



**AAFC RESEARCH BRANCH
Research Project Final Report**

Developing Innovative Agri-Products Program (Vote 10 Funding)

Project Title:	Activity B.4: To develop integrated pest management strategies for insect pests of canola in Eastern Canada
Start Date (yyyy-mm-dd):	2011-04-01
Expected End Date (yyyy-mm-dd):	2013-03-31
Actual End Date (yyyy-mm-dd):	2013-03-31
Principal Investigator (PI):	Geneviève Labrie
Short Executive Summary of report:	
<p>Insect pests can cause serious damage to crops and significantly reduce yield. The insect species attacking canola in Eastern Canada are not as well documented as in Western Canada. New exotic invasive insects such as the cabbage seedpod weevil (<i>Ceutorhynchus obstrictus</i> have been found to attack canola in Eastern Canada since 2000. The impacts of flea beetles (<i>Phyllotreta crucifera</i> and <i>Phyllotreta striolata</i>) and, recently, European corn borer (<i>Ostrinia nubilalis</i>) are not well documented in this part of Canada. Climatic growing conditions in Eastern Canada are different, which results in different levels of insect pressure on canola. Knowledge on their incidence and economic threshold in relation to these conditions is lacking. The overall objective of this project was the development of integrated pest management strategies for insect pests of canola in Eastern Canada. Resistant lines of canola were tried against cabbage seedpod weevil and root maggot, with some lines showing good resistance. Management practices such as late seeding dates were effective in reducing flea beetle and cabbage seedpod weevil numbers. However, yield was better at the earliest seeding dates, even with the presence of high numbers of cabbage seedpod weevil. Fertilization and tillage did not have major impacts on populations of insect pests of canola. Biological control of cabbage seedpod weevil increased in the two years of the project and parasitoids were observed in Northern areas. Low populations of European corn borer observed in the two years of this study did not cause yield losses in canola. Insecticides were, however, effective in reducing damage by this species and could be used, if populations are high in some years. Insecticides are efficient to reduce populations of cabbage seedpod weevil. However, no differences in yield were observed in any trials in producers' treated fields. Furthermore, introduction of high numbers of cabbage seedpod weevil in cages with canola did not allow calculation of threshold levels against this pest as there was no relationship between yield losses and the percent of pods damaged in either year of this study. More experiments with higher levels of infestation are necessary to calculate these thresholds. This project resulted in improved understanding of the major pests of canola in Eastern Canada and strategies which can be used to reduce their impact on canola yield. More years of study, with different environmental conditions and different levels of insect pressure are needed to give strong recommendations to producers.</p>	

A. Research Progress and Accomplishments (to date in relation to expected milestones and deliverables / outputs)

- Include brief summary of:
 - Introduction, literature review, objectives, milestones and deliverables / outputs.
 - Approach / methodology (summary by objectives).
- Include results and discussion (overview by objectives and milestones), next steps and references.

Introduction

Environmental and agronomic conditions in Eastern Canada are different from Western Canada in terms



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of temperature, humidity and growing periods. Insect pest species are also different. The main pest of *Brassica* crops (Brassicaceae) in North America is the European cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera, Curculionidae), which was first observed in British Columbia in 1931 (McLeod 1962). In Eastern Canada, cabbage seedpod weevil was reported for the first time in Quebec in 2000, and in Ontario in 2001 (Brodeur et al. 2001; Mason et al. 2003). Adults feed on flower buds, causing their destruction (bud-blasting), and larvae feed within seedpods. Each larva consumes five to six seeds to complete its development, causing losses of 18-20% (Dmoch 1965; Dossdall and Dolinski 2001; McCaffrey et al. 1986; Buntin and Raymer 1994). In Ontario and Quebec, high levels of infestation were observed during the first years of appearance (28%-75% of pods infested in Ontario and Quebec respectively) (Brodeur et al. 2001; Mason et al. 2003). Since that time, populations have been monitored each year in the province of Quebec and spread to the eastern area was noted (Couture et al. 2008; Couture and Parent 2010). However, the impact of this insect on canola in Eastern Canada is not well defined. Other insects, such as root maggot or flea beetles, can also significantly reduce canola yield (Lamb 1984). European corn borer has recently been recorded attacking canola in Eastern Canada, but there is no information on its economic impact, population density, economic threshold or control measures that could be used in canola production. An increase in canola production is expected to result in an increase in populations of insect pests (Carcamo et al. 2008) and insect pest management strategies have to be adjusted before this increase happens.

Canola production in PEI has increased over the past few years; between 2010 and 2011, an increase from 2500 acres to 3000 acres was noted and this increase in acreage is expected to continue. With increases in canola acreage, increased insect pest pressure should be expected. Insect pests can cause serious damage to the crop and significantly reduce yield. It is important to identify the insect species that can cause crop damage in order to develop control strategies to minimize damage and maximize yield. There are several insect pests that attack canola in PEI, one being the European corn borer (ECB), *Ostrinia nubilalis* (Lepidoptera: Crambidae). The European corn borer was introduced to North America from Europe in the early 20th century and has spread to all corn producing areas in North America. In Canada, it is found in all provinces except British Columbia and is presently regulated by the Canadian Food Inspection Agency for domestic and import movement of corn (CFIA 2006). European corn borer adults emerge in late spring to early summer and the female begins laying eggs on the stem and leaves of its host plant. The larvae hatch within a week and remain on the foliage for one to two days before burrowing into the stem. Once inside the stem, the larvae feed on the pith, phloem and xylem tissue, restricting the flow of water and nutrients to the terminal parts of the plant. Damage results in wind breakage, water loss and invasion of pathogens (Anderson et al. 1981). Precise timing of an insecticide spray is essential, because once inside the stem, the larvae are protected (Nault and Kennedy 1996). This insect is generally a pest on corn where it can cause significant yield reduction (Hudon and LeRoux 1986). However, in the Maritimes, it has moved into potatoes where insecticide control measures are required to protect the crop and reduce yield losses (Boiteau and Noronha 2007, Noronha and Carragher 2006). The European corn borer has only recently been found attacking canola in PEI. Because it is a new pest of canola, there is no information on the economic impact of damage caused by larval feeding or potential control measures that could be used in this crop.

Strategies for control in Western Canada could not be adapted to Eastern Canadian conditions and there is a need to evaluate and develop integrated pest management strategies for Eastern Canada, which include development of resistant canola cultivars, best management practices, identification of biological control agents and development of economic thresholds.

The base of an integrated pest management strategy is to develop cultivars with resistance to pest species which allow high yield and quality. While there are cultivars with this type of resistance for Western Canada, they could not be adapted to Eastern Canadian conditions. Thus, it is necessary to test and develop such varieties for Eastern Canada. As part of this DIAP proposal, the most damaging insect pests on canola in Eastern Canada were to be investigated. Among these are cabbage seed pod weevil (*Ceutorhynchus* sp.), root maggot (*Delia* sp.) and flea beetle (*Phyllotreta* sp.). The University of Guelph's part of this proposal was to generate breeding lines with some resistance to these pests. Resistance was



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introgressed into canola by classical crossing methods from the weedy relative *Sinapis alba*. Several publications describe this research progress (Shaw et al. 2009; Dossdall & Kott 2006; Ekuere et al 2005; Kott & Dossdall 2004). Over the past number of years, as a routine, these traits were introgressed into the University of Guelph's breeding lines and some of these lines were offered to ECODA DIAP collaborators for field testing during 2011 and 2012.

Management practices such as seeding dates, rotation, fertilization regimes and tillage are adapted for specific local conditions. These practices can influence development and abundance of insect pest species. Late planted fields have been observed to escape cabbage seedpod weevil damage in Western Canada (Dossdall et al. 2008a). It has also been demonstrated that increased fertilization can increase damage by cabbage seedpod weevil larvae (Dossdall et al. 2008a). Direct seeding can reduce abundance and damage by flea beetles because of increased moisture under crop residues (Milbrath et al. 1995). The influence of such management practices on insect pest species of canola in Eastern Canada has not been evaluated.

Biological control agents of insect pests in canola are well known in Western Canada (Dossdall et al. 2009). However, knowledge is lacking for Eastern Canada. Some parasitoid wasps of cabbage seedpod weevil have been recently discovered in Quebec (Labrie et al. 2010; Mason et al. 2011), but the incidence of these natural enemies is not known.

Chemical control is necessary when populations of a pest reach the economic threshold level. Economic thresholds for cabbage seedpod weevil or flea beetles have been established for Western conditions. No economic threshold levels are available for European corn borer in canola. Economic thresholds have to be adapted and the efficacy of insecticides needs to be evaluated for Eastern Canadian conditions.

Objectives

General Objective: In order to improve production and increase the competitiveness of this industry, the **general objective of the project was to develop integrated pest management strategies for insect pests of canola in Eastern Canada.**

Specific Objectives:

- 1) Develop resistance of canola to major insect pest species in canola;
- 2) Develop management practices for prevention and control of insect pest species in canola;
- 3) Evaluate the incidence of biological control on major insect pest species of canola; and
- 4) Determine economic threshold levels and evaluate insecticide performance for major insect pest species of canola.

Milestones

Milestone 1: Resistance of canola to insects

- Protocol for identifying biochemical markers associated with root maggot developed, based on glucosinolates & HPLC technology;
- Number of selected DH lines identified with resistance to root maggot based on field resistance;
- Number of biochemical compounds involved with root maggot repulsion or attraction;
- Identification of biochemical compounds active in the cotyledons by HPLC or GC;
- Development of biochemical screening methods to identify flea beetle resistance;
- Selection of a number of individual winter canola breeding lines identified in field and lab with superior resistance;
- Identification of molecular markers associated with field resistance to cabbage seedpod weevil;
- Identified canola lines with resistant trait;

Milestone 2: Management practices' impact on insect pests

- Identification of tillage regime (conventional or direct seeding) which reduces abundance and/or damage by flea beetles and cabbage seedpod weevil in canola;
- Identification of fertilization regimes which reduce populations and/or damage of flea



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beetles and CSW in canola;

- Development of recommendations on tillage and fertilization regimes for increased yield of canola under reduced pressure by insects
- Identification of seeding dates which reduce abundance and damage by flea beetles, and CSW;
- Identification of combinations of seeding date and insecticide use which reduce impact of insects in canola and increase yield of canola;

Milestone 3: Biological control of insect pests in Eastern Canada

- Emergence number of parasitoids wasps from larvae of cabbage seedpod weevil in Ontario, Québec and Maritimes;
- Identification of species of cabbage seedpod weevil parasitoids in Eastern Canada;
- Identification of potential biological control programs with parasitoids to control cabbage seedpod weevil;

Milestone 4: Chemical control of insect pests in canola

- Incidence of damage in canola under specific insect pressure (number of insects introduced in cages);
- Correlation between yield and known abundance of insects;
- Economic evaluation of the impact of each insect on canola in relation to yield, seed price and treatment costs;
- Reduction of insect numbers following insecticide treatments;
- Evaluation of canola yield increase following insecticide treatments;
- Identification of best insecticide treatments to control insect pest species to a level below economic thresholds.

Outputs/Deliverables

- Identification of biochemical methods for detection of root maggot resistance; Field and laboratory test results validating the presence of the resistance trait in selected germplasm;
- Introgression of the resistance traits into elite canola lines;
- Development of a biochemical screening method to identify flea beetle resistance using HPLC profiles to identify resistant DHs early for rapid breeding purposes;
- Reduction of abundance and damage by insects in canola under specific tillage (conventional vs. direct seeding) and fertilization regime;
- Reduction of abundance and damage by insects in canola under specific seeding dates combined with insecticide use;
- Identification of main biological control agents of cabbage seedpod weevil in Eastern Canada;
- Determination of economic impact of cabbage seedpod weevil and European corn borer;
- Determination of economic threshold levels for cabbage seedpod weevil and European corn borer

Methodology

4.1 Resistance of canola to root maggot and cabbage seedpod weevil (L. Kott, University of Guelph)

The **methodology** involved standard crossing of canola lines with resistance (P1) with new breeding lines (P2) in order to transfer the trait to more adapted and elite germplasm. F1s were produced for all three insect pests separately and were subjected to microspore culture for the extraction of doubled haploid lines. This activity generated DHs and assured 100% homozygosity of all traits including the resistance traits. Double haploids were then either tested in the field when time allowed or were tested in the lab using previously developed methods that identified specific HPLC profiles that indicated resistant lines. Some of these methods are published and others are available, if required. Among the DHs produced (e.g. 477 DHs in 2011), lines were selected for blackleg and stem rot during the culture period where time allowed. Seed of the best DH selections were increased for field trials and although most of



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the seed was forwarded to ECODA DIAP collaborators in PEI and Quebec (2011 and 2012), a small root maggot trial was carried out in Elora Research Station (University of Guelph) in 2012 as well.

Deliverables: Development of the cabbage seedpod weevil resistant DH lines was at a more advanced stage and, therefore, seed of these lines was already available for field testing in 2011. Sixteen resistant lines of cabbage seedpod weevil (2011 and 2012) were sent to Dr. Anne Vanasse (U. Laval) and 23 root maggot resistant lines were sent to Dr. Denis Pageau (AAFC Normandin) and to Dr. Richard Martin (AAFC Charlottetown) in 2012.

The following 16 lines, selected for **cabbage seedpod weevil** resistance, were sent for field screening to Dr. A. Vanasse (2011 and repeated 2012).

CSPW/05-255 CSPW/05-268 CSPW/06-341 CSPW/06-351 CSPW/06-363 CSPW/06-371
CSPW/06-373 CSPW/07-424 CSPW/07-426 CSPW/07-428 CSPW/07-442 CSPW/07-446
CSPW/08-512 CSPW/08-514 CSPW/08-515 CSPW/08-517

The following 23 lines were sent to Dr. D. Pageau and Dr. R. Martin for field screening for **Root Maggot** damage (2012).

1362-02, 1362-03, 1362-04, 1362-05, 1362-06, 1362-07, 1362-08, 1362-10, 1362-11, 1362-12, 1362-13, 1362-14, 1362-15, 1362-16, 1362-17, 1362-18, 1362-19, 1362-20, 1362-21, 1362-22, 1362-23, 1362-24, 1362-26

Biomarker development objectives (root maggot resistance identification) (L. Kott, University of Guelph) were to examine biochemical compounds contained in the third and fourth leaves of root maggot field tested canola plants that are most likely involved in plant-insect interaction. This involved 1) production of HPLC profiles from a larger number of pre-screened canola lines in the field (identified as either resistant R or susceptible S); 2) analysis of the HPLC peak profiles to identify biochemical markers; and 3) use mass spectrometry characterization of these compounds.

1 & 2) **Marker development.** HPLC profiles of third (markers denoted with 'M') and fourth (markers denoted with 'R') leaves of 40 lines were analysed giving a sufficient number of observations for logistic regression for marker development. Using two subpopulations (resistant & susceptible), logistic regression procedure was applied to the HPLC data. That statistical approach is applicable also for predictive analysis, so as a result the most significant factors (peak areas or markers) are involved in the plant-insect interaction and there is a model to test newly developed lines for resistance. Trying various methods to select the minimum number of markers, probably containing compounds directly involved in the plant-insect interaction on which to build the model that predicts and explains the plant-insect interaction, the branch-and-bound algorithm was used, testing different marker combinations to find a specified number of models with the highest likelihood score (chi-square) statistic (table 1).

Table 1. Regression models selected by score criterion

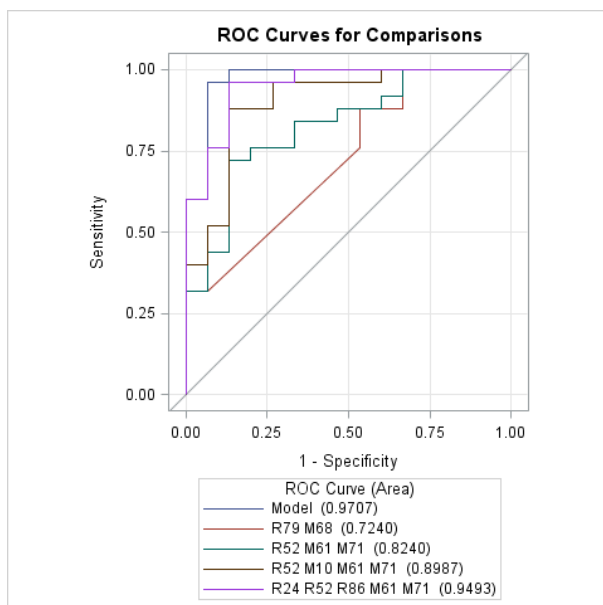
Number of Variables	Score Chi-Square	Variables Included in Model
2	8.792	R79 M68
3	12.2196	R52 M61 M71
4	16.3577	R52 M10 M61 M71
5	19.5063	R24 R52 R86 M61 M71



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Since adding more markers did not improve the model significantly (figure 1), the model is based on three markers (AUC=0.8240).

Figure 1. Comparison of different models by using AUC (area under the curve) of ROC curves, better models are with AUC closer to one, weaker models are with AUC closer to 0.5 (the diagonal)



3) **Identification of biochemical compounds.** The objectives of mass spectroscopy study were 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 ESI-LC/MS, which allowed the identification of compounds mainly from known chemical classes, such as glucosinolates. Then, using the Bruker AmaZon SL *ion trap* LC-MSn system it was possible to link the previous results, ion-by-ion, and to determine the MS patterns of the compounds of interest. To date, the available markers to specific compounds were linked based on chemical structure.

Marker	probable compound
R52	Proanthocyanidin A1
M61	Kaempferol-3-O-glucose-7O-glucoside 3' methoxy quercetin-3- O glucoside-7 Oglucoside
M71	Glucobrassicin

4.2 Management practices (A. Vanasse - U. Laval, D. Pageau - AAFC Normandin. G. Labrie - CÉROM)

4.2.1 Tillage and fertilization

In 2011, canola was sown in Saint-Augustin-de-Desmaures (May 18th) and Normandin (June 21th) in split-plots with four replications. The main plot was the soil tillage (no-till and conventional tillage). The subplots were composed of three nitrogen sources (urea, calcium ammonium nitrate (CAN) and slow-release fertilizer (SRF)) at four rates (0-50-100-150 kg N/ha). The total number of experimental plots was 96. In Normandin, experimental plots had to be planted twice: all plots were first consumed by flea beetles, and incorrect herbicides were applied the second time, so the conventional tillage plots were



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planted again on June 21th. Evaluation of defoliation by flea beetles was done by visual observation of three plants at three different locations in each plot, between cotyledon and five leaves. Insect sampling was done by three sweeps at two stations per plot during flowering period. Insects were identified directly in the field. On July 27th and August 5th at Saint-Augustin-de-Desmaures, 100 pods were randomly collected from each plots, observed for emergence holes of CSW, and put in emergence boxes for evaluation of parasitism of CSW (section 4.3).

In 2012, canola was sown in Saint-Augustin-de-Desmaures (May 12th) and Normandin (June 12th) in split-plot with four replications. The main plot was the soil tillage (no-till and conventional tillage). The subplots was composed of nitrogen (urea with ammonium sulfate) at four rates (50-100-150-200 kg N/ha) and sulfur (ammonium sulfate) at three rates (0, 20, 40 kg S/ha). The total number of experimental plots was 96. Fertilizers were applied on May 12th at Saint-Augustin-de-Desmaures and on June 12th at Normandin. Evaluation of defoliation by flea beetles was done by visual observation of three plants at three different locations in each plot, between cotyledon and five leaves. Insect sampling was done by three sweeps at two locations per plot during the flowering period. Insects were identified directly in the field. Canola was harvested on August 21th at Saint-Augustin-de-Desmaures and on September 24th at Normandin.

4.2.2 A) Impact of seeding date and insecticide on flea beetle (D. Pageau, Normandin, QC)

In 2011, canola was sown in Normandin on three dates: May 20th (D1); May 30th (D2) and June 9th (D3). Plots were eight rows by six meters long. There were three insecticide treatments: No application (F0), one application (F1) and two applications (F2) of Matador[®] (a.i. lambda-cyhalothrin, 83ml/ha, 40 lb psi). Each treatment was replicated four times. Maturity of canola was reached on August 31th for D1 and D2 and on September 18th for D3. Evaluation of defoliation by flea beetles was done by visual observation of three plants at three different locations in each plot, between cotyledon and five leaves (June 6th to June 29th).

In 2012, canola was sown in Normandin on three dates: May 18th (D1); May 28th (D2) and June 5th (D3). Plots were eight rows by six meters long. There were three insecticide treatments: No application (F0), one application (F1) and two applications (F2) of Matador[®] (a.i. lambda-cyhalothrin, 83ml/ha, 40 lb psi). Each treatment was replicated four times. Evaluation of defoliation by flea beetles was done by visual observation of three plants at three different locations in each plot, between cotyledon and five leaves (June 1st to June 22th). Canola was harvested between September 13th and 24th.

4.2.2 B) Impact of seeding date and insecticide on insects during flowering period (A. Vanasse, Saint-Augustin-de-Desmaures, QC)

In 2011, canola was planted in Saint-Augustin-de-Desmaures, on three dates: May 12th (D1); May 18th (D2) and May 25th (D3). The last seeding date did not emerge due to soil crusting and results were not considered for this date. For each treatment, three plots of nine rows by six meters long were seeded. There were two insecticide treatments: No application or one application of Matador[®] (a.i. lambda-cyhalothrin, 83ml/ha, 40 lb psi) at the 10% flowering stage. Each treatment was replicated four times. Insecticides treatments were done on July 1st (D1) and July 5th (D2). Cabbage seedpod weevil populations were evaluated by three sweeps at two locations inside two plots of each treatment between July 4th and July 12th. At stage 5.2, 200 pods per plot were collected and the number of holes caused by emergence of CSW was noted. Yield was evaluated on one of the three plots (undisturbed by sweep net).

In 2012, canola was planted in Saint-Augustin-de-Desmaures on three dates: May 2th (D1); May 14th (D2) and May 25th (D3). For each treatment, three plots of nine rows by six meters long were seeded. There were two insecticide treatments: No application or one application of Matador[®] (a.i. lambda-cyhalothrin, 83ml/ha, 40 lb psi) at the 20% flowering stage (June 21th, July 1st and July 7th). Each treatment was replicated four times. Canola was harvested between August 13th and 28th. Abundance of insects was evaluated by three sweeps at two locations inside two plots/treatment before the insecticide treatment and two days after the treatment. At stage 5.2, 200 pods per plot were collected and the number of holes caused by emergence of CSW was noted. Yield was evaluated on one of the three plots (undisturbed by



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sweep net).

4.3 Presence and incidence of parasitism on CSW (G. Labrie – CÉROM)

Damage by CSW, parasitoid presence and parasitism rate was evaluated in 38 sites in Eastern Canada in 2011 (Québec: 30 sites; PEI: 4 sites; New Brunswick: 3 sites; and Ontario: 1 site) and in 41 sites in 2012 (Québec: 37 sites; PEI: 3 sites; and Nova Scotia: 1 site). On each site, 1000 pods were collected two weeks before maturity and placed in rearing containers (Dosdall et al. 2006). The containers were maintained under continuous light at room temperature. A 2-cm-diameter hole at the end of the boxes allowed insects to exit into plastic collection containers. Collection containers were inspected daily during six weeks. The boxes were then opened and pod samples were examined under stereoscopic microscope for the presence of additional parasitoid adults that had not moved to the plastic containers.

4.4 Economic threshold for European corn borer and cabbage seedpod weevil and efficacy of insecticides

4.4.1 European corn borer (C. Noronha, AAFC Charlottetown)

A) Economic threshold

In 2011, canola plots were established at AAFC's research farm in Harrington PEI on June 17th. Sixteen plots, 6m x 3m in size, were planted with the variety InVigor at a rate of 5kg/ha seeding rate. Fertilizer (N:P:K, 20:10:10) at 500kg/ha was applied at seeding. Planting was delayed due to a very wet spring. Pheromone traps were placed at the end of June to monitor moth emergence and flight activity. Wild moths were allowed to lay eggs in the canola plots over the summer. In early October, 50 plants each from the left and right side of each plot were inspected for damage for a total of 100 plants/plot. Damage was recorded as the number of holes on each stem. The center of the plot (1.55m x 6m) was left undisturbed and was harvested to determine yield. Because of the low average temperatures, insect activity was very low over the summer and damage was much lower than expected. All plots were harvested in mid-October and seed yield was estimated. A regression analysis was used to determine the relationship between holes and yield.

B) Efficacy of insecticides against ECB

In 2012, canola plots were established at AAFC's research farm in Harrington on June 17th. The plots, 6m x 3m in size, were planted with the variety InVigor at a rate of 5kg/ha seeding rate. The plots were set up in a randomized block design with four treatments and four replicates per treatment. The four treatments consisted of 1) untreated check, 2) Coragen (chlorantraniliprole) at 300ml/ha, 3) Rimon 10EC (novaluron) at 600ml/ha, and 4) Success 480SC (spinosad) at 120 ml/ha. Fertilizer (N:P:K, 20:10:10) at 500kg/ha was applied at seeding. Planting was delayed due to a very wet spring. Pheromone traps were placed at the end of June to monitor moth emergence and flight activity. When moths were found flying in mid-July and eggs masses were detected, two insecticide sprays were applied one week apart (July 15 and 25) to the respective treatment plots. Plant growth was slow because of below average temperatures over the summer. In early October, 50 plants each from the left and right side of each plot were inspected for damage for a total of 100 plants/plot. Damage was recorded as the number of holes on each stem. The center of the plot (1.55m x 6m) was left undisturbed and was harvested to determine yield. Because of the low average temperatures, insect activity was very low over the summer and damage was much lower than expected. All plots were harvested in mid-October. Data collected were analyzed using an ANOVA and differences between means were estimated by Duncans Multiple Range test.

4.4.2 Cabbage seedpod weevil (G. Labrie, CÉROM)

A) Economic threshold

To evaluate the relationship between the number of pods damaged by CSW and yield, CSW was introduced in cages at the CEROM site in 2011 and 2012. Cages of muslin (1m x 1.5m x 2m) were installed at cotyledon stage in canola plots at the research centre. Three different introduction rates were used in the experiments in 2011: two, four and 10; and in 2012: four, 10 or 20 CSW per cages, with a



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control with no introduction. Each treatment was replicated three times. These introductions corresponded to 0.5 CSW/sweep (2), 1.4 CSW/sweep (4), 3.5 CSW/sweep (10) and 7 CSW/sweep (20). At maturity, 100 pods were collected and examined for CSW emergence holes. Canola was harvested in each cages in August 2012 and weighed for yield evaluation.

B) Efficacy of insecticides against CSW

Insecticide efficacy against CSW was evaluated at 1) CÉROM (Beloil, Quebec) and 2) three producers sites.

- 1) CÉROM (Beloil; 2011). Canola plots were established at the CEROM experimental site at Beloil, QC on May 23, 2011. The plots were planted with the variety 5030LL on eight replications of 10x10m. Four plots were considered control plots and four were treated with Matador on July 8th 2011. In 2011, it was impossible to harvest canola after the passage of tropical storm "Irene". Insect pressure was so low in 2012 that this trial was conducted.
- 2) Producers sites (2011 only). For this part, 16 sites were monitored in two regions around Québec City to find canola fields that reach threshold for insecticide treatments. Three sites in the National Capital region of Quebec were above the threshold for CSW at the beginning of flowering period (P1: three CSW/sweep net; P2: two CSW/sweep net; P3: 25 CSW/sweep net). In each of these fields, insecticide treatment with Matador was done at the 20% flowering stage. One hectare of each field was left untreated. At maturity, 200 pods were collected at four locations in each section (control and treated). Before commercial harvest of the fields, four 1m² samples of canola were harvested for yield evaluation.

In 2012, no canola fields were found above thresholds and no insecticide treatment were done.

Results & Discussion

4.1 Resistance of canola to root maggot and cabbage seedpod weevil

A) Root maggot field results from Elora (ON) & Normandin 2012 only

A number of **selected DH lines** identified with resistance to root maggot from the 477 doubled haploid lines generated from 36 crosses between RM resistant lines and elite breeding lines were also selected *in vitro* for *Sclerotinia* resistance during the microspore culture procedure and screened for blackleg resistance. The best 48 lines were then tested by HPLC for RM resistance or susceptibility and approximately 30 DH lines were available for field trialing. Twenty-three lines had enough seed for field tests.

Root Maggot resistant lines compared between Elora and Normandin locations:

The 23 DH lines selected for root maggot resistance along with InVigor 5440 as the check were field trialed in Normandin in the 2012 season. From each of the three replications, 25 plants were scored for maggot damage based on a 0 to 4 scale, where 0 represented no damage and 4 represented severe root damage with large areas of tunneling on the root surface. Ultimately for the summary, results were used only from replications two and three due to uneven and poor development of plants in replication one.

The seed sent was treated with Helix XTra as requested by Dr. Pageau. To combat flea beetle attack, the trial was treated with various pesticides, namely: Pounce on May 25th, June 6th and June 8th; Malathion on June 12th; and Sevin XLR on June 15th. All insecticides were applied at early stage of canola (between cotyledon and fourth leaf).

Figure 2 summarizes the results showing the SD bars and mean scores of all lines. Due to the heavy application of insecticides during the growing season, it was not possible to determine which, and if any, lines had greater tolerance to root maggot pressure. The check, InVigor 5440, was 17th among the twenty-four entries. Although the graph shows that there are clear differences between the means of many of the entries, the *p*-value for this data set was 0.37 indicating that there were no real statistical differences between the lines. Lines were ranked by mean damage ranging from a mean of 0.08 to 0.24 on the rating scale, which is almost a flat line since the whole range is over only 0.16 scoring units. Some



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lines had damage scores with extremely large standard deviations, which is reflected in the high *p*-value. These statistics indicate that the insecticide application had varying effects on the different lines, but in general and as expected, all lines in this trial clearly showed an improvement in damage ratings over the non-treated root maggot trial in Elora in a comparable field trial. Clearly, for the identification of root maggot resistance in these lines, insecticides should not have been used on this trial.

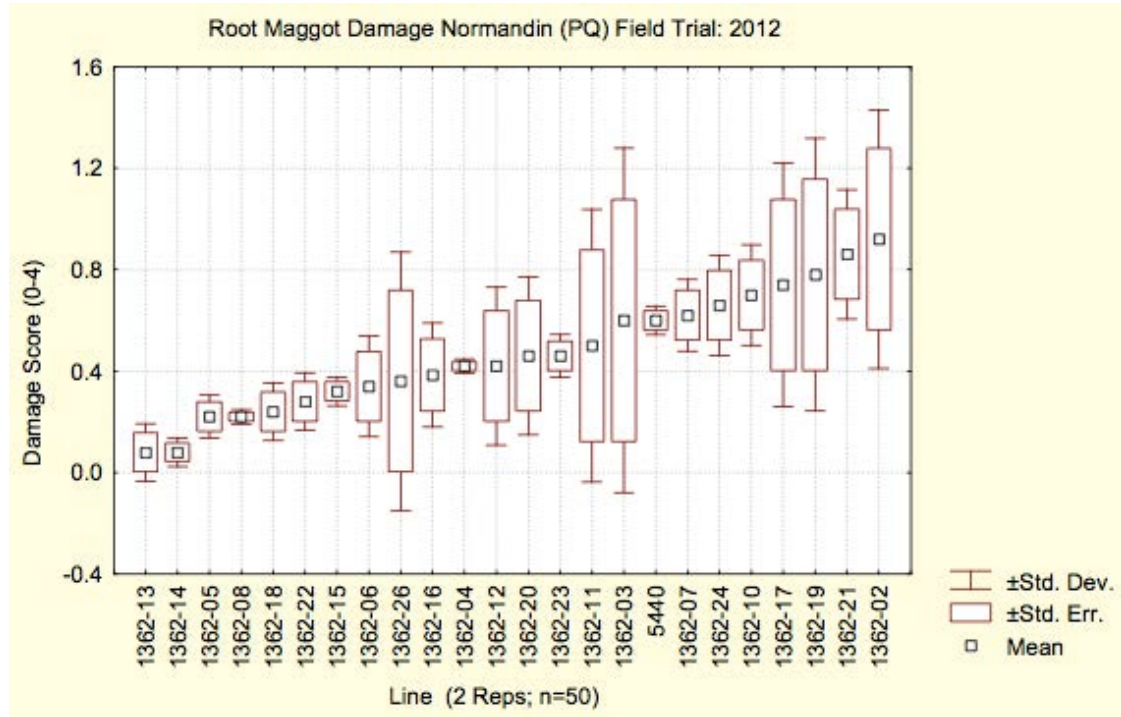


Figure 2.

Twenty-five DH lines from the same seed source were used in a field trial at Elora, using the lines where residual seed was available. For this reason the Normandin and Elora trials have slightly different entry sets, although many lines are common. The Elora trial consisted of four rows by six meters plots in two replicates. The RM damage was assessed on 15 roots from each plot/rep, 30 roots per entry. The canola hybrid checks were 45H21, 45H28 and 46H02. Neither initial seed treatment nor post emergence insecticides were used in this trial and, therefore, flea beetle damage on plants and seed yield was considerable, but root maggot damage could clearly be assessed. The root maggot damage scoring method was the same (0-4) as in the Normandin trial, which allowed damage comparison between the sites. Figure 3 summarizes the Elora trial where damage ranged from 1.68 to 3.13 on the maggot damage score, indicating relatively high insect pressure at this site. The *p*-value for this data set indicated that there are strong statistical differences between lines.

The four DH lines with the best RM damage scores were 1362-21, 1362-28, 1362-17 and 1362-11. Some of these DH lines could be used in the future to develop RM tolerant lines for Eastern Canada. One check, 45H21, scored well (ranked fourth) on the damage scale. This hybrid was bred for clubroot resistance, while the other two hybrid checks clearly were poor for RM resistance (ranking 25th and 28th). The insecticide treatments on the Normandin trial were done precisely at the time when the female maggot flies were selecting plants for oviposition. The critical timing for this appears to be around the three to four leaves stage, when flies respond to olfactory stimuli from the young leaves as observed in the laboratory studies. The results reflect the near absence of maggot damage in the treated trial at Normandin.



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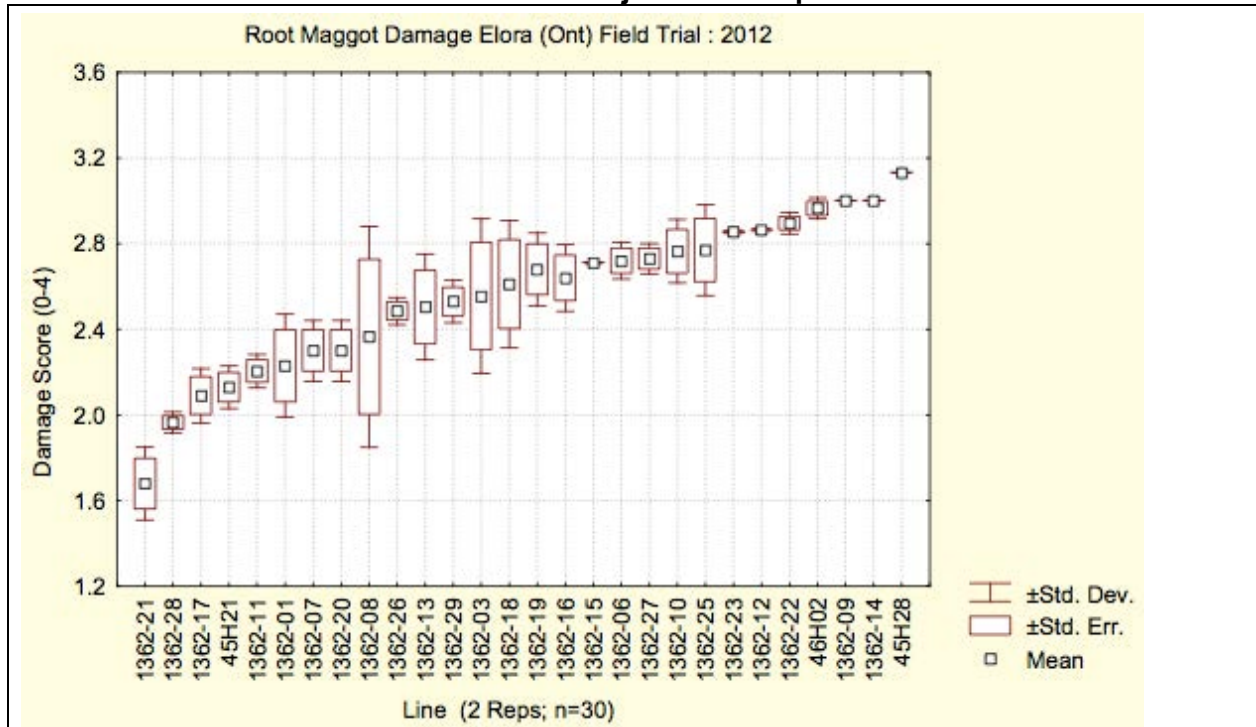


Figure 3.

For both RM trials yield data was determined, and although the Elora yields (mean of all lines 111 kg/ha) were low due to flea beetle and maggot damage in comparison to the sprayed Normandin trial (mean of all lines 282 kg/ha), where the Quebec yields were about 2.5 times higher than the Ontario yields. The DH lines showing the best RM scores were all in the upper third of the ranked yields, whereas the two worst DH lines for RM damage (1362-09 and -14) were lowest and fourth lowest in yield. In the treated trial, three of the DH lines with the best damage scores were in the top-yielding group. All raw field data is available, if required.

Biomarker development

This section of the project was not included in the original proposal, but became a possibility during the life of this project. The usefulness of an HPLC laboratory method could be foreseen when applied as a pre-field screen that would reduce time and effort to test all potential root maggot resistant lines, generated in a breeding population in the field. An HPLC method based on similar principles for the screening of cabbage seedpod resistant lines (Shaw et al. 2009) and root maggot resistant rutabaga lines (Malchev et al. 2010) was developed previously. The results of this work are incorporated in the 'Methodology' section (above). In summary, three biochemicals, identified as R52, M62 and M71, were found to be useful biomarkers for identifying resistant lines.

B) Cabbage seedpod weevil field trials from St-Augustin-de-Desmaures in 2011 and 2012

In 2011, all the lines were planted in four replicates of four rows by five meters long on May 20, 2011 at St-Augustin-de-Desmaures, QC. A control line (5030 LL) was used for comparison. Canola was harvested on September 3rd and 14th (one line). During the flowering period, three sweeps per plot were done on July 5th for evaluation of cabbage seedpod weevil populations. The number of insects varied between zero and four adults/sweep. In each plot, 100 pods were collected for evaluation of CSW damage (number of holes/100 pods). Damage varied between 0.75% and 5.5% of pods. The control line



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had the least damaged pods (0.75%) and CSPW/08-515 presented the highest percentage of damaged pods (5.5%). However, no experimental lines exceeded the economic threshold level of 25% of pods.

In 2012, all the lines were planted in four replicates of four rows by five meters long on May 5, 2012 at St-Augustin-de-Desmaures. Another control line (5440 LL) was used for comparison. Canola was harvested between August 16th and 28th. During the flowering period (between June 28th and July 6th), three sweeps per plot were done for evaluation of cabbage seedpod weevil populations. The number of insects varied between 0.33 and 8 adults/sweep (figure 4). Most lines were above threshold levels of two to four CSW per sweep. In each plot, 100 pods were collected for evaluation of CSW damage (number of holes/100 pods). Damage varied between 1% and 7% of pods. Six lines presented the least damaged pods (1-1.5%) (figure 5) and CSPW/08-515 presented the highest percentage of damaged pods (7%). This line was also the most damaged line also in 2011. However, no experimental lines exceeded the economic threshold of 25% of damaged pods.

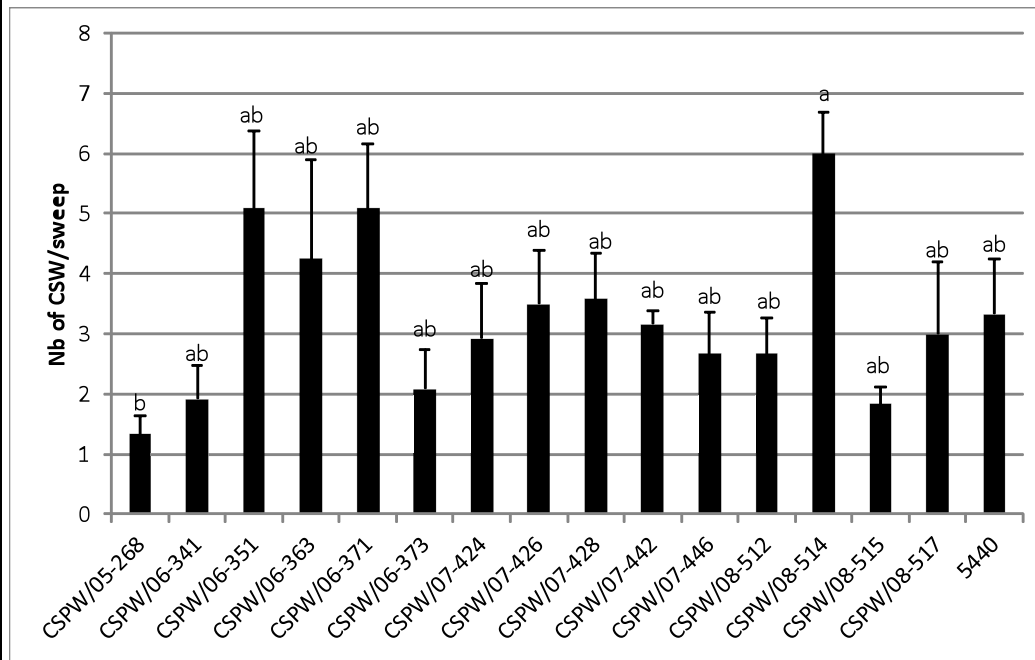


Figure 4. Mean number of cabbage seedpod weevil (CSW) on resistant lines during flowering period (June 28 to July 6, 2012) at Saint-Augustin-de-Desmaures. Note: different letters (a, b) indicate significant differences.



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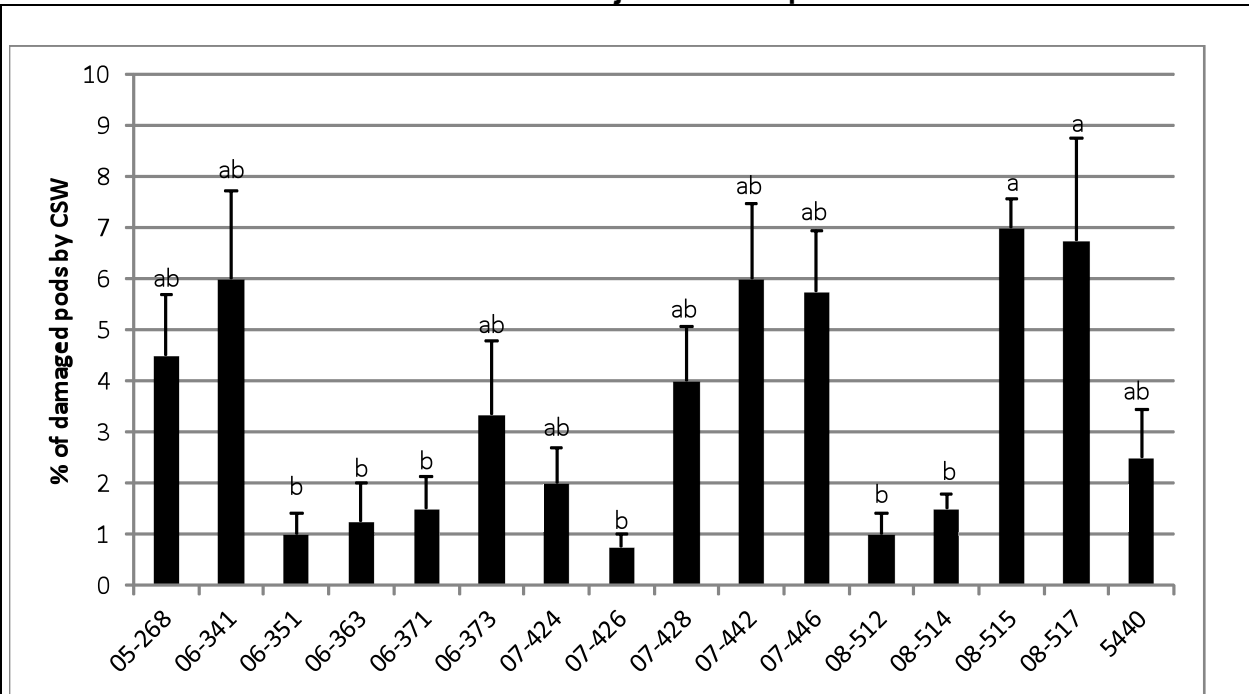


Figure 5. Percentage of pods damaged by cabbage seedpod weevil (CSW) in resistant lines during summer 2012 at Saint-Augustin-de-Desmaures. Note: different letters (a, b) indicate significant differences.

Overall discussion

Six lines were quite resistant to cabbage seedpod weevil in field trials and were significantly different from two lines. Four of these lines were also repeatedly screened at Lethbridge, AB and were resistant to CSW (351, 363, 371, 426). Two lines (512, 514) were never tried in field, but presented good resistance. Unexpected results came out from observation of CSW populations during flowering period and from damaged pods, which were negatively correlated. There was highest abundance of CSW on more resistant lines, which presented less damage. These results were the opposite of other work on resistant lines, where those lines presented less attractive chemicals for ovipositing females (Shaw et al. 2009). Higher abundance in these plots could be link to differences in flowering dates between all lines, and females could visit all plots equally to search adequate oviposition sites. More work has to be done on the resistant lines before this strategy could be used by producers.

4.2 Management practices

4.2.1 Tillage and fertilization

A) Saint-Augustin-de-Desmaures site (U. of Laval)

In 2011, there was an impact of date x nitrogen rate on CSW at 5% flowering stage, with highest number observed at 0 and 50 kg N/ha. There were also higher CSW numbers at 5 and 30% flowering stage in conventional plots than in no-till plots. However, there was no impact of treatment on damaged pods and number of seeds consumed by CSW, with 22.4% of damaged pods and 19.6% of seeds consumed by larvae observed in all plots. There was no impact of CSW on yield. The highest yield was observed in plots with the highest rate of nitrogen (figure 6), with no differences between sources of nitrogen.



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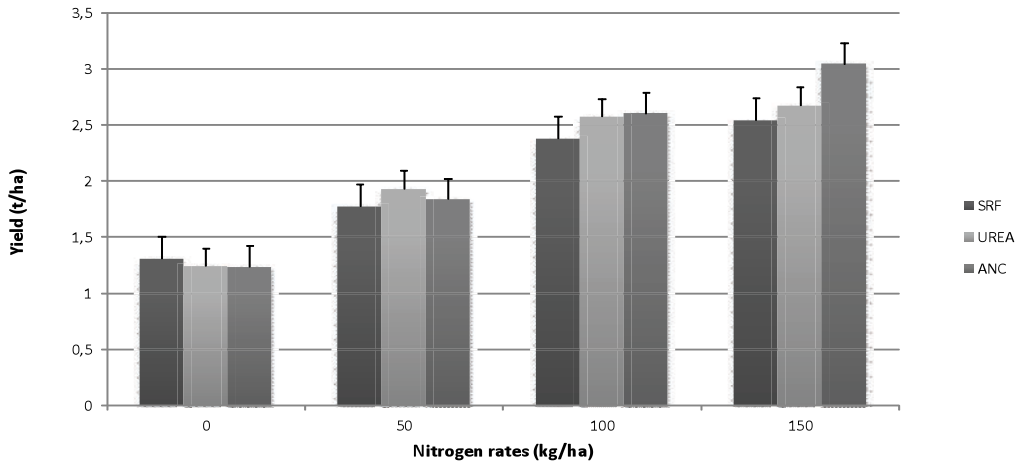


Figure 6. Yield of canola (t/ha) for three sources of nitrogen and four rates at Saint-Augustin-de-Desmaures in 2011. Note: SRF : slow release fertilizer; CAN: calcium ammonium nitrate.

In 2012, contrary to 2011, there were no effects of fertilization or tillage on cabbage seedpod weevil numbers. The lowest number of pods damaged by cabbage seedpod weevil occurred at 50 kg/ha nitrogen ($F_{3,95} = 3,61; P = 0.02$; figure 7). However, the number of damaged pods at 100, 150 and 200 kg N/ha did not exceed the economic threshold of 25% of pods (figure 7).

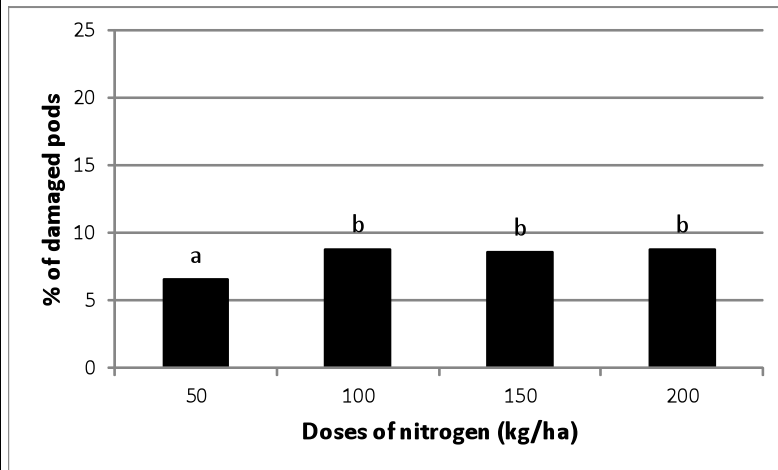


Figure 7. Percent of pods damaged by cabbage seedpod weevil for rates of nitrogen (kg/ha) at Saint-Augustin-de-Desmaures during summer 2012. Note: different letters (a, b) indicate significant differences.



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There was a difference in yield between tillage regimes ($F_{1,95} = 13.8$; $P = 0.004$; figure 8A) and rates of nitrogen ($F_{3,95} = 9.53$; $P < 0.001$; figure 8B), with no interactions between all variables. Highest yield was observed in conventional tillage and at 200 kg N /ha.

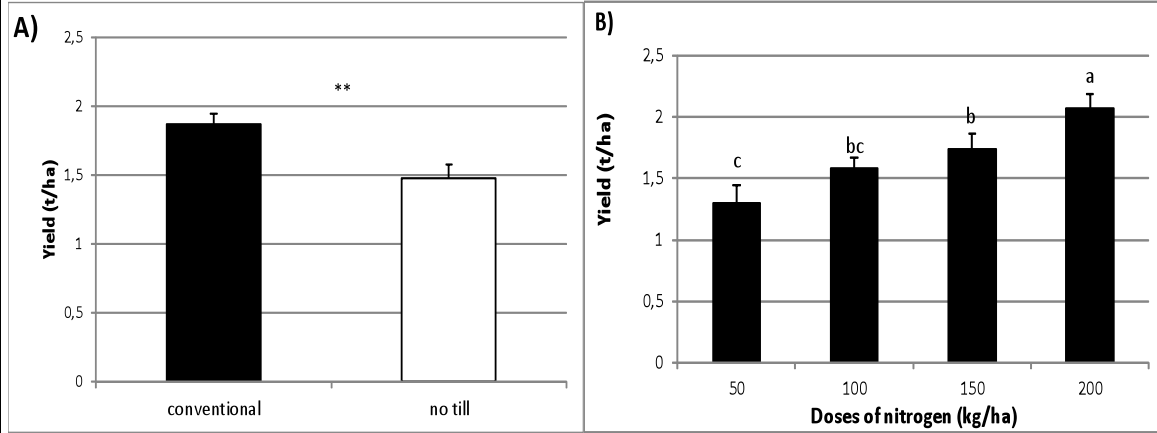


Figure 8. Yield of canola (t/ha) for different tillage regimes (A) and nitrogen at four rates (B) at Saint-Augustin-de-Desmaures in 2012. Note: different letters (a, b, c) indicate significant differences.

B) Normandin

In 2011, highest defoliation by flea beetles was observed on conventional tillage at one to two leaf (16/06) and three to four leaf (20/06) stages ($F_{2,138} = 6.09$; $P = 0.0002$; figure 9).

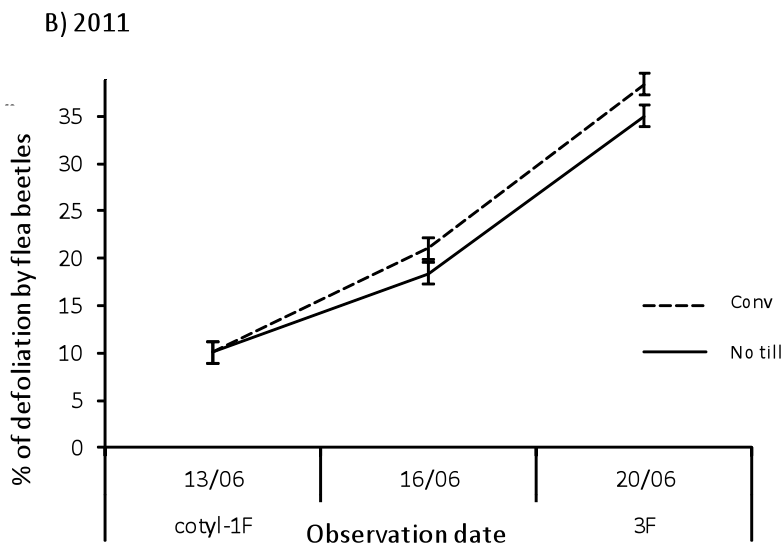


Figure 9. Defoliation by flea beetle following tillage (conventional or no-till) between cotyledon and four leaf stages at Normandin in 2011.



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In 2011, highest yield was observed in calcium ammonium nitrate (CAN) and slow-release fertilizer (SRF) at 100 kg N/ha, with a mean yield of 2.81 and 2.82 t/ha. With urea, the highest yield was observed at 0 and 150 kg N/ha, with a mean yield of 2.78 and 2.76 t/ha.

In 2012, defoliation by flea beetles did not differ among fertilization treatments. Less than 10% defoliation was observed in all treatments between cotyledon and four leaf stage.

Only 31 cabbage seedpod weevils were observed during the entire summer of 2012 in fertilization plots at Normandin, therefore data were not analysed.

Pollen beetles were abundant in fertilization trials in 2012. There was an effect of sulfur rates and tillage on abundance of pollen beetle during flowering period, with higher abundance at 0 kg/ha sulfur than at 20 or 40 kg/ha ($F_{2,645} = 6,37; P = 0,002$; figure 10) and at no till ($F_{1,645} = 19,03; P < 0,001$; figure 11).

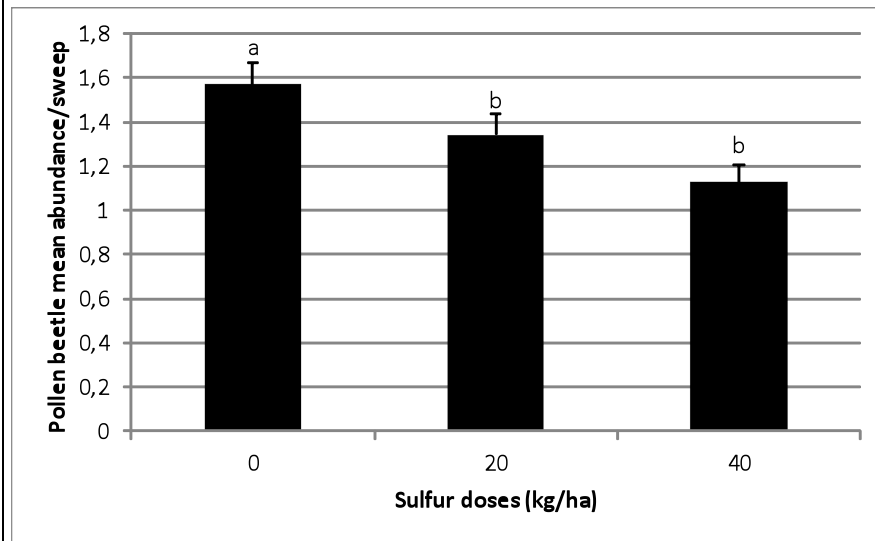


Figure 10. Mean abundance of pollen beetles following three sulfur doses during flowering period in 2012 at Normandin. Note: different letters (a, b) indicate significant differences.

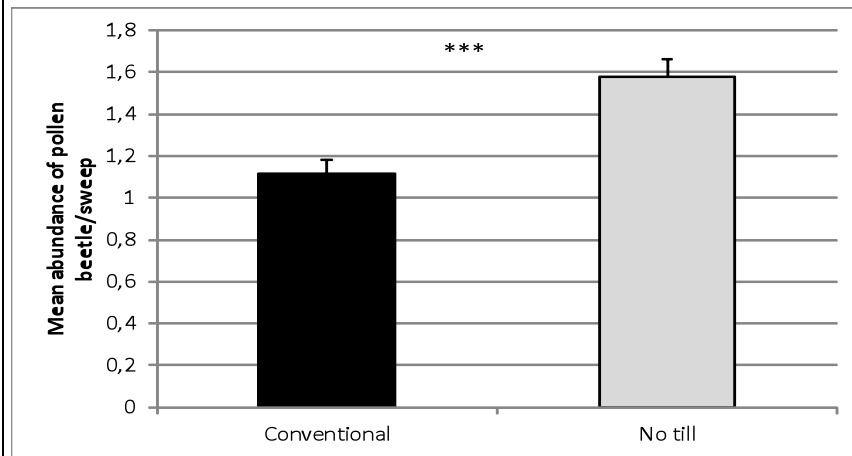


Figure 11. Mean abundance of pollen beetles in conventional and no till plots during flowering period in 2012 at Normandin. There was a difference in yield between tillage regime ($F_{1,645} = 22,81; P < 0,001$).



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figure 12A), rates of urea ($F_{3,95} = 11.16$; $P < 0.001$; figure 12B) and rates of sulfur ($F_{2,95} = 28.80$; $P < 0.001$; figure 12C), with no interactions between all variables. Highest yield was observed in conventional tillage, at 200 kg N /ha and 20 kg/ha sulfur.

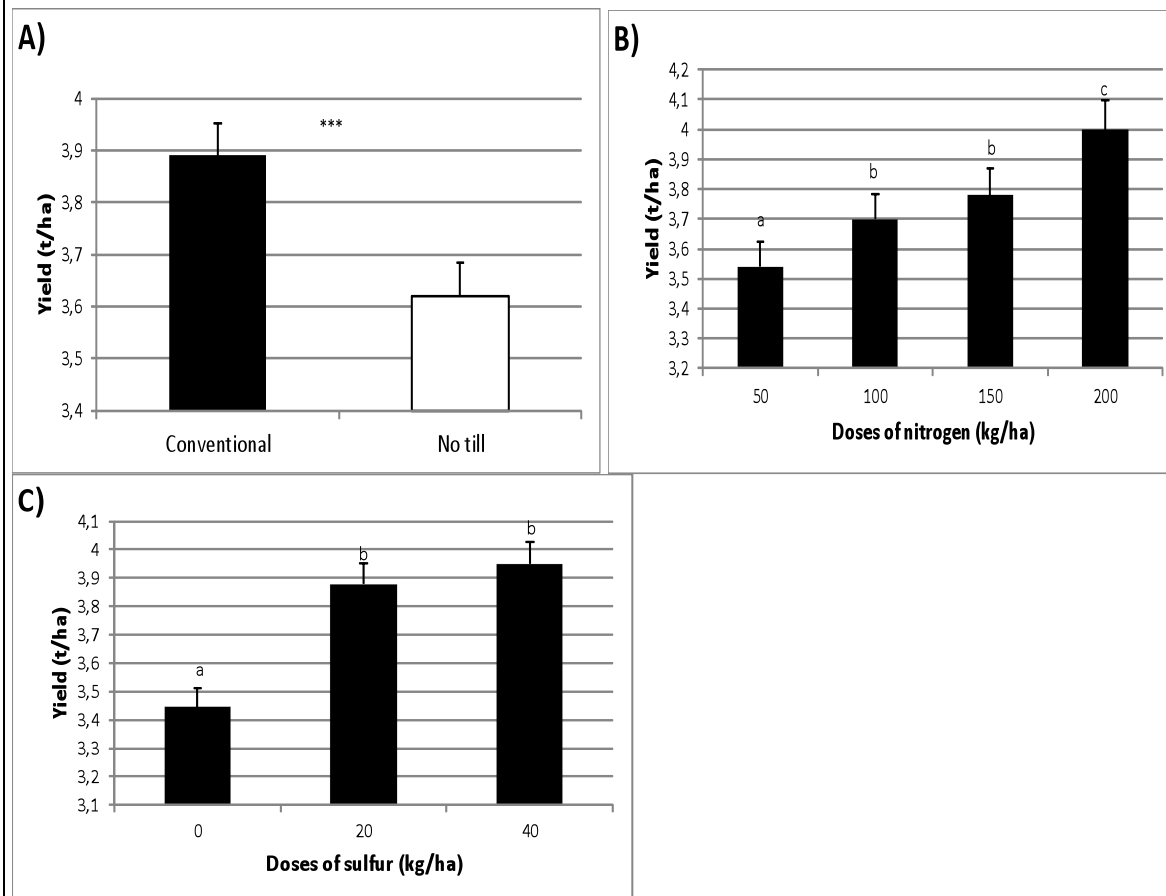


Figure 12. Yield of canola (t/ha) for tillage regime (A), nitrogen at four rates (B) and sulfur at three rates (C) at Normandin in 2012. Note: different letters (a, b, c) indicate significant differences.

Overall discussion

An impact of tillage on flea beetles was observed only in 2011 at Normandin. Dossdall et al. (1999) observed less flea beetle in no-till plots. The same results were observed at Normandin, with reduced numbers of flea beetles in no-till plots, which could be due to higher soil moisture. Flea beetles prefer dry soil conditions for oviposition, which is observed more often in conventional tillage than in no till. Soil moisture could be the same in conventional and no-till plots, or emergence of canola could be delayed in no-till plots, attracting flea beetles in these plots when conventional plots were more advanced.

The impact of fertilization on cabbage seedpod weevil was different than in the study by Dossdall et al. (2008a), where the highest fertility regime demonstrated higher numbers and higher damage to canola by this pest. In 2011, the highest numbers were observed at 0 and 50 kg N/ha on one site, with no impact on the number of pods damaged. Blake et al. (2010) also demonstrated an attraction and higher oviposition by cabbage seedpod weevil females at the lowest rate of nitrogen and higher rate of sulfur. Sulfur fertilization can result in increases in sulfur-containing glucosinolate compounds in plant tissue (Zhao et al. 1994; Kim et al. 2002), and the breakdown products of these compounds are attractive to



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adult weevils (Evans and Allen-Williams 1992; Blight et al. 1995). As a result, weevils tend to cluster in fields where sulfur content is high in plant tissue. In two years, the highest damage on canola pods was observed at the highest nitrogen rates only in 2012 and no interaction with sulfur was observed. Furthermore, there was no impact by insects on canola yield, with highest yield at 100 and 150 kg N/ha and 200 kg N/ha in 2011 and 2012, respectively.

Other studies demonstrated that the number of pollen beetles, *Meligethes* spp., cabbage seedpod weevil and cabbage root fly, *Delia radicum* (L.) (Diptera, Anthomyiidae), increase in plots fertilized with sulfur (Aljmli 2007). While an increase in sulfur is associated with an increase of compounds attractive to cabbage seedpod weevil, an increase in nitrogen is, however, associated with production of compounds repulsive to this pest (Kim et al. 2002; Blight et al. 1995; Smart and Blight 1997). The 2012 trials did not show any impact from the addition of sulfur on cabbage seedpod weevil abundance. However, lowest sulfur rates attracted more pollen beetles on one site in 2012. These results support the plant stress hypothesis where ovipositing females will prefer hosts with lower levels of N and S, because these plants would have higher concentrations of free amino acids resulting from reduced protein synthesis (White 1984).

On the agronomic side, highest fertilization in nitrogen and sulfur provide the highest canola yield. Given these results, no changes to current fertilizer management practices can be suggested for canola production in areas infested annually with high population densities of cabbage seedpod weevil.

4.2.2 A) Impact of seeding date and insecticide on flea beetle (D. Pageau, Normandin)

In 2011, flea beetle damage reached 25% in D1 at the cotyledon stage, but only 10% in D2 and in D3 (figure 13). The first seeding date reached the economic threshold of 25% of defoliation. The first seeding date was, thus, more attractive to flea beetle than the two other dates. However, there was no significant difference in seed yield between the three seeding dates or between no, one or two applications of insecticides (figure 14).

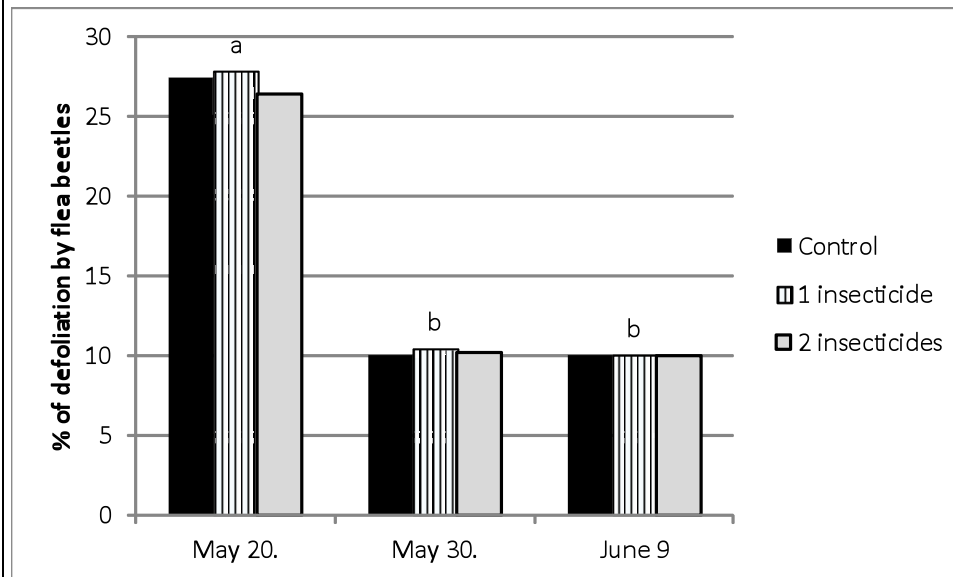


Figure 13. Defoliation by flea beetles on cotyledon stage of canola for three seeding dates and two insecticide treatments at Normandin during summer 2011. Note: different letters (a, b) indicate significant differences.



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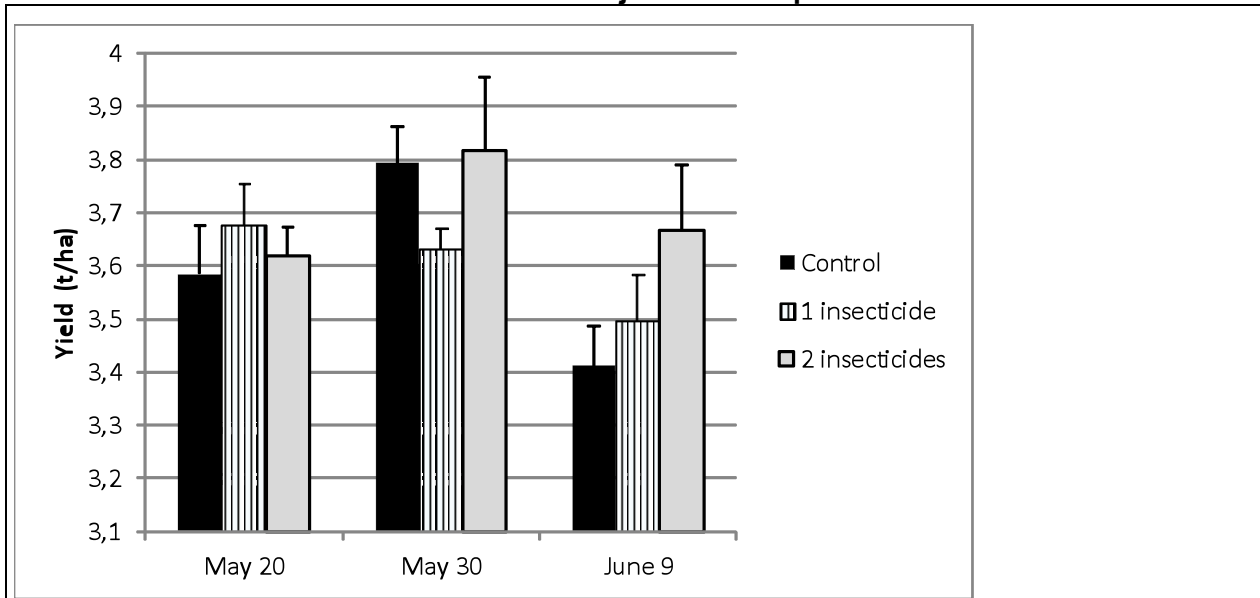


Figure 14. Yield of canola (t/ha) for three seeding dates and two insecticide treatments in Normandin, 2011.

In 2012, flea beetle damage at the cotyledon stage reached 9% on the first date, 32% on the second date and 13% on the third date (figure 15). The second seeding date was above the economic threshold level of 25% defoliation. This defoliation was also caused by slugs, which were very abundant in the experimental plots in 2012. In contrast to 2011, the second date of planting was more attractive to flea beetles than the two other dates at the cotyledon and one to two leaf stage (figures 15, 16, 17 and 18). In contrast to 2011, there was a significant difference in seed yield between the three seeding dates or between no, one or two applications of insecticides. However, there was no interaction between seeding date and insecticide treatment. The highest yields were obtained at the first and third seeding dates ($F_{2,35} = 26,71$; $P < 0,001$; figure 19A) and with two insecticide treatments ($F_{2,35} = 17,26$; $P < 0,001$; figure 19B), respectively.

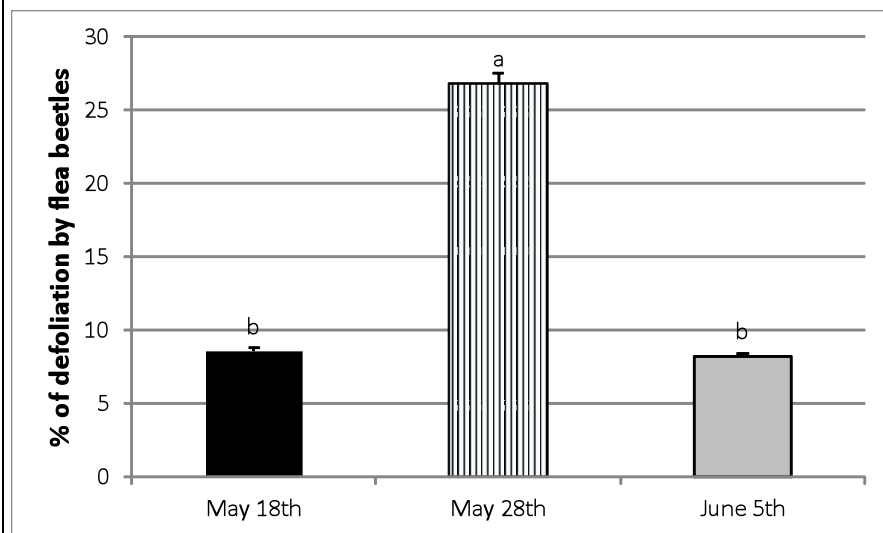


Figure 15. Defoliation by flea beetles on cotyledon stage of canola for three seeding dates at Normandin during summer 2012. Note: different letters (a, b) indicate significant differences.



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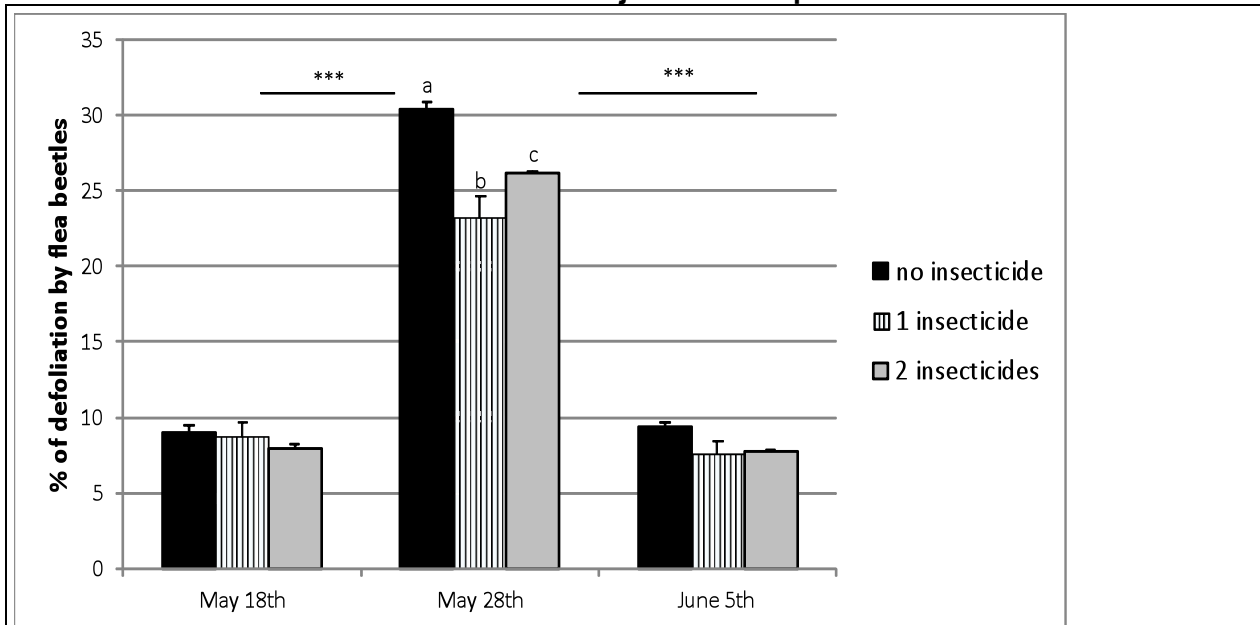


Figure 16. Defoliation by flea beetles at cotyledon stage of canola for three seeding dates, with no insecticide, one or two insecticide treatments (Matador®) at Normandin during summer 2012. Note: different letters (a, b, c) indicate significant differences.

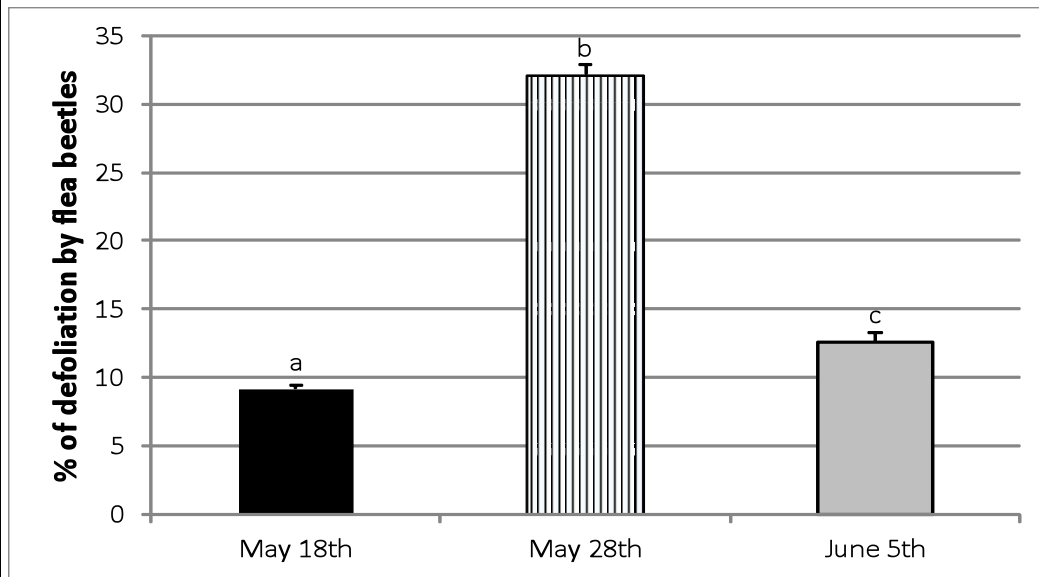


Figure 17. Defoliation by flea beetles on one to two leaf stages of canola for three seeding dates at Normandin during summer 2012. Note: different letters (a, b, c) indicate significant differences.



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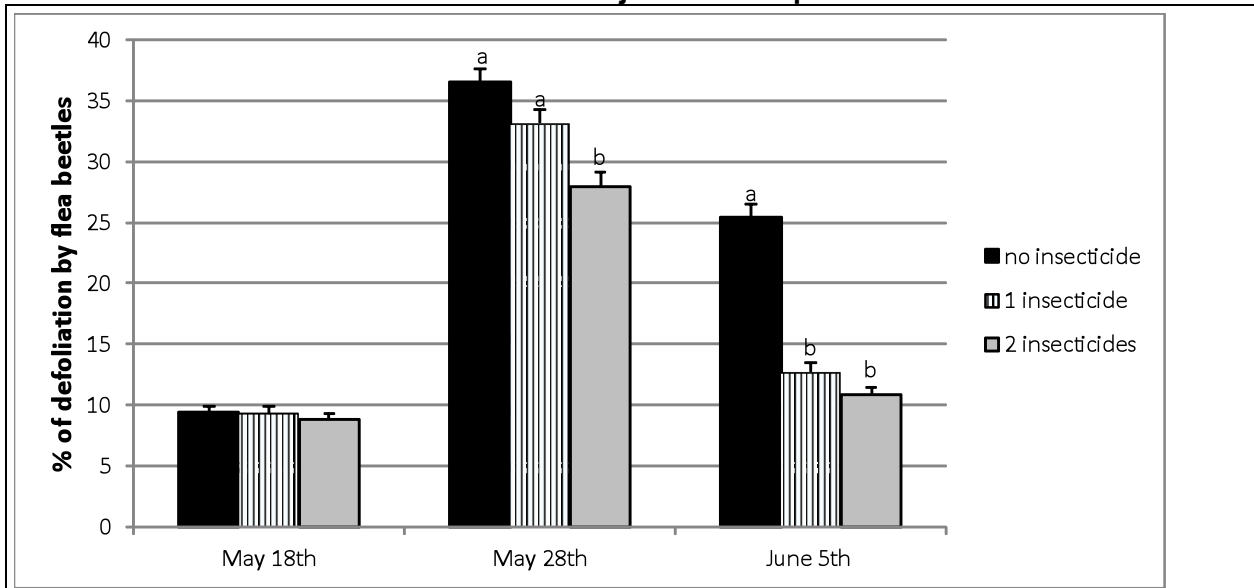


Figure 18. Defoliation by flea beetles at one to two leaves stage of canola for three seeding dates; with no insecticides, one or two insecticide treatments (Matador®) at Normandin during summer 2012. Note: different letters (a, b) indicate significant differences.

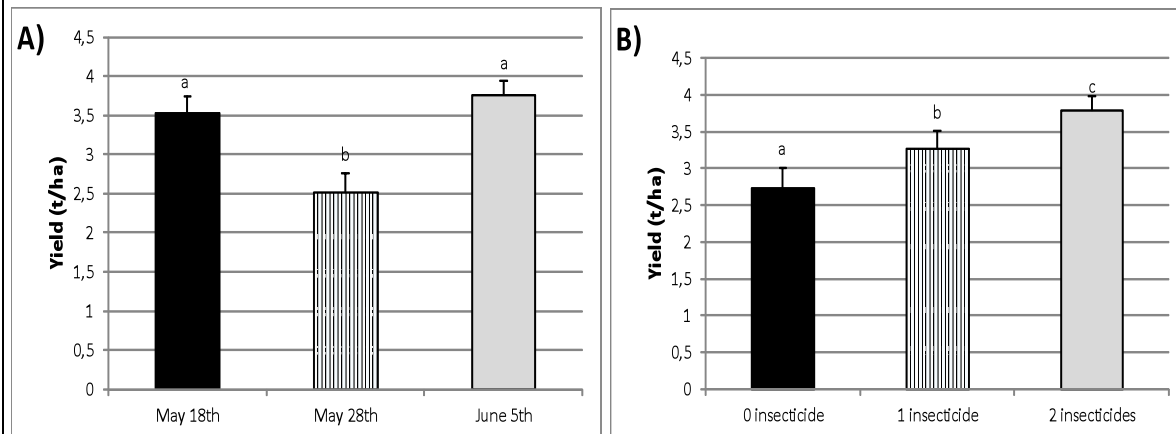


Figure 19. Yield of canola (t/ha) for three seeding dates (A) and three insecticide treatments (B) at Normandin in 2012. Note: different letters (a, b, c) indicate significant differences.

Overall discussion

The contrasting results for the impact of seeding dates on flea beetle damage could be explained by environmental conditions and the abundance of slugs. Effects of seeding date on flea beetle damage appear to vary among regions and are also likely to vary within a region depending on weather, because flea beetle damage may increase in early spring with hot, dry conditions (Carcamo et al. 2008). In 2011, temperatures at Normandin varied between 14 and 27°C on May 20th, while in 2012, temperature varied between 2 and 18°C on May 18th. Some studies have shown that flea beetles fly only when temperatures exceed 14°C (Lamb 1983). Temperatures on the first seeding date in 2011 was, thus, optimal for flea beetle flight and damages were high, while in 2012, slugs were very abundant with wet and cold conditions on the second date.

In 2011, flea beetle damage was not correlated with yield in the study. These results are similar to the study by Carcamo et al. (2008), where no yield differences were observed between early and normal



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seeding dates. Dosdall and Stevenson (2005) also observed no association between insecticide treatment for flea beetles and yield of canola, which could have been due to low soil moisture availability. The summer of 2011 was very dry in Normandin, which could explain the results. Other studies also showed that canola plants can compensate for defoliation levels of around 20% (Gavloski and Lamb 2000), and can tolerate up to 50% defoliation provided the growing tip is not damaged (Nowatzki and Weiss 1997). Damage by flea beetles in 2011 was around 25% defoliation. However, in 2012, damages were higher for the second seeding date (around 35% defoliation) and were correlated to yield losses. Insecticide treatments were, thus, effective in reducing flea beetle damage and yield losses at this date.

Some studies suggested that planting canola as early as possible is the best strategy to adopt when environmental conditions are optimal, but that additional control by insecticides will be required some years when planting early is correlated with high temperature (Carcamo et al. 2008; Knodel et al. 2008). More studies are necessary in this area to recommend the best strategy to use against flea beetles.

4.2.2 B) Impact of seeding date and insecticide on insects during flowering period (A. Vanasse, Saint-Augustin-de-Desmaures)

In 2011, there was a significant difference in CSW numbers for the first seeding date ($F_{1,26} = 49.99, P < 0.001$), with 9.05 ± 1.21 CSW/sweep in the control plot compared to 2.96 ± 0.36 CSW/sweep in treated plots two days after treatment. These numbers are above the threshold level of two CSW/sweep used in Western Canada. No differences were observed in CSW numbers on the second date. The number of pods damaged by CSW were, however, not different between treated and untreated plots for both seeding dates and were far below the economic threshold of 25% of pods damaged (D1- control: 4.7 % damaged pods; D1 – Treated: 3.21%; D2 – control: 3.86%; D2 – treated; 4.77%).

There were no differences in yield between control and treated plots for both seeding dates (figure 20).

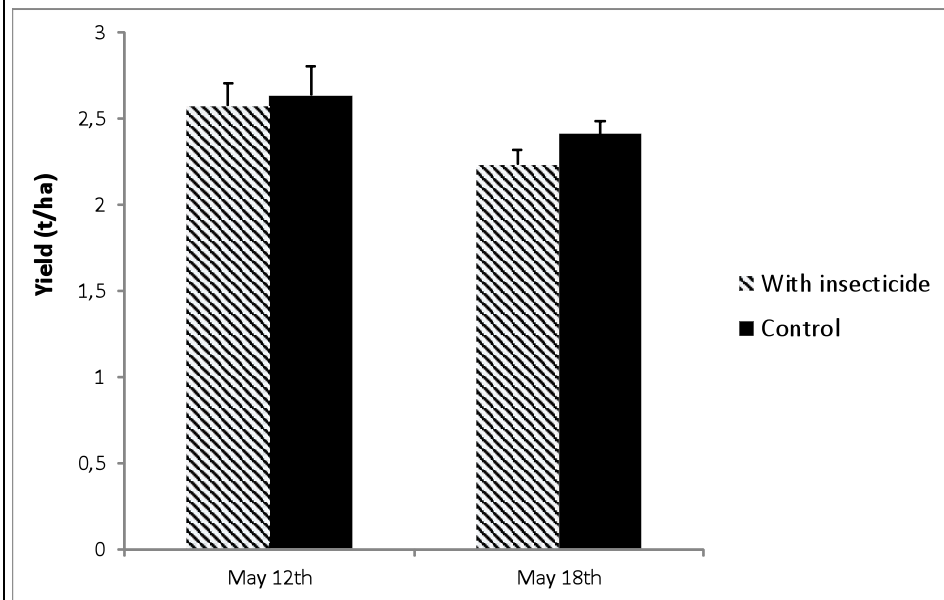


Figure 20. Yield of canola (t/ha) for two seeding dates and with insecticide treatment at Saint-Augustin-de-Desmaures in 2011.

In 2012, the economic threshold for cabbage seedpod weevil was reached during the first and second seeding dates, before insecticide treatment. Highest numbers of cabbage seedpod weevil occurred during the first seeding date ($F_{2,47} = 103.38; P < 0.001$; figure 21). There was a lower number of cabbage seedpod weevils in treated versus in control plots during first seeding date ($F_{1,17} = 4.45; P = 0.03$; figure



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21).

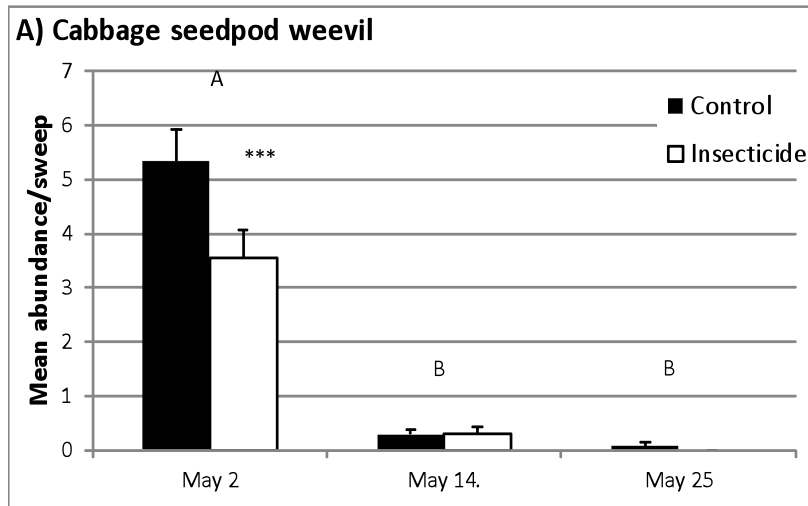


Figure 21. Mean number of cabbage seedpod weevils in sweep net after insecticide treatment for three seeding dates at Saint-Augustin-de-Desmaures during summer 2012. Note: Capital letters represent significant differences between seeding dates, while asterisks represent significant differences between control and treated plots for each seeding date.

There was no difference between treatments in the number of pods damaged by cabbage seedpod weevil ($F_{8,23} = 1,15$; $P = 0,39$; figure 22) even when the threshold was reached.

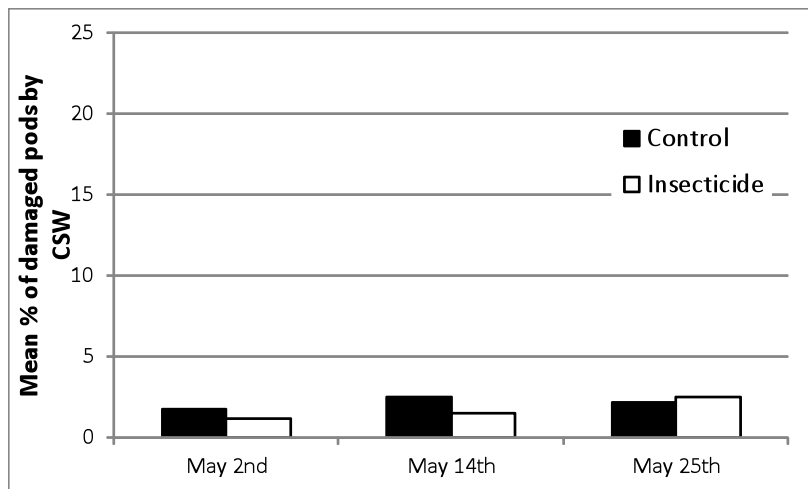


Figure 22. Mean percentage of pods damaged by cabbage seedpod weevil for three seeding dates and insecticide treatments at Saint-Augustin-de-Desmaures during summer 2012.

The highest yield occurred for the first seeding date ($F_{2,23} = 43,04$; $P < 0,001$; figure 23A) and in general, with insecticide treatments ($F_{1,23} = 5,20$; $P = 0,04$; figure 23B).



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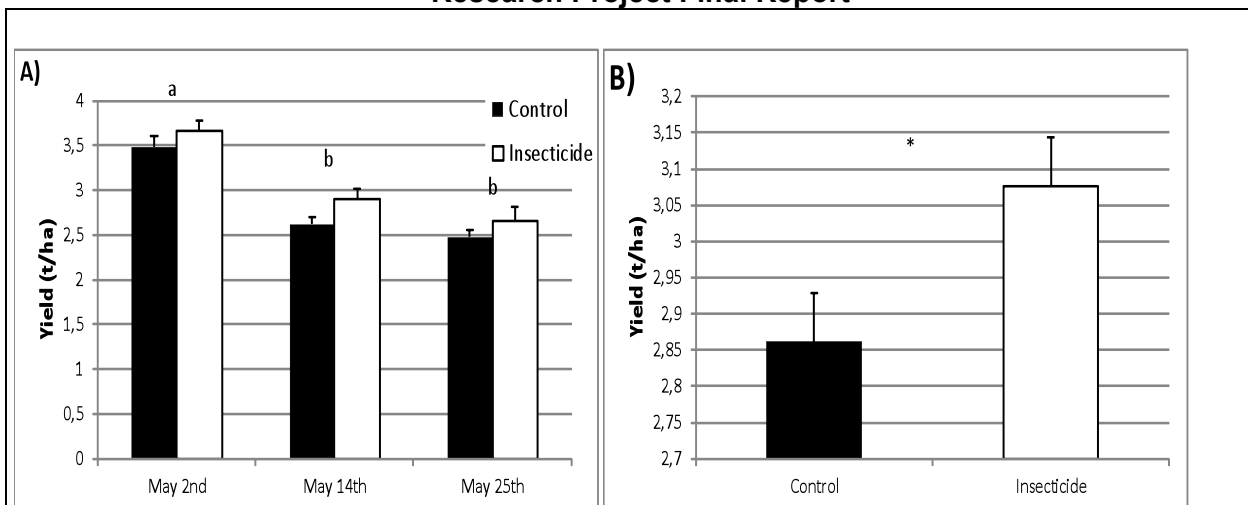


Figure 23. Yield of canola (t/ha) for three seeding dates (A) and insecticide treatment (B) at Saint-Augustin-de-Desmaures in 2012. Note: different letters (a, b) indicate significant differences.

Overall discussion

The highest abundance by cabbage seedpod weevil was observed in the first seeding date of both years of this study. Insecticide treatment was successful in reducing the numbers to below threshold levels. However, no significant damage on pods were observed in control or treated plots for all seeding dates during either year of the study. The highest yield was observed in 2012 on the first seeding date. These results are in contrast to studies of Dossall et al. (2004; 2008a), where highest damage and yield losses were observed on the first seeding date. Observation of parasitism in 2012 on these plots demonstrated that between 87.5 and 100% of pods infested by cabbage seedpod weevil were parasitized, which could explain the lack of correlation between cabbage seedpod weevil numbers and yield. Natural control is effective in reducing the impact of this pest, and the use of insecticides on these plots was, therefore, unnecessary. In fields where parasitoids are not present, seeding early could attract cabbage seedpod weevils and some yield losses may appear. However, seeding early also provides the highest yields, so more study on the seeding date will be necessary to provide recommendations to producers.

4.3 Biological control

In 2011, no pod damage by cabbage seedpod weevil was observed in PEI, New Brunswick and Ontario. The amount of damaged pods in Québec varied between 0.5% and 37%. Two sites were above the economic threshold of 25%, at Maskinongé (31%) and Bécancour (37%) (figure 24).

Both sites were sown with winter canola, which could be more attractive to cabbage seedpod weevil that just emerged from overwintering sites. Parasitoids were observed on 11 different sites in Quebec, located in six different regions. A total of 689 parasitoids was observed (figure 25).

However, one site provided 609 specimens of parasitoids, on a total of 19,200 pods observed (site for experiment 4.2.1 at U. of Laval). Parasitoids were observed for the first time in the north of the province (Normandin). Parasitism rates varied between 7.69% and 100% (figure 26).



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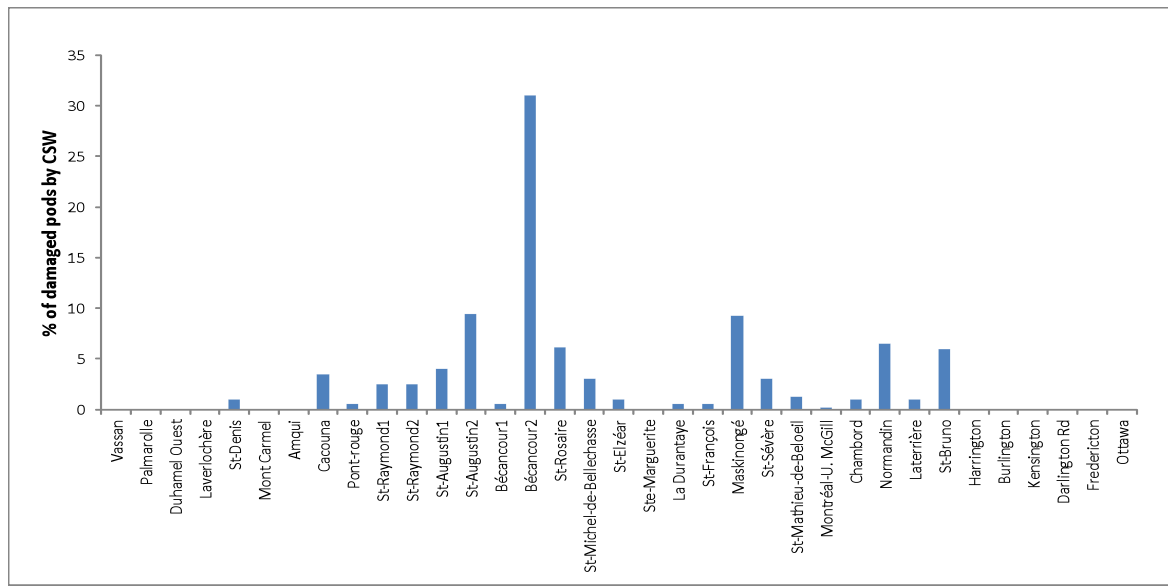


Figure 24. Percentage of pods damaged by cabbage seedpod weevil in different regions of Quebec, Ontario, Prince Edward Island and New Brunswick in 2011.

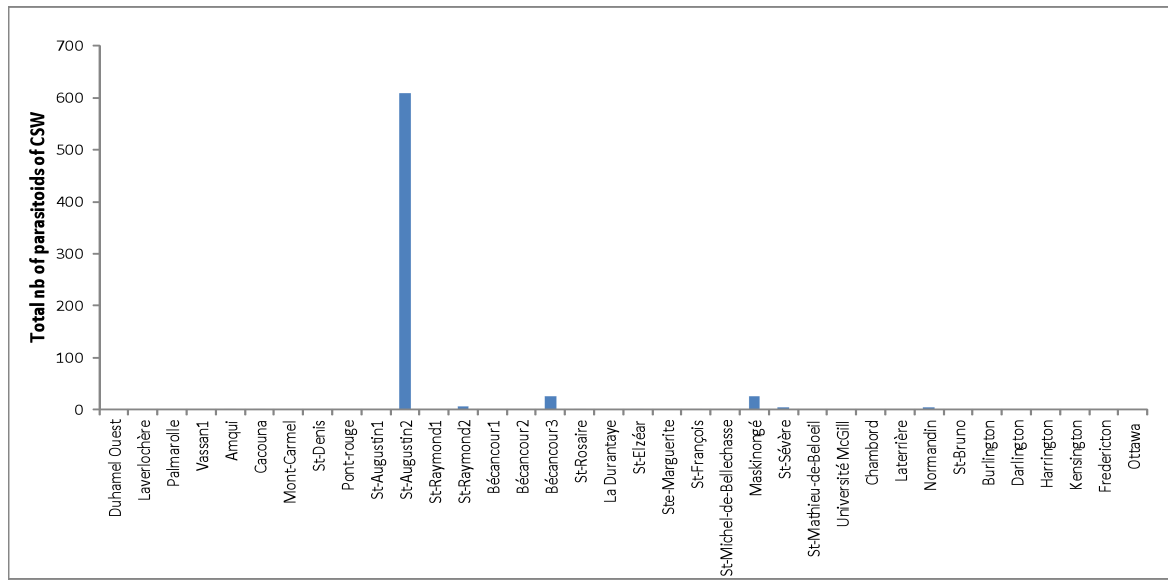


Figure 25. Total numbers of parasitoids in different regions of Quebec, Ontario, Prince Edward Island and New Brunswick in 2011.



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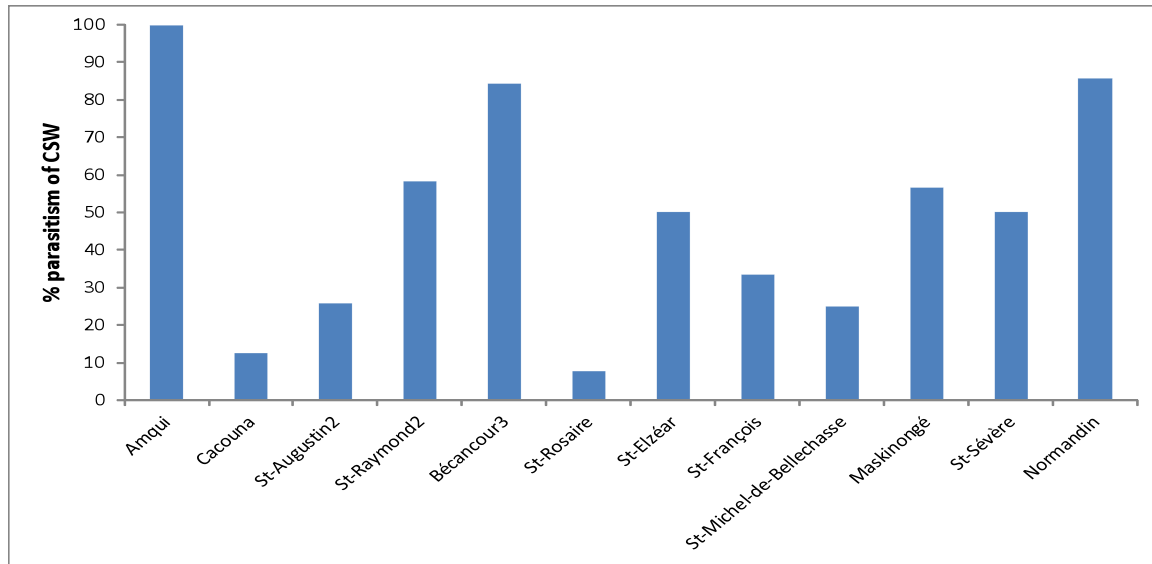


Figure 26. Parasitism rate on cabbage seedpod weevil (no. parasitoids/no. parasitoids + no. larvae of CSW) in different regions of Quebec in 2011.

In 2012, no pods damaged by cabbage seedpod weevil were observed in PEI and Nova Scotia (figure 27).

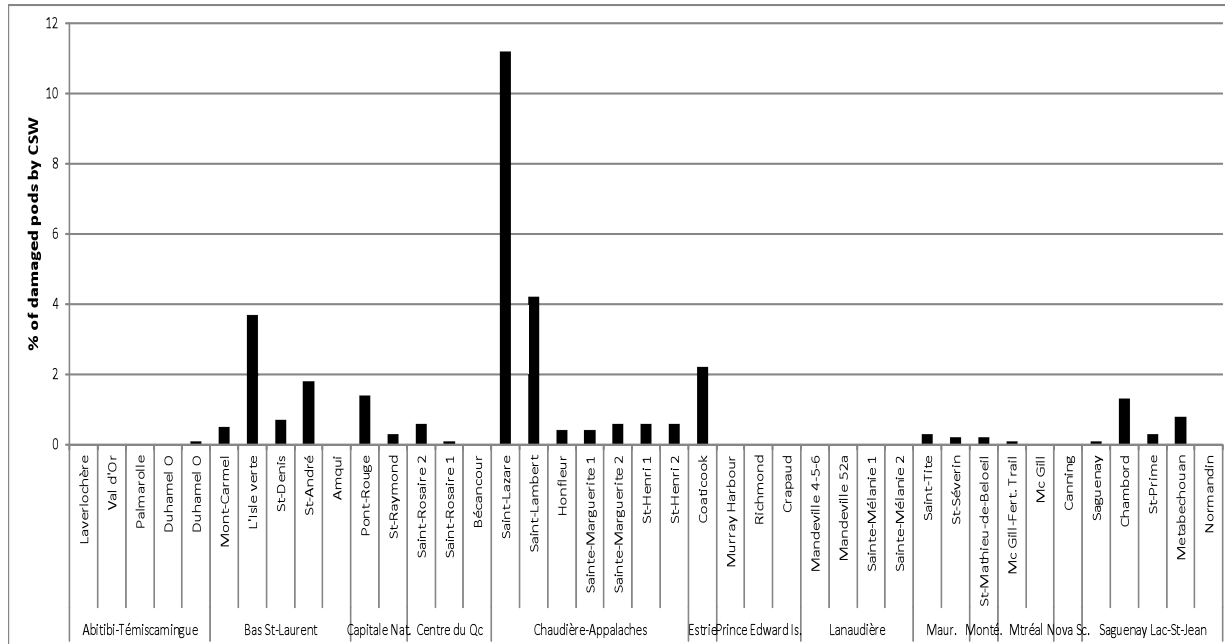


Figure 27. Percentage of pods damaged by cabbage seedpod weevil in different regions of Quebec, Prince Edward Island and Nova Scotia in 2012.



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The number of pods damaged in Québec varied between 0.1% and 11.2% (figure 28). No site was above the economic threshold of 25% of pods damaged. Parasitoids were observed on 22 different sites in Quebec, located in 11 different regions (figure 28). A total of 148 parasitoids was observed (figure 28). Additionally, 1,457 parasitoids were observed in the fertilization trial at Saint-Augustin-de-Desmaures and nine others at Normandin. Parasitism rates varied between 25 and 100% (figure 29).

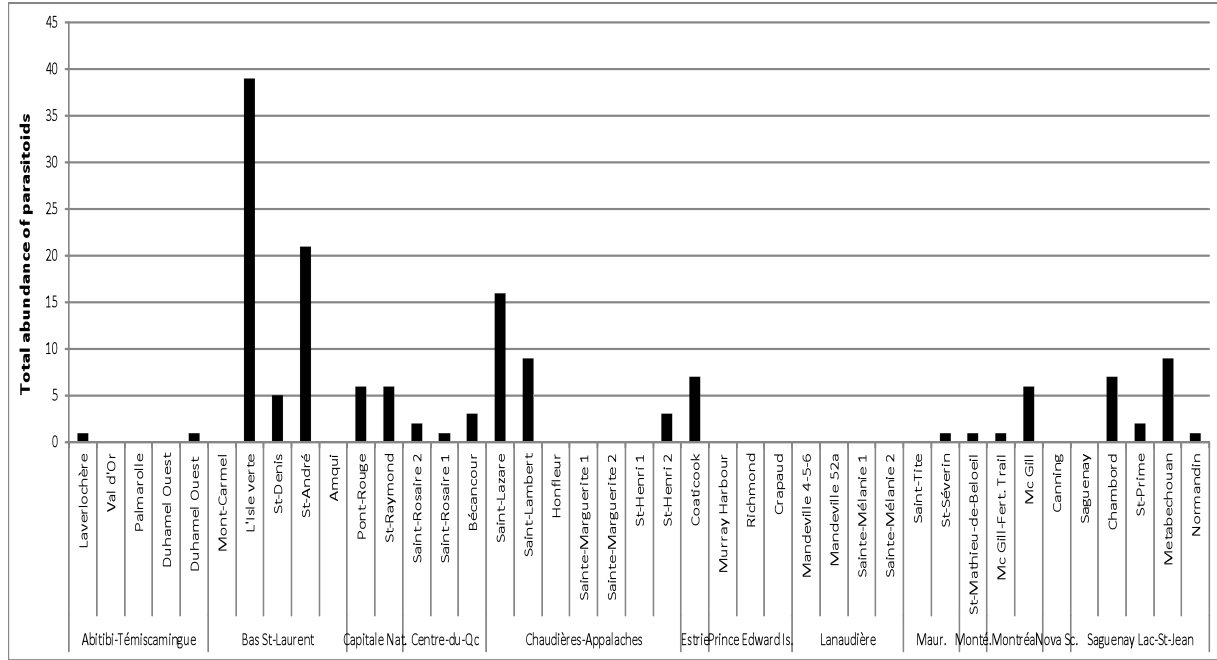


Figure 28. Total numbers of parasitoids in different regions of Quebec, Prince Edward Island and Nova Scotia in 2012.

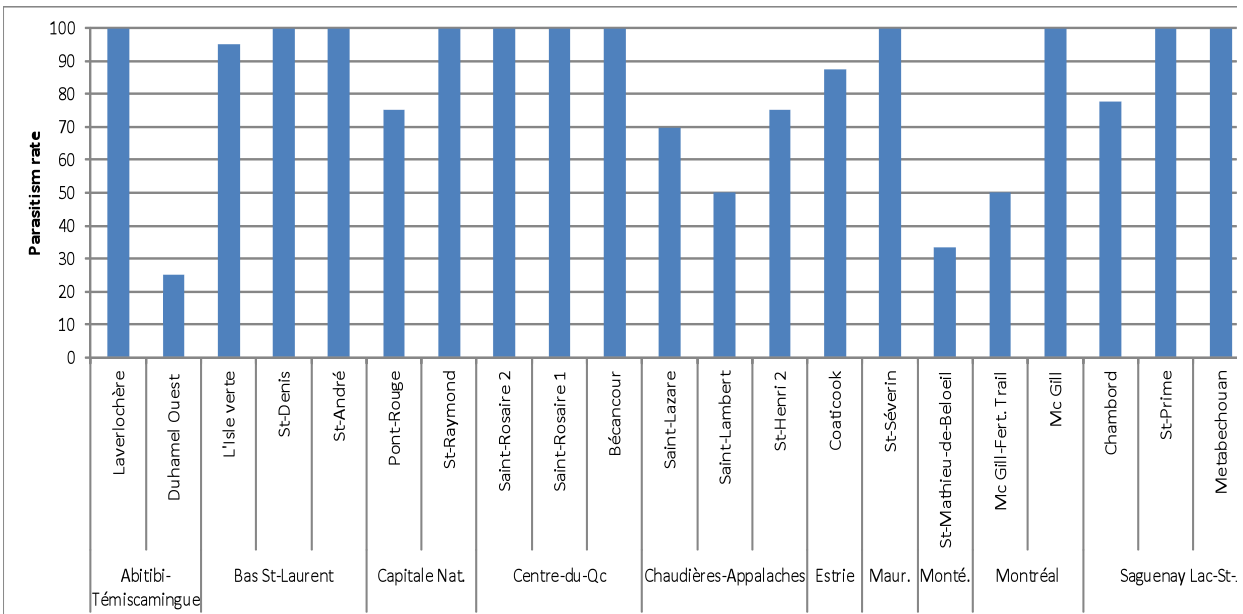


Figure 29. Parasitism rate of cabbage seedpod weevil (no. parasitoids/no. parasitoids + no. larvae of CSW) in different regions of Quebec in 2012.



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Overall discussion

There is clearly an expansion of parasitoids of CSW in the province of Quebec since 2009, where *Trichomalus perfectus* was observed for the first time on three sites in two regions (Labrie et al. 2010; Mason et al. 2011). In 2011 parasitoids were observed at 11 sites, while in 2012, they were observed on 22 sites. Furthermore, parasitoids were observed in Normandin for the first time in 2011 on one site, while in 2012, they were discovered in four different sites in this region. Other studies also observed an increase in parasitoid numbers and parasitism rates in two or three years of study after the first observation of their presence (Kevvař et al. 2006; Dosdall et al. 2008b).

In Europe, the cabbage seedpod weevil is host to 31 species of parasitoids (Ulber et al. 2010), with *T. perfectus* being the most important parasitoid responsible for reducing *C. obstrictus* abundance (Williams 2003). While identification to species for all parasitoids was not completed in this study, many of them are first identified as *T. perfectus*.

Estimates of parasitism by *T. perfectus* in Europe range from 10 to up 95%, and can be high even at low pest densities (Büchi 1991; Buntin 1998; Kulhmann et al. 2006; Lerin 1987; Murchie et al. 1997; Murchie and Williams 1998). In this study, parasitism rates ranged between 7 and 100%, with a mean parasitism rate of 42% in 2011 and 84% in 2012. These rates are similar to European sites, where almost no chemical control is needed against cabbage seedpod weevil. Biological control by parasitoids in Quebec seems, thus, effective in controlling cabbage seedpod weevil in many regions and sites. However, parasitoids are not present in all canola fields monitored and some of these fields could be at risk of damage by this pest. Enhancement and preservation of these parasitoids is of great concern for the future as insecticides could affect the parasitoids and reduce natural control.

4.4 Economic threshold for European corn borer and cabbage seedpod weevil and efficacy of insecticides

4.4.1 European corn borer (C. Noronha, AAFC Charlottetown)

A) Economic threshold

No significant relationship was found between damage by the European corn borer and yield in 2011 and 2012 (figure 30, 31 and 32). A trend towards decreasing yield with higher number of holes was seen in 2011 and at one site in 2012, however, the low R sq value decreases the confidence in this prediction. In both years natural ECB populations were very low.

In 2011, unseasonably low temperatures over the summer in PEI contributed to low insect populations in general.

In 2012, two sites were observed and the number of plots was doubled, however, once again because of low populations, stem damage ranged from 0-18 holes per 100 plants, a level of infestation considered to be extremely low for this pest. In 2012, the moisture content of the seed and the oil quantity and quality were estimated. No significant differences were found in the moisture content, oil content and oil quality (percent oleic and linolenic components) irrespective of the level of damage (table 2). This data was not collected in 2011. The low level of damage, maximum of 15-18 holes per 100 plants, would explain the reason why no significant differences in yield and oil quantity or quality were found.



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Table 2. Average yield, seed moisture, dry matter oil, % oleic and linolenic content in canola plots with different levels of damage in 2011 and 2012.

holes /100 plants	Yield t/ha	Moisture	Oil	Oleic	Linolenic
0.00	-	-	-	-	-
1-5	1.99	-	-	-	-
6-10	2.48	-	-	-	-
11-15	1.67	-	-	-	-
Site 1, 2012					
0.00	2.77	6.99	48.83	66.84	8.84
1-5	3.03	6.88	48.8	66.88	9.01
6-10	2.78	6.91	4.81	66.96	8.94
11-15	3.01	6.94	48.41	66.7	8.95
Site 2 , 2012					
0.00	2.41	6.42	51.50	68.30	8.54
1-5	2.22	6.32	51.58	68.07	8.51
6-10	2.38	6.33	51.76	68.13	8.79
11-15	2.27	6.48	51.96	67.91	8.98

Studies on potatoes found that ECB damage did not affect total yield, but higher numbers of tubers or small tubers were found. It is unclear whether canola plants compensated for damage by increasing the number of pods per stem - a phenomenon common in plants attacked by insects (Williams and Free 1979; Tatchell 1983). Over the two years of this study, there was no major wind storm during the growing season which could have resulted in lodging and subsequent yield loss, because ECB damage weakens stems making them more susceptible to lodging in a wind storm.

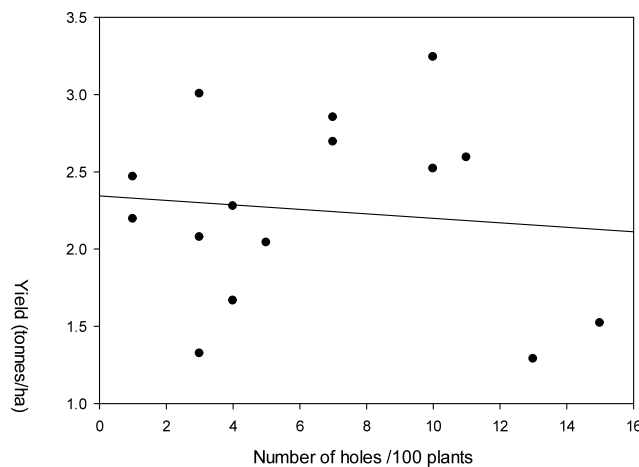


Figure 30. Relationship between European corn borer damage and canola yield (t/ha) in 2011 (Rsquared=0.01)



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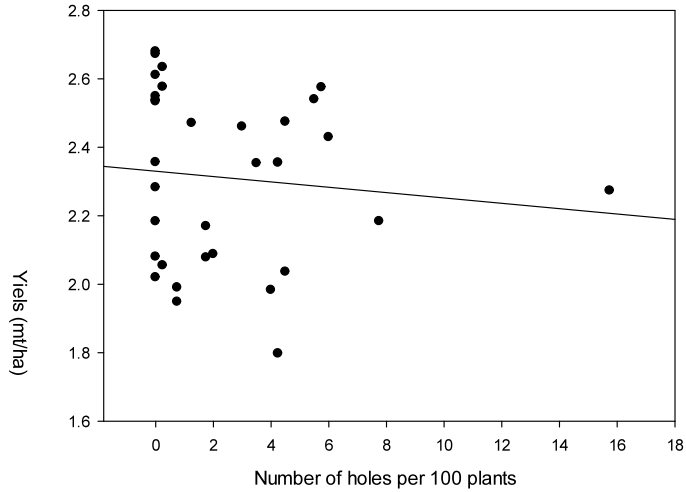


Figure 31. Relationship between European corn borer damage and canola yield (t/ha). Site 1, 2012 (Rsq=0.01)

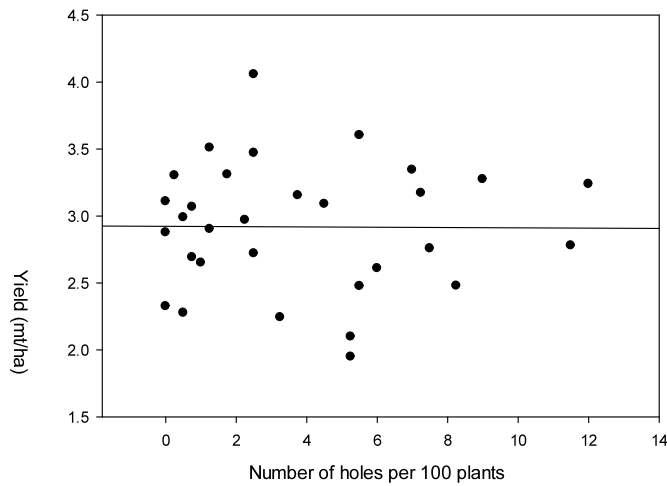


Figure 32. Relationship between European corn borer damage and canola yield (t/ha). Site 2, 2012 (Rsq=0)

B) Efficacy of insecticides against ECB

A significant reduction in the number of holes per plant was recorded for all three insecticide treatments when compared to the untreated control. In 2011, the total of holes in the untreated check was 11 per 100 plants as compared to no holes per 100 plants in the insecticide treated plots (table 3).



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Table 3. Efficacy of three foliar insecticides in controlling European corn borer damage and its effects on yield in 2011.

Insecticide	Rate (product/ha)	Holes/100 plants	Yield (t/ha)
Untreated check	-	11 a	1,79 a
Coragen	300 ml	0 b	1,89 a
Rimon 10EC	600 ml	0 b	1,86 a
Success 480SC	120 ml	0 b	1,82 a

Note: different letters (a, b) indicate significant differences.

Similarly in 2012, the highest number of holes was found in the untreated check plot (a mean of 24.5 holes in 400 plants) compared to no holes in the Coragen, two holes in the Rimon and one hole in the Success treated plots (table 4). In both years no significant difference was found in the total yield between the untreated check and the treated plots. In 2012, the seed moisture levels, the oil content and oil quality were evaluated for each treatment. No significant differences were found between the untreated check and the insecticide treated plots for seed moisture, dry matter oil content or oil quality, based of percentage of oleic and linolenic oil content (table 4). Although populations recorded in both years were lower than usual resulting in fewer eggs and lower than expected damage, the presence of zero holes in the insecticide treated plots compared to the untreated check in this study shows that these insecticides tested are effective in reducing damage by the European corn borer in canola in PEI without any phytotoxicity to the canola plant.

Table 4. Efficacy of three foliar insecticides in controlling European corn borer damage and its effects on yield, seed moisture, and oil content and quality in 2012.

Treatment	Rate (product/ha)	Total holes / 400 plants	Yield t/ha	Seed moisture	Oil (DM)	% Oleic (DM)	% Linolenic (DM)
Check	-	24.5 a	2.74 a	6.7 a	48.02 a	66.33 a	9.07 a
Coragen	300 ml	0 b	2.61 a	6.7 a	47.92 a	66.17 a	9.14 a
Rimon 10EC	600 ml	2 b	2.50 a	6.8 a	47.80 a	66.24 a	8.88 a
Success 480SC	120 ml	1 b	2.71 a	6.7 a	47.83 a	66.06 a	8.86 a

Note: different letters (a, b) indicate significant differences.

Overall Discussion:

The results indicate that low populations of European corn borer do not have an economic impact on canola yield and oil quantity and quality in the absence of high wind and stem breakage. The European corn borer causes damage by feeding and forming tunnels inside the stem; this larval activity does not only weaken the stem, but also acts as an entry source for disease organisms in the field. Weakened stems can also break in severe weather such as wind storms during the growing season, resulting in lodging. In 2011, populations of ECB were lower than expected, a phenomenon noted for several insect species, mainly because of lower than usual temperatures throughout the region. These low populations in 2011 may also have contributed to low populations noted in 2012. In this study on the economic importance, no relationship was observed between the number of holes and yield loss, suggesting that low infestation levels do not impact canola yield. In 2012, the plots were infected with the fungus *Alternaria*, but no association between the infection and crop damage was noted. Studies on potatoes show that high ECB infestation levels do not show a reduction in overall yield, but tuber size is effected with an increase in smaller tubers. In this study, the moisture content of the seed was evaluated mainly



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to determine if tunnelling through the stem influences the moisture content of the seed. Oil content and oil quality were evaluated as well. Results show that none of these factors were economically affected by the damage level in the plots.

Results from the insecticide efficacy trial show that the insecticides Coragen, Rimon 10EC, and Success 480SC applied at egg hatch are effective in controlling the European corn borer in canola. Because of human health and environmental concerns with insecticide use, these trials were done with newer, reduced risk chemicals, which are known to be effective against ECB in potatoes (Noronha and Carragher 2006; Boiteau and Noronha 2007). Rimon10EC is an insect growth regulator having very low mammalian toxicity (Cutler et al. 2006) and Success 480SC (Spinosad) is derived through the fermentation of naturally occurring bacteria and is a potent larvicide (Salgado 1998). Results demonstrated that all three products tested gave excellent control of the European corn borer when compared to the untreated check.

Thus, based on the results from these studies, it can be concluded that ECB populations should be monitored using pheromone traps, and if populations are low, an insecticide application is not required. However, in years when populations are high, growers could use one of the three tested insecticides which will give excellent control.

4.4.2 Cabbage seedpod weevil (G. Labrie, CÉROM)

A) Economic threshold

- 1) Three producers sites in the National Capital region of Quebec (2011)

No significant relationship was found between pods damaged by CSW (%) and yield (kg/ha) of canola ($F_{1,21} = 2,27$, $p = 0,14$; $R^2 = 0,1$; figure 33). Abundance of CSW during the flowering period was very high, varying between one and 25 CSW/sweep net. However, even with levels above threshold (two to four CSW/sweep), no relationship between numbers of this pest and yield was observed in these fields in 2011.

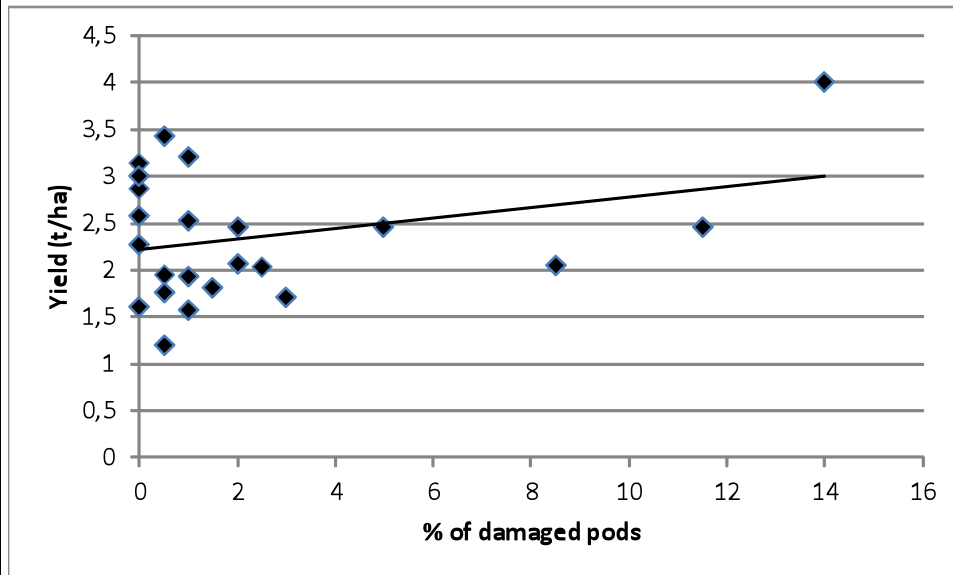


Figure 33. Relationship between cabbage seedpod weevil damage (% pods with emergence holes of CSW) and canola yield.



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2) Introduction of CSW in cages at CÉROM (2011 and 2012)

In 2011, some of the cages suffered from environmental conditions, so only 12 cages were considered for analysis. No significant relationship was observed between the percentage of pods damaged by CSW and PMG (weight (grams) per 1000 seeds) in the cages ($F_{1,10} = 0,29$, $p = 0,60$; $R^2 = 0.02$; figure 34).

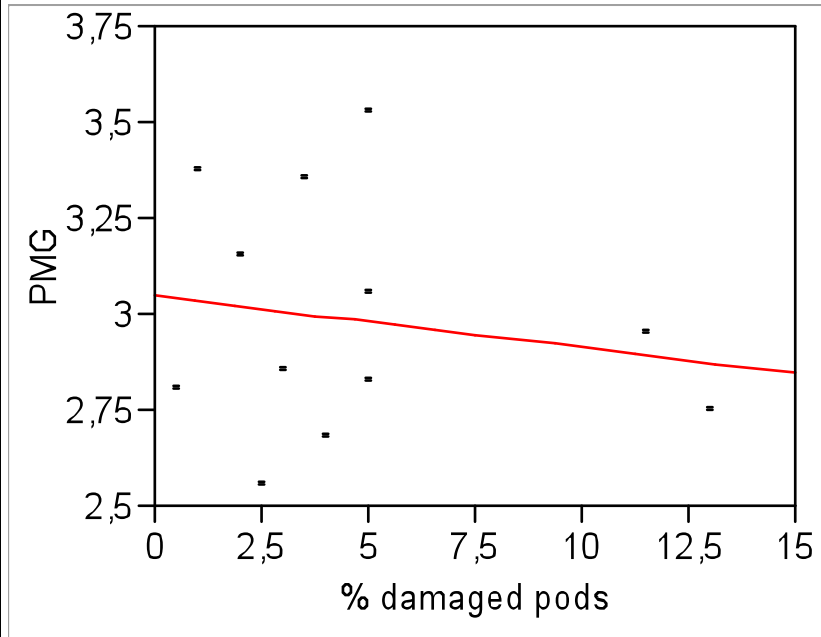


Figure 34. Relationship between cabbage seedpod weevil damage (% pods with emergence holes of CSW) and PMG (weight (g) per 1000 seeds) in 2011.

In 2012, a higher percentage of damaged pods in cages occurred with 20 CSW per sweep than with 10, four or no CSW per sweep ($F_{3,11} = 15,59$; $P = 0.001$; figure 35). There were, however, no yield differences between treatments ($F_{3,11} = 0,50$; $P = 0.69$; figure 36). There was also no significant relationship between the percentage of pods damaged by CSW and the percentage of yield loss (i.e. difference between yield on treated and control cages) ($F_{1,8} = 3,82$; $P = 0.09$; figure 37).



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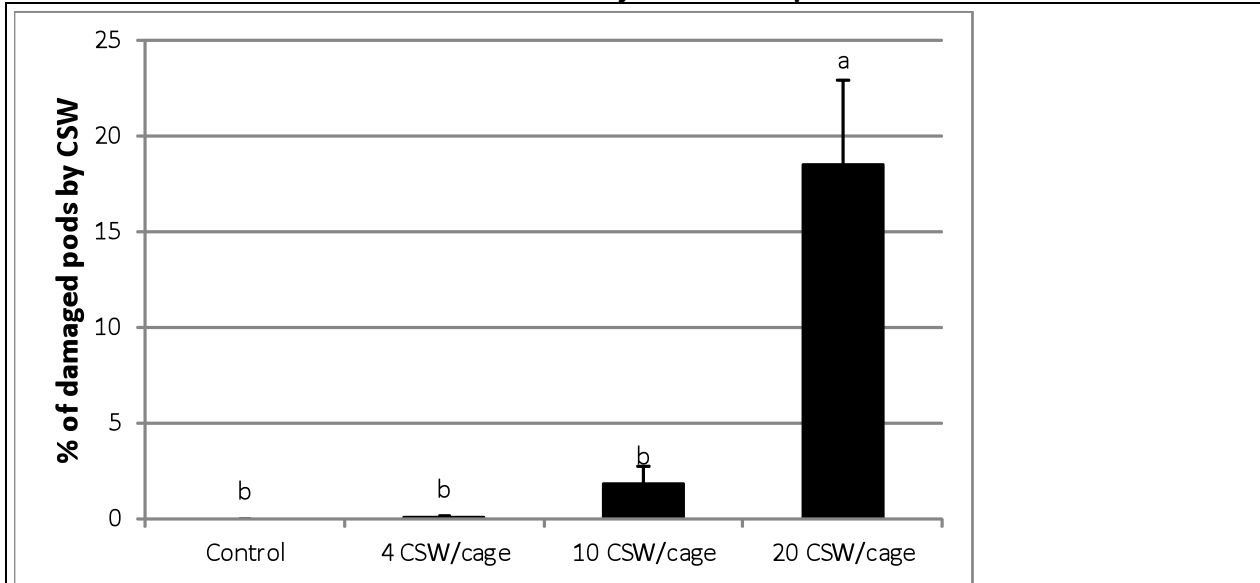


Figure 35. Percentage of pods damaged by CSW in cages with introduction of 0, 4, 10 or 20 CSW during flowering period in 2012. Note: different letters (a, b) indicate significant differences.

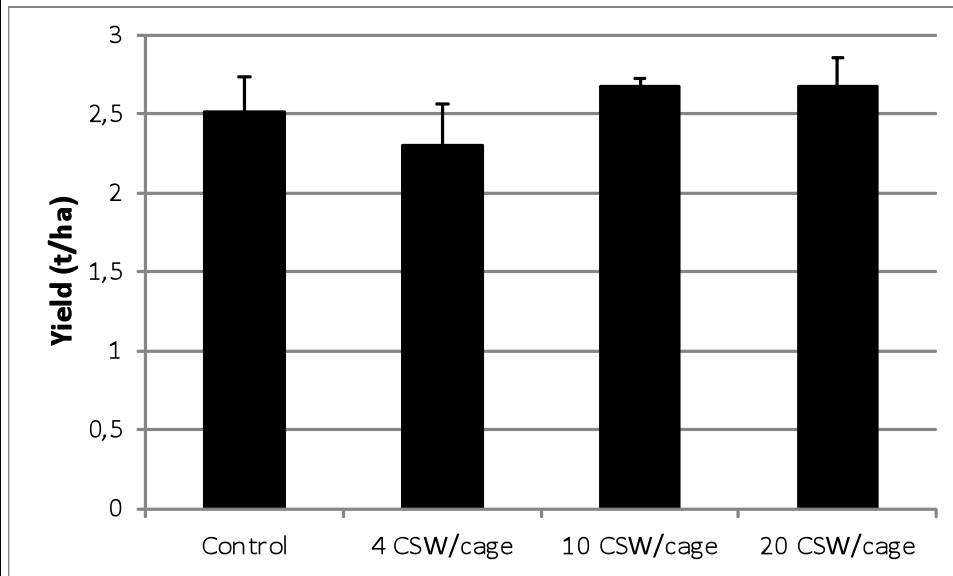


Figure 36. Yield of canola in cages with introduction of 0, 4, 10 or 20 cabbage seedpod weevils during flowering period in 2012.



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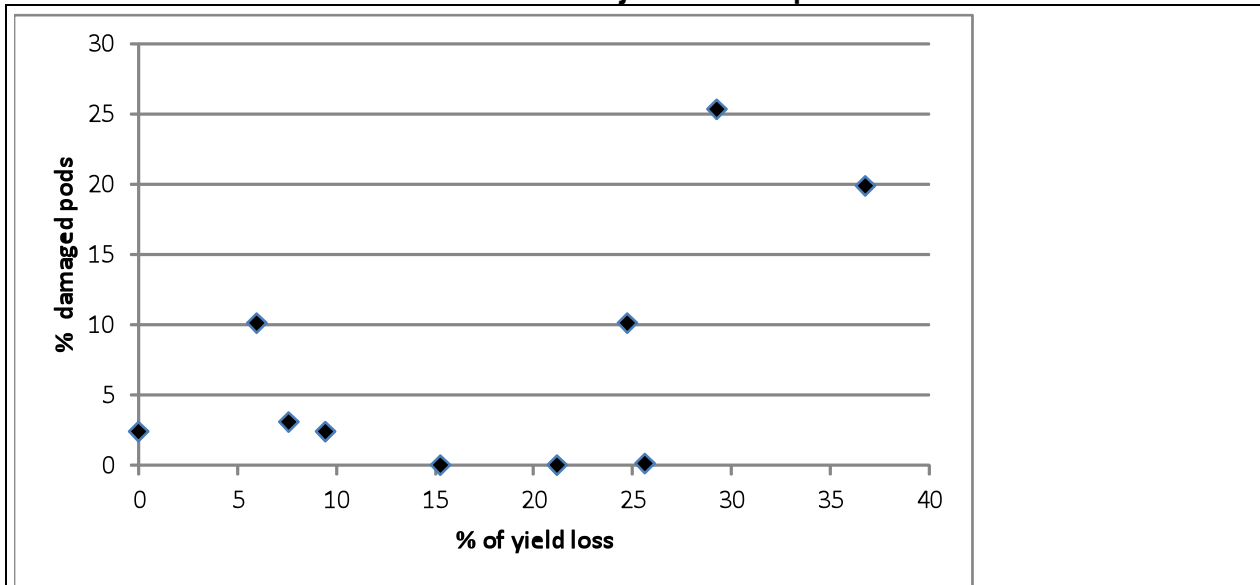


Figure 37. Relationship between % of yield loss and pods damaged by cabbage seedpod weevil in cages during summer 2012.

B) Efficacy of insecticides against CSW

Insecticides efficacy against CSW was evaluated at 1) CÉROM and 2) three producer's sites.

- 1) CEROM. Canola plots were established on the CEROM experimental site at Beloeil on May 23, 2011. The plots were planted with the variety 5030LL on eight replications of 10x10m. Four plots were considered control plots and four were treated with Matador on July 8th. After the passage of tropical storm "Irene", it was not possible to harvest any canola.
- 2) Producers' sites. For this part, 16 sites were monitored in two areas around Québec City to find canola fields that reached the threshold for insecticide treatments. Three sites in the National Capital region of Quebec were above the CSW threshold levels at the beginning of the flowering period (P1: three CSW/sweep; P2: two CSW/sweep; P3: 25 CSW/sweep). In each of these fields, insecticide treatment with Matador was done at the 20% flowering stage. One hectare of each field was left untreated. At maturity, 200 pods were collected at four locations in each section (control and treated). Before commercial harvest of the field, four 1m² samples of canola were harvested for yield evaluation.

There was a significant difference in the number of pods damaged by CSW on the third site (P3) between control and treated plots ($F_{5,17} = 18,42$, $P < 0,001$; figure 38). There was, however, no significant difference in canola yield between control and treated plots for the three sites ($F_{5,17} = 1,37$; $P < 0,29$; figure 39).



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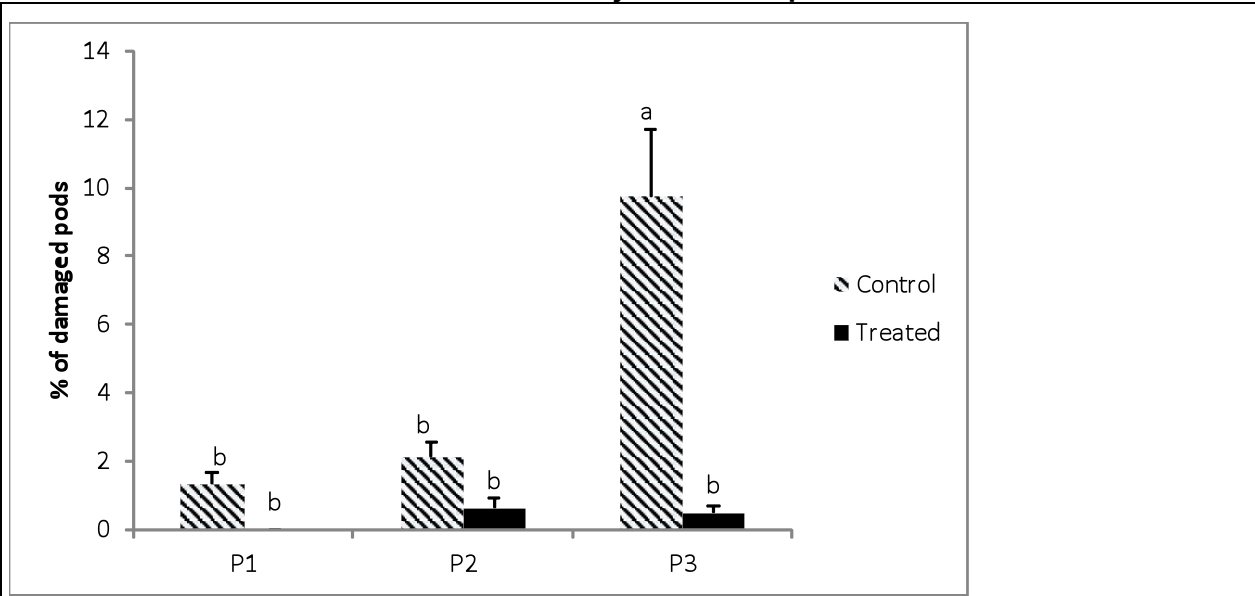


Figure 38. Impact of insecticide treatment on pods damaged by cabbage seedpod weevil in three canola sites in the National Capital region, Québec in 2011. Note: different letters (a, b) indicate significant differences.

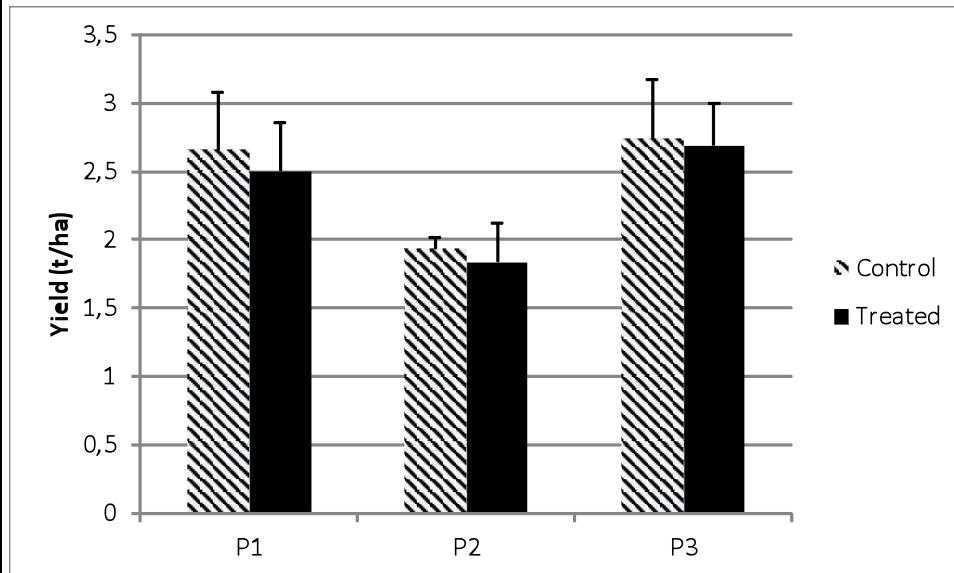


Figure 39. Impact of insecticide treatment on canola seed yield (t/ha) in three canola sites in the National Capitale region, Québec in 2011.

In 2012, no canola fields monitored were above the threshold and no fields were treated with insecticide for cabbage seedpod weevil.

Overall discussion

The results in producers' sites and cage experiments demonstrated no impact of cabbage seedpod weevil on yield in either year of study, even when no natural enemies were present in the cages. These results contrast greatly with all studies on the impact of cabbage seedpod weevil in Western Canada and



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in other countries (Buntin 1999; Free and Williams 1978; Free et al. 1983). In Europe, an infestation of 59% of pods by cabbage seedpod weevil would lower grain yield by 10-11% (Free et al. 1983). In some studies (Tulisalo et al. 1976; Carcamo et al. unpublished data), two weevils per plant reduced yield between 40 to 64%, and at higher densities, seed weights were considerably lower than in uninfested controls. Other studies (Lerin and Rivault 1984; Buntin 1999) determined that canola pod infestation levels below 26% (as indicated by exit hole counts) have no measurable effect on yield. In this study, no heavy infestation levels were observed in any fields or experimental plots in two years of study. High infestation levels were observed in 2001 when cabbage seedpod weevil was first observed in Quebec (Brodeur 2002). These low levels of infestation observed in the fields in recent years could explain why it was not possible to demonstrate any effects of insecticides or to validate the threshold used in Western Canada. More studies will be necessary to evaluate more precisely the economic injury level for this pest.

Next steps

To develop integrated pest management strategies against insect pests of canola in Eastern Canada, the work on resistance to cabbage seedpod weevil and root maggot needs to be continued. Also, adapting the seeding dates could be a good strategy against flea beetles or cabbage seedpod weevil, however, more study on conditions under which this strategy could be used, is necessary. Natural control of cabbage seedpod weevil by parasitoids is an important discovery in this study and more observations are required to understand the relationship between this pest and its natural enemy. The economic threshold for cabbage seedpod weevil seems different from Western Canada and more research on this issue needs to be performed.

Other insects such as flea beetles and pollen beetles will need more study to develop integrated pest management strategies. Flea beetle damage increased greatly in recent years in some areas of Quebec and pollen beetles were more abundant in many fields. These pests can cause heavy damage on canola and solutions need to be found to reduce their impacts.

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B (I). Funded Collaborators (Co-PI, AAFC, other federal scientists)

- Include the name of scientist / organization.

Ivan Malchev, Technician, Plant Agriculture, University of Guelph, ON
HPLC Technician; developed HPLC method to identify biochemical profiles for identifying resistant root maggot and cabbage seedpod weevil canola lines.

Geoff Worthington, Field Technician, Plant Agriculture, University of Guelph, ON
Performed field trials for all new selected lines; scored agronomic data and monitored seed increase



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plots; developed field plot designs, field preparation, seeding, plot maintenance, bagging, scoring, harvesting, seed cleaning, and data analysis.

Kathy Perry, Doubled Haploid Technician, Plant Agriculture, University of Guelph, ON
Carries out production of all DHs in the breeding program; haploid/diploid plant maintenance; crossing; indoor seed increases etc.

Geneviève Labrie, Researcher; Centre de recherche sur les grains Inc. (CÉROM), Beloeil, QC
Elaboration of protocols, analysis of data, conferences and report.

Jennifer De Almeida, Research Professional; Centre de recherche sur les grains Inc. (CÉROM)
Performed cage trials for economic threshold and evaluation of parasitism rates on pods received from all canola fields.

Anne Vanasse, Researcher; Laval University, QC
Elaboration of protocols, analysis of data, conferences and report.

Valérie Bélanger, Research professional, Université Laval
Performed field trials in 2011 at Saint-Augustin-de-Desmaures (research station of U. Laval): field design, field preparation, planting, plot maintenance, agronomic and entomological data, harvesting, seed cleaning, data analysis.

Marie-Ève Bernard, Research professional, Université Laval
Performed field trials in 2012 at Saint-Augustin-de-Desmaures (research station of U. Laval): field design, field preparation, planting, plot maintenance, agronomic and entomological data, harvesting, seed

Denis Pageau, AAFC Normandin, QC
Elaboration of protocols, analysis of data, conferences and report.

Christine Noronha, AAFC Charlottetown, PEI
Elaboration of protocols, analysis of data, conferences and report.

B (II). Acknowledgement of non-funded collaborators (who provide support, e.g. access to other laboratory or other facilities and equipment input / advice / guidance / assistance, etc).

- For research supported by targeted funding programs (e.g. DIAP, Clusters, etc.) please list any collaborators who are receiving Contribution Vote 10 funds (e.g., university and industry collaborators). In addition, please list separately the participants who support your project but are not receiving any funding through the program.
- Include name of scientist / organization.

Raymond Lee, PDF, Plant Agriculture, University of Guelph
Molecular geneticist; developed molecular markers for CSW and RM resistance; identified biochemical compounds of interest using the Mass Spec.

Ronald Fletcher, PDF, Plant Agriculture, University of Guelph
Plant biochemist; research on biochemical fingerprints that could predict the resistance or susceptibility of lines against flea beetle.

Julian Heath, Ph. D. student; working full time at Pioneer Georgetown (L. Kott supervisor)
Developing molecular markers for flea beetle resistance; working with various + or – resistant canola lines.

Jean-Michel Delage, agronomist, Club Fertior, St-Bernard, QC
Sampling of CSW and of plants for yield evaluation in canola fields in Chaudière-Appalaches and Capitale Nationale area in Québec.

Brigitte Duval, agronomist, MAPAQ Nicolet, Québec
Sampling of canola pods in winter and spring canola at different dates for parasitoids

Maryse Provancher, agronomist, MAPAQ Trois-Rivières, Québec
Sampling of canola pods in winter and spring canola at different dates for parasitoids

Isabelle Morasse, AAFC Normandin



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Performed field trials for trials at Normandin: field design, field preparation, planting, plot maintenance, agronomic and entomological data, harvesting, seed cleaning

C. Variance Report (if applicable, describe how the work differs from the proposed research)

- Include changes to objectives and project work plan / budget, changes to the team, other constraints.

1) Resistance to insect pest

The death of Dr. Kott's biochemist colleague halted activities pertaining to the identification of biochemical fingerprints for resistance in canola against flea beetles, and since Dr. Kott has now retired, she did not refill this position. This position was not supported by the ECODA DIAP project. Some molecular marker work in flea beetle is still being done by a PhD student (not supported by this DIAP project), but the research is not yet completed.

2) Fertilization trial

Changes to the protocol between 2011 and 2012 were done to add sulfur into the protocol.

D. Impact Assessment (if applicable, describe how the variance factors above will impact project continuation)

- Include changes to the objectives, changes to the project work plan / budget, changes to performance (i.e. meeting targets).

1- Resistance to insect pest

Due to the passing of Dr. Kott's biochemist colleague, she was unable to continue with biochemical fingerprinting and identification of canola lines resistant to flea beetles. For this reason, no DH breeding lines were provided to the ECODA DIAP project collaborators for field testing.

E. Achievements (include only those related to this project)

- Include innovations, publications / conferences, technology transfer, capacity building, success stories, media, recognition and other outputs.

1) Resistance to insect pests

Four doubled haploid lines were identified in the field as having significantly reduced root maggot damage from the Elora field trial, namely lines 1362-21, -28, -17 and -11.

The HPLC research related to identification of biochemicals that could be used by breeders in the future to determine resistance to root maggot is novel research and will appear in a publication, once the work is finalized.

2) Management practices

Vanasse, A. 2013. Essais de régie sur le canola. Journée d'information INPACQ sur le canola. Bécancour, Québec. 26 février 2013.



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Pageau, D., Labrie, G. et Vanasse, A. 2013. Gestion des maladies et des insectes du canola. Conférence présentée lors du Symposium sur les Céréales et oléagineuses de l'Atlantique. Frédéricton, N.B. 13-14 février 2013.

3) Biological control

Duval, B. and Labrie, G. 2013. Ravageurs du canola : portrait de la situation. Journée d'information INPACQ sur le canola. Bécancour, Québec. 26 février 2013.

4) Economic threshold

Duval, B. and Labrie, G. 2013. Ravageurs du canola : portrait de la situation. Journée d'information INPACQ sur le canola. Bécancour, Québec. 26 février 2013.


F. Lessons learned (self-evaluation of project)

1) Resistance to insect pests

Dr. Kott probably should have stressed the method of field trial management more clearly, as the lines generated for screening for insect resistance were inadvertently (or intentionally) treated with pesticides. This did not allow identification of level of resistance/susceptibility among the new lines.



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Research Project Final Report**

Geneviève Labrie	<i>May 30th 2013</i>	
PI Name	Date	Signature

Note: After completion and signature, this report must be provided to the appropriate Science Director for assessment. A PDF copy of this report will be sent to Science Operations by the Science Director's office along with the project assessment.