The assessment of *Aleochara bipustulata* as a potential biological control agent of *Delia radicum*.

By

Kimberley Riley

A thesis Submitted to the Faculty of Graduate Studies of
The University of Manitoba
In Partial Fulfillment of the Requirements for the Degree of

Master of Science

Department of Entomology
University of Manitoba
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THE UNIVERSITY OF MANITOBA

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Abstract

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Aleochara bilineata (Gyllenhal) and *A. bipustulata* (L.) first instar larvae are important pupal ectoparasitoids of *Delia radicum* (L.) in many *Brassica* crops in Europe. In North America, however, *A. bipustulata* does not occur and therefore is a potential candidate for biological control against *D. radicum* in Canadian canola. Prior to its introduction, further ecological data such as how the two species interact with each other, as well as methods to implement the introduction of *A. bipustulata,* are needed. Field cages studies determined that the two species occurring together would increase parasitism of *D. radicum* compared to each species occurring individually. Parasitism levels were higher when three pairs of each of the two species were present than when six pairs of either of the species were present alone. Laboratory studies showed that, regardless of whether there was choice, the *Aleochara* species inside a puparium, or the instar of the parasitoid inside a puparium, first instar larvae of *A. bipustulata* seldom entered a previously parasitized *D. radicum* puparium. Frequency of entry of *A. bilineata* larvae into a previously parasitized *D. radicum* puparium was higher particularly in the absence of choice of an unparasitized puparium. Frequency of entry of *A. bilineata* into a parasitized puparium was less if the parasitoid inside was a conspecific and was inversely related to the instar of the parasitoid inside. Mustard seed meal applied to canola was attractive to *A. bipustulata* but not attractive to *A. bilineata.* Y-tube olfactometer tests showed that mustard seed meal was attractive to *A. bipustulata* adults, regardless of whether the meal was wet or dry. In the field, catches of adult *A. bipustulata* parasitism were enhanced by mustard meal mulch. Mustard meal did not affect *A. bilineata*. It is concluded that *Aleochara bipustulata* potentially could coexist with *A. bilineata* in the Canadian Prairies. Larval-larval and adult-adult interactions have shown that *A. bipustulata* adults are not aggressive toward *A. bilineata* and since the first larval instar prefers to parasitize unparasitized puparium they do not pose a major threat against *A. bilineata* larvae. The application of mustard meal to canola could enhance the release and establishment of *A. bipustulata* and allow them to locate *D. radicum* hosts as well as finding other *A. bipustulata* with which to mate.

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Chapter 1: General introduction

Delia radicum (L.) (Diptera: Anthomyiidae), the cabbage root maggot, is a pest of economic importance in both Europe (Finch 1989) and North America (Liu & Butts 1982; Liu 1984; Griffiths 1986; Soroka *et al.* 1999, 2002). This European pest was accidentally introduced into eastern North America in the 19th century (Schoene 1916; Griffiths 1991; Biron *et al*. 2000; Soroka *et al.* 2002) and is now commonly found across Canada (Griffiths 1991), attacking vegetable *Brassica* crops such as broccoli, cauliflower, rutabaga, and turnip (Soroka *et al.* 2002). The larvae of *D. radicum* feed on the surface tissue of *Brassica* roots, and at high infestation levels cause wilting, stunted growth, and limitations on water and nutrient uptake.

In Europe, *D. radicum* is considered a minor pest of canola. The majority of canola grown in continental Europe is winter canola (sown in late August to early September) rather than summer canola (sown in early May to mid-June) (Ekbom 1995, Alford *et al.* 2003). The life cycle of *D. radicum* is not synchronized with germination of winter canola in the field, and so there is little impact on the crop (Lamb 1989).

In the Canadian prairies, cultivars of *Brassica napus* and *B. rapae* are grown using a summer canola production system. Since the early reports of detection in the crop (Allen 1964), *D. radicum* populations have grown in density and area of infestation, and in the last 20 years, have become a major constraint to canola production systems (Soroka *et al.* 2004). In Manitoba, 12% of surveyed canola fields had *Delia radicum* infestations in a 1985-1988 (Turnock *et al.* 1992) and in Saskatchewan in (1983-1987) 19% of fields were infested (Burgess & Weeger 1988), however, in surveys from 1995 to 1998 96% of fields were infested in both provinces (Soroka *et al.* 2004). In Alberta, percent of infested fields increased from 90.6% in 1984 (Liu 1984) to 99.8% in 2001 (Soroka *et al.* 2004).

Delia radicum larvae feed on the roots of canola plants creating tunnels along the surface of the root. When plants are heavily infested, feeding damage to the root can cause wilting, stunted growth, lodging, and reduced numbers of flowers; severe infestations can lead to plant death (McDonald & Sears 1992). Tunnels can be entry points for bacterial and fungal pathogens. Yield losses as high as 50% have been recorded for *Brassica rapa* and 20% for *Brassica napus* (Canola Council of Canada 2009).

In Canada, no insecticides are registered to control *D. radicum* in canola and cultural control methods, such as cultivating prior to seeding, altering seeding rates, row spacing and cultivar selection, have been suggested (Dosdall *et al.* 1994, 1996, 1998). Although many methods have been developed for controlling *D. radicum* in *Brassica* vegetables (Finch 1987, 1989, 1993; Gehringer & Goldstein 1988, Finch & Collier 2000), few are recommended for canola production systems in the Canadian Prairies (Soroka *et al.* 2002). With limited methods available for control of *D. radicum*, this alien invasive species is an ideal target for classical biological control, which should be incorporated into an integrated pest management program for *D. radicum*. The goal of introducing *A. bipustulata* for classical biological control is to decrease the population growth rate of *D. radicum* in canola so that the current trend for population increase is halted or reversed.

Classical biological control of *D. radicum* was previously attempted in eastern Canada for *Brassica* vegetables beginning in 1949 with no success (Soroka *et al.* 2002). The recent invasion of *D. radicum* into canola in the Canadian Prairies, has prompted a reassessment of potential biological control agents that might be effective in the novel cropping system. In the mid-1990s, Soroka *et al.* (2002) conducted an extensive literature survey of parasitoids and predators associated with *D. radicum* in *Brassica* crops in Canada. This survey identified four main parasitoids, *Aleochara bilineata*, *A. verna* Say. *Trybliographa rapae* Westwood (Hymenoptera: Figitidae) and *Phygadeuon trichops* Thomson (Hymenoptera: Ichneumonidae), which seemed to have potential as biological control agents of *D. radicum*. Soroka *et al.* (2002) also identified a second *Aleochara* species *A. bipustulata* which was they referred to as a European biotype of *A. verna*.

To develop a classical biological control program, surveys were conducted to examine parasitioid communities in the place of origin (Europe) as well as in the place of invasion (Canadian Prairies) (Hemachandra *et al.* 2007). Surveys in the Canadian Prairies revealed that four parasitoid species were commonly associated with *D. radicum* puparia: *A. bilineata*, *T. rapae*, *A. verna*, and a *Phygadeuon* sp. (Hemachandra *et al.* 2007). Assessments of the parasitoid community of *D. radicum* in summer canola in Europe revealed seven parasitoids: *T. rapae*, *A. bilineata, A. bipustulata*, *A. brevipennis* Gravenhörst, *P. trichops*, and an undescribed *Phygadeuon* species (Hemachandra *et al.* 2007). After evaluating the two parasitoid communities, *A. bipustulata* was identified as a potential classical biological control agent that could be added to the present parasitoid community in canola in the Canadian Prairies. Before a potential agent can be introduced, studies of potential risks and benefits of the proposed introduction are necessary (van Lenteren *et al.* 2006). The research for this thesis examined whether there are likely to be benefits, in terms of increased *D. radicum* mortality, from adding *A. bipustulata* to the parasitoid community Canadian Prairies and also addressed methods of manipulating populations of *Aleochara* in canola. The thesis is divided into five chapters. Chapter one is the general introduction, and is followed by a literature review in chapter two. Chapter three addresses the use of mustard seed meal as an attractant of *Aleochara* spp. and has been published (Biocontrol Science and Technology, 2007; 17(3):273-284.) Chapter four examines the interactions of *A. bipustulata* and *A. bilineata* and is in the format of a research paper. The final chapter is the general discussion. Dr. L.J. Donald performed the volatile analysis reported in chapter three. With that exception, all research reported in this thesis was done by me.

Chapter 2: Literature review

This chapter summarizes the biology and characteristics of *D. radicum*, the damage it causes to canola, and the current state of pest management. Steps from implementing a classical biological control strategy are then reviewed and finally current knowledge about the natural enemies present in canola infested with *D. radicum* is summarized.

The pest - *Delia radicum*

Delia radicum (L.) belongs to order Diptera (true flies), family Anthomyiidae and subfamily Anthomyiinae. This species has gone through many name changes it was first named *Musca radicum* L., described by Linnaeus in 1756 (Pont 1981). Among names used since are *Anthomyia brassicae* (Bouché), *Chortophila floccose* (Macquart), *Phorbia brassicae* (Bouché), *Anthomyia radicum* (L.) *Anthomyia ruficeps* (Meigen), *Pegomya brassicae* (Bouché), *Chortophila brassicae* (Wiedemann), *Hylemyia brassicae* (Bouché), *Erioischia brassicae* (Bouché) *Delia brassicae* (Hoffmannsegg & Wiedemann) and *Hylemya brassicae* (Weidemann) before finally being designated as *Delia radicum* (L.) (Grittiths 1991).

Delia radicum anatomy

Adults

The adult fly is an average of 6 mm in length (Smith 1927). The adult male is dark grey and covered with bristles (Schoene 1916). Females tend to be lighter in colour than males with a shade of brown on the body and legs (Brooks 1951). Male compound eyes are holoptic and female compound eyes are dichoptic (Brooks 1951).

Eggs

Eggs range from 0.93 to 1.2 mm in length (Schoene 1916; Smith 1927; Hughes & Salter 1959) and are white in colour. Each egg is cylindrical in shape with longitudinal ridges along the dorsum (Brooks 1951; Miles 1952). The anterior end of the egg is depressed at the micropyles and has several aeropyles on the posterior end (Schoene 1916; Hinton & Cole 1965).

Larvae

Delia radicum have three larval instars. The first instar is about 1.5 mm in length, with knob-like posterior spiracles and no anterior spiracles (Griffiths 1986). The second instar is 2 to 4 mm in length (Schoene 1916; Smith 1927) and has both posterior and anterior spiracles (Schoene 1916). The third larval instar is 2 to 8 mm in length (Schoene 1916; Smith 1927) with both anterior and posterior spiracles. The posterior spiracles each have three slits on each spiracle (Schoene 1916; Brooks 1951).

Puparium

Delia radicum larvae go through two stages of pupation. As the larva goes through its metamorphic changes to adult, the exoskeleton of the third instar larva forms a puparium in which a pupa develops, later becoming an adult fly. At the beginning of the pupal stage, the puparium is milky white and later becomes reddish-brown in colour as it ages. The puparium is sub-elliptical with smoothly round sides (Miles 1952). The length ranges from 3.5 to 7 mm (Schoene 1916; Miles 1952). The surface of the puparium is made up of transverse ridges (Royer *et al.* 1998), which are formed when longitudinal muscular contractions shorten the length of the larva by one quarter during puparium formation (Fraenkel & Bhaskaran 1973).

Life cycle

The number of generations of *D. radicum* in Canada ranges from one to three depending on the region where it occurs (Turnock & Boivin 1997). *Delia radicum* overwinters in the puparium in the soil and emerges as an adult in the spring. In Manitoba in rutabaga, there are one or two generations of *D. radicum*, adults emerge from overwintering puparia in the middle of June and a second generation may emerge as adults at the end of August. (Bracken 1988). After mating, each female has a four to six day pre-oviposition period (Read 1965). Mated females lay between 80 and 100 eggs each (Finch 1971;Nair & McEwen 1976) over a period of 5 to 6 weeks (Hughes & Salter 1959; Whistlecraft *et al.* 1985a, Griffiths 1991). Eggs are laid on the soil surface or in cracks and on the stems of *Brassica* plants (Coaker & Finch 1971). The larvae hatch in 3 to 7 days, (Whistlecraft *et al.* 1985b) and burrow into the soil towards the roots of a *Brassica* plant. The larvae feed on the roots of canola (McDonald & Sears 1991). The feeding duration of the second larval instar is around 6 days (Hughes & Salter 1959; McDonald 1985) and 8 to 12 days for the third larval instar (Schoene 1916). After feeding for 3 to 4 weeks, larvae leave the plant roots and pupate in the soil about 5 to 20 cm away (Fraenkel & Bhaskaran 1973). Pupation of non-diapausing pupae lasts for 15 to 35 days (Fraenkel & Bhaskaran 1973).

Host range

Delia radicum attacks mainly vegetable Brassicaceae crops such as *Brassica oleracea* (L.) (cabbage and cauliflower), *Brassica oleracea* var. *botrytis* subvar. Cymosa (L.)

(broccoli), *Brassica oneous* var. *gemmifera* (L.) (Brussel sprouts), *Brassica napus var. napobrassica* (L.) (rutabaga), and *Raphanus sativus* (L.) (radish). *Brassica napus* L., *Brassica rapa* (L). (canola), *Armoracia rusticana* (L.) (horseradish) and *Thlaspi arvense* (L.) (pennycress) are alternative food plants.

Delia radicum and canola

Delia radicum was first detected on canola in western Canada in 1958 and is now common in almost all provinces in Canada (Griffiths 1991). *Delia radicum* was first observed in canola in Manitoba in 1958 (Allen 1964), and in Alberta in 1981 (Liu & Butts 1982; Liu 1984). Over the last 20 years, infestations of *D. radicum* in the Canadian Prairie Provinces have increased and are now a major constraint on canola production (Soroka *et al.* 2004). In the early 1980s, canola roots were infested in 12% of surveyed fields in Manitoba (Turnock *et al.* 1992) and 19% of those in Saskatchewan (Burgess & Weeger 1988); these levels had increased to 96% by the time of surveys from 1995 to 1998 (Soroka *et al.* 2004),. In Alberta, percent of infested fields increased from 90.6% (Liu 1984) to 99.8% (Soroka *et al.* 2004). Higher infestation of *D. radicum* in Alberta may be associated with the predominance of *Brassica rapa*, which contains higher levels of glucosinolate and erucic acid, compared to *B. napus* which is grown in the eastern Prairies (Turnock *et al.* 1992). *Delia radicum* adults are attracted to 2-phenethyl isothiocyanate and this compound is released by *B. rapa* at 4-5 weeks after planting, when the plant is between onset and stem elongation (Cole 1980; Finch 1978).

Feeding damage on canola

Delia radicum larvae feed on the roots of canola plants creating tunnels along the surface of the root. When plants are heavily infested, feeding injury to the root can cause the plant to wilt, have stunted growth, and have reduced number of flowers and seeds (McDonald & Sears 1991). Tunnels on the roots can be entry points for bacterial and fungal pathogens (Klein-Gebbinck & Woods 2002). Yield losses as high as 52% for *Brassica rapa* and 20% for *Brassica napus* have been recorded (Canola Council of Canada 2002). *Delia radicum* infestations and canola yield losses are highest when a cool wet spring precedes planting. Surveys to assess the damage by *D. radicum* found that in years with higher amounts of precipitation damage by *D. radicum* was significantly higher (Turnock *et al.* 1992; Griffiths 1986; Dosdall *et al.* 1996). However, Soroka *et al.* (2004) did not find current year's precipitation to be a significant factor/predictor of the percentage of infested plants to have an effect on damage levels.

Control methods for *Delia radicum*

To compare with economic injury levels, methods of assessing populations of *D. radicum* have been developed for cauliflower, broccoli, and cabbage (El Titi 1977; Bligaard 1999). Population assessment is mainly through the use of felt traps that monitor the oviposition of *D. radicum* (Bligaard 1999). Although many management strategies have been developed for controlling *D. radicum* in *Brassica* vegetables (Finch 1987, 1989, 1993; Gehringer & Goldstein 1988; Finch & Collier 2000), they are not practical for canola production systems in the Canadian Prairies (Soroka *et al.* 2002). In Canada, no insecticides are registered to control *D. radicum* in canola and cultural control methods, such as cultivating prior to seeding, altering seeding rates, row spacing and cultivar selection, are suggested as potential strategies (Dosdall *et al.* 1994, 1996, 1998). In canola, the threat of economic yield losses does exist with the occurrence of optimal environmental conditions for *D. radicum* flight activity that result in high infestation levels (Alford *et al.* 2003; Soroka *et al.* 2004). With limited strategies available to control *D. radicum*, this invasive species is an ideal target for a classical biological control approach that could be incorporated into an integrated pest management program.

Classical biological control

There has been an increasing demand for alternative methods to control invasive alien pests in many agricultural and forest crops. In the past, chemical control was the dominant method to control exotic pests as well as native pests. With the deregistration of pesticides, increasing development of resistance to current pesticides as well as the negative impact and perception of chemical control (Sheppard *et al.* 2006), much research and interest has been generated in alternative control methods such as classical biological control. When a pest species invades a habitat or region outside its place of origin, an agent or combination of agents are selected from the pest's place of origin and released in the new habitat to enhance the control of the target pest species (Van Driesche *et al.* 2008). Classical biological control has been used since the early 1800s (Waage & Mills 1992); recently however, classical biological control and biological control in general has been criticized for not considering the potential negative direct and indirect impacts of release an exotic or native biological control agent (Messing *et al.* 2006). There have been many efforts within the biological control community to work together to establish uniform methods of releasing and establishing native and especially exotic invertebrate biological control agents (Hunt *et al.* 2007). From this point on, the focus will be on the release of exotic invertebrate biological control agents.

Steps in introducing an exotic biological control agent

Van Driesche *et al.* (2008) summarizes the twelve steps for reviewing a potential exotic biological control agent. The steps are similar both in insect or weed biological control. 1) evaluate the target pest and recruit interest and support; 2) obtain taxonomic clarification of the target pest; 3) identify the target pests place of origin; 4) conduct surveys of the natural enemies associated with the target pest in the place of invasion; 5) survey for natural enemies in the target pests place of origin; 6) assess potential candidates; 7) develop colonies of potential biological control agents for quarantine studies; 8) estimate the host range; 9) apply for a petition for release; 10) release and establish the agent; 11) re-evaluation of risk assessment testing and 12) re-evaluation of the agents efficacy since the release and economic value.

Before a classical biological program can be implemented one must ensure research, technical, industrial and funding support is available for the project. The impact of the target pest on the crop or commodity should be thoroughly evaluated to ensure there is enough evidence to enable one to find support from different sources such the research, industry and financial communities. Once the support is in place the next critical step is to correctly identify the pest, as well as the major damage and symptoms (Coppel & Mertins 1977). The need for correct taxonomic identification of the pest and of potential agents has resulted in biocontrol researchers working more closely with taxonomists, particularly in classical biological control. The correct taxonomic identification of the species is essential because it allows researchers to review the literature on the pest and also provides insight into potential biological control agents and the natural enemies that may be associated with related species (Sabrosky 1955, DeBach & Rosen 1991). An extensive literature search is then conducted to determine the pests' native range, and to search for potential biological control agents. This review also provides insight into the biology and ecology of the pest, which can help determine the potential range in the area of invasion (Coppel & Mertins 1977).

The next stage for a classical biological control program is reviewing the literature and conducting surveys both in the place of origin as well as the place of invasion. A thorough literature review determines the natural enemies associated with the target pest in its place of origin, their impact on the target pest (Waage & Mills 1992), and clarifies the taxonomy, biology, efficacy and potential non-target impacts (Hunt *et al.* 2007). To verify information in the literature, surveys conducted in the area of origin as well as in the area of invasion are key. Potential natural enemies can be collected using a variety of methods such as pitfall traps and sweep nets and by collecting a variety of stages from eggs to adults. These samples are then shipped to a quarantine facility where they are processed and a list of potential biocontrol agents identified.

Once the candidate agent is chosen, studies on the host-specificity of agent are required (van Lenteren *et al.* 2003). Host specificity testing is used to identify potential hosts of the agent to determine if it will pose a threat in its new habitat to non-target species in and around the habitat (van Lenteren *et al.* 2003). The host range of the potential agent or the impact on non-target species has become one of the most important and critical parts of pre-release studies. To minimize the risks, regulatory agencies are becoming more critical of the evaluation process to evaluating a potential agent and now require an agent to have a high degree of host-specificity (Kuhlmann *et al.* 2000). To assess the host-specificity of a potential agent one must investigate many different aspects to determine the potential host range.

Gathering information from literature records about the local ecology in the source locality of the potential agent and all known field host records provides some indication of host range. These findings should be confirmed with taxonomic experts as misidentification issues can occur. To verify information provided by the literature or if there is limited information available, surveys should be conducted to assess the species that are associated with the potential agent. Behavioural studies are also a requirement because they provide valuable information about the host/parasitoid biology and interactions occurring between them such as searching, oviposition and host discrimination (van Lenteren *et al.* 2006).

Kuhlmann *et al.* (2006) developed protocols that can be used for generating and narrowing down a list of test species that covers the ecological host range, phylogenetic/taxonomic issues, and beneficial/endangered species impacts. When analyzing the field host records of the target species and any close relatives in the native range there are three categories that should be considered. The first is ecological similarity. Any species that live in similar or adjacent habitats should be considered for testing. The second is phylogenetic and taxonomic affinity. Any species of insect that is closely related to the target species of the potential agent should be added to the list for consideration for testing. The third is a safeguard consideration; here beneficial insects such as pollinators, and other biocontrol agents as well as endangered species should be evaluated.

The initial test species list could contain more than 50 species, which is an impractical and cost prohibitive number for many classical biological control programs. To create a more suitable and reasonable list, Kuhlmann *et al.* (2006) recommend two filters. The first filter examines attributes that overlap with the target pest species such as geographic distribution, climate requirements, synchronization with the pest species, and host size. The second filter considers the availability and accessibility of test species. If sufficient numbers of one particular species are not available then that species should not be considered further. For this narrowed and revised list, a combination of well-designed and carefully planned controlled experiments will generate meaningful information about the host range of the potential biological control agent.

van Lenteren *et al.* (2006) proposed a method of testing the selected non-target species. The first step is to test if the potential agent will attack the non-target species in a small arena no-choice black-box test. In this test, the number of hosts killed and not killed, the number of parasitized and unparasitized hosts, and host suitability are tested. If the non-target species is not attacked by the candidate agent it is considered not to be threatened.

If attacks were observed on the non-target then one would conduct a test in a small arena no-choice behavioural test. This test assesses the same three measures as the black box test but also includes: encounter and attack rate over time to determine possible increases in pressure and latency time to first attack. No attacks on the non-target species would result in the species not being affected; however, if significant amounts of attacks were observed then the species would be moved into a large arena choice behavioural test. At this point in the testing, the potential agent is given a choice between the target host and the non-target host. Here the potential agent is assessed for: the number of target and non-target hosts killed and not killed, and the number of target and non-target hosts parasitized and unparasitized. If the non-target species is still attacked then the potential agent is tested in a field test to determine if the agent will attack the non-target in its natural habitat. If the potential agent continues to attack the non-target species in the field test situation then this suggests that the potential agent will pose a risk to the non-target species if released against the target pest species. The above steps are outlined in the context of a candidate biological control agent that is a parasitoid; the same procedures, but with variables for measurement adapted appropriately, should be used for predators

Another important factor is the ability of the potential agent to coexist with the current parasitoid community and ensure there are no indirect impacts of the adding the potential agent. Petri-dish testing can provide some initial observations on how different species will interact with each other and reduces the host finding aspect (Wang & Messing 2003).

Once all the pre-release data are collected, evaluated and it is determined that it is safe to release the agent; the last step in a classical biological program is to release the biocontrol agent. After releasing the agent(s), it is important to conduct follow up studies to see if the populations are establishing on the target pest, determine if effective control is being achieved and continue to monitor to ensure that non-targets species are not threaten (Barratt *et al.* 2006). Post-release surveys reveal if the agent became established or not, it can show the rate of spread in its new and surrounding habitats and also show if it has potentially been moved by accidental human activity (Van Driesche *et al.* 2008). Successful establishment is considered when the agent is recovered in three consecutive years after first release. The last part of the final stage is to evaluate the success of the program.

***Delia radicum* and biological control**

A classical biological control program for *D. radicum* was previously implemented in eastern Canada for *Brassica* vegetables in 1949. *Aleochara bilineata* and *Trybliographa rapae* were imported from Europe and released to control *D. radicum.* It was later determined after surveys were conducted that *A. bilineata* and *T. rapae* were already present in Canada. *Aleochara bilineata* had been misidentified as *Baryodma* (*Aleochara*) Casey (Soroka *et al.* 2002). The life cycle of *A. bilineata* is not as well synchronized with *D. radicum* populations in eastern Canada due to overwintering adult emerging later than overwintering adult *D. radicum* (Soroka *et al.* 2002). The adults of *A. bilineata* emerge several weeks after oviposition by *D. radicum*; therefore, they are unable to suppress the first generation of *D. radicum* (Nair & McEwen 1975) and are only effective at inflicting mortality on the second *D. radicum* generation. Studies conducted to determine if there was a difference in parasitism rates between Europe and Canada concluded that they were similar. With both *A. bilineata* and *T. rapae* present in Canada it was concluded that there was no beneficial effect with the addition of more parasitoids, thus the program was then terminated in 1954 (Soroka *et al.* 2002). This recent invasion of *D. radicum* into canola on the Canadian Prairies and the lack of alternative control strategies warrant the reassessment for a new potential for biological control.

Aleochara* parasitoids of *Delia radicum

Aleochara bilineata and *A. bipustulata* play an important role in the suppression of *D. radicum* in Europe (Andreassen *et al.* 2007). Both species are larval ectoparasitoids of *D. radicum* pupae. *Aleochara bilineata* is already present in canola on the Canadian Prairies. The addition of *A. bipustulata* to the existing parasitoid community could provide additional population suppression of *D. radicum*. The adults of both *Aleochara* species are predators of *D. radicum* eggs and larvae (Fuldner 1960). Female *Aleochara* oviposit their eggs singly in areas where host pupae or larvae are present (Fournet et al. 2001). Female *A. bilineata* are able to distinguish between parasitized and unparasitized puparium and prefer to oviposit in areas with unparasitized puparia and plants infested with *D. radicum* (Fournet *et al.* 2001). The mobile first instar larvae of both *A. bipustulata* are responsible for host selection. After selecting a host puparium, the larva gnaws an entrance hole in the puparium, then begins to feed, and develops within the host puparium as a solitary ectoparasitoid (Fuldner 1960).

Aleochara bilineata

Anatomy of A. bilineata

Egg. The eggs of *A. bilineata* have a smooth chorion , are ellipsoid in shape and creamy white at the beginning of development. The eggs are also hydrophilic and increase in volume due to water absorption. The egg increases in length by 75 µm and in width by 60 µm (Gauvin et al. 2001).

Larva. *Aleochara bilineata* has three larval instars and the development as well as the morphology has been well documented (Wadsworth 1915; Colhoun 1953; Fuldner 1960). The first instar is pale yellow with the intersegmental areas a creamy white (Wadsworth 1915). It is campodeiform, with mobile legs allowing the larva to search for a host puparium. It ranges from 1.25 to 1.64 mm in length and 0.12 to 0.25 mm in width prior to feeding on a host (Wadsworth 1915; Colhoun 1953; Fuldner 1960). The head of the first instar is flat with many sensory bristles (Fulnder 1960), 0.015 mm diameter eyespots on each side of the side of the head (Wadsworth 1915) and three-segmented antennae (Fuldner 1960). The abdomen consists of 10 segments with the last two segments heavily sclerotized. On the last segment is the pygopodium, which aids in mobility and anchors the insect when parasitism of a host occurs. Once the larva has fed on the host, its size increases from 2.3 to 2.5 mm in length and 0.37 mm in width by the end of the first instar (Colhoun 1953; Fuldner 1960).

The second larval instar undergoes hypermetamorphosis and becomes eruciform. As eruciform larva they are less mobile, with rudimentary legs and reduced bristles. The cuticle is lightly sclerotized. The larva begins at 2.3 to 3 mm in length (Colhoun 1953;

Fuldner 1960), and as it feeds on the host within the host puparium, it grows to as long as 4 mm (Colhoun 1953; Fuldner 1960).

The third instar is also eruciform but with a more sclerotized cuticle and more prominent mandibles. Its size ranges from 5.0 to 7.6 mm in length and 1.64 to 2.0 mm in width (Wadsworth 1915; Colhoun 1953; Fuldner 1960).

Pupa. The pupa initially is a creamy white colour but as development progresses, the tip of the abdomen begins to turn brown and eventually turns black by the end of pupation. The pupa fills the length of the host, puparium averaging between 4.25 to 4.66 mm in length (Wadsworth 1915; Colhoun 1953).

Adult. Adult *A. bilineata* have a shiny black slender body, with short elytra, and lack the red elytral spots on those of *A. bipustulata* (Fuldner 1960). The abdomen is pointed with the last segment bearing the pygopodium. The adult beetles are between 2.0 to 6.0 mm in length (Klimaszewski 1984). Beetle size has been associated with the size of the host puparium from which it develops (Wadsworth 1915; Langlet et al. 1998).

Life history of A. bilineata

Adult *A. bilineata* are predators of *D. radicum* eggs and larvae are parasitioids of *D. radicum* puparia (Colhoun 1953; Read 1962; Fuldner 1960; Bromand 1980). Under greenhouse conditions, Bromand (1980) observed that pairs of *A. bilineata* can consume approximately 2400 *D. radicum* eggs or 250 *D. radicum* third instar larvae during their lives.

Female *Aleochara bilineata* are synovogenic, so they produce eggs continuously over their life time, and they mate with more than one male. During mating, the male raises and bends his abdomen over his head with his claspers to expose the aedeagous. Mating lasts for 20 to 65 seconds (Colhoun 1953). The preoviposition period is between 36 to 96 hours (Wadsworth 1915; Colhoun 1953) after which the female oviposits between 8 to 15 eggs per day with a total of 500 to 700 oviposited in her lifetime (Read 1962; Bromand 1980; Fournet et al. 2000).

Female *A. bilineata* oviposit in the soil near *Brassica* plants. Females select sites based on mating, foraging and oviposition potential (Tomlin *et al.* 1992). Both male and females respond to cues from damaged brassica plants as well as from the frass and larvae of *D. radicum* (Fournet *et al.* 2001).

Upon hatching, each *Aleochara* larva contains fat globules with a limited quantity of nutrients (Fuldner 1960). *Aleochara bilineata* can live six to eight days without parasitizing a host (Colhoun 1953; Fuldner 1960). Once a host puparium is selected, the larva secures itself to the puparium using the adhesive pygopodium. It then gnaws a circular entrance into the cuticle using its mandibles. Larvae frequently chew an entrance holes on the dorsum of the host puparium (Royer *et al.* 1998). Entrance hole formation takes from 12 to 72 hours to complete (Colhoun 1953; Fuldner 1960; White & Legner 1966). Several factors can influence the formation of entrance holes: Brunel & Langlet (1994) compared the rate of penetration of *Delia radicum* and *D. antiqua* (Meigen) by first instar *A. bilineata* larvae while varying the physiological state of host puparium, temperature and humidity. They determined that the optimal conditions for *A. bilineata* penetration are between 20 and 25°C and 50% relative humidity, and host puparia that had pupated two days earlier (Brunel & Langlet 1994).

Once the larva has entered the puparium, it crawls to the anterior end of the pupa and pierces the vertex of the host head to feed on the haemolymph. In between feeding periods, *A. bilineata* seals the entrance hole by excreting contents from their malphigian tubules (Fuldner 1960; Bromand 1980). After a short feeding period, the larva moults into the second instar. The larva continues to feed and the second instar lasts approximately 5 days (Colhoun 1953; Fuldner 1960). Feeding continues for about10 days through the third larval instar, until the host is completely consumed except for the cuticle (Colhoun 1953). In preparation for pupation, the larva excretes a substance over the inside of the host puparium; this takes about 36 hours (Colhoun 1953; Fuldner 1960). Pupal development lasts for approximately 34 days (Colhoun 1953; Fuldner 1960; Bromand 1980) and the adult emerges using its mandibles to cut a ventro-cephalic hole through the puparium. Total development time from egg hatch to adult is an average of 33 days (Fournet et al. 2000). *Aleochara bilineata* overwinters as a first larval instar inside the host puparium on a diapausing host pupa (Colhoun 1953; Bromand 1980; Whistlecraft *et al.* 1985a).

Habitat and host range

The host range and habitat of *A. bilineata* has been thoroughly investigated by Maus *et al.* (1998). The species is found through out Western Europe, Scandinavia, parts of Eastern Europe and North America in habitats such as rotting plant material, agricultural crops, and faeces (Klimaszeski 1984; Maus *et al.* 1998). *Aleochara bilineata* develops in puparia of the genus *Delia* (Wishart 1957; Maus *et al.* 1998) and has a narrow host range in comparison to *A. bipustulata*. Besides attacking fly puparia within the Anthomyiidae, *A. bilineata* also parasitizes puparia from species of Lonchaeidae and Muscidae (Maus *et al.* 1998; Andreassen *et al.* 2009).

Aleochara bipustulata

Anatomy of A. bipustulata

The external anatomy of *A. bipustulata* is very similar to that of *A. bilineata*. The egg and larval stages are all morphological similar to *A. bilineata*. The main anatomical differences are within the adult *Aleochara.*

Adult *A. bipustulata* are stout, broad, robust and cylindrical in body shape. They are a shiny black with short elytra; however unlike *A. bilineata*, there is one red spot on each elytron. The body of an adult *A. bipustulata* is pubescence with spines on the pro and mesotibiae (Maus et al. 1997).

Life history of A. bipustulata

Aleochara bipustulata has a very similar life cycle to that of *A. bilineata*. The main distinction is that *A. bipustulata* overwinters as an adult or a pupa (Heydmann 1956) whereas *A. bilineata*, as stated previously, overwinters as a first instar larva within the host puparium (Whistlecraft *et al.* 1985a). Observations by Fuldner (1960) suggested that *A. bipustulata* females seldom oviposit on or near *D. radicum* puparia in vials in the laboratory. The optimal temperature for larval emergence is between 22 to 25°C (Fournet *et al.* 2000). *Aleochara bipustulata* larvae have been observed chewing a hole on the dorsal surface of the puparium. Larvae attach themselves to the surface of puparia with the aid of their pygopodium (Fuldner 1960) and use their mandibles to break through the puparial case creating a U-shape hole. Fuldner (1960) observed that puparial penetration required a period of 12 to 36 hours and takes place mainly on the dorsal side of the puparium; either medially or dorsolaterally (Fuldner 1960). Upon entering the puparium, like *A. bilineata*, *A. bipustulata* feeds on the host pupa and then begins to seal the hole in the host puparium (Fuldner 1960).

Once the larva has sealed the entrance hole, it crawls along the back of the thorax and positions its head between the eyes of the fly pupa or within the surrounding area. The *A. bipustulata* larva bites into the neck of the pupa resulting in a constant flow of liquid nutrition. After a short feeding period, the larva moults to the second instar and continues to feed and digest the contents of the host pupa. By the third instar the *A. bipustulata* larva has grown and covered the entire dorsal surface of *D. radicum* pupa. Just as the third instar larva is about to finish feeding, the malpighian vessels become swollen and white; this is followed by the coloration phase and pupation. Two days after the host *D. radicum* pupal contents have been consumed, A bipustulata defecates a white substance which lines the inner wall of the *D. radicum* puparium (Fuldner 1960). The total developmental time from larval entrance to pupal emergence of *A. bipustulata* is 33 to 34 day at 20°C and 25 to 27 days at 25°C (Fuldner 1960, Fournet *et al.* 2000).

Fuldner (1960) observed that after pupation 55% of *A. bipustulata* individuals failed to emerge from hosts due to rotting of the residual *D. radicum* puparia. *Aleochara bipustulata* has been found more frequently in smaller Diptera species, such *Delia platura* (Meigen) (Ahlström-Olsson 1994). Fuldner (1960) found that 49.3% of adult *A. bipustulata* emerged from *D. radicum* puparia in the size range of 5.1 to 5.5 mm. Ahlström-Olsson (1994) found that larvae of *A. bipustulata* when offered a puparia, in the range 4 to 8 mg, had a parasitism levels is 57% compared with puparia that weighed 15 to 23 mg where parasitoid emergence was only 11%. Larvae of *A. bipustulata* larva offered *D. radicum*, *D. platura*, or *D. antiqua* puparia were equally parasitized (Ahlström-Olsson 1994). ;

The total fecundity of *A. bipustulata* is considerably higher than that of *A. bilineata*. A female *A. bipustulata* lays an average of 1139 eggs in her lifetime, which is significantly higher than the 637 eggs laid by *A. bilineata* (Fournet *et al.* 2000). Although *A. bipustulata* has a higher fecundity than *A. bilineata*, Royer *et al.* (1999) also demonstrated that *A. bipustata* has a lower multi-parasitism level than *A. bilineata*, suggesting that *A. bipustulata* larvae have a lower chance of finding a host puparium. Higher fecundity may be a compensation for lower success of *A. bipustulata* larvae in finding unparasitized hosts.

Habitat and host range

Puparia containing *A. bipustulata* have been collected from agricultural habitats (such as brassica vegetables, potatoes, seed flax, beans, sugar beet and wheat) as well as in dung, carrion, bark and algae (Maus *et al.* 1998). In Europe, *A. bipustulata* has been recorded from Norway, Finland, Sweden, England, Holland, Denmark, Belgium, Scotland, Switzerland, France, Lativa, Romania, Russia and Hungary (Maus *et al.* 1997). Initially it was thought that *Aleochara bipustulata*'s host range as a parasitoid was considered to be broader than *A. bilineata* (Maus *et al.* 1997). The physiological host range of *A. bipustulata* has been documented in families such as Anthomyiidae, Muscidae, Calliphoridae, Ulidiidae, Piophilidae, Lonchaeidae and Sacrophagidae (Maus et al. 1997). Andreassen *et al.* (2009) demonstrated the ecological host range of *A. bipustulata* is more restricted as none of the tested non-target species living in dung were suitable hosts.

Chapter 3: Can mustard seed meal increase the abundance of *Aleochara* sp. attacking *Delia* sp. in canola?

Introduction

Delia radicum L. (Diptera: Anthomyiidae), the cabbage root maggot, is a pest of economic importance in vegetable brassica crops in Europe (Finch 1989) and North America (Soroka *et al.* 2002). In canola, larvae of *D. radicum* feed on the roots, and at high infestations, the injury limits water and nutrient uptake leading to reduced flowering and seed set (McDonald & Sears 1991). In Europe, *D. radicum* is an occasional pest of spring-seeded canola (Alford *et al.* 2003). In the prairie provinces of Canada, where spring-seeding is the norm, level of damage and percentage of canola fields infested by *Delia* spp. have increased markedly in recent decades (Soroka *et al.* 2004). In some parts of the prairies, root maggots significantly limit canola production (Griffiths 1991) and losses during years of high populations may be substantial (Soroka *et al.* 2004)

Although there are many options for controlling *D. radicum* in vegetables (Finch 1987, 1989, 1993; Gehringer & Goldstein 1988, Finch & Collier 2000), they are not practical for canola production systems in the Canadian Prairies, and so classical biological control is being considered (Soroka *et al.* 2002). Two staphylinid beetles, *Aleochara bilineata* (Gyll.) and *A. bipustulata* (L.) are important parasitoids of *D. radicum* (Finch 1989). *Aleochara bilineata* contributes to *D. radicum* mortality in canola in both Europe and Canada (Hemachandra 2004). *Aleochara bipustulata* does not occur in North America (Hemachandra *et al.* 2005) and we are evaluating its potential as an agent for classical biological control in Canada.

The adults of both *Aleochara* species are predators of *D. radicum* eggs and larvae (Fuldner 1960) and a pair can consume 50 eggs per day (Langlet & Brunel 1996). Females oviposit in the soil near *D. radicum* puparia and brassica plants (Fournet *et al.* 2001). The first instar parasitoid larva searches for a host puparium. After selection of a puparium, the larva gnaws an entrance hole enters the puparium, and feeds externally on the host pupa (Fuldner 1960). There are three larval instars within the host puparium, and pupation occurs inside the puparium (Fuldner 1960).

In brassica vegetables in Sweden, white mustard seed meal mulch attracted *Aleochara* spp. and suppressed *D. radicum* populations (Ahlström-Olsson & Jonasson 1992). The mechanism of attraction was not identified but it was suggested that either *Aleochara* were directly attracted to the mustard seed meal, or to its decomposition products, or that the attraction was based on the stem rot sometimes associated with the mulch, or that the mulch attracted alternative hosts for the parasitoids early in the season so that at the time of *D. radicum* attack a local population of parasitoids was present. The ability to manipulate *Aleochara* parasitism of *Delia* attacking canola could be a useful pest management tool, and so we performed field studies in Switzerland to determine the effectiveness of the technique, and laboratory studies to investigate mechanisms of attraction.

Our study aimed to determine if mustard seed meal attracts *Aleochara* spp. in canola and to investigate the mechanisms for any attraction observed.

Materials and methods

Insect rearing

Delia eggs and puparia were supplied by Agriculture and Agri-Food Canada in London, Ontario, Canada. A colony of *A. bipustulata* was established using adults emerged from parasitized *D. radicum* puparia collected from organic vegetable *Brassica* fields in the Province of Skåne, southern Sweden. An *A. bilineata* colony was established using adults emerged from parasitized *D. radicum* puparia collected in organic rutabagas near Winnipeg, Canada. Adult *Aleochara* were maintained in cages designed by (Herdveldt *et al.*, 1984) for egg collection and fed second and third instar *D. radicum* larvae. *Aleochara* eggs were collected each week and incubated on moist filter paper in Petri dishes. Newly-hatched larvae were provided with *D. radicum* puparia in moistened sand: vermiculite mixture in a Petri dish.

Field studies

Attraction of mustard seed meal to *Aleochara* spp. was evaluated in 2003 and 2004, on an organic farm, near Galmiz (46° 51′ 47.7″ N, 7° 8′ 46.4″E), Switzerland. Each year, field plots were seeded with canola (*Brassica napus* cv. Express) and two treatments, with and without mustard seed meal, were applied in a randomized block design.

In 2003, there were four rows of canola planted in a bed 57 m long and 1 m wide. The bed was divided into three replicate blocks, each 15 x 1 m and consisting of two 6 x 1 m plots separated by a 3 m buffer. There were 3 m buffers between each replicate and at each the end of the bed. All buffers were bare soil. Canola was seeded in early June and the mustard seed meal treatments were applied on 27 June when the canola was at the early bloom stage, and larvae of the second generation of *D. radicum* were present in the plot. White defattened mustard seed (*Sinapis alba* L.) meal (Kräuterpflug, Kiel, Germany) was applied to the soil between the plants of the mustard meal treatment plots at a rate of 20 g/m^2 using a hand-held flour sifter.

To assess the occurrence of *Aleochara* species, one pitfall trap, 10.5 cm diameter, was placed at the centre of each plot and emptied every week for four weeks following the application of the treatments. Each week, 30 canola roots were collected from each plot and dissected to determine the stage of *D. radicum* larval development. When *D. radicum* reached the puparial stage, roots from the final weekly sample were collected and the number of puparia collected from 30 roots was used to determine if there was a difference between meal treated and untreated plots. The following week, all remaining canola plants in each plot were pulled up, and the soil shaken from their roots. Puparia were separated from soil by floatation in water (Langer 1996). Puparia were reared in groups in Petri-dishes lined with moist filter paper until *D. radicum* or parasitoid emergence.

In 2004, the experiment was repeated three times, once with each generation of *D. radicum* attacking brassica vegetables. For each generation of *D. radicum,* a 75 x 4 m field of canola was planted, and divided into four replicate blocks, each 15 x 4 m. Replicate blocks were subdivided into paired treatment plots, each 6 x 4 m and separated by a 3 m buffer of bare soil. The canola for the three experiments was seeded on 8 April, 4 May and 1 July. In the first and second experiments in 2004, the natural population of

D. radicum was supplemented. Eggs of *D. radicum* from London, Ontario were mixed with corn meal and applied at a rate of 5/plant in a 1 cm deep trench adjacent to the canola row and covered with soil. In 2004, the mustard seed meal treatments were applied at the rosette stage (12 May, 9 June and 10 August respectively). In 2004, puparial collections were made on two occasions for each experiment. Canola plants in half of each plot were uprooted for puparial collection when puparia formation was first detected (the equivalent time to the 2003 collection). Puparia from the remaining half of the plots were collected 2 weeks later; at this time canola roots from the final collection were rated for *D. radicum* injury using the damage scale of Dosdall *et al.* (1994).

Analysis of variance was performed on arcsine-transformed percentages of *Aleochara* parasitism of puparia and untransformed root ratings. Friedman non-parametric analysis of variance was used to analyse pitfall trap catches. Statistical analyses were conducted using Systat (Systat, 2004); an α level of 0.05 was used throughout.

Olfactometer studies

The response of *A. bipustulata* and *A. bilineata* to mustard seed meal was investigated in laboratory experiments using an array of 12 Y-tube olfactometers. Each olfactometer was constructed of 2 cm diameter glass tubing, with an entry arm 15 cm long. A fan forced air into each Y-tube arm through an activated charcoal filter and a humidification chamber containing water. Sources of volatiles to be tested were suspended above the water in the humidification chamber. Volatile sources were compared in three pair-wise tests: 15 g dry mustard seed meal vs clean air control, 15 g dry mustard seed meal vs 15 g dry sterile soil, 15 g dry mustard seed meal vs 15 g mustard seed meal to which 20 ml of water had been added 2 days previously. All experiments were conducted at 22±1°C under two Cool White 36 W fluorescent lights.

Responses of individual naive 5-9 day old adult *A. bipustulata* and *A. bilineata* were evaluated in the olfactometer. Each beetle was allowed 3 min to respond to the choice of volatiles by entering one of the source arms. For each of the tested volatile, choices of 36 responding adults were recorded. Between tests, Y-tubes were thoroughly washed. The frequency of choice of each olfactometer arm was compared with the expectation of there being no differential preference between the test volatiles using χ^2 tests.

Volatile analysis

Volatiles produced by mustard seed meal were collected on activated carbon-impregnated fabric, then characterized using, a gas chromatograph - mass spectrometer (GC-MS). Strips (5.6 x 5.6 cm) of ACC C-TEX knitted fabric (Siebe Gorman & Co. Ltd, GB) were washed four times in HPLC grade methylene dichloride, then air dried on a glass plate gradually heated to 300°C. After 30 min each strip was transferred to a clean 25 mL Erlenmeyer flask, which was then sealed with a butyl rubber septum.

Four carbon-impregnated strips were prepared simultaneously. One remained sealed in an Erlenmeyer flask as a control. Each of the remaining three flasks was connected by a glass tube to a flask containing a volatile source, and laboratory air was pumped through the source flask to the flask containing the strip for a period of 24 - 26 h. In two of the source flasks, there was 1 g of mustard seed meal; the third source flask was empty to provide a control of laboratory air. Just before GC-MS analysis, 1 mL of methylene dichloride was added to the flask containing the monitor strip, agitated thoroughly, and then the liquid was removed to a smaller sample tube. Aliquots of 1 μL of the liquid were analysed in a Hewlett Packard 5890 gas chromatograph with column HP - 5 MS (30 m x 2.5 mm x 0.25 μm film), interfaced to a VG 7070E mass spectrometer. The column temperature was held at 80°C for 5 min, then increased at 10° C /min to 250°C with a 30 min hold time. A solvent only flush was used between samples to assure there was no carry-over. Spectra were analysed by MASSPECII and compared to the NIST database. The process was repeated four times. Before each experimental run, all glassware soaked in chromic acid solution for 24h, then thoroughly rinsed in deionized water and air dried.

Results

Field studies

In all experiments, pitfall trap catches of adult *A. bipustulata* were higher in plots treated with mustard seed meal than in untreated plots (Figure 1). The differences in catch were significant in the first and second experiments in 2004 (Experiment 1: Friedman S = 4.0, P = 0.045; Experiment: 2: S = 4.0, P = 0.045). In 2003, and in the third experiment in 2004, the trend was not significant (2003 Experiment: S = 0.3, P = 0.6; 2004 Experiment 3: S = 2, P = 0.16). No *A. bilineata* were caught during 2003, but adult *A. bilineata* were caught in all three 2004 trials. In none of the 2004 experiments were there significant treatment effects on catches of *A. bilineata* (Figure 1) (Experiment 1: S = 0.3, P = 0.6; Experiment 2: S = 0, P = 1.0; Experiment 3: S = 0.3, P = 0.6).

Total *Aleochara* parasitism levels were not significantly different between mustard meal treated and untreated plots with exception of the third experiment in 2004 where there was a significantly higher parasitism level in the meal treated plots (2003 Experiment: $F = 0.7$, df = 1,2, $P = 0.5$; 2004 Experiment 1: $F = 0.3$, df = 1,3, $P = 0.6$; 2004 Experiment 2: $F = 2.2$, df = 1,3, $P = 0.2$; 2004 Experiment 3: $F = 27.9$, df = 1,3, $P = 0.01$). Parasitism of *D. radicum* puparia by *A. bipustulata* was significantly higher in mustard seed meal plots than in untreated plots in all early puparial collections (Figure 2) (2003 Experiment: $F = 137.7$, df = 1,2, $P = 0.007$; 2004 Experiment 1: $F = 10.1$, df = 1,3, $P = 0.05$; 2004 Experiment 2: $F = 24.2$, df = 1,3, $P = 0.01$; 2004 Experiment 3: $F = 55.9$, df = 1,3, $P = 0.005$). In the late puparial collections in 2004, parasitism by *A. bipustulata* was significantly higher in the mustard seed meal treatment only in Experiment 3 (Experiment 1: $F = 0.3$, df = 1,3, $P = 0.6$; Experiment 2: $F = 4.2$, df = 1,3, $P = 0.1$; 2004 Experiment 3: $F = 33.8$, df = 1,3, $P = 0.01$). Parasitism by *A. bilineata* tended to be higher in control plots than mustard seed meal plots in all early puparial collections although this was significant only in the second experiment of 2004 (2003 Experiment: $F = 0.5$, df = 1,2, $P = 0.5$; 2004 Experiment 1: $F = 2.4$, df = 1,3, $P = 0.2$; 2004 Experiment 2: $F = 56.8$, df = 1,3, $P = 0.005$; 2004 Experiment 3: $F = 4.7$, df = 1,3, $P = 0.1$). In the late puparial collections in 2004, parasitism by *A. bilineata* was never significantly affected by the treatment (Experiment 1: $F = 0.1$, df = 1,3, $P = 0.8$; Experiment 2: $F = 0.7$, df = 1,3, $P = 0.5$; 2004 experiment 3: $F = 7.3$, df = 1,3, $P = 0.07$).

Over all four experiments, only experiment 2 in 2004 had significantly higher *D. radicum* puparium numbers in the control plots (2004 Experiment 2:$F = 16.7$, df = 1,3, $P = 0.03$). There was no significant difference in *D. radicum* puparial numbers between treated and untreated plots (2003 Experiment: $F = 0.7$, df = 1,2, $P = 0.5$; 2004 Experiment 1: $F = 0.9$, df = 1,3, $P = 0.4$; 2004 Experiment 3 $F = 0.03$, df = 1,3, $P = 0.9$). Root rating assessments in 2004 were never significantly affected by treatment. Root ratings were very low in Experiments 2 and 3 (Table I).

Olfactometer studies

Aleochara bipustulata adults were attracted to the olfactometer arm with dry mustard seed meal volatiles when the alternative was a clean air (control) or dry soil (Figure 3). Whether volatiles were from wet or dry mustard meal did not influence the response of *A. bipustulata.* In contrast, *A. bilineata* exhibited no significant preference for any of the choices (Figure 3).

Volatile analysis

All four experiments in which volatiles were captured and analysed by GC-MS gave very similar results. In the trace from the gas chromatograph, 16 major peaks were evident (Figure 4), and mass spectrometry allowed positive identification of 14 compounds. None of these peaks were present in the gas chromatograph traces from the two sorts of control sample types. The most abundant volatile was limonene (peak 2), and several other peaks (peaks 1, 5, 6, 7, 13) represent structurally similar compounds. In addition to matching with the NIST database, the identity of the limonene peak was verified by running a limonene standard diluted in methylene dichloride under the same GC-MS conditions.

Discussion

Aleochara bipustulata occurred in higher numbers and higher parasitism levels were observed in *D. radicum* in field plots treated with mustard seed meal. Although not significant in all experiments, the results are consistent with the observation that a mustard seed meal mulch treatment attracts *A. bipustulata* (Ahlström-Olsson & Jonasson 1992). The only significant treatment effect on *A. bilineata* in field plots was that on parasitism in the early collection of experiment 2 in 2004; however most other measures showed a non-significant tendency for *A. bilineata* to be in the control plots over the meal-treated plots. Our results contrast with those of Ahlström-Olsson & Jonasson (1992), who recorded higher numbers of *A. bilineata* adults in pitfall traps in the meal treatment. The pattern we observed could be the result of competition for oviposition sites between the two *Aleochara* species. Female *A. bilineata* avoid parasitized host puparia when selecting a potential oviposition site (Fournet *et al.* 2001). First instar larvae of both *A. bipustulatua* and *A. bilineata* prefer not to enter *D. radicum* puparia previously parasitized by a conspecific or another *Aleochara* species (Royer *et al.* Fournet *et al.* 1999).

Mustard seed meal applied to a canola cropping system, produced no significant difference in root rating levels and, with the exception of the second experiment in 2004, overall there was no difference in *D. radicum* puparial numbers between treated and untreated plots. In 2004, the *D. radicum* infestation levels were low for all three trials conducted that year. The average root rating for meal treated plots was 1.4, and for untreated plots it was 1.5. Soroka *et al.* (2004) surveyed canola plants from 1994 to 2001, and even when 75% of plants were infested in 2001, the root rating level was < 1.5. In laboratory studies, when the damage exceeded 50% of the root surface of canola (root rating ≥4), there were fewer racemes, lower seed productivity, lower total biomass, and reduced seed yield (McDonald & Sears 1991). However, as *D. radicum* damage levels in this study were below 50% of root surface in both treated and untreated plots, we would not expect a difference between treated and untreated plots. Further studies are necessary to determine if the application of mustard seed meal at higher infestation levels of *D. radicum* would lower the damage caused by *D. radicum* larvae.

Olfactometer studies with mustard seed meal suggest that attraction of *A. bipustulata* to mustard meal plots in the field experiment resulted from a direct attraction of *A. bipustulata* to volatiles of mustard seed meal. *Aleochara bipustulata* moved toward the mustard seed meal volatiles more often than to clean air or sterile soil to meal. The absence of discrimination between dry mustard seed meal and mustard seed meal that has been wet for two days, suggests that the attraction is due directly to the volatiles from mustard seed meal rather than to the breakdown products. *Aleochara bilineata* showed no preference among olfactometer choices, which is consistent with our findings in the field.

The application of mustard seed meal has demonstrated some potential positive benefits. The application did not increase or decrease the number of *D. radicum* infesting the plots. Although there was only one trial, 2004 experiment 3, which showed significantly higher parasitism levels in meal treated plots, the trend was towards higher overall parasitism levels in meal treated plots. *Aleochara* adults from both species consume up to 50 eggs per day (Langlet & Brunel 1996), which could have a significant predatory impact on the egg mortality of *D. radicum.* At the same time *Aleochara* larvae also function as parasitoids on *D. radicum* puparia. This dual action of *A. bipustulata* and *A. bilineata* may have a synergistic or additive effect on the parasitism and mortality of *D. radicum* populations.

There are two challenges to improve the use of mustard seed meal in a canola production system. First, an efficient application method is required and secondly, timing of the application needs to be perfected. Mustard seed meal should be placed at the base of the canola plant to attract *A. bipustulata* where *D. radicum* eggs have been laid. Canola is planted in rows similar to vegetable brassicas but with less space between plants. Equipment such as fertilizer spreaders and harrowers, have been designed for vegetable brassica to place fertilizers and turnover soil to reduce weeds near vegetable brassica seedlings. However, there is no equipment used in between rows of canola after the crop has become established. Modifications to the current equipment would be needed.

Another challenge is to determine when the application of mustard seed meal should occur. *Delia radicum* is attracted to canola during the early bolting stage of plant growth

(Griffiths 1986) and the most effective time to apply mustard seed meal should coincide with the oviposition period of *D. radicum* to ensure that female *A. bipustulata* are present early on to feed on *Delia* eggs and select high quality oviposition sites. Multiple applications of mustard seed meal during the growing season would also increase the effectiveness of *A. bipustulata*. It has been suggested that the effects of mustard seed meal are gone after five weeks (Jonasson 1995) and with current application methods, *A. bipustulata* is attracted to the treated plots only during the oviposition and early larval instar stages of the *D. radicum*. Multiple applications of mustard seed meal during the growing season could extend the attraction *A. bipustulata* into canola for the entire *D. radicum* life cycle increasing predation and parasitism by *A. bipustulata.* Applications of mustard seed meal could be done at the early rosette stage and just before flowering to ensure that *A. bipustulata* is present during all the developmental stages of *D. radicum*. However, it may be impractical for a farmer to make a second application of mustard meal with the current equipment available.

Some of the volatiles detected by GC-MS emitted from mustard seed meal are similar to those emitted from damaged *Brassica* plants, which emit volatiles such as limonene, camphor, nonanal, copanene and caryophyllene (Mattiacci *et al.* 1994;, Geervliet *et al.* 1997; Mattiacci *et al.* 2001; Shiojiri *et al.* 2001). In nature, *A. bipustulata* may use a variety of stimuli to locate hosts. Volatiles produced directly from the damaged *Brassica* plants may provide long-range signals for potential feeding and oviposition sites; such volatiles not only give information that the host's food plant is present, but also that it is under herbivore attack. Our mustard seed meal treatment may be mimicking these signals. After responding to these long-range cues, *A. bipusulata* may arrive at locations where it can detect short range visual or olfactory cues produced directly by its host (Dicke *et al.* 1990; Turlings *et al.* 1991; Vet & Dicke 1992). *Aleochara bilineata* demonstrated no response to mustard seed meal in the laboratory experiments, which was consistent with our field experiments. Neveu *et al.* (2002) demonstrated that when the roots of *Brassica* plants attacked by *D. radicum,* the plants systematically emit volatiles which attract *Trybliographa rapae* Westwood (Hymenoptera: Figitidae), another parasitoid of *D. radicum*.

To effectively release and use a biological control agent, a method to attract and maintain populations of natural enemies of *D. radicum* would greatly enhance its effectiveness. The application of mustard seed meal to canola has attracted higher numbers of *A. bipustulata* as well as increasing levels of parasitisms in treated plots. This method of applying mustard seed meal to canola and manipulating the populations of *A. bipustulata* may be incorporated into an integrated pest management system.

Table 1. Root damage ratings (Dosdall *et al.* 1994) of canola roots that were collected at the end of *D. radicum* larval development in 2004 field experiments.

Experiment	Root rating (Mean ± SEM)		*F* (df = 1,3), *P*
	Mustard seed meal	Control	
1	2.7 ± 0.1	3.0 ± 0.1	2.6, 0.2
2	0.6 ± 0.1	0.8 ± 0.2	1.8, 0.3
3	0.8 ± 0.2	0.7 ± 0.1	0.1, 0.7

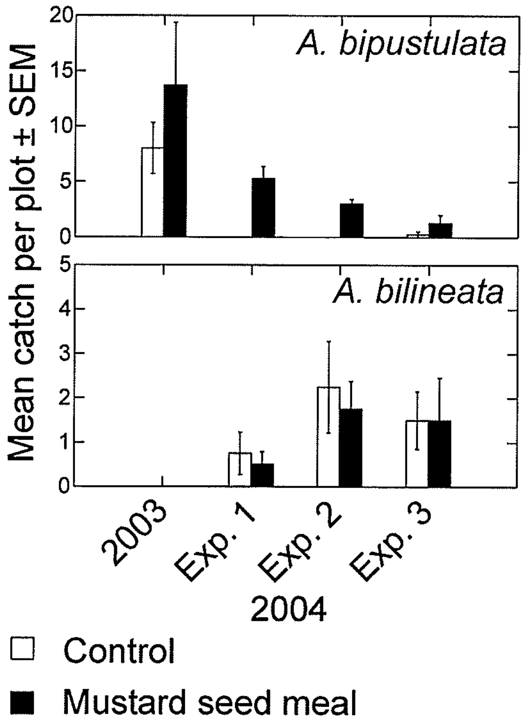

Figure 1: Mean (± SEM) number adult *A. bipustulata* and *A. bilineata* in pitfall trap catches in plots treated with mustard seed meal and in untreated control plots, in the 2003 experiment (second generation of *D. radicum*) and the three experiments (generations 1, 2, and 3 of *D. radicum* respectively) conducted in 2004.

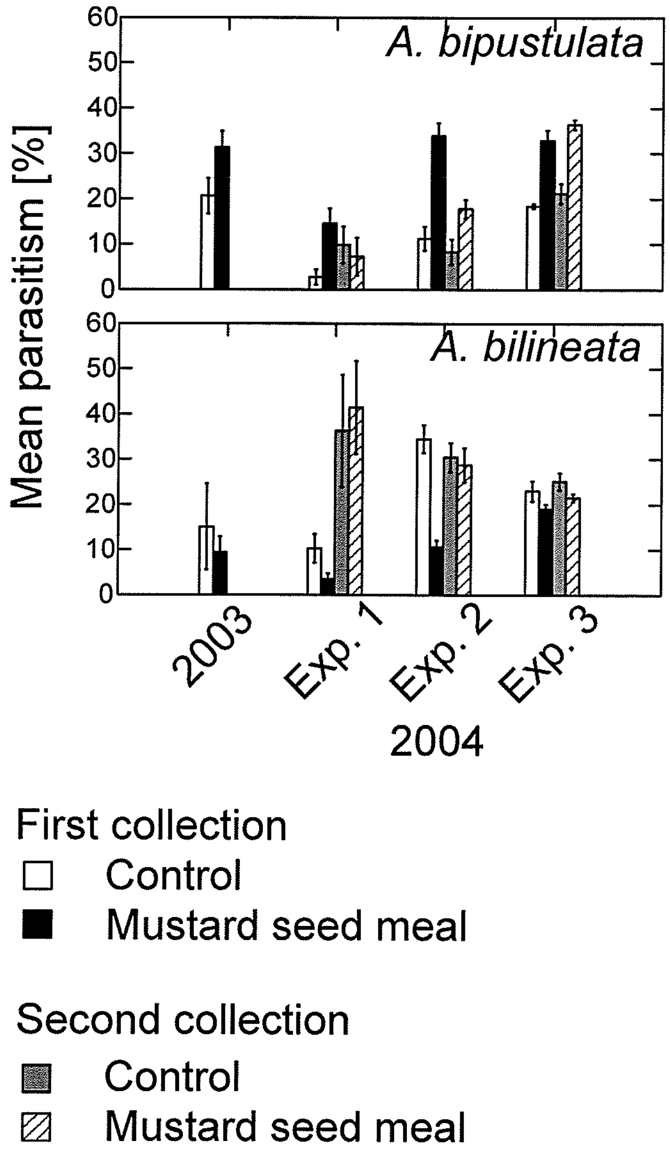

Figure 2: Mean (± SEM) percent parasitism by *A. bipustulata* and *A. bilineata* in samples of >100 *D. radicum* puparia collected from plots treated with mustard seed meal and from untreated control plots. First collections were performed in all four experiments at the time pupariation was first detected; second collections were performed two weeks after the first collections in the 2004 experiments. The 2003 experiment concided with generation 2 of *D. radicum* and the three experiments in 2004 with generations 1, 2, and 3 of *D. radicum* respectively.

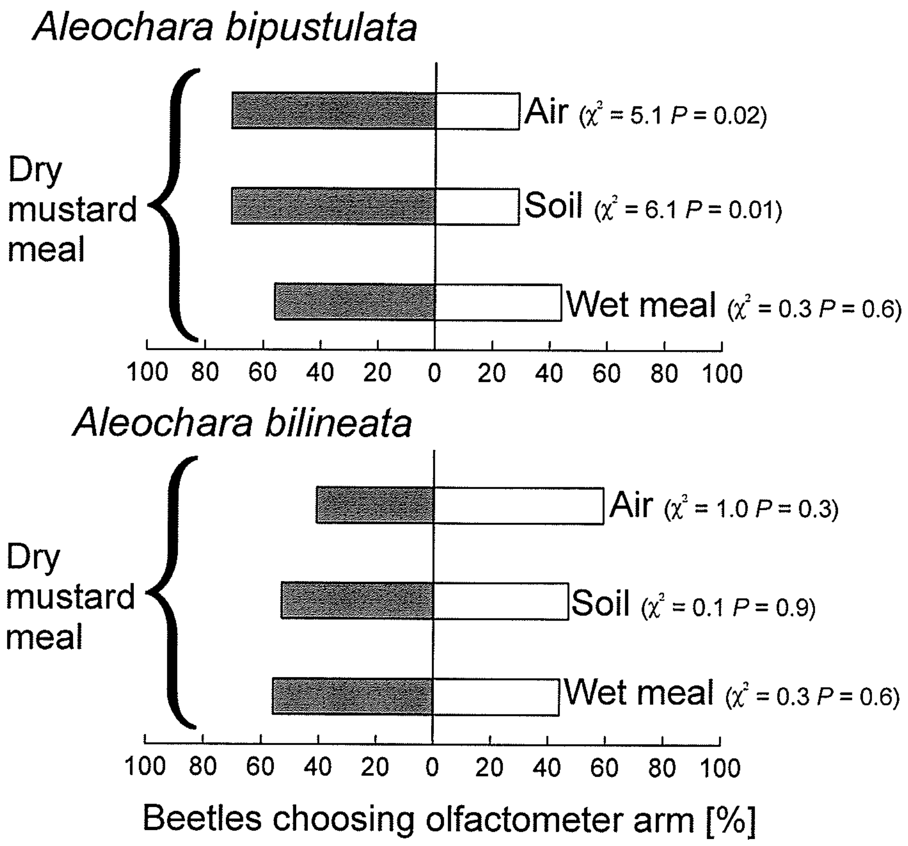

Figure 3: Percentage of responding adult *A. bipustulata* and *A. bilineata* choosing each arm of Y-tube olfactometer in pair-wise tests of dry mustard seed meal against alternatives.

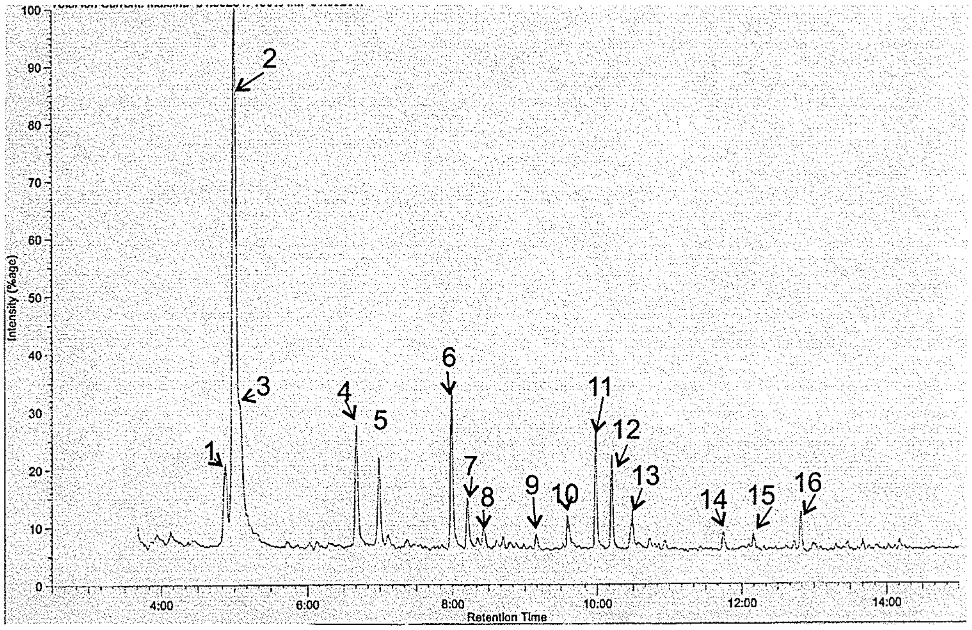

Figure 4: Total ion gas chromatogram from mustard seed meal volatiles. Identities of numbered peaks are: 1. Benzene, 1-methyl-2-(1-methylethyl)-, 2. Limonene, 3. Eucalyptol, 4. Bicyclo[2.2.1]heptan-2-one, 1,3,3-trimethyl-, 5. Terpineol, 6. Camphor, 7. Cyclohexanone, 5-methyl-2-(1-methylethyl)-, 8. Cyclohexanone, 5-methyl-2-(1-methylethyl)-, 9. Dodecane, 10. Benzothiazole, 11. 2-cyclohexen-1-one, 2-methyl-5-(1-methylethenyl)-, 12. ?, 13. Nonanal?, 14. Cyclohexene, 3-methyl-6-(1-methylethylidene)-, 15. Copaene, 16. Caryophyllene.

Chapter 4: Does the addition of *Aleochara bipustulata* to a system with *A. bilineata* increase the parasitism of *Delia radicum?*

Introduction

Delia radicum (L.), the cabbage root maggot, is of European origin (Biron *et al.* 2000), and a pest of vegetable brassicas and canola in Europe (Erichsen & Huenmoerder 2005) and Canada (Turnock *et al.* 1992; Dosdall *et al.* 1994; Soroka *et al.* 2002). Currently there are no registered insecticides to control *D. radicum* in canola in Canada (Manitoba Crop Protection Guide 2008), and cultural control methods have limited effectiveness; therefore, biological control is being considered (Soroka *et al.* 2002; Hemachandra 2004). In Europe, three species of *Aleochara* (*A. bilineata* (Gyll.), *A. bipustulata* (L.) and *A. verna* (Say)) are pupal parasitoids associated with *Delia radicum* (L.) in many brassica crops (Soroka *et al.* 2002). *Aleochara bilineata* and *A. verna* are present in North America but *A. bipustulata* is not (Hemachandra *et al.* 2005) and so may be a potential candidate for biological control against *D. radicum* in Canadian canola (Hemachandra 2004, Hemachandra *et al.* 2007).

Prior to introducing an exotic biological control agent, the biology, host range, and potential indirect effects of the exotic agent must be investigated (Messing *et al.* 2006). Indirect effects may include competition, displacement and secondary interactions of the exotic agent with the members of the natural enemy community already present (Messing *et al.* 2006). Before *A. bipustulata* can be released in the Canadian Prairies, studies are needed, including those to assess its interactions with the existing natural enemy community of *D. radicum*. Of particular concern, is the potential for competition between *A. bipusulata* and *A. bilineata* (Fournet *et al.* 1999). Such competition could prevent the introduction of *A. bipustulata* from increasing the overall mortality of *D. radicum* and, at worst the introduction could decrease total mortality because of interference with existing mortality caused by *A. bilineata*.

Both *A. bipustulata* and *A. bilineata* have a similar life cycle. Females lay their eggs in the soil, near infested plants and near puparia of hosts (Fournet *et al.* 1999). The first instar larva crawls through the soil in search of *D. radicum* puparium. After selecting a suitable host puparium, it gnaws an entrance hole in the cuticle of the puparium, where it enters, feeds and develops within the host (Fuldner 1960). The larva goes through three larval instars and pupates within the host puparium. Only one larva can successfully develop on the host pupa (Fuldner 1960). Selection of oviposition sites by female *Aleochara* may decrease the probability of two or more larvae entering the same host puparium (Fournet *et al.* 2001), and searching first instar larvae also may avoid entering puparia containing conspecific or congeneric larvae (Fournet *et al.* 1999, Royer *et al.* 1999). It has also been suggested that differences in preferred puparial size may result in niche separation between *A. bilineata* and *A. bipustulata* (Ahlstöm-Olsson 1994; Jonasson 1994).

The objectives of this study were to determine how the addition of *A. bipustulata* to a system containing *A. bilineata* will affect the parasitism inflicted by the latter species, *A. bilineata* on puparia of their shared host, *D. radicum*. Field experiments were conducted to examine the overall effect on *D. radicum* parasitism of the intra- and interspecific interactions, and laboratory experiments were conducted to investigate in detail the interactions between adult and larval congeners.

Materials and methods

Insect rearing

A colony of *A. bilineata* was established using adults emerged from *D. radicum* puparia collected in organic rutabagas near Winnipeg, Canada. A colony of *A. bipustulata* was established using adults emerged from parasitized *D. radicum* puparia collected from fields of organic vegetable *Brassica* in the Province of Skåne, southern Sweden. Both *Aleochara* species were reared on *D. radicum* following the methods of Whistlecraft *et al.* (1985a); adults were maintained in cages designed by Herdveldt *et al.* (1984) for egg collection and fed second and third instar *D. radicum* larvae. *Aleochara* eggs were collected weekly and incubated on moist filter paper in Petri dishes. Newly hatched larvae were provided with *D. radicum* puparia in a mixture of moist sand and vermiculite in a Petri dish. *Delia radicum* was cultured according to the methods of Whistlecraft *et al.* (1985b).

Outdoor cage experiments

Four field cage experiments were set up to investigate the overall effect of the interaction of *A. bilineata* and *A. bipustulata* on levels of parasitism of *D. radicum*. These were conducted during the 2005 field season at CABI Bioscience Switzerland Centre Delémont, Switzerland. Two field cage experiments were run simultaneously starting on 11 April 2005 and the other two were started on 16 July. In each experiment, there were

16 cages arranged in a randomized block design allowing four replications of four treatments. Cages were constructed on raised wooden beds which were 60 cm x 60 cm x 30 cm deep. The bottom of each cage was lined with a black mesh to allow for drainage and to retain insects. Cage tops were 60 cm x 60 cm and 137 cm high and consisted of a prefabricated collapsible aluminum frame covered by dark green UV-resistant polyester netting (Bioquip 14150).

The bed was filled with a mixture of pasteurized soil and sand and, on 11 April and 16 July 2005, transplanted with nine rows of summer canola seedlings (6 cm between rows and 6 cm between plants within rows) for a total of 81 plants per cage. Immediately after transplanting, the cage tops were placed over the beds and sealed to the bed with duct tape to prevent natural oviposition by *D. radicum.* Once the canola had reached the rosette stage, each cage was artificially infested with two *D. radicum* eggs per plant (162 eggs per cage) to simulate the oviposition period of *D. radicum*. To ensure *D. radicum* puparia were present for the *Aleochara* larvae, 2 sentinel puparia were buried near the roots of each plant on 13 June and 15 August, 2005. *Aleochara* adults were introduced into the cages immediately after the placement of sentinel puparia. Each field cage received one of four treatments: 1) six female and six male *Aleochara bilineata*, 2) six female and six male *Aleochara bipustulata*, 3) three female and three male *Aleochara bilineata* and three female and three male *Aleochara bipustulata*, and 4) no *Aleochara* (control). The *Aleochara* were left in the cages for two weeks. Then the *D. radicum* puparia and *Aleochara* adults were collected by removing the soil from the wooden bed and separating the insects from the soil by floatation. The number of puparia recovered was recorded, and puparia were then placed individually into vials of moist sand and vermiculite and reared until parasitoid emergence. Total percent parasitism for each *Aleochara* species was arcsine transformed and subjected to analysis of variance using all experiments with blocks nested within experiments. Analysis were performed using Systat (Systat, 2004); an α level 0.05 was used throughout.

Larval no choice experiment

A no choice experiment was performed to assess whether a first instar *Aleochara* larva searching for a host, hereafter called a "responding larva" would enter a previously parasitized *D. radicum* puparium and whether entry was influenced by the species and the age of the *Aleochara* larva inside the host puparium. A single *D. radicum* puparium parasitized by one *Aleochara* species (*A. bilineata* or *A. bipustulata*) in the first, second or third instar was placed into a Petri dish and covered with 2 cm moistened soil. A first instar responding larva of the same or the other *Aleochara* species to that in the puparium was released on the surface of the sand and held in the Petri dish for 72 h. The larval instar inside the *D. radicum* puparium was determined by timing since the puparium was attacked and by microscopic investigation: puparia with first instar larvae inside had been held for 72 h after release of the parasitizing *Aleochara* larva; puparia with second instar larvae had been held for 96 h since releasing of the larva; parasitize puparia that contained a third instar larvae had been held for 178 h old since release of the parasitizing larva.

After the 72 h exposure, the host puparium was examined under a dissecting microscope to determine if the responding larva had entered. Puparia entered by the responding larva were reared individually until a parasitoid or adult fly emerged. Each combination of instar and species inside the puparium was replicated about thirty times for each responding *Aleochara* species. The frequency of entry of the responding larva under different treatment conditions was analyzed using contingency tables.

Larval choice experiment

A choice experiment was performed to determine the frequency with which responding *Aleochara* larvae enter previously parasitized *Delia* puparia, when simultaneously offered an unparasitized puparium. One puparium parasitized either by either first, second or third instar *A. bilineata* or *A. bipustulata* larva, and one unparasitized puparium were placed in a Petri dish and covered with 2 cm moistened sand. A first instar *Aleochara* larva was confined to the Petri dish for a72 h period. At the end of the 72 h, the puparia were recovered, examined and entry into the puparium was recorded. The experiment was replicated approximately 30 times. Exposures in which neither puparium was entered were not included in the analyses. The frequency of the responding larvae entering parasitized puparia was compared for different treatments, and with the frequency of entering unparasitized puparia using χ^2 tests.

Interaction among adults

Two naïve *Aleochara* adults, either of the same or different species, were confined to a test arena consisting of a plastic bowl in the form of a segment of a sphere; the bowl was

11 cm deep and 21 cm in diameter. A piece of rutabaga was placed in the center of the bowl. The interior of the bowl was divided into approximately quadrilateral areas by lines of longitude, radiating from the center of the bowl and 2 cm apart at the rim, and lines of latitude, which were 2 cm apart and parallel to the rim. Two *Aleochara* adults were placed in the test area and observed for thirty minutes, during which encounters and the distance of displacement after each encounter was recorded. Three pair-wise combinations of *Aleochara* adults were tested: a) *Aleochara bipustulata* and *A. bilineata*, b) *A. bilineata* and *A. bilineata*, and c) *A. bipustulata* and *A. bipustulata*. For each combination, twenty tests were conducted. In each test, naive adults were used. For tests with mixed species, the number of encounters and displacement after encounters was compared between species using t-tests. Analysis of variance was used to compare numbers of encounters among types of test; and t-tests were used to compare displacements after encounters in the different types of test.

Results

Outdoor cage experiments

Over the four experiments, the average number of *D. radicum* puparia recovered was 122 ± 3 per cage and the minimum ever recovered from a cage was 73. Puparia from control cages were never parasitized and puparia in single species treatments were never parasitized by the *Aleochara* sp. which had not been introduced. Since the effect of retaining treatments with no parasitism and no variance in the analysis was to underestimate error variance and contravene the assumptions of analysis of variance, these treatments were excluded from the final analyses. Among treatments in which parasitoids were introduced, the effect of treatment on total *Aleochara* parasitism was not significantly different among experiments ($F = 0.5$, df = 6, 24, $P = 0.8$) so it was justifiable to pool all experiments for the final analysis. The effect of treatment on total parasitism was highly significant (Fig. 5) ($F = 14.2$, df = 2,24, $P < 0.001$); most of the variance associated with treatment was attributable to the difference between the treatment with both species, and the single species treatments ($F = 26.2$, df = 1,24, $P < 0.001$), and total parasitism did not differ between the *A. bilineata* and *A. bipustulata* treatments ($F = 2.1$, df = 1,24, $P = 0.16$). Mean parasitism in the treatment with both species was 56 ± 4% and in single species treatments 32 ± 1%.

In the two treatments in which *A. bipustulata* occurred, the effect of treatment on *A. bipustulata* parasitism was almost significant ($F = 3.8$, df = 1,15, $P = 0.07$). *Aleochara bipustulata* parasitism in cages in which six pairs of *A. bipustulata* were introduced was 29 ± 1%; in cages with both species, of which three pairs were *A. bipustulata*, *A. bipustulata* parasitism was 24 ± 2%. The estimated number of emerging *A. bipustulata* in each cage was divided by the number of *A. bipustulata* adults introduced to the cage to provide a measure of *A. bipustulata* increase. The effect of treatment on *A. bipustulata* increase was highly significant ($F = 23.6$, df = 1,15, $P < 0.001$); the increase in the single species treatment was 3.8 ± 0.1 adults/adult and in the treatment with both species was 6.4 ± 0.5 adults/adult.

In the two treatments in which *A. bilineata* occurred, treatment did not significantly affect the level of *A. bilineata* parasitism ($F = 1.7$, df = 1,15, $P = 0.2$). *Aleochara bilineata* parasitism was 36 ± 1% in cages where six pairs of *A. bilineata* were introduced and 32 ± 2% in cages with both species of which three pairs were *A. bilineata*. In the single species treatment, *A. bilineata* increase was 4.8 ± 0.1 adults/adult, whereas in cages with both species increase was 8.7 ± 0.7, a highly significant difference (F = 36.2, df = 1,15, P < 0.001).

Larval no choice experiment

Responding larvae of *A. bipustulata* were more likely to remain in the substrate than to enter parasitized *D. radicum* puparia; over the whole trial, 7% of *A. bipustulata* entered parasitized puparia. This frequency of entry by responding *A. bipustulata* was not significantly affected by the instar of the responding larva (LR χ^2 = 3.2, df = 2, P = 0.2) or by the species of *Aleochara* larva inside the puparium (LR χ^2 = 0.30, df = 1, P = 0.6) (Table 2). Notwithstanding the non-significance of these tests, frequency of entry of responding larvae tended to decline with increasing instar inside and tended to be lower when the larva inside was conspecific.

About 28% of responding *A. bilineata* larvae entered parasitized puparia; thus the probability of entering a parasitized puparium was greater for *A. bilineata* than for *A. bipustulata* (LR χ^2 =31.0, df = 1, P < 0.001). The frequency of entry was affected by the instar of the larva inside (Table 2) (LR χ^2 = 12.4, df = 2, P = 0.02), and was 42, 28 and 14% for instars 1, 2 and 3 respectively. Entry by *A. bilineata* was significantly influenced by the species of the larva inside the puparium (LR χ^2 = 5.0, df = 1, P = 0.02). When the puparium was occupied by a conspecific, only 21% of responding *A. bilineata* larvae entered, whereas when the occupant was an *A. bipustulata* larva, 36% of *A. bilineata* larvae entered.

Larval choice experiment

Responding larvae of *A. bipustulata* were more likely to enter unparasitized puparia than to enter parasitized puparia when offered a choice (LR $\chi^2 = 173.8$, df = 1, $P < 0.001$). In the choice test, 7% of responding *A. bipustulata* larvae that entered a puparium entered the parasitized puparia, which did not differ from the frequency of entry of responding *A. bipustulata* larvae in the absence of choice (LR $\chi^2 = 0.0$, df = 1, $P = 1.0$). Larvae of *A. bilineata* also were more likely to enter unparasitized puparia (LR $\chi^2 = 94.6$, df = 1, $P < 0.001$) when offered a choice. In choice experiments 16% of responding *A. bilineata* larvae entered the parasitized puparium compared to 28% when there was no choice (LR $\chi^2 = 10.5$, df = 1, $P < 0.001$). In the choice tests, entry into parasitized puparia by responding *A. bilineata* larvae was significantly more frequent than for *A. bipustulata* (LR $\chi^2 = 7.1$, df = 1, $P = 0.008$).

In choice tests, frequency of entry by responding *A. bipustulata* larvae into parasitized puparia (Table 3) was influenced by the instar of the parasitoid larva inside the puparium (LR $\chi^2 = 7.8$, df = 2, $P = 0.02$); frequency of entry into parasitized puparia was 13, 8, and 1% when the parasitoid occupant was in instar 1, 2 and 3 respectively. As with the no-choice experiment, the frequency of entry of *A. bipustulata* larvae into the parasitized puparium was not significantly affected by the species it contained (LR $\chi^2 = 2.5$, df = 1, $P = 0.1$); nevertheless, as for the no-choice experiment, the frequency of entry when the parasitized puparium contained a conspecific (4%) tended to be lower than when it contained *A. bilineata* (10%).

For those responding *A. bilineata* larvae entering a puparium, the frequency of entry into the parasitized puparium was significantly affected by the parasitoid instar inside (LR $\chi^2 = 9.2$, df = 2, $P = 0.01$); entry was 20, 23 and 5% into puparia containing instars 1, 2 and 3 respectively. Species of the parasitoid within the puparium also influenced the frequency of entry (LR $\chi^2 = 6.2$, df = 1, $P = 0.013$): 9% of *A. bilineata* larvae entered puparia containing a conspecific, but when the occupant parasitoid was *A. bipustulata*, 23% were entered.

Interaction Among Adults

No aggressive behaviour was observed in adults of either species of *Aleochara*. In trials in which both species were present, about 40% of encounters between adults involved both insects moving towards each other. In the remaining 60% of encounters, the adult of one species advanced while that of the other was stationary. In these encounters, an *A. bipustulata* adult advanced towards a stationary *A. bilineata* more frequently than the alternative (Table 4) (paired $t = 2.85$, df = 19, $P = 0.01$). In trials with both species, the displacement following an encounter (Table 4) did not differ between the species (paired $t = 0.48$, df = 19, $P = 0.6$).

The total number of encounters was affected by the type of trial ($F = 9.7$, df = 2,57, $P < 0.001$); total encounters was significantly lower in mixed species trials than in single species trials ($F = 18.5$, df = 1,57, $P < 0.001$), but did not differ between conspecifics in single species trials. The distance of displacement of an adult *A bipustulata* after an encounter with an *A. bilineata* in a mixed species trial did not differ significantly from displacement after encountering a conspecific in a single species trial ($t = 0.35$, df = 58. $P = 0.7$). Similarly, the distance of displacement of adult *A. bilineata* after an encounter with an *A. bipustulata* in a mixed species trial did not differ from that after an encounter with another *A. bilineata* in a single species trial ($t = 1.55$, df = 58, $P = 0.1$).

Discussion

This study investigated the interactions between *A. bipustulata* and *A. bilineata* to determine if the introduction of *A. bipustulata* would have an impact on the parasitism of *D. radicum* by *A. bilineata* which is already present in the Canadian Prairies. There are four possible outcomes for *Aleochara* parasitism levels from the introduction of *A. bipustulata* to the Canadian Prairies. The first is a synergistic effect where the total parasitism by two agents is greater than the sum of their individual effects. Second, additive effects may occur where total parasitism by the two agents combined is equal to the sum of the individual effects. Third, antagonist effects could occur, in which total parasitism by the two agents are less than the sum of parasitism levels of each operating separately. Finally, if the two together produce total parasitism levels that are is less then when either agent acted on its own this would be an example of reduced antagonist effects.

The majority of the studies focusing on multiple natural enemy interactions found antagonist effects (Ferguson & Stiling 1996; Rossi 2004); however in some studies

(Gutierrez *et al.* 1988; Kindlmann & Ruzicka 1996) introductions of multiple natural enemies have resulted in additive effects, therefore providing more effective control. The field cage experiments indicated that when *A. bipustulata* and *A. bilineata* occurred together in a treatment higher level of parasitism resulted compared to treatments containing only a single *Aleochara* species. As the total number of adult parasitoids was the same in mixed and single species treatments, the results suggest a synergistic effect. Each introduced pair of each *Aleochara* spp. was more successful in inflicting mortality on the host in the combination treatment, than in the corresponding single species treatment. To understand how the synergistic effects are occurring, it is necessary to investigate the interactions between *Aleochara* adults and between *Aleochara* larvae.

Two types of competition could have occurred in the field cage experiments. The first is interspecific competition or intraspecific competition. Interspecific competition occurs when two different species compete with each other (Gordh & Headrick 2001); however, in the field cage experiments the two species of *Aleochara* provide a higher parasitism rate than the individual species alone. If interspecific competition is less intense than intraspecific competition, it might be expected that *A. bipustulata* and *A. bilineata* could coexist together in the Canadian Prairies. Intraspecific competition occurs when two individuals of the same species compete with each other (Gordh & Headrick 2001). In the literature, many cases intraspecific competition occur due to host availability. Larvae of both *Aleochara* species need a host puparium to complete their development (Fuldner 1960). If two or more parasitoid larvae of the same species enter a puparium, superparasitism occurs. If parasitoid larvae of different species enter a puparium, the result is multiparasitism (Gordh & Headrick 2001). In our field cage experiment, host availability was not a limiting factor and therefore intraspecific competition occurred as a result of the two *Aleochara* species avoiding each other. As the adult *Aleochara* were searching for a mate, as well as an oviposition site, avoidance between the two species could occur, resulting in females of one species ovipositing in areas away from the other *Aleochara* species. When the *Aleochara* larvae hatch and search for a host to parasitize, they would be competing with larvae of their species instead of larvae of the other species for a host puparium.

Some parasitoids are capable of discriminating between parasitized and unparasitized hosts (Pijls *et al.* 1995). Most studies on host discrimination and larval competition have focused on interactions between adult females and often where two species are related (Agboka *et al.* 2002; McBrien & Mackauer 1990; Turlings *et al.* 1985; Vet *et al.* 1984). Royer *et al.* (1999), demonstrated that both *A. bipustulata* and *A. bilineata* are capable of intraspecific and interspecific host discrimination. One method of host discrimination is using pheromones and in hymenopteran parasitoids markings may be internal or external (Hofvang 1990). Lizé *et al.* (2007) and Fuldner (1960) suggested that, for *A. bilineata*, the substance plugging the entrance hole is an external marking used for host discrimination. Host discrimination has also been shown in adult *A. bilineata* where females will make decision about oviposition and number of eggs laid by using cues associated with *D. radicum* as well as brassica volatiles produced by the brassica plant (Fournet *et al.* 2001). These methods of host discrimination from both the larvae and adults increase the probability of successfully parasitizing a host puparia.

Fournet *et al.* (1999) and Royer *et al.* (1999) both reported what appear to be the results of the same study, in which *A. bilineata* larvae were more likely to multiparasitize than superparasitize, although levels of multi- and super-parasitism did not differ significantly for larvae of *A. bipustulata.* The no choice and choice tests revealed similar results demonstrating *A. bipustulata* discriminates more thoroughly between parasitized and unparasitized puparium. Royer *et al.* (1999) suggested that because encounters with puparia containing conspecifics usually do not result in superparasitism, high levels of parasitism result in increased searching time, a pseudo-interference effect (Free *et al.* 1977) that would become more pronounced as more puparia are utilized by conspecifics. Reduced searching efficiency from pseudo-interference may diminish ultimate levels of parasitism by an *Aleochara* species more than does superparasitism. If this is so, then the higher probability of multi-parasitism than super-parasitism that we observed would suggest that search efficiency of an *A. bilineata* first instar larvae was depressed less in the presence of congeners than conspecifics.

This phenomenon could contribute to the synergistic effects observed in the field cages. Larvae of *A. bipustulata* would search for an unparasitized puparium for as long as they could survive, rather than risk multiple parasitism where their survival rate is low. Multi- and superparasitism were avoided in the choice test with 93% choosing the unparasitized puparium. Other studies have found similar results showing *A. bipustulata* avoiding multi and superparasitism (Fournet *et al.* 1999; Royer *et al.* 1999). This suggests *A. bipustulata* may have a high search efficiency and is very selective when searching for a host puparium. With a high search efficiency, *A. bipustulata* would continue to search for an unparasitized host puparium and maintain a high parasitism level as long as host numbers are not limiting (Fournet *et al.* 1999).

Our larval no choice and choice results, as well as those from Fournet *et al.* (1999), suggest that *A. bilineata* first instar larvae have a competitive advantage over *A. bipustulata*. With *A. bilineata* having a higher probability of multi- and superparasitism, the time to when the *A. bilineata* decides to parasitize a puparia does not limit the survival of the first larval instar; however, the prospects for successfully developing into an adult in a previously parasitized puparium are not high. A first instar *A. bilineata* larva can reduce its search efficiency because if there are no unparasitized puparia available it will either super- or multiparasitize a puparium. Conversely, the timing of attack on a *D. radicum* puparia is more crucial for *A. bipustulata* because their preference is to avoid multi- or superparasitism of puparia and if they continue to search for an unparasitized puparium their search efficiency is increased and their likelihood of finding an unparasitized puparia is decreased.

Interactions among *Aleochara* adults may also have influenced the outcome of the field cage study. Although *A. bipustulata* was more likely to approach *A. bilineata*, this did not cause one species of *Aleochara* to move further away than the other. Encounters between adults resulted in displacements away from the site of the site of encounter, and the greater number of encounters in tests with conspecifics suggests that more time would be spent in encounters and displacement movement in a single species population than where the same density of adults of two species occurred. How this would affect efficiency of parasitism is not clear from our study. There was no determination whether the *Aleochara* adults were males or females. If interactions between females in search of oviposition sites are similar to those we observed, efficiency of searching for oviposition sites might be higher in mixed species situations than when all females are of the same species.

Aleochara bilineata females are more likely to oviposit in sites with unparasitized *D. radicum* puparia than in sites with puparia parasitized by conspecifics (Fournet *et al.* 2001); such behaviour could result in pseudo-interference depressing search efficiency when high populations of *A. bilineata* occur. It is not known how *A. bipustulata* oviposition site selection is influenced by conspecific parasitism, nor is it known whether oviposition site choice is influenced by parasitism by congeners. If pseudo-interference is stronger when gravid females encounter puparia containing conspecific larvae, it is likely that this behaviour would contribute to the synergistic parasitism observed in the field cages.

This study provides further information required to determine if *A. bipustulata* is a suitable biological control agent for release on the Canadian Prairies. The field cage experiments demonstrated that the combination of *A. bilineata* and *A. bipustulata* provides a syngeristic effect on levels of parasitism. Laboratory experiments suggest that the mechanism involves pseudo-interference, which has a stronger effect in single species populations than when the same density of two species occurs. The field cage studies do not precisely mimic the situation of classical biological control, in which *A. bipustulata* would be added to an existing population of *A. bilineata* presumably with a net increase in total *Aleochara* density. However, they do show that, even if the total density of *Aleochara* were not increased by introduction of *A. bipustulata*, there would probably be elevated levels of *D. radicum* mortality, because the two *Aleochara* species appear able to coexist and to interact synergistically in inflicting mortality on their host.

Table 2: Percentage of responding first instar *Aleochara* larvae that entered a previously parasitized *D. radicum* puparium in a 72 h no choice test in relation to the species and instar of the *Aleochara* parasitoid inside the puparium.

Responding larva	*Aleochara* sp. inside the puparium	Larval instar inside puparium		
		1	2	3
A. bipustulata	*A. bilineata*	10 (31)[a]	9 (34)	6 (35)
A. bipustulata	*A. bipustulata*	10 (30)	9 (35)	0 (35)
A. bilineata	*A. bipustulata*	53 (30)	37 (30)	17 (30)
A. bilineata	*A. bilineata*	31 (35)	20 (35)	12 (34)

[a] Percentage of responding larvae entering: number of responding larvae tested is in parentheses.

Table 3: Percentage of responding first instar *Aleochara* larvae that entered a previously parasitized *D. radicum* puparium in a 72 h a choice test in relation to the species and instar of the *Aleochara* parasitoid inside the puparium. Responding larvae chose between entering a parasitized and an unparatized puparium; those that entered neither were excluded from the results.

Responding larvae	*Aleochara* sp. inside the puparium	Larval instar inside puparium		
		1	2	3
A. bipustulata	*A. bilineata*	19 (32)[a]	13 (30)	0 (40)
A. bipustulata	*A. bipustulata*	6 (31)	3 (30)	3 (35)
A. bilineata	*A. bipustulata*	21 (24)	41 (29)	7 (29)
A. bilineata	*A. bilineata*	19 (31)	6 (33)	3 (31)

[a] Percentage of responding larvae that entered a previously parasitized puparium: number of tests where one of the puparia was entered is in parentheses.

Table 4: The average number of encounters and average displacement after an encounter when two adult *Aleochara* interacted in a laboratory arena for 30 minutes.

Responding species	Experiment type	Number of encounters		Displacement after encounter (cm)
		Total	Where responding species advanced	
A. bipustulata	Mixed species	6.5 ± 0.7	2.4 ± 0.3	1.3 ± 0.3
	Single species	10.6 ± 0.7	–	1.4 ± 0.2
A bilineata	Mixed species	6.5 ± 0.7	1.5 ± 0.3	1.2 ± 0.3
	Single species	9.6 ± 0.7	–	1.7 ± 0.2

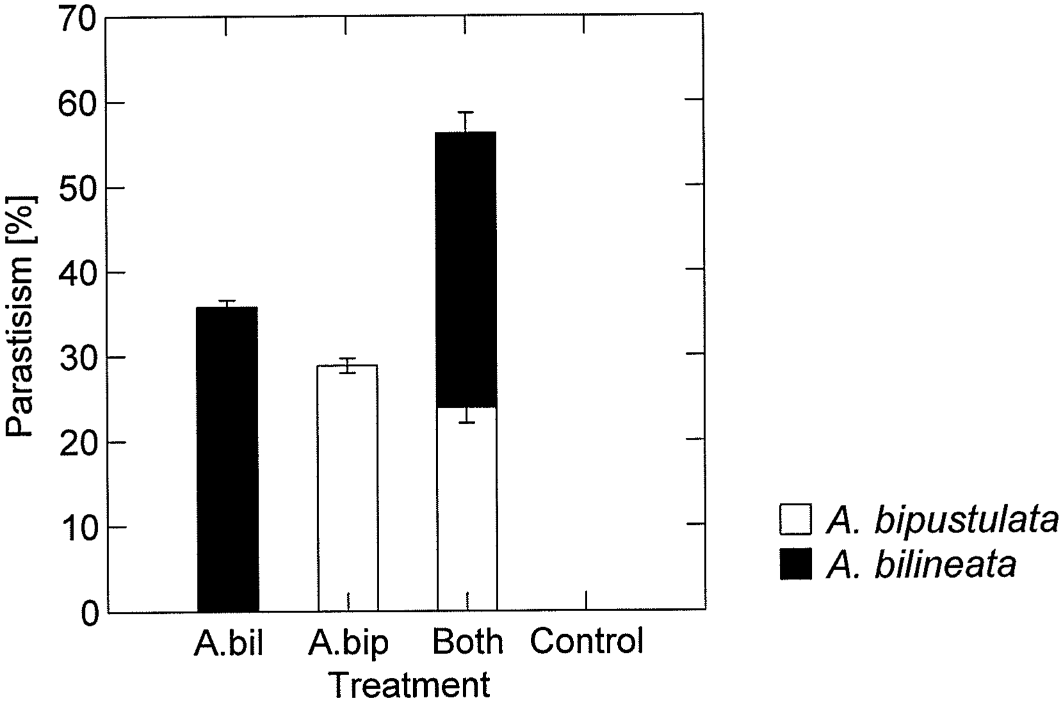

Figure 5: Mean parasitism levels by *A. bilineata* and *A. bipustulata* of *D. radicum* puparium when 6 male and 6 female parasitoids were confined with 181 sentinel puparia of *D. radicum* for two weeks. Treatments were: "A. bil": 6 male and 6 female *A. bilineata*; "A. bip": 6 male and 6 female *A. bipustulata*; "Both": 3 male and 3 female *A. bilineata* and 3 male and 3 female *A. bipustulata*; "Control": no *Aleochara.*

Chapter 5: General discussion

Initiating a classical biological control program for *D. radicum* in summer canola involved an in-depth evaluation of the parasitoid community in summer canola in Canada and brassica vegetable as well as summer and winter canola in Western Europe. Surveys conducted in Canada by Hemachandra *et al.* (2007) revealed *T. rapae* and *A. bilineata* are found in the highest numbers in summer canola. Other parasitoids, such as *A. verna* and a *Phygadeuon* species, were identified but occurred in smaller numbers (Hemachandra *et al.* 2007). Numerous studies have confirmed that in Europe, *T. rapae*, *A. bilineata* and *A. bipustulata* are the major parasitoids present in both summer canola and vegetable brassicas (Wadsworth 1915; Wishart *et al.* 1957; Bromand 1980; Finch & Skinner 1980, Finch & Collier 1984; Hemachandra *et al.* 2007; Jonasson 1994, 1995; Jonasson *et al.* 1995; Brunel & Fournet 1996). Although in previous studies *A. bipustulata* has been reported to be found in North America, it was later determined by Hemachandra *et al.* (2005) that these specimens were misidentified. Thus, as *A. bipustulata* is the only major parasitoid not present in North America, it was selected as a candidate for biological control in summer canola (Hemachandra *et al.* 2007; Andreassen *et al.* 2007).

Very little is known about the interactions between the three common parasitoids found to attack *D. radicum*. Two studies confirmed that larvae of *A. bilineata* avoid *D. radicum* puparia previously parasitized by *T. rapae* (Bromand 1980; Reader & Jones 1990). *Trybliographa rapae* parasitizes the larval stages of *D. radicum* where it is an endoparasitoid. When the *D. radicum* larva undergoes pupation, *T. rapae* emerges from the pupa and becomes an ectoparasitoid inside the puparial case, and at this stage *A.*

bilineata larvae searching for a host are able to discriminate between a puparium containing a *T. rapae* and a unaparasitized puparium (Reader & Jones 1990). When *A. bilineata* multi-parasitizes a puparium containing an endoparasitic *T. rapae*, both parasitoids may die, but if they do not, it is usually the *A. bilineata* that survives. If multiparasitism occurs after the *T. rapae* has reached the ectoparasitic stage, the *T. rapae* is more likely to survive than the *A. bilineata* (Reader & Jones 1990). Hemachandra *et al.* (2007) determined that in Europe, larvae of *A. bilineata* are synchronized with the timing of *D. radicum* pupation, resulting in *T. rapae* entering its ectoparasitic stage before *A. bilineata* larvae are ready to parasitise *D. radicum* puparia. However in Canada, *A. bilineata* is not as synchronized with *D. radicum*, resulting in larvae of *A. bilineata* potentially parasitizing puparia while *T. rapae* is in the endoparasitic stage. There have been no studies of the interactions of *A. bipustulata* and *T. rapae*, or of the three species interaction of *A. bilineata*, *A. bipustulata* and *T. rapae* together.

Our laboratory studies of the inter- and intraspecific interactions of *A. bilineata* and *A. bipustulata* (both no-choice and choice) demonstrated that *A. bilineata* was quite likely to enter a parasitized puparium already containing a first instar *A. bilineata* larva but *A. bipustulata* was more likely to search for another host, thereby risking death. Thus, *A. bipustulata* appeared to be the less aggressive of the parasitoids.

Interspecific competition (both choice and no-choice) experiments suggested that *A. bipustulata* has a higher search efficacy that *A. bipustulata*, and when confronted with a parasitized puparium, was more likely to seek a different host than was *A. bilineata,* which was quite likely to enter the parasitized puparium regardless of whether the occupant was an *A. bipustulata* or conspecific. Although our laboratory experiments suggested certain behavioural characteristics between and within each *Aleochara* species, we still needed to conduct field experiments to gain insight on how they would coexist together if they were released in the Canadian Prairies.

The Lotka-Volterra competition model is a useful model to begin to understand the coexistence of *A. bipustulata* and *A. bilineata*. This model is based on two equations of population growth for two competing species to predict the outcome of competition over time. If an individual of one species has a negative effect on population growth of the other species which is equal to the negative effect of a conspecific individual, then the alpha in the equation equals 1. When alpha is higher than 1, the negative effect on population growth of a species is greater for an individual competitor of the other species than for a conspecific individual. If the negative effect on population growth on a species is less for an individual of the other species than it is for a conspecific individual, alpha is less than one (Irwin 2008). The results of the field cage experiment showed that adult parasitoid production per pair of parasitoids introduced was greater for both *Aleochara* species in the treatment with three pairs of each species, than when there were of six pairs of one *Aleochara* species. Therefore, population growth rates were increased for both species when interspecific competition replaced some of the intraspecific competition, and so for both *Aleochara* species alpha was less than 1.

There are three potential outcomes predicted by the Lotka-Volterra competition model. In one type of outcome, one species out-competes the other species and the result is predictable; regardless of starting densities, only one species will survive and the identity of that species is determined by the carrying capacities, and values of the competition coefficients. With these results one species is regulated by intraspecific competition, while the population of the other is destroyed by interspecific competition. A second type of outcome also involves the population of one species persisting and the other not; however, the identity of the surviving species is not fixed, but depends on the starting densities. In this situation interspecific competition is stronger than is intraspecific competition resulting in one species excluding the other. In a third type of outcome coexistence occurs and neither species is excluded. Coexistence is predicted to occur when, for each species, intraspecific competition inhibits population growth more than does interspecific competition, that is, the alpha for each species is less than 1, the situation that occurred in the field cages.

Our interaction cage experiment suggests coexistence of the two *Aleochara* species was occurring when six pairs of *A. bipustulata* and six pairs of *A. bilineata* are released. Our arena adult interaction studies suggest that *A. bilineata* and *A. bipustulata* adults have less encounters than *Aleochara* adults of the same species, both species of *Aleochara* were avoiding each when selecting oviposition sites in our cage experiments. As a result of this avoidance and potentially each species of *Aleochara* ovipositing in their own section of canola this would result in one species being at higher density in one area of the cage and the other species higher in the other area of the cage, resulting in intraspecific competition occurring during the search for *D. radicum* hosts. Hassell & May (1986) suggest that in order for coexistence to occur between two biological control agents, one agent must be a generalist and be more efficient at searching for hosts than the competing agent, which is a specialist. These conditions have been shown with *T. rapae* being the specialist agent with a lower search efficiency than the generalist *A. bilineata* (Jones & Hassell 1988). Although our results suggest that coexistence occurred in our cage experiments, one must further investigate the interactions between *T. rapae* and *A. bipustulata* as well as the interactions between all three parasitoids (*T. rapae*, *A. bipustulata* and *A. bilineata*) to determine what may cause the intraspecific competition and if it would continue when three parasitoids occur together.

Although our field cage studies suggest the two *Aleochara* species can coexist together our mustard seed meal field results provide us with some different insight. Our mustard seed meal experiments suggested that there was an increase in the population of *A. bipustulata* with application of mustard seed meal and a decreased number of *A. bilineata* present in our plot. These results are inconsistent with the results from our field cage experiment which showed when both species of *Aleochara* were present there was an increase in parasitism of *D. radicum* puparia.

One possible explanation for these results is both species of *Aleochara* are avoiding each other. For instance, in the adult arena study, when one *A. bipustulata* adult and one *A. bilineata* adult were released, the rate of the two species encountering each other was lower than when two *Aleochara* of the same species encounter each other. Also *A.*

bipustulata was more likely to approach *A. bilineata* than vice versa. With *A. bilineata* willing to avoid *A. bipustulata* more, this could have resulted in the mustard seed meal experiment where *A. bilineata* avoided plants with higher numbers of *A. bipustulata* creating a dominance of *A. bipustulata.* This could not occur in the cage experiments as insects could not leave the cage.

There are many factors that can influence intraspecific competition such as host density, and parasitoid density. Lane and Mills (2003) found an increase in host density reduced the developmental and growth rate of the host, which resulted in a higher pupal mortality rate as well as lower fecundity in adults of the next generation in *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) (Mediterranean flour moth). The study also found that at low host densities there was no effect on population growth rates of *E. kuehniella.* Similar host density effects might occur in *D. radicum* populations and may have impacts on *A. bipustulata* and other parasitoids in the community. Royer *et al.* (1999) demonstrated that parasitism of a conspecific increases as host density decreases; however, no work has been conducted on the effects of *D. radicum* densities on parasitoid intraspecific competition. Mukerji (1971) observed the population dynamics of *D. radicum* in Canada and determined that the only density dependent mortality occurred in the pupal stage and was due to parasitism by *A. bilineata.* Bonsall *et al.* (2004) further investigated the interaction or coexistence of *D. radicum*, *T. rapae* and *A. bilineata.* Their study showed that the density of *T. rapae* was independent of the density of *D. radicum* and suggested that *A. bilineata* was a generalist predator because it had no dependence on host density. As well, there was no relationship between parasitoid efficiency and host density. Understanding the effect of host density on the population of *A. bipustulata* and *A. bilineata* and exploring the coexistence of *D. radicum*, *A. bilineata*, *A. bipustulata* and *T. rapae* will provide valuable information about what would happen when a parasitoid is added at high, medium and low host densities.

Although female parasitoids are capable of discriminating between parasitized and unparasitized hosts, under laboratory conditions or when host availability is low, parasitizing a previously parasitized host may be adaptive if the probability of finding an unparasitized host is low and the probability of survival to adulthood of an egg laid in a parasitized host is greater than zero. This scenario may have occurred in our field cage experiments and to fully understand the effect of each *Aleochara* species on the populations of *D. radicum* further studies are needed to determine the minimum parasitoid density at which intraspecific competition occurs, and the effects of intraspecific competition on characteristics of the parasitoid's biology such as fecundity and development time.

Once an effective parasitoid density is determined a method to enhance and maintain the parasitism and population of *A. bipustulata* and *A. bilineata* would be beneficial to the release of *A. bipustulata* in the Canadian Prairies. Our field experiments as well as laboratory experiments confirmed the observations of Ahlström-Olsson & Jonasson (1992) that *A. bipustulata* is found in higher numbers with mustard seed meal than without mustard seed meal. Ferry *et al.* (2007) isolated the volatile dimethyl disulfide (DMDS) from winter canola infested with *D. radicum*, which attracted both *A.*

bipustulata and *A. bilineata* in field and laboratory experiments. DMDS potentially could be a substitute for mustard seed meal. If this chemical compound could be applied easily in an established summer canola field, it could provide a similar effect to the mustard seed meal and could enhance the introduction of *A. bipustulata* into the Canadian Prairies. Although DMDS could be a good alternative, the compound is hazardous to handle. Both species of *Aleochara* had a strong attraction to the *D. radicum* infested roots (Ferry *et al.* 2007), therefore DMDS may not be the best alternative.

Although our study has provided some insight on the use of mustard seed meal in summer canola, as well as the interactions between *A. bipustulata* and *A. bilineata*, there are many follow up studies needed before considering the release of *A. bipustulata* into the Canadian Prairies. Very little is known about the interactions that occur between *A. bipustulata* and *T. rapae* and between *A. bipustulata*, *A. bilineata* and *T. rapae*. Field and laboratory experiments similar to those reported here will provide valuable information about these interactions. Much more work is also needed on target and non-target host range testing both as a parasitoid and a predator as well as its suitability to survive in the Canadian Prairies before any decision can be made about the release of *A. bipustulata* is selected as a biological control agent.

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