FINAL PROJECT REPORT
Canola Agronomic Research Program (CARP)

The Annual Final Report should fully describe the work completed for the year and note the personnel involved. It should also note any deviations from the original plan and next and/or corrective steps as may be required if deviations are noted. The report should also provide an update on the status of the Project including forecasted date of completion. A complete statement of expenses should be included. In the event major changes are anticipated within the budget supporting notes along with a proposed budget should also be included. The report should also capture a complete summary of activity for the year.

Project Title: **Classical biological control of root maggots in canola with *Aleochara bipustulata***

Research Team Information

Lead Researchers:		
Name	***Institution***	***Expertise Added***
N.J. Holliday	University of Manitoba	Principal investigator, Crop protection, biological control, population dynamics
Research Team Members		
Name	***Institution***	***Expertise Added***
U. Kuhlmann	CABI Swiss Centre	Biological control
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Project Start Date: **1 April 2009**

Project Completion Date: **31 March 2012**

Reporting Period: **December 1, 20_______ to March 31, 20_______ Final cumulative report**

CARP Project Number: **2009-3**

Instructions: This Final Project Report shall be completed and submitted on or about March 31st of the fiscal year that the agreement is in effect (upon completion of the project). The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a detailed account upon completion of the project.. Final project financial reporting should be provided at this time.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to your appropriate CCC contact.

1. Date of Completion:

CARP funding for this three year project ended on 31 March 2012.

Laboratory and field research for objectives 2 and 3 are complete. Most of the work for objective 1 is complete. However, as described below, a small amount of laboratory work on objective 1 remains to be completed, as we need to collect experimental material from the field. We anticipate that the experimental work will be completed by August 2012.

Objectives 1 and 2 are the topic of a Ph.D. thesis expected to be submitted in summer 2012, final results and their interpretation will not be completely available until then. Analysis of data for objective 3 is now complete and a manuscript reporting the results of this objective is almost ready for submission to a scientific journal, and will also be a major component of an M.Sc. thesis.

The major final product of the project is to be a petition for introduction of *Aleochara bipustulata* for biological control of root maggots in canola. It is envisaged that this petition will be prepared in September 2012.

2. Status of Activity: (please check one)

_____ Ahead of Schedule __X___ On Schedule _____ Behind Schedule _____ Completed

Comment:

See comments in section 1. Publications relating to the research funded by CARP will continue to emerge for at least two years.

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

Objective 1. To characterize the risk to non-target species of feeding by adult *Aleochara bipustulata*.

Aleochara bipustulata is the candidate biological control agent that we are testing for feasibility of introduction for control of root maggots in canola. All laboratory studies of feeding on non-target species are complete. Field collections for studies of prey consumption by adult *A. bipustulata* are also complete. Development of a quantitative PCR-based molecular technique for identifying target and non-target species in the gut of adult *A. bipustulata* has proven problematic. Before the beginning of field work in 2011, we had a method that seemed to work, but when samples were processed in fall 2011, it became apparent that the technique was not as specific as we first thought. In fall 2011, issues of primer specificity and DNA recovery were addressed and new primers selected. For the target insect, we were able to verify the adequacy and efficacy of the new primers, and we conducted successful feeding trials in January 2012 to determine the duration following feeding during which target DNA can be detected. Following that, we have so far processed 410 field-collected *A. bipustulata* to determine whether they had eaten the target insect. To validate primers for the non-target insects of interest, we must also conduct feeding trials, and these cannot be started until those insects become available in May 2012. We will then need to establish a colony of the insects, and complete the feeding trials. If these are successful, the testing of field-collected material should be complete by August 2012.

Objective 2. To study the interactions of *A. bipustulata* with existing major Canadian natural enemies of root maggots.

Laboratory and field studies for this objective are completed, and data are being analysed.

Objective 3. To study the host and prey selection cues of *A. bipustulata*.

Studies for this objective are completed, data have been analysed and a publication and thesis are being prepared to report the results.

With the exception of the difficulties mentioned under objective 1, no major issues or variances between planned and actual activities have occurred or are envisaged.

4. Significant Accomplishments

The overall goal of the project was to obtain the data necessary to petition the Canadian Food Inspection Agency for introduction of *Aleochara bipustulata* for the biological control of root maggots in canola. When the final components of objective 1 have been completed, this will have been achieved, and a petition will be readied in September 2012 for submission.

Our major findings, to date, are:

1. That the majority of non-target invertebrates in canola fields are not eaten by *A. bipustulata*, but that there may some risk of predation of carabid beetles (still being investigated).
2. That *A. bipustulata* does eat eggs and larvae of the target pest, both in the laboratory and the field, and that the number eaten is influenced by egg placement, and the density of eggs available.
3. That the introduction of *A. bipustulata* may not increase the number of root maggot eggs eaten, as there is evidence of competition between it and other predators that may limit total egg consumption by the guild of predators.
4. That *A. bipustulata* is an effective parasite of root maggot pupae, and its introduction will likely increase total levels of pest mortality due to parasitism, thus providing an important additional check on the population growth of root maggots.
5. That the chemical cues involved in host and prey finding by *A. bipustulata* are quite complex and include the simple organic molecule dimethyl disulphide (DMDS) and one or more volatiles given off by mustard seed meal.
6. Mustard seed meal mulch is an effective and specific method of attracting *A. bipustulata* to locations and can be used to increase predation and parasitism by *A. bipustulata*. The basis of this activity is not DMDS.
7. DMDS affects both *A. bipustulata* and its Canadian relative *A. bilineata* in similar ways. The responses of these species are dependent upon age and stage of the insect, but are likely to result in parasitism of only those maggot pupae associated with canola and other brassicas and with a few other habitats. Additional constraints imposed by habitat preference and larval choice among hosts greatly restrict the risks to non-target species.

In addition to conference presentations both locally, nationally and internationally, the research funded through the CARP program will result in completion of one M.Sc. thesis and one Ph.D. thesis, and an estimated seven refereed scientific publications. In addition to training of two graduate students, five undergraduate research assistants have received training in basic research techniques during the course of the project.

5. Research and Action Plans

Abstract in lay language

Root maggots, principally cabbage maggots, are a constraint on canola production in prairie Canada. The larval root maggots invade the roots of canola plants in the middle of the growing season and cannot be controlled with insecticides. Cabbage maggots originate in Europe, where they have specialized natural enemies that are not present in Canada. The purpose of the project was to gather data to support a petition for the introduction of one of these natural enemies, the rove beetle *Aleochara bipustulata*, for biological control of root maggots in canola in Canada. Larvae of *A. bipustulata* parasitize the pupal stage of cabbage maggots, and adult *A. bipustulata* are predators. To complete the package needed for petition, we need information not only on how

effective *A. bipustulata* is at killing the target pest (the cabbage maggot), but also whether it might kill non-target insects, whether it might interfere with existing natural enemies of root maggots, and how it finds the habitats it lives in and the organisms it kills. We already know that the range of insects that *A. bipustulata* can kill by parasitism is quite narrow. In the project we were able to show that the range is narrowed further by preference for open habitats where pupal stages of flies are associated volatile chemicals given off by maggot-infested canola or other brassicas, members of the onion family, or dung or carrion. In the laboratory and field, *A. bipustulata* readily eats immature cabbage maggots, and the number of cabbage maggot eggs eaten is dependent upon egg density and placement. Few non-pest organisms in canola fields were eaten by *A. bipustulata*; one beneficial group that is eaten in the laboratory is carabid beetles, and we are investigating the extent to which these beneficial insects are eaten in the field: we expect to have answers to this question by August 2012. Because of competition between egg predators, the level of predation on root maggot eggs may not increase if *A. bipustulata* is introduced; however our studies of interactions of *A. bipustulata* with other parasites of cabbage maggots suggest that levels of parasitism would increase if *A. bipustulata* were successfully established. Elevated levels of parasitism, particularly if integrated with cultural controls and host plant resistance, could reverse the trend of increasing root maggot abundance and severity that has been observed over the last three decades. It is planned to develop the petition for introduction of *A. bipustulata* in September 2012.

Introduction

Root maggots, principally the cabbage maggot, first began to cause economic loss in canola in Alberta in the early 1980s, and since then maggots have increased in incidence and damage severity in all three Prairie Provinces (Soroka et al. 2004). Currently, estimates of average annual losses from root maggot damage range from $20 million to $75 million; in years of severe infestation, losses may reach $100 million. In canola, root maggots cannot be controlled with insecticides. The cultural control of higher seeding rates and long rotation intervals can reduce damage somewhat, but current trends in the canola industry are to lower seeding rates and shorter rotations. Although work is under way to develop host plant resistance in canola, the mechanisms of resistance are based on behavior of the egg-laying female, and so are unlikely to confer absolute immunity. Methods of increasing effectiveness of naturally-occurring predators, and methods of reducing egg-laying by maggot females by increasing vegetational diversity are being researched at the University of Alberta. However it appears that although increasing vegetational diversity by undersowing with wheat or delaying herbicide applications does reduce maggot damage, it also reduces crop yield; furthermore, it reduces the effectiveness of the maggots' natural enemies. Thus, there is no single solution to the control of root maggots in canola, and additional methods of control are needed.

The cabbage maggot is originally a European insect, and arrived in North America before 1835. At the same time as its arrival, it is believed that two of its specialized natural enemies from Europe were also introduced (Biron et al. 2000). However, other specialized natural enemies did not come with the pest, and part of the reason for the current high populations of root maggots in canola may be the absence of the complete range of specialized natural enemies that are present in the native habitat. The idea of classical biological control is to seek the specialized natural enemies in the area of origin of an alien pest and introduce them to the invaded area, thus restoring the original complement of natural enemies of the pest, and hopefully suppressing the pest population. The absence of effective alternative control measures and the European origin of cabbage maggot are factors that make investigation of classical biological control worthwhile. A successful classical biological control program for root maggots, particularly if coupled with other control tactics such as host plant resistance and cultural methods, could change the ongoing upward trend of root maggot damage into a downward trend, subsequently reaching a sustained equilibrium level where economic losses from root maggots are rare, and no ongoing inputs are required to maintain the population below economically damaging levels. Such an outcome would improve profitability of canola production and remove an impediment to the plans for industry growth.

Since 1999, we have been working in collaboration with scientists from CABI–Europe Switzerland and Agriculture and Agri-Food Canada (AAFC) on a program aimed at controlling root maggots in canola using a biological control agent introduced from Europe. We began by comparing the community of specialized natural enemies of cabbage maggot in the Prairie Provinces with the community in Europe, and so identified a promising candidate agent, currently not present in Canada, for introduction (Hemachandra et al. 2005, 2007).

The agent is a small rove beetle, *Aleochara bipustulata.* Adults of this beetle eat eggs and larvae of root maggots. When pupae of root maggots become available, female *A. bipustulata* lay eggs in the vicinity of the pupae. These eggs hatch and the newly-hatched larva seeks out a pupa, chews through its pupal case, and enters. Inside it feeds on the root maggot pupa, ultimately killing it. The larva then pupates inside the maggot pupal case, and an adult *A. bipustulata* emerges from it. The predation on maggot eggs and young larvae could provide protection of the current crop from maggot damage; however, we believe the parasitism, which may kill 40% of maggot pupae in crops in Europe (Brunel and Fournet, 1996) may be more important. Although the parasitism occurs too late in the season to provide current year protection, its long term effect on population dynamics may be sufficient to reverse the current trend of population increase.

Our program is following the Canadian Food Inspection Agency (CFIA) protocols for developing a petition to implement classical biological control of root maggots with *A. bipustulata*. The funding from CARP was to support research to gather the data necessary for a petition to CFIA. Satisfying the CFIA protocols is expected to ensure that the benefits of biological control will accrue without jeopardizing environmental integrity. CFIA requirements fall under four general categories: (1) demonstrating that there is a need for classical biological control, (2) showing that the agent is likely to be effective in suppressing the target pest population, (3) showing that the agents potential to harm non-target organisms and the environment is within acceptable limits, and (4) showing that the agent will not interfere with existing natural enemies in a way that might reduce their effectiveness. We believe there is already sufficient information to show that there is a need for classical biological control of root maggots and that the environmental risks are minimal. So our research has focused on the likely effectiveness of the agent, its potential to harm non-target organisms, and the likelihood that an introduction would have negative effects on the efficacy of natural enemies that already exist in Canada.

At the beginning of the current CARP project, we had already made considerable progress beyond the initial identification of the candidate agent towards satisfying CFIA requirements. In particular, we had assessed the range of hosts that are successfully attacked by the parasitic larvae of *A. bipustulata* by laboratory trials of parasitic capability. These studies were augmented by field assessments of the types of habitats in which *A. bipustulata* lives, as exposing potential hosts to parasites in the laboratory shows only the theoretical capability to parasitize a non-target host: if the non-target host and the parasite do not coexist in the same habitats in the field, the non-target host is not at risk. As a result of this work, we have characterized host range sufficiently to be able to predict risk of parasitism of non-target organisms by the parasitic larvae of *A. bipustulata* (Andreassen et al. 2007, 2009). We had also made progress on the cues that *A. bipustulata* uses to find habitats where it may predate or parasitize the target pest (Riley et al. 2007); knowledge of these cues may allow us to manipulate introduced *A. bipustulata* populations to make them establish better and make them more effective, and such knowledge also assists in understanding how the insect chooses habitats, hosts and prey and so what non-target species are most at risk.

The objectives of the CARP-funded research were to address the remaining areas in which CFIA requires data. They were:

1. **To characterize the risk to non-target species of feeding by adult *A. bipustulata*.**
2. **To study the interactions of *A. bipustulata* with existing major Canadian natural enemies of root maggots.**
3. **To study the host and prey selection cues of *A. bipustulata*.**

Apart from general literature statements that adult *A. bipustulata* eat immature root maggots, there was no information available on other components of the diet of *A. bipustulata* or indeed on the capacity of the insect to consume root maggot eggs and larvae in realistic field situations. Hence objective 1 was essential. Some work on the interactions of *A. bipustulata* with its Canadian relative *A. bilineata* was already available (e.g. Fournet et al. 1999, Riley 2009); however, these focused only on interactions through parasitism, and were conducted in very simple laboratory systems. Objective 2 was designed to examine interactions among predators of cabbage maggot, and to attempt to elucidate parasitic interactions in more complex systems that might be closer to those in which the insects operate in the field. Although our work on mustard seed meal (Riley et al. 2007), showed that mulches of this material can be used to manipulate *A. bipustulata*, in that study we were not able to identify all the active compounds among the volatiles given off by mustard seed meal. Almost simultaneously with the publication of Riley et al. (2007), a French research group published a paper (Ferry et al. 2007) on dimethyl disulphide (DMDS), which is given off by maggot-infested brassica roots, and which attracts adult *A. bipustulata*. Therefore, our work for objective 3 focused on the role of DMDS in the biology of *A. bipustulata*, and how DMDS is related to the volatiles from mustard seed meal.

CFIA regulations allowed us to perform some laboratory studies of *A. bipustulata* at the University of Manitoba, in a containment facility. Until the petition is granted, no field research on the insect can be conducted in Canada, and so all field research was performed in Switzerland.

Objective1. To characterize the risk to non-target species of feeding by adult *A. bipustulata*

Initial studies for this objective involved characterizing the non-target organisms that occur in the same habitats as *A. bipustulata* in field conditions in brassica (mostly canola) fields in Europe. Simple laboratory studies were then carried out to determine which of these non-target organisms is at risk of being eaten. Techniques have now been developed to allow detection of whether these “at risk” species were actually eaten in the field.

The list of non-target organisms for laboratory testing was derived from the literature, and from the organisms found in a series of soil samples taken in canola fields. Individuals of the selected non-target groups were exposed in a Petri dish to an adult *A. bipustulata* for 24 h. Whether or not the potential prey was consumed within the first 10 minutes, or within 24 h, was recorded. The target number of replicates was 20 for each non-target species. The non-insect groups tested were two species of Annelida (segmented worms including earthworms), five species of mites (Acarina), one spider (Araneae), two species of centipedes (juveniles, Chilopoda), two species of millipedes (Myriapoda), and one species of sowbug (immature, Isopoda). Insects tested included two species of springtails (Collembola), one species of ant (Formicidae), two species of aphids (Aphidae), diamondback moth caterpillars (Lepidoptera), the larvae and pupae of the target cabbage maggot and three other species of true flies (Diptera), and three species of beetles (Coleoptera) including eggs, larvae and pupae of the carabid beetle *Amara similata*.

Adults of *A. bipustulata* readily ate the target cabbage maggot in its larval stage, but the pupal stage was not eaten. Pupae are protected by a hard pupal case and this prevents consumption: we use pupae as food for *A. bipustulata* in the laboratory, but the pupal case must be broken open to allow the pupa to be eaten. We did not test cabbage maggot eggs in Petri dishes, as we know from our earlier studies and those of others (e.g. Fournet et al. 2000) that *A. bipustulata* adults consume many maggot eggs per day. Over two years, 28 non-target groups were tested. Non-target groups shown to be “at-risk” in these studies are mostly soft-bodied fly or beetle larvae that resemble the target root maggot larvae; however, most of these were not consumed in the first 10 minutes of the trials, but only after 24 h during which they had with no alternative food. An exception was the carabid beetle, *A. similata*: eggs and pupae of this species were frequently consumed within the first 10 minutes of the test, and after 24 h, more than half the eggs and pupae and one third of the larvae of this species had been consumed. Some carabids are known to be predators of cabbage maggot eggs (e.g. Wishart et al. 1956). Many carabids are thought to be beneficial predators of pest insects, and in addition *Amara* species are predators of weed seeds (Larochelle and Larivière 2003). It should be born in mind that the Petri dish method can overestimate risk to a non-target organism as that organism cannot escape and the adult *A. bipustulata* is provided with no other food. However, the trial does indicate a need to do further evaluations of risk to carabid beetles. The soft bodied larvae of the other species that were consumed in Petri dish trials were not favored food of *A. bipustulata*. Most would be considered pests if they were in a canola crop, so we did not follow up with more realistic testing of these species.

We are following up the laboratory studies with DNA-based assessments of the extent to which *A. bipustulata* adults eat the target pest and carabid beetles in the field. In preparation for these assessments, we collected adult *A. bipustulata* in Swiss brassica fields in each of summers 2009–2011. Collections were made in pitfall traps that were emptied every 4 h and the insects immediately transferred to acetone; this technique preserves the DNA of the beetles and of its gut contents, and minimizes the time after trapping during which digestion of gut contents can occur. The pitfall trap collections at 4 h intervals were made for 24 h periods. Dates of collection were chosen to allow us to compare the indicators of maggot consumption with what stages of cabbage maggot were available in the field to be eaten. Over 630 adult *A. bipustulata* (>400 in 2009, 127 in 2010 and 101 in

2011) were collected and successfully preserved, frozen in acetone, for DNA analysis. Data from 2009, when most beetles were collected, suggest that adult *A. bipustulata* are most active in the afternoon and evening, particularly from 8:00 PM to midnight.

We have developed primers that allow detection of *A. bipustulata* DNA and cabbage maggot DNA. The primers are specific to the two types of DNA, and through PCR (polymerase chain reaction) amplification, are sensitive enough to allow detection of cabbage maggot DNA inside the gut of an adult *A. bipustulata*. In laboratory feeding trials we could always detect DNA from a single cabbage maggot egg eaten by an adult *A. bipustulata*, if the egg had been consumed within the last 4 h; detection rate was very high up to 8 h after consumption and but by 24 h after eating a maggot egg, DNA of the maggot could be detected only 33% of the time. We have so far successfully tested the gut contents of 310 adult *A. bilineata* collected in 2009, and of these, 65 (21%) were positive for cabbage maggot DNA. For 2010, 64 beetles have been tested so far, and of these 5 (8%) were positive for cabbage maggot DNA. Testing of the remaining beetles is in progress, and once complete, the results will be interpreted in light of the information on duration of detectability from the laboratory feeding trials, and our knowledge of the stages of cabbage maggot in the field at the time of *A. bipustulata* collection. This type of assessment of the level of predation of immature cabbage maggots by *A. bipustulata* is an important innovation, as estimates of egg predation by beetles like *A. bipustulata* vary wildly from negligible to 90% of eggs eaten (Finch and Elliott 1999 and references therein), and none of them have been performed using natural maggot populations in field conditions.

We have developed a primer system that will allow detection of carabid beetle DNA in the gut of *A. bipustulata*, so that we can follow up on the finding that immature stages of *A. similata* were readily consumed in the laboratory. As mentioned in section 3 of this report, specificity of primers has been a problem, one which we think we have now overcome for carabid beetle DNA. Before we can test field collections of *A. bipustulata*, it is necessary to perform feeding trials to determine how long the DNA of the carabid can be detected in the gut of an adult *A. bipustulata* after it has eaten a carabid egg. For these trials we need eggs of carabid beetles, and unfortunately finalization of the primers occurred after carabid beetles became unavailable in the field in fall 2011. When carabid beetles become active in spring 2012, adults of a common species will be collected to establish a colony. Eggs from the colony will be used in the feeding trials and, once these are satisfactorily completed, the field-collected material will be tested to provide estimates of consumption of carabid beetles in the field. We expect to have completed this process by August 2012, so that the information is available for the preparation of the petition to CFIA, scheduled for September 2012.

Objective 2. To study the interactions of *A. bipustulata* with existing major Canadian natural enemies of root maggots

To satisfy this objective, we examined the interactions of *A. bipustulata* with Canadian natural enemies of root maggots that are either predators or parasitoids. As there is already some literature on parasitic interactions, most of the initial work focussed on predatory interactions between *A. bipustulata*, its close relative, *Aleochara bilineata*, and the carabid beetle *Bembidion quadrimaculatum.* Adults of the three species hunt for food on the surface of the soil, and both *A. bilineata* and *B. quadrimaculatum* are considered to be important predators of cabbage maggot eggs in Canada and in Europe. Our approach to studying the potential interactions of these species was to begin with the simple systems in which predation of individual adults of a single species is characterized, and then move to a simple laboratory system in which combinations of adults can interact, and finally move to experiments in field cages in which the adults interact in a more realistic system containing growing canola plants.

Predation by each of the three species was studied in 5 cm diameter Petri dishes containing soil and 20 cabbage maggot eggs. An individual adult of one of the three species was introduced into the dish, and the eggs that remained after 24 h of exposure were counted; there were 20 replicates for each species and egg location. When the eggs were exposed on the soil surface, the number consumed averaged 15 for *A. bipustulata*, 19 for *A. bilineata*, and 14 for *B. quadrimaculatum*. When the eggs were covered with a very thin layer of soil, the number consumed was much less: 2 for *A. bipustulata*, 6 for *A. bilineata*, and 2 for *B. quadrimaculatum*. The next level of complexity, still using a single individual of a single species of predator, involved exposing eggs for 24 hours in a covered plant pot containing a canola plant. In this system, eggs were either exposed or under a thin layer of soil, and the number and arrangement (grouped or scattered) of eggs was varied. There were eight replicates of each combination of predator species and egg density and position. Again, the number eggs consumed was much lower when the eggs were covered by a thin layer of soil. Particularly when eggs were buried, consumption of eggs at low egg densities was very small. At intermediate egg densities, consumption of eggs increased with increases in the egg density. As egg densities increased above about 20, consumption tended to plateau, so that the number of eggs consumed was generally no greater when 35 eggs were available than when 20 were available; again numbers consumed were lower when the eggs were covered with a layer of soil. Our results are consistent with a well-known pattern of predator response in short term experiments like this: at low densities of prey, predators are not interested in searching for prey; at intermediate densities they search actively and find prey in proportion to prey availability; at high prey densities, prey consumption is limited by the amount of time required to process (digest or handle) each prey item. We have a large data set that is still undergoing analysis to shed further light on predator behaviour. Of particular interest is the consumption when eggs are buried, as this is the normal situation in the field.

Interactions between the predators were studied in the laboratory in Teflon-coated 30 cm diameter pie plates with a layer of soil and a total of 50 cabbage maggot eggs covered slightly with soil. Beetles were then released on the soil, and left to forage for 24 h. The following combinations were compared: 1 or 2 pairs of *A. bipustulata*, 1 or 2 pairs of *A. bilineata*, 1 or 2 pairs of *B. quadrimaculatum*, 1 pair of *A. bipustulata* and 1 pair of *A. bilineata*, 1 pair of *A. bilineata* + 1 pair of *B. quadrimaculatum*, and 1 pair of *A. bipustulata* + 1 pair of *B. quadrimaculatum*. Results of the 20 replicates of each treatment are shown in the table on the next page.

In all experiments, there were eggs left at the end of the experiment. In the single species treatments, by comparing the number of eggs consumed by one pair with that consumed by two pairs, we can assess whether there is competition for eggs. In the absence of competition, we expect two pairs to consume twice what one pair consumes. For *A. bipustulata* and *A. bilineata* it appears that the expectation is met, so there is no evidence of competition between adults of these species. However, two pairs of *B.quadrimaculatum* consumed less than twice the number of eggs consumed by one pair of *B. quadrimaculatum*, which suggests competition occurs between individuals in this species. The same arguments can be applied to treatments with two species: if there is no competition between the species, we expect the number of eggs consumed by one pair of each species to be the same as the sum of the consumption by one pair of each species separately. Although considerable further analysis of these data is needed and is in progress, the initial impression is that the treatments with two species have lower egg consumption than predicted, and so competition between species is occurring, and is particularly strong when *A. bilineata* is one of the species involved.

Predators	Mean eggs consumed /50 (s.e.m.)
1 pair *A. bipustulata*	11.6 (2.54)
2 pairs *A. bipustulata*	24.6 (3.52)
1 pair *A. bilineata*	9.3 (2.30)
2 pairs *A. bilineata*	22.4 (3.33)
1 pair *B. quadrimaculatum*	11.7 (2.83)
2 pairs *B. quadrimaculatum*	7.1 (2.10)
1 pair each of *A. bipustulata + A. bilineata*	11.4 (4.03)
1 pair each of *A. bilineata + B. quadrimaculatum*	11.5 (4.78)
1 pair each of *A. bipustulata + B. quadrimaculatum*	17.0 (5.42)

A similar experiment was conducted in field cages, each 60 x 60 cm and 2 m tall, and planted with 25 canola plants. At the beginning of the experiment, 125 cabbage maggot eggs were placed in the cages and different combinations of the three predator species were introduced. In this experiment, the basic unit was 6 individuals of a species, and these were present either alone or with 6 individuals of a second species or, in one treatment, there were 6 individuals of each of the three species. Cages were left for 2 days and then the predators removed and the eggs counted. Cages with no predators at all showed virtually no egg loss, and cages with predators always showed significant egg loss. There were 12 replicates of each treatment. In this study, the introduction of 6 individuals of a second or third species never increased the consumption of eggs to the level predicted by summing the consumption of 6 individuals of each species separately. Thus, at the densities involved in this experiment, there is evidence of competition among the predators. Because it is difficult to measure density of the highly mobile predatory insects in the field, we do not know whether the densities we used in the field cage (6 individuals per cage is equivalent to 17/m^2, so our three species treatment had 51 insects/m^2) are higher than what would typically be found in the field. If the densities are similar to those in the field system, the results suggest that root maggot predation would not be increased by introduction of *A. bipustulata*.

Interactions of *A. bipustulata* through parasitism involve *A. bilineata* and a parasitic wasp, *Trybliographa rapae*. Both *Aleochara* species have a two stage host-selection process: females lay eggs near maggot pupae, and larvae that hatch from those eggs select a pupa to attack, chew through the pupal case and enter it, and then after feeding for a little while, plug the entrance hole, and remain inside the pupal case for the remainder of their development to adulthood. In contrast the wasp female lays an egg directly into a maggot larva, and the egg hatches into a first stage wasp larva inside the maggot; this is known as the endoparasitic stage. Development of the wasp larvae does not continue until after the maggot pupates. About a week after maggot pupation, the wasp larva exits from the maggot pupa and occupies space outside the maggot pupa but inside the pupal case; this is known as the ectoparasitic stage. Eventually the wasp larva pupates within the pupal case of the maggot, and later an adult wasp emerges from the pupal case. Because of the complexity of the parasitic interactions, most studies examine only parts of the process. Thus, we know that egg-laying female *A. bilineata* avoid laying eggs near maggot pupae that are already parasitized by *A. bilineata* larvae (Fournet et al.2001), and assume that female *A. bipustulata* may show the same behaviour. Our previous work (Riley 2009) and a published study (Fournet et al. 1999), show that larvae of the two *Aleochara* species avoid entering a maggot pupal case that already contains a larva of one or other of the *Aleochara* species. This discrimination probably uses chemical cues emanating from the plug with which the first larva closed its entrance hole into the puparium (Royer *et al*., 1999; Lizé *et al*., 2010). Interactions between *A. bilineata* larvae and maggot pupae parasitized by *T. rapae* have also been studied. When the *T. rapae* larva is in the endoparasitic stage, *A. bilineata* will attack the maggot pupa; although the *A. bilineata* is more likely to survive than the *T. rapae*, survival of both parasites is low when two parasites occupy the same pupa. Larvae of *A. bilineata* tend to avoid attacking maggot pupae when they contain *T. rapae* larvae at the ectoparasitic stage; if the *A. bilineata* larva does attack, it dies and the wasp generally survives.

All the above work was done with single individual parasites. To add to this body of knowledge, we examined interactions when there were multiple *Aleochara* larvae and many maggot pupae to choose from. In a soil-filled laboratory arena, 30 maggot pupae were exposed to either 10 or 20 larvae of *A. bilineata* or *A. bilineata*, or 10 larvae of each species. The proportion of pupae parasitized was the same whenever 20 larvae were added, and the number of pupae parasitized per larva was the same in the mixed species treatment as in most others, with only 10 *A. bipustulata* being higher. This suggests that while *A. bipustulata* larvae may compete with other larvae of their own species, *A. bilineata* larvae do not compete with other larvae of the same species, and there is no competition between larvae of different species.

Interactions between *A. bipustulata* and *T. rapae* were studied in the laboratory by presenting maggot pupae that were parasitized by *T. rapae* to larvae of *A. bipustulata* for them to parasitize. When maggot pupae contained *T. rapae* larvae at the endoparasitic or ectoparasitic stage, *A. bilineata* larvae attacked them readily, but when the maggot pupa contained a *T. rapae* pupa, *A. bipustulata* larvae avoided making an attack if there was an alternative of an unparasitized pupa. Attacks by *A. bipustulata* larvae on maggot pupae containing any stage of *T. rapae* usually resulted in the death of both parasites and of the maggot.

We hoped to gain an overall understanding of interactions among parasites by introducing adult *A. bipustulata* and *A. bilineata* into 60 x 60 cm field cages 2m tall and containing 25 canola plants. Either six pairs of *A. bipustulata* or six pairs of *A. bilineata*, or three pairs of each of these species were introduced into each cage. Maggot pupae, some of which were previously parasitized by *T. rapae* were introduced into the cages in small trays. The experiment proceeded for 20 days, with pupae being added and removed at intervals to allow different stages of *T. rapae* to be exposed, and to give time for *Aleochara* larvae to construct the plugs that signal to other larvae that a pupa is previously parasitized. For interactions among the two *Aleochara* species, seven replicates were successfully completed, and levels of parasitism were not significantly different among the three treatments: three pairs of each species gave approximately the same level of parasitism as six pairs of either species alone. In fact the level of parasitism was marginally higher in the treatment with the two species than with each species alone, a result also obtained in a similar pilot experiment. It was difficult to get clear information about interactions with *T. rapae* in this system because it was not possible to be sure whether a maggot pupa was actually parasitized by *T. rapae* before it was placed in the cage, and diagnosis afterwards was imprecise. It was noted that in treatments with *T. rapae* and one or both *Aleochara*, mortality of the maggots was higher than in the absence of *T. rapae*.

Overall, our results suggest that adding *A. bipustulata* to the parasites that afflict cabbage maggots in Canadian canola will increase mortality from parasitism. There appear to be no negative effects of *A. bipustulata* on parasitism by *A. bilineata*, which is the main parasite of root maggots in canola in the Prairie Provinces. There may be some negative effects on *T. rapae*, which is far less common in canola on the prairies, but even so, the combination of *A. bipustulata* and *T. rapae* kills more maggots than either does alone.

Objective 3. To study the host and prey selection cues of *A. bipustulata*

Our studies are focusing on the biological significance of a simple organic compound, dimethyl disulphide (DMDS). DMDS is a breakdown product of sulphur-containing compounds, such as the plant chemicals of brassicas. DMDS is given off by maggot infested brassicas, and increases the catch of *A. bilineata*, *A. bipustulata* and *B. quadrimaculatum* adults in pitfall traps (Ferry et al. 2007). We originally thought that DMDS might be the active ingredient in the mustard seed meal that can be used to increase levels of parasitism (Riley et al. 2007) and predation (Ahlström-Olsson & Jonasson 1992) of cabbage maggots by *A. bipustulata*. However, manipulation with DMDS has not successfully elevated cabbage maggot mortality (Ferry et al. 2009) and, unlike DMDS, which attracts multiple species of predators, mustard seed meal is specific to *A. bipustulata*. Therefore, in addition to investigating the biological significance of DMDS, we examined the relationships of DMDS to the volatiles given off by mustard seed meal.

In an arena with still air, larvae of both *Aleochara* species responded when close to a source of DMDS by being arrested (reducing their level of movement); this behaviour occurred both in light and in simulated darkness; 30 replicates were performed in each condition. In moving air, in which insects choose to move towards air flows bearing different odours, larvae did not make a choice between DMDS and alternatives: they were arrested by the DMDS and did not move. In a sand-filled arena with two cabbage maggot pupae, DMDS was released below one pupa, and the other had no such release. In this system, larvae of both *A. bipustulata* and *A. bilineata* preferred to attack the pupa in the vicinity of the DMDS: significantly higher levels of parasitism resulted. In a similar arena, larvae were presented with a choice between a cabbage maggot pupa and a pupa of a less preferred host species, neither of which was associated with DMDS. We then repeated this study, but released DMDS close to the unpreferred pupa. The presence of DMDS around the unpreferred pupa did not increase the level of parasitism of this pupa, but reduced the parasitism of the cabbage maggot pupa. Our results suggest that DMDS is an important part of the cues that larvae use to find cabbage maggot pupae to parasitize; the arrestment effect causes them to search intensively in locations where maggot feeding has occurred. However, DMDS is not the only cue involved in accepting a host, and will not, by itself, cause acceptance of an unpreferred host.

We performed tests of the responses of adult *A. bipustulata* and *A. bilineata* to DMDS in still and moving air, and initially did these on unmated adults that had emerged from the pupae less than 2 days previously, then allowed these adults to mate, and repeated the tests 10 days later. In general, adult responses to DMDS in still air were rather subtle. The only significant effect was that newly-emerged *A. bipustulata* adults avoided areas close to DMDS droplets. In moving air, newly emerged adults of both *Aleochara* species avoided DMDS and chose the alternative. After 10 days during which mating was allowed, females of both species are attracted to DMDS and males, while not attracted, no longer avoided DMDS so strongly. In males, at least, the switch in behaviour was related to mating status rather than to age.

To examine the relationship of mustard seed meal volatiles to DMDS, aliquots of the original samples of mustard seed meal we used for Riley et al. (2007) and of newly purchased samples from the same source, were placed in a vial and the headspace sampled with a solid phase micro-extraction fibre (SPME). The SPME was then placed in a gas chromatograph-mass spectrometer for analysis. From this, it was determined that mustard seed meal does not give off DMDS.

Our major aim for objective 3 were to understand better the processes by which *A. bipustulata* makes choices about what to kill; this has implications for non-target effects of an introduction of this species. A subsidiary aim was to investigate whether chemicals could be used to manipulate *A. bipustulata* populations. Clearly manipulations can be done with mustard seed meal, as we previously demonstrated (Riley et al. 2007). DMDS is not present in mustard seed meal volatiles, and is not a substitute for it, and so is not a useful tool for manipulations, as Ferry et al. (2009) demonstrated. The reasons for this are probably related to the complexity of responses to DMDS. Adult responses are primarily manifested in moving air, and that fits with our knowledge that adults fly readily and are active on the surface of the soil, where they would experience air movement. Larvae mostly operate below the ground surface where air does not move. The larval responses clearly will tend to increase their chances of finding a cabbage maggot pupa that is closely associated with the damaged brassica root in which it fed as a larva. Whether a larva enters a pupa and successfully parasitizes it is not strongly influenced by DMDS, but by pupal size and morphology (Andreassen et al. 2009) and the presence of a plug from a previously entered *Aleochara* larva (Royer et al 1999). However, larval range is of the order of a few metres at most, and so choices of habitats are not made by larvae. Selection of the habitats in which larvae will occur is the result of females choosing egg-laying sites, and the attraction of mated females to DMDS will tend to bring egg-laying females to sources of DMDS. Such sources are damaged brassicas and onions, decaying carrion, and dung, and the recorded host range of *A. bipustulata* includes hosts from these habitats (Maus et al. 1998; Andreassen et al. 2007, 2009). The avoidance of DMDS by newly-emerged umated adults probably allows adults to disperse away from the DMDS-rich location in which they developed. Experimental work and analysis of these results is now complete, and a manuscript is being prepared for publication.

General Discussion

Once work has been completed on the levels of predation of carabid beetles in the field, we will have completed the assembly of data that we believe is necessary for a petition to CFIA for introduction of *A. bipustulata* for classical biological control. It is planned to write that petition in September 2012. As the principal investigator will, by then, be retired and so not eligible to be the petitioner, it has been arranged that the formal petitioner will be an Agriculture and Agri-Food Canada Research Scientist, who will, if the petition is successful, be responsible for coordinating introductions and post-introduction assessments.

Given that predatory insects tend to choose food items mostly on the basis of size and ability to be handled by the predator, the range of prey consumed in our Petri dish studies was remarkably narrow, and with one exception gave no cause for concern that a beneficial or desirable organism in a canola field would be eaten by *A. bipustulata* in significant quantities. The one exception, a carabid beetle, is of concern; much depends upon the results of our molecular study. If that shows that carabid DNA is frequently found in the gut of *A. bipustulata* there may be significant resistance to granting of petition without further study of the issue.

Our studies, plus information in the literature provide reasonable grounds for a prediction that if *A. bipustulata* can be established in Canada, it will contribute to mortality of root maggots, primarily through its parasitism. Interactions with other parasites do occur, but the overall effect of the addition of *A. bipustulata* will likely be an increase in parasitism. In habitats such as canola on the Canadian prairies where the current major parasite is *A. bilineata*, negative effects on existing parasitism are likely to be negligible. In our studies, competition by predators for eggs limited egg consumption, and if such competition occurs in the field, the addition of *A. bipustulata* is unlikely to increase egg predation significantly.

Our studies of the influence of chemical cues on *A. bipustulata* are in accord with the view that, if this species is introduced into Canada, it will mostly be confined to brassica crops or onions (where it is likely to attack the onion maggot), and to carrion and dung. Carrion and dung will likely only be a habitat if it is in open areas. In these sorts of habitats a range of fly pupae may be attacked, but Andreassen et al. (2009) showed that the range that are killed and can successfully support development of *A. bipustulata* is limited by pupal size, morphology and other factors. Thus, responses to chemical cues and other limitations of habitat choice provide significant safeguards for many non-target species.

A future introduction of *A. bipustulata* could, by itself, reverse the trend of increasing severity of root maggot infestations in canola in the Prairie Provinces. However this is not a likely scenario if rotation intervals shorten and seeding rates are reduced, as both these agronomic choices favour root maggot population growth. However, the additional mortality from *A. bipustulata*, in the context of an IPM approach to root maggot management, may cause the desired pest population reductions. Host plant resistance in canola and compatible cultural control methods should also be part of the IPM program for root maggots that includes biological control by existing natural enemies and *A. bipustulata*.

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List of publications and presentations on the project topic during the duration of funding

Publications in Refereed Journals

Holliday AE, Pak DM, Holliday NJ. *Submitted.* The *Aleochara* (Coleoptera: Staphylinidae) attractant in mustard seed meal is not dimethyl disulphide. Proceedings of the Entomological Society of Manitoba.

Andreassen LD, Kuhlmann U, Whistlecraft JW, Soroka JJ, Mason PG, Akinremi OO, Holliday NJ. 2010. Spring emergence of Canadian *Delia radicum* (Diptera: Anthomyiidae) and synchronization with its natural enemy, *Aleochara bilineata* (Coleoptera: Staphylinidae). The Canadian Entomologist 142, 234B249.

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Other publications

Holliday NJ, Andreassen LD, Dixon PL, Kuhlmann U. *Submitted. Delia radicum* (L.), Cabbage Maggot (Diptera: Anthomyiidae). Chapter in Mason P & Gillespie D [eds]. Biological Control Programmes in Canada 2001B2010. CABI Publishing, Wallingford, UK.

Holliday NJ. 2011. Classical biological control of cabbage maggot revisited. IOBC/NRS Newsletter 33(3): 5.

Conference presentations

Andreassen LD, Whyard S, Kuhlmann U, Mason PG, Holliday NJ. 2011. Development of molecular markers to study the natural diet of a predacious biological control agent. Presented paper. Annual Meeting, Entomological Society of Canada, Halifax, November.

Du J, Holliday NJ. 2011. Responses of *Aleochara bilineata* and *A. bipustulata* (Coleoptera: Staphylinidae) to dimethyl disulphide. Presented paper. Annual Meeting, Entomological Society of Canada, Halifax, November.

Du J, Holliday NJ. 2011. Responses of *Aleochara bilineata* and *A. bipustulata* (Coleoptera: Staphylinidae) to dimethyl disulphide. Presented paper. Annual Meeting, Entomological Society of Manitoba, Winnipeg, October.

Simon FL, Andreassen L, Haye T, Holliday NJ, Mason PG, Kuhlmann U. 2010. Do *Aleochara bipustulata* (Coleoptera: Staphylinidae) larvae prefer *Delia radicum* (Diptera: Anthomyiidae) puparia over smaller host species? Poster paper. Annual Meeting, Entomological Society of Canada, Vancouver, November.

Andreassen L, Kuhlmann U, Mason PG, Holliday NJ. 2010. Assessment of *Aleochara bipustulata* (Diptera: Staphylinidae) as an additional mortality factor of *Delia radicum* (Diptera: Anthomyiidae) eggs in prairie canola. Presented paper. Annual Meeting, Entomological Society of Canada, Vancouver, November.

Du J, Holliday NJ. 2010. Responses of *Aleochara bilineata* and *Aleochara bipustulata* (Coleoptera: Staphylinidae) to dimethyl disulphide. Presented paper. Annual Meeting, Entomological Society of Canada, Vancouver, November.

Andreassen LD, Kuhlmann U, Mason PG, Holliday NJ. 2010. Assessment of *Aleochara bipustulata*

(Coleoptera: Staphylinidae) as an additional mortality factor of *Delia radicum* (Diptera: Anthomyiidae) eggs in prairie Canola. Presented paper. Annual Meeting, Entomological Society of Manitoba, Winnipeg, October.

Du J, Holliday NJ. 2010. Responses of *Aleochara bilineata* and *Aleochara bipustulata* (Coleoptera: Staphylinidae) to dimethyl disulphide. Presented paper. Annual Meeting, Entomological Society of Manitoba, Winnipeg, October.

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Anticipated future publications arising from this project

Du J, Andreassen LD, Holliday NJ. Behavioural responses to dimethyl disulphide by *Aleochara bilineata* and *A. bipustulata* (Coleoptera: Staphylinidae). Complete manuscript draft for submission to Physiological Entomology.

Du J. The role of DMDS in the biology of two *Aleochara* parasitoids of *Delia radicum*. M.Sc. Thesis, anticipated submission date, April 2013. The above manuscript is a major component of this thesis.

Andreassen LD. Assessment of *Aleochara bipustulata* as an agent of classical biological control for *Delia radicum*. Ph.D. thesis, anticipated submission date July 2012. Approximately five refereed publications are expected to be derived from this thesis.

6. Final Project Budget and Financial Reporting

Detailed financial statements have been requested from Financial Services, University of Manitoba, and will be sent as soon as the account closure process is finalized.

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