by Tansey et al. (2010b). Individual male or female flies, 7 to 14 days old, were introduced to the apparatus. The position of flies in the arm of the apparatus subjected to air streams from a control chamber containing a pot of soil with no plant, or from a chamber containing a potted test plant, was recorded after 10 minutes. Responses of at least 10 novice male and approximately 20 novice female flies were evaluated per plant genotype. Responses of no more than five flies per individual plant were assessed before the plants were replaced with ones of the same age and genotype. The apparatus was washed with soapy water and rinsed with 70% ethanol between tests of individual plants.

To assess the potential for associational resistance between susceptible and resistant canola plants, we field-planted monocultures of *B. napus*, *S. alba*, and two of the new resistant hybrid genotypes in 4 x 4 m plots at a seeding rate of 5 kg per ha and row spacing of 20 cm, resulting in 20 plant rows per plot. We also performed mixed plantings of each genotype so that we attained ratios of 25% resistant plants:75% susceptible plants; 50% resistant plants:50% susceptible plants; and 75% resistant plants:25% susceptible plants. The experiment was a randomized complete block, performed at two sites in 2010 (Vegreville and Ellerslie, AB). Due to flooding damage, only data from one site could be used in 2011 (Vegreville, AB).

At the end of the season, 40 plants from each plot were removed, and the roots washed and scored for the degree of root maggot feeding damage according to the method of Dosdall et al. (1994). Damage ratings were subjected to analysis of variance using the Mixed procedure of SAS (SAS Institute 2010). Site and planting scheme were considered main effects; block and the interaction of block and plot nested in site were random factors. Multiple comparisons were conducted using *t*-tests.

In our research to identify the key biochemical compounds associated with host acceptance or rejection, different plant organs (first, second, third, and fourth true leaves, stems and roots) were tested using High Performance Liquid Chromatography (HPLC), enzyme assays, and mass spectrometry. A key focus of our research centered on glucosinolate compounds because these are known to cause behavioral responses in both adults and larvae of root maggots. Third and fourth true leaves of canola plants were found to be the best sources of useful discrepancy for characterizing field-tested resistant and susceptible plants.

The plant population was separated into two subpopulations, resistant and susceptible to root maggot attack. HPLC profiles of third and fourth leaves of 40 genotypes were used in logistic regression for marker development. The statistical approach used was also applicable for predictive analysis, so that markers for root maggot resistance could be identified that can be used in newly developed lines. Several methods were used to select the minimum number of markers, representing plant compounds directly involved in the plant-insect interaction, on which to build the model that predicted and explained the interaction. Initially methods such as 'purposeful selection' were used which involved implementing a SAS macro to determine which markers were appropriate for logistic regression, and later the 'branch-and-bound' algorithm was used to test marker combinations to find a specified number of models with the highest likelihood (chi-square) statistic.

A mass spectrometry study was undertaken to identify the chemical nature of the compounds that constituted the marker peaks. The study was completed in two phases. Initially the study was done by Electrospray Ionization Liquid Chromatography/Mass Spectrometry (ESI-LC/MS), which allowed us to identify compounds from known chemical classes, such as glucosinolates. In the second phase of the study, we used the Bruker AmaZon SL ion trap system. On that system, we were not able to run the same chromatographic method used for HPLC and ESI-LC/MS; however, we could link our previous results, ion-by-ion, to the results we had already obtained. That way we could determine the mass spectrometry patterns of the compounds of interest. We do not have access to a mass spectrometry database, so we determined the likely chemical structures of compounds by searching for previously discovered close ion-fragment patterns in the plant kingdom, or various species of *Brassica*, through the published literature.

4. Results, discussion and conclusions (max 8 pages)

Research conducted by my collaborating researcher, Dr. L. Kott of the University of Guelph, Guelph, ON, was directed toward completion of Objectives 2 and 3 of the submitted full proposal:
Objective 2: to develop biochemical markers in canola germplasm associated with root maggot resistance; and
Objective 3: to identify the major biochemical pathway(s) associated with root maggot resistance or susceptibility.

Developing markers associated with root maggot resistance is a crucial step in this research because it is only possible to determine the biochemical pathways associated with resistance or susceptibility after the key compounds responsible for resistance are identified. Selection of a suitable (susceptible) canola host plant by female root maggots begins when the fly lands on the leaves or stems, followed by tarsal and antennal actions that sense whether or not the plant is a suitable host for its offspring. This is followed by either rejection of the plant in the case of a resistant genotype, or in the case of a susceptible genotype, egg deposition near the plant stem-root interface (Kostal and Finch 1994). In research to identify the key biochemical compounds associated with host acceptance or rejection, different plant organs (first, second, third, and fourth true leaves, stems and roots) were tested using High Performance Liquid Chromatography (HPLC), enzyme assays, and mass spectrometry. A key focus of research centered on glucosinolate compounds because these are known to cause behavioral responses in both adults and larvae of root maggots (Gouinguené and Städler 2006; van Dam and Raaijmakers 2006).

The third and fourth true leaves of canola plants were found to be the best sources of useful discrepancy for characterizing field-tested resistant and susceptible plants. The plant population was separated into two subpopulations, resistant and susceptible to root maggot attack. HPLC profiles of third and fourth leaves of 40 genotypes were used in logistic regression for marker development. The statistical approach used was also applicable for predictive analysis, so that markers for root maggot resistance could be identified that can be used in newly developed lines. Several methods were used to select the minimum number of markers, representing plant compounds directly involved in the plant-insect interaction, on which to build the model that predicted and explained the interaction. Initially methods such as 'purposeful selection' were used which involved implementing a SAS macro to determine which markers were appropriate for logistic regression, and later the 'branch-and-bound' algorithm was used to test marker combinations to find a specified number of models with the highest likelihood (chi-square) statistic.

The model was based on three markers because they were constant and were linked with root maggot resistance. Adding more markers did not significantly improve the model. Table 1 below shows the parameter estimates of the model developed in this study. Negative values represent markers that when present in higher amounts increase the probability for the genotype to be resistant (markers R52 and M61). Positive values (marker M71) indicate that when the marker is present in a higher amount it decreases the probability for the line to be resistant. Odds ratio analysis indicated that R52 and M61 were almost equally present in resistant lines while M71 was more likely to occur in the susceptible lines.

Parameter	Estimate	95% Confidence Limits	
Intercept	2.3368	0.7809	4.3483
R52	-8.52E-7	-1.71E-6	-1.57E-7
M61	-1.84E-6	-3.52E-6	-5.88E-7
M71	9.796E-6	1.906E-6	0.000021

Table 1. Parameter estimates and profile-likelihood confidence limits of three biochemical markers associated with canola germplasm resistant to infestation by root maggots.

A mass spectrometry study was undertaken to identify the chemical nature of the compounds that constitute the marker peaks. The study was completed in two phases. Initially the study was done by Electrospray Ionization Liquid Chromatography/Mass Spectrometry (ESI-LC/MS), which allowed us to identify compounds from known chemical classes, such as glucosinolates. In the second phase of the study, we used the Bruker AmaZon SL ion trap system. On that system, we were not able to run the same chromatographic method used for HPLC and ESI-LC/MS; however, we could link our previous results, ion-by-ion, to the results we had already obtained. That way we could determine the mass spectrometry patterns of the compounds of interest. We do not have access to a mass spectrometry database, so we determined the likely chemical structures of compounds by searching for previously discovered close ion-fragment patterns in the plant kingdom, or various species of *Brassica*, through the published literature. These studies determined that the probable compound for marker R52 is Proanthocyanidin A1, a flavonoid. The probable compound for marker M61 is kaempferol-3-O-glucose-7-O-glucoside, and marker M71 appears to be glucobrassicin.

The identification of biochemical markers associated with canola germplasm resistant and susceptible to root maggot infestation enabled improved understanding of the biochemical pathways associated with root maggot resistance or susceptibility. Since markers R52 and M61 are flavonoids with antioxidant activity, their increased presence in resistant lines can be explained by their interaction with the by-products formed from the myrosinase-glucosinolate complex, which represent the actual insect attractants and the mechanism by which crucivores (like root maggots) detoxify glucosinolate compounds that can be toxic to some insect species (Ratzka et al. 2002). The increased concentrations of antioxidants can diminish the concentration of isothiocyanates, and hence result in less susceptibility to root maggot attack because these insects are highly attracted to isothiocyanates (Hardman and Ellis 1978). Marker R52, a proanthocyanidin, is yellow in color and therefore can be a leaf-color modifier. It is known that adult root maggots (*Delia radicum* L.) are attracted by the greener (healthier-looking) plants (Prokopy et al. 1983), so more proanthocyanidin can lead to yellowish-looking leaves which are less attractive for the female flies.

Flavonoids, such as those of markers R52 and M61, are sequestered differently from host plants in the insects that feed on them. Concentrations in the insects are positively associated with the amounts of flavonoids in the plant tissues that they consumed. Compounds close to, or similar to, the compounds in M61 are selectively sequestered and metabolized by another crucifer specialist, larvae of the cabbageworm *Pieris brassicae*, while other flavonoids such as myricetin derivatives, flavones and isoflavonoids were mostly excreted (Ferreres et al. 2007). As there is no similar study previously undertaken to correlate the flavonoid content in root maggot larvae with that of their host

plants, it will be of great interest to test root maggot larval preferences to flavonoids in future research.

Research conducted at the University of Alberta focused on completion of Objectives 1 and 4 of the submitted full proposal:
Objective 1: to confirm resistance to root maggots in intergeneric hybrid genotypes developed from crosses of *Sinapis alba* x *Brassica napus*, and backcrossed with the *B. napus* parent for several generations; and
Objective 4: to determine the mechanism(s) of resistance to root maggot attack through evaluations of behavioral differences preceding egg laying in resistant and susceptible germplasm by mated, gravid females.

For Objective 1, several doubled haploid lines with previously identified robust root maggot resistance were crossed with elite canola cultivars to produce an F_1 plant population. These hybrids were pollen donors for microspore culture and were used to generate a new population of root maggot-resistant lines. In 2011, the genotypes were seeded in plots at the Universities of Alberta and Guelph to assess susceptibilities to root maggot attack under field conditions. Results indicated that segregation of resistant and susceptible doubled haploids had occurred within this new population. We used root damage assessments to score nearly all plants (Dosdall et al. 1994), and this strategy identified lines with greatest root maggot resistance. Biochemical screening confirmed the results obtained in the field assessments.

Considerable research was conducted to determine the mechanism of root maggot resistance, as proposed in Objective 4. These studies can be grouped into three general categories: 1) visual tests, to determine light reflectance properties of the resistant and susceptible genotypes, and the responses of adult flies to visual cues elicited by leaves and flowers; 2) olfactory tests, to determine responses of adult flies to odors elicited by resistant and susceptible plants; and 3) associational resistance to determine root maggot responses associated associated with field interplantings of root maggot-resistant and -susceptible plants.

1) Visual Tests: Root maggot adults respond to visual cues elicited by their host plants, and these affect whether or not certain plants can then be explored further by the flies as hosts for egg-laying (Hopkins et al. 1999). We measured the reflectance properties of leaves and flowers using a dual-beam spectrophotometer operating between 200 and 1000 nm. Visual responses of root maggot females to resistant and susceptible lines were assessed using a six-way choice arena constructed of acrylonitrile butadiene styrene pipefittings. To minimize the potential influence of olfactory cues confounding the visual data, we capped each end tube of the arena with a clear cellophane film. Light was provided with a Hortilux - Blue™ daylight metal halide lamp positioned 1.2 m above the arena, as this lamp provides a more balanced light spectrum that more closely approximates natural sunlight than conventional florescent lighting. We first placed plants of the different genotypes (at the 5-6 true leaf stage) in front of the chambers, then we added a root maggot adult. After 20 mins we recorded the position of the fly within the chamber and considered that the end tube in which it was located represented a preference for that particular genotype. Many replicate flies were assessed.

There were significant differences between genotypes in the diffuse foliar reflection of the ultraviolet and green areas of the spectrum, with all genotypes reflecting less light in these regions than the *S. alba* resistant parent. Our photographic measurements of foliar area indicated that *S. alba* typically had a smaller foliar area than two resistant intergeneric hybrids, although the effect of genotype on leaf area was not significant overall.

Among the intergeneric hybrid genotypes, male flies did not demonstrate a significant overall effect of genotype in their responses ($P > 0.05$), although males were significantly more attracted to plants of the susceptible *B. napus* parental genotype than to the resistant *S. alba* parent ($P < 0.05$)

(Fig. 1). By contrast, females exhibited substantial differences in their responses to genotype, with responses to the *B. napus* parent significantly greater than those to *S. alba* ($P < 0.05$). Responses of female flies to two resistant intergeneric genotypes (Genotypes 229 and 308) were similar and did not differ significantly from responses of females to the *S. alba* resistant parent ($P > 0.05$), but responses to two more visually susceptible hybrid genotypes (Genotypes 1299 and 38) were significantly greater than responses to *S. alba* ($P < 0.05$) (Fig. 1).

Our studies on visual properties as a mechanism to explain resistance to root maggots in resistant germplasm indicated that ultraviolet and green light reflected from plant foliage was related to responses of adult flies, and that different visual responses by female flies is influenced in part by reflectance properties. On the basis of visual assessments alone, intergeneric Genotypes 229 and 308 were least attractive to female root maggots.

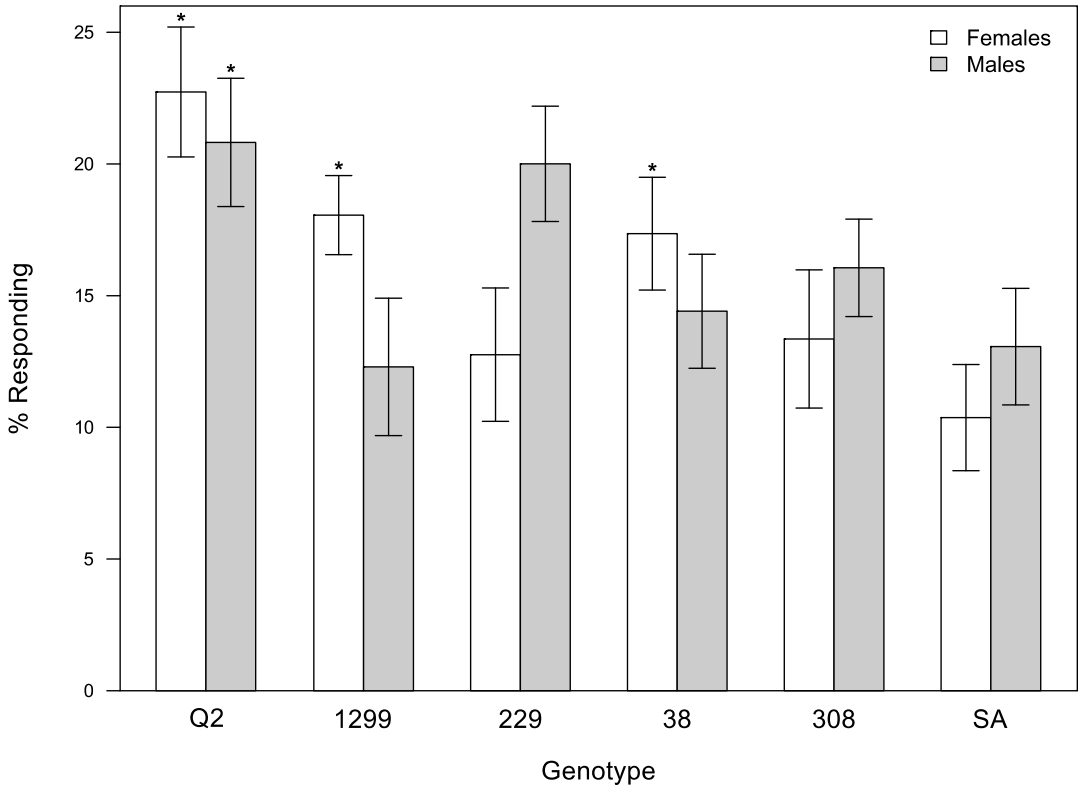

Figure 1. The responses of males and females of the root maggot, *Delia radicum*, to plants of different genotypes within the visual choice arena. Q2 = *Brassica napus*, SA = *Sinapis alba*, and 1299, 229, 38, and 308 = intergeneric hybrid genotypes. Within each sex an asterisk indicates a significant contrast ($\alpha = 0.05$) with the mean of *S. alba*.

2) Olfactory Tests: Olfaction is a key component in host plant location by root maggots (Finch 1978). Attractive stimuli from olfactory sources while the insect is in flight can cause it to eventually land and initiate contact with a potential host plant, and this is followed by post-alighting behavior that can lead to either host acceptance or rejection (Thorsteinson 1960; Jones 1992; Singer et al. 1992). We assessed the role of olfactory cues in reduced infestation levels of some intergeneric hybrids by root maggots.

Responses of *D. radicum* to the olfactory cues associated with *S. alba*, *B. napus*, and the intergeneric hybrids were evaluated in a Y-tube olfactometer as described by Tansey et al. (2010b). Individual male or female flies, 7 to 14 days old, were introduced to the apparatus. The position of flies in the arm of the apparatus subjected to air streams from a control chamber containing a pot of soil with no plant, or from a chamber containing a potted test plant, was recorded after 10 minutes. Responses of at least 10 novice male and approximately 20 novice female flies were evaluated per plant genotype. Responses of no more than five flies per individual plant were assessed before the plants were replaced with ones of the same age and genotype. The apparatus was washed with soapy water and rinsed with 70% ethanol between tests of individual plants.

Both male and female root maggots responded most frequently to plants of the *B. napus* parental genotype. Significant differences were observed among the responses of females to the different genotypes, with responses to *B. napus* and two of the intergeneric hybrid genotypes (1299 and 308) greater than positive responses for *S. alba* ($P < 0.05$) (Figure 2). Two genotypes (229 and 38) elicited behavioral responses of female flies that were similar to responses to the resistant parent, *S. alba* ($P > 0.05$) (Fig. 2). On the basis of olfactory assessments alone, intergeneric Genotypes 229 and 38 were least attractive to female root maggots.

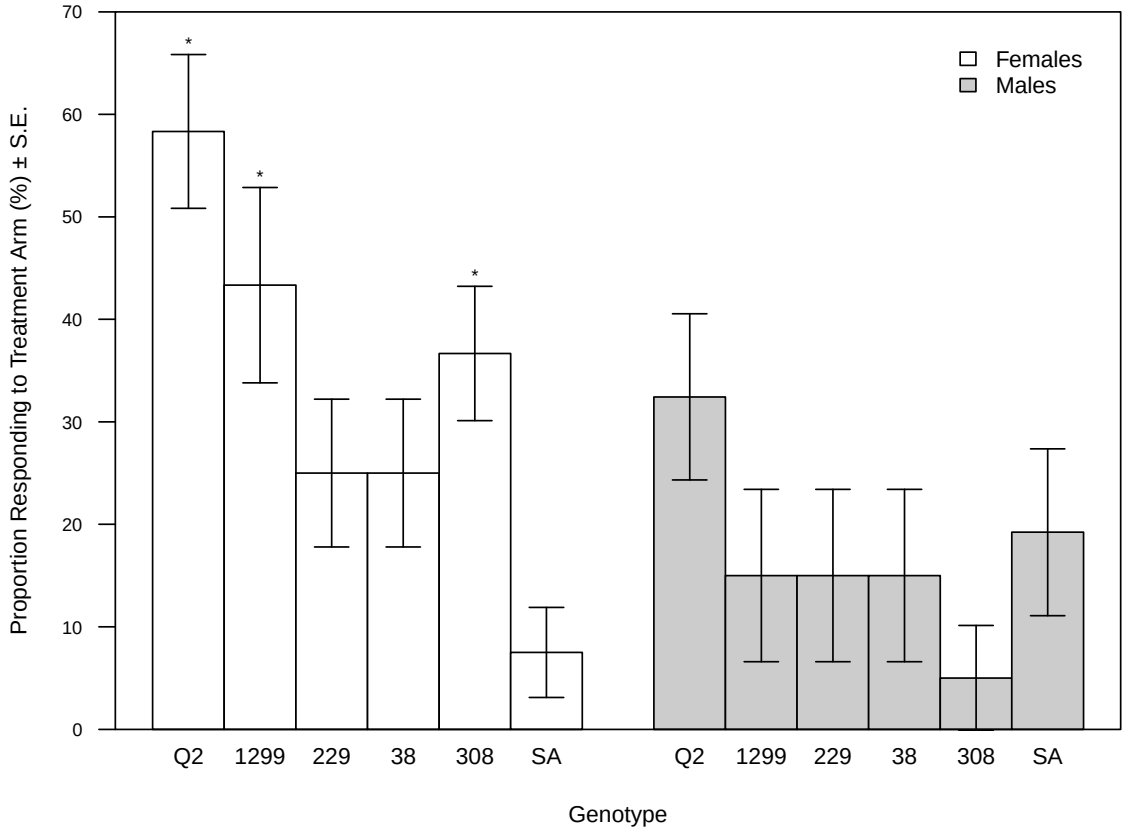

Figure 2. The proportion of the total numbers of females and males of the root maggot, *Delia radicum*, introduced to the olfactometer and responding to the treatment arm for each genotype. Q2 = *Brassica napus*, SA = *Sinapis alba*, and 1299, 229, 38, and 308 = intergeneric hybrid genotypes. Within each sex an asterisk indicates a significant contrast ($\alpha = 0.05$) with the proportion responding to *S. alba*.

3) Associational Resistance Assessments: Growing monocultures of resistant plants is not sustainable over the long term: this cropping practice increases selection pressure on insects to develop resistance, and hence resistant genotypes can become susceptible in a relatively short time. Because root maggot larvae are relatively sedentary, interspersed refuges can be an effective strategy for reducing selection pressure of novel resistance traits in crop plants (Bernal et al. 2004).

We field-planted monocultures of *B. napus*, *S. alba*, and two of our new resistant hybrid genotypes in 4 x 4 m plots at a seeding rate of 5 kg per ha and row spacing of 20 cm, resulting in 20 plant rows per plot. We also performed mixed plantings of each genotype so that we attained ratios of 25% resistant plants:75% susceptible plants; 50% resistant plants:50% susceptible plants; and 75% resistant plants:25% susceptible plants. The experiment was a randomized complete block, performed at two sites in 2010 (Vegreville and Ellerslie, AB), and due to flooding damage in 2011, only data from one site could be used in 2011 (Vegreville, AB).

At the end of the season, 40 plants from each plot were removed, and the roots washed and scored for the degree of root maggot feeding damage according to the method of Dosdall et al. (1994). Damage ratings were subjected to analysis of variance using the Mixed procedure of SAS (SAS Institute 2010). Site and planting scheme were considered main effects; block and the interaction of block and plot nested in site were random factors. Multiple comparisons were conducted using *t*-tests.

A significant effect of site-year was apparent. Damage ratings were: Vegreville 2011 > Vegreville 2010 > Ellerslie 2010 ($P < 0.001$ for all comparisons). Planting scheme was also influential ($F_{12, 357} = 7.64$; $P < 0.001$). Least root maggot damage occurred in *S. alba* monocultures. For Genotype 308, damage to all plants per planting scheme was comparable (at $\alpha = 0.05$) to *B. napus* monocultures. However, for Genotype 229, we found low root maggot damage levels when grown in monoculture, comparable to those achieved with *S. alba* monocultures. Similarly, damage was reduced in plantings of 75% *S. alba* and 25% Genotype 229 relative to *B. napus* monocultures. Damage increased to *S. alba* and to Genotype 229 with increases in the proportion of *B. napus* in the interplantings.

The intergeneric hybrid Genotype 229 demonstrated promise for conferring root maggot resistance in susceptible plants in these field trials. Results indicated that damage to plants from feeding by larvae of these pests decreased with the proportion of resistant plants in the plot.

In summary, our research investigated germplasm of canola with resistance to root maggot attack. Biochemical markers associated with the resistance trait were identified and validated. The markers pointed to the presence of flavonoid compounds in the resistant material that alter concentrations of compounds highly attractive to root maggots. Behavioral studies confirmed that the novel resistant germplasm reflected green and ultraviolet light differently than in susceptible lines, and the odors emitted by resistant lines were less attractive to gravid female flies than those of susceptible genotypes. When the resistant germplasm was planted in the field in a mixture with a susceptible cultivar, it conferred greater resistance to the susceptible genotype than occurred in a monoculture of the susceptible line.

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Tansey, J.A., L.M. Dosdall, B.A. Keddie, R.S. Fletcher, and L.S. Kott. 2010b. Responses of *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae) to olfactory cues

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6. Benefits to the industry (max 1 page)

a) Describe the impact of the project results on Alberta's agriculture and food industry (results achieved and potential short-term, medium-term and long-term outcomes).

This project has contributed toward improving agri-food knowledge. Previous developments of insect resistance in crop plants have relied on a transgenic approach where one or more foreign genes are introgressed to host plant genomes. For example, Bt corn and Bt cotton production involve inserting genes into crop plants from the bacterium, *Bacillus thuringiensis*. The genes code for synthesis of a toxin causing mortality of insects that ingest it. This approach has met with opposition because it has been considered unnatural and potentially damaging to biodiversity if the transgenes are accidentally transferred to other sexually compatible non-crop species. Our approach of using existing genes for resistance to root maggots from a relative of canola (*Sinapis alba*) avoids this criticism, and consequently imparts more sustainable control.

In many instances where crop resistance has failed over the long term, crop resistance has been used in isolation, as the only control strategy practiced by growers. Our host plant resistance approach does not attempt to provide a 'silver bullet', single strategy for controlling root maggot infestations, but rather will provide one key component among a suite of cultural and biological strategies. Consequently this approach integrates very well with other root maggot cultural and biological control approaches already in place or being developed, and so can enhance sustainable, long-term control of this pest. Our determination of the mechanism of resistance, can aid in decisions of how best to maintain the resistance trait in the canola population for as long as possible so that insect resistance does not develop, or develops only very slowly in the root maggot population. Our identification of biochemical markers associated with compounds that induce germplasm resistance to root maggot attack can be used as a basis for introgression of the traits to elite commercial genotypes of canola, and so enhance canola productivity in regions of western Canada where root maggots cause severe economic damage.

b) Quantify the potential economic impact of the project results (*e.g.*, cost-benefit analysis, potential size of market, improvement in efficiency, etc.).

In western Canada, the canola industry is valued at approximately $15 billion annually to the economy of our country (Canola Council of Canada 2013). Root maggots can cause yield losses as high as 20% to *Brassica napus* (and 50% to crops of *Brassica rapa*) (Soroka et al. 2004). Assuming that only 2% yield losses occur averaged throughout the entire region of Canadian canola production due to root maggot attack, this still amounts to $333 million annually. Our resistant germplasm should reduce those crop losses substantially. We acknowledge that our results require further steps in the seed commercialization process. However, through our validation that specified germplasm does possess consistent and constant resistance to root maggot infestations, the identification of markers that are associated with resistant plants, and through our determinations of the mechanisms of resistance, the results determined in this project provide crucial foundation proof-of-concept information that can be pursued by commercial interests.

7. Contribution to training of highly qualified personnel (max ½ page)
Specify the number of highly qualified personnel (*e.g.,* students, post-doctoral fellows, technicians, research associates, etc.) who were involved in the project.

This project has contributed substantially to the training of highly qualified personnel. Mr. Dan Stanton has been working to conduct research related to the development of root maggot-resistant canola that will form the main component of his Ph.D. thesis. One post-doctoral fellow (Dr. James Tansey) was trained through this project for Years 1 and 2 of the study at the University of Alberta. Dr. Tansey was an ideal researcher for this work because he had recently completed his Ph.D. on studies to determine the mechanism of resistance in novel germplasm resistant to the cabbage seedpod weevil. At the University of Guelph, the project contributed to the training of post-doctoral researcher Dr. Ron Fletcher who worked in the lab of Dr. Laima Kott. Following the departure of Dr. Tansey from the University of Alberta to assume a managerial position with Dow AgroSciences, Mr. Adam Blake assisted with the final experiments associated with the study for Year 3. Mr. Blake had recently completed his M.Sc. training under my supervision at the University of Alberta, and he has now moved on to undertake Ph.D. training at Simon Fraser University in British Columbia. At the University of Guelph, Ph.D. student Mr. Ivan Malchev gained training in many techniques associated with determination of biochemical markers associated with root maggot resistance in canola.

The project also contributed to the training of a research technician, Mr. Ravi Subramaniam, who learned techniques associated with screening novel genotypes of canola for visual and olfactory cues associated with host plant resistance to insect herbivores. Finally, the project contributed to the training of several (ca. 10) summer students. The summer students were all in various stages of earning B.Sc. degrees at the University of Alberta or the University of Guelph, and training in this research project provided invaluable experience for their future careers in biology.

8. Knowledge transfer/technology transfer/commercialisation (max 1 page)

Oral Presentations:

Tansey, J.A., and L.M. Dosdall. 2010. Antixenosis and antibiosis resistance to and olfactory responses of root maggots associated with lines developed through *Sinapis alba* L. x *Brassica napus* L. Presented at the Joint Meeting of the Entomological Society of Alberta and the Western Forum on Pest Management, Lethbridge, AB.

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Tansey, J.A., and L.M. Dosdall. 2010. Evaluation of root maggot-resistant canola germplasm. Joint Meeting of the Entomological Societies of Canada and British Columbia, Vancouver, BC.

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Dosdall, L.M. 2011. Challenges and opportunities in the integrated management of insect pests of canola or oilseed rape. 13th International GCIRC Rapeseed Congress, Prague, Czech Republic. This was an invited plenary address.

Tansey, J.A., and L.M. Dosdall. 2011. Differential responses by some insect pests to novel insect-resistant *Brassica napus* L. germplasm. Presented at the 13th International GCIRC Rapeseed Congress, Prague, Czech Republic.

Stanton, D., L.M. Dosdall, and R.C. Yang. 2011. Understanding host preference of root maggots (*Delia* spp.) (Diptera: Anthomyiidae) in *Brassica napus*. Joint Meeting of the Entomological Societies of Canada and Acadia, Halifax, NS.

Stanton, D., L.M. Dosdall, and R.C. Yang. 2012. Evaluation of associational resistance in mixed stands of crucifers for breeding canola (*Brassica napus*) resistant to infestation by root maggots, *Delia* spp. Joint Meeting of the Entomological Societies of Canada and Alberta, Edmonton, AB.

Dosdall, L.M. 2012. Integrated insect management: Challenges and opportunities. Agronomy Update Conference, Red Deer, AB.

Dosdall, L.M. 2012. Insect pest management research supported by the Alberta Crop Industry Development Fund. Poster presentation prepared for an informal meeting of ACIDF grant recipients, Delta Hotel, Edmonton, AB.

Scientific Journals:

Soroka, J.J., and L.M. Dosdall. 2011. Coping with root maggots in prairie canola crops. *Prairie Soils and Crops* 4: 24-31.

Blake, A.J., J.A. Tansey, L.M. Dosdall, and R. Subramaniam. The role of visual cues in root maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae), responses to resistant genotypes of canola (*Brassica napus* L.). *Pest Management Science*, in press.

Popular Press:

Soroka, J.J., and L.M. Dosdall. 2011. Coping with root maggots in prairie canola. *Top Crop Manager*, November edition.

Tours:

Tour of research plots and laboratory facilities given to farmers, agrologists and members of the press, 2010 and 2011.