Investigation of *Aleochara bipustulata* (Coleoptera: Staphylinidae) adult diet and community interactions

by

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Abstract

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The exotic cabbage maggot (CM) infests canola on the prairies, feeding on roots in its larval stage, which disrupts the uptake of nutrients and water and provides an entry point for fungal plant pathogens. The European staphylinid, *Aleochara bipustulata* L., may be introduced for control of CM, but only if the risk to other species is low and if *A. bipustulata* has demonstrable potential to increase mortality already caused by natural enemies in Canada. *Aleochara bipustulata* could contribute to pest management as a predator of CM eggs and larvae, and as a parasitoid of CM puparia; however, it could affect non-pest species in the same two ways. A variety of invertebrates that share the soil of *Brassica* fields with immature CM were screened in laboratory no-choice assays to determine what adult *A. bipustulata* eat. In these assays, immobile or barely mobile invertebrates were accepted regularly and could be at risk. The majority of groups were seldom or never consumed. Also, a molecular assay developed to test for CM DNA in the guts of field-collected *A. bipustulata* revealed its high potential as a predator, and a similar assay developed for two carabid beetle species showed these to be seldom if ever consumed. Laboratory and field cage assays with other CM egg predators showed *A. bipustulata* has potential to disrupt other species, particularly the closely related *A. bilineata* Gyllenhal, as they seem to forage in similar microhabitats. Measurements of field-collected beetles indicate CM is unlikely to be the primary host in Europe, so introducing *A. bipustulata* to Canada may bring risks to non-target Diptera species. This was observed even though a series of laboratory experiments demonstrated CM is a superior and preferred host relative to the smaller, acalyptrate cheese skipper.

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Chapter 1

Introduction

This thesis is concerned with research for continuation of a classical biological control effort directed at managing the cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae) in Canada. this exotic pest had its range expanded accidentally in the 19th century when individuals from north western Europe were introduced, doubtless as pupae in ballast soil (Biron et al. 2000). In classical biological control, exotic pests of this sort are reunited with at least some of the coevolved natural enemy species that exist in the pests' area of origin (Van Driesche et al. 2008). A previous attempt with the same objective undertaken 60 years ago was stopped after it seemed the species being brought from Europe already were present in Canada (Andreassen et al. 2007; Soroka et al. 2002). Interest was renewed after *D. radicum* and related species were noted to be extremely prevalent and injurious to canola crops on the prairies, there being no chemical control options available in canola at this time (Andreassen et al. 2007; Soroka et al. 2004). A comparative assessment of the parasitoid communities on the prairies and in Europe was done, and the results indicated *Aleochara bipustulata* L. (Coleoptera: Staphylinidae) to be the only parasitoid commonly associated with *D. radicum* in Europe that was not already present in Canada (Hemachandra et al. 2005; Hemachandra et al. 2007a), and so this species has been selected for further evaluation.

Adult *A. bipustulata* are predators of *D. radicum* eggs and larvae, and *A. bipustulata* are parasitoids of *D. radicum* puparia (Fuldner 1960). This species can contribute to *D. radicum* mortality and to reductions in population size in two ways. However, there is always a risk that species other than the pest will be negatively affected,

whenever natural enemies are introduced to new regions (Barratt et al. 2010; Howarth 1983; Howarth 1991). It is worthwhile, therefore, in addition to host specificity testing to determine which non-target species are most likely to be a risk (van Lenteren et al. 2006b), to confirm natural enemies have potential before they are introduced (McClay and Balciunas 2005). In the case of *A. bipustulata*, while it may contribute to *D. radicum* control as both a predator and as a parasitoid, it could affect non-target species in both ways as well.

As predators, apart from their association with the eggs and larvae of their Diptera hosts, *Aleochara* species including *A. bipustulata* remain poorly understood. It is not known what else they eat, although records for the family as a whole (Good and Giller 1991) indicate the diet potentially is quite broad. It is not even certain *D. radicum* eggs are normally consumed, since adults seem not well able to locate eggs when they are slightly buried in soil, as the majority of eggs are (Finch et al. 1999). In addition, some coleopteran predators of *D. radicum* are known also to interfere with one another and reduce per capita rates of consumption (Prasad and Snyder 2004); it would not do to disrupt the beneficial activities of beetles already in Canada, especially if overall levels of egg mortality drop as a result of introduction. The range of prey consumed by adults requires investigation, as does their impact as predators, alone and when foraging in the presence of other beetle species that share the resource and exploit it in a similar way.

Attention already has been devoted to *A. bipustulata*'s range as a parasitoid, and the risks to non-target species of parasitism. The only calyptrate Diptera family tested representative species of which consistently supported complete development in the laboratory was the Anthomyiidae, to which *D. radicum* belongs (Andreassen et al. 2009).

Several acalyptrate species consistently did support development; however, adult *A. bipustulata* from these hosts were relatively quite small, and it was suggested these might not be attacked in nature if the consequences of being so small are substantial (Andreassen et al. 2009). These consequences require investigation, as does the size of adults collected in nature, for more thorough assessment of risk to non-target Diptera species.

Finally, it is of interest to investigate *A. bipustulata*'s potential as a parasitoid complement to *A. bilineata* (Gyllenhal), another parasitoid of *D. radicum* with very similar habits (Fuldner 1960), but that already is in Canada. In theory, two parasitoid species that share a host are expected to reduce host density more than one species would on its own if the parasitoid niches differ such that different portions of the host resource are attacked in preference by each species (Pedersen and Mills 2004). Puparial mass, within a species such as *D. radicum*, is one dimension along which the resource is believed to be partitioned (Jonasson 1994). Host species use is another, and may include *A. bilineata*'s avoidance of acalyptrate species, and *A. bipustulata*'s avoidance of larger species of Anthomyiidae. The outcome will be influenced by the tendency of larvae of both species to avoid puparia already containing another larva; this tendency does break down as larvae age (Royer et al. 1999), however, and so more hosts may attacked by multiple larvae when fewer puparia are available to each larva.

This thesis contains four chapters. The first chapter is the general introduction. The second chapter is a review of the literature. The third chapter describes the research, and has four sections. The first section covers two objectives: to investigate the range of prey consumed by *A. bipustulata* adults, especially in the laboratory, and to confirm

immature *D. radicum* are consumed by adult *A. bipustulata* in nature. The second section has but one objective, to determine if the rate of *D. radicum* egg predation when these are exposed to multiple predator species is predictable based on the consumption rates of individual species, or if the species interfere. The third section has again two objectives: to compare *A. bipustulata* and *A. bilineata* in their ability to parasitize three host species, including a range of sizes of each host; and, to determine if the host puparium resource is partitioned by the two species with respect to host mass or to host depth in soil. The fourth section had four objectives: to determine some of the consequences of host size and host species selection on rates of growth and development; to bring these together in a matrix model to determine their effects on rate of population increase; to study the preferences of *A. bipustulata* larvae between two host species; and, to compare the size of field-collected *A. bipustulata* to these expectations.

Chapter 2

Literature Review

If biological control is the study and manipulation of beneficial organisms for regulation of pest population densities (DeBach 1964b), then its notion arose shortly after the domestication of plants and animals for food. Around 10,000 years ago, the people of Mesopotamia started to harbour cats, *Felis sylvestris* (L.), for management of the rodent pests of their grain (Driscoll et al. 2007), and the practice spread with adoption of agriculture (Faure and Kitchener 2009). In Egypt, male cats came to symbolize the sun god Amon-Re, female cats to symbolize the fertility goddess, Bast, and cats were so sacred that armies sometimes were dispatched to enforce prohibition on their export (Baldwin 1975). Today we are less fanatical, but the biological control principle has endured and spread across the world.

In Canada, biological control of insect pests began when the first Director of the Dominion Experimental Farms, William Saunders, released *Trichogramma minutum* Riley (Hymenoptera: Trichogrammatidae) from New York State into Ontario in 1882 (Glen 1956). W. Robin Thompson helped to establish network of centres, now called CABI (Blight 2011), and this network is the primary source of insects introduced to Canada today.

Classical biological control of insect pests in the three Canadian prairie provinces has had a poor start, in which most species introduced have failed to establish, and in which few of the established species contribute to reductions in pest population sizes. The first such introduction was in 1930, of *Collyria coxator* Villers (Hymenoptera: Ichneumonidae) [as *C. calcitrator* (Gravenhorst)], for management of the western wheat-

stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae) (Smith 1931). The parasitoid established temporarily in some locations, but not permanently, although half a million wasps were released over a decade (Turnbull and Chant 1961). In fact, only one species of 19 intentionally introduced to the prairies for biological control of insect pests has established (De Clerck-Floate and Carcamo 2011). This is *Platygaster tuberosula* Kieffer (Hymenoptera: Platygasteridae), a parasitoid of the orange wheat blossom midge, *Sitodiplosis mosellana* (Géhin) (Diptera: Cecidomyiidae) (Olfert et al. 2003). There are additional exotic natural enemy species that contribute to pest management on the prairies after having arrived by accident or by dispersal from regions in which they had been intentionally introduced. Another parasitoid of *S. mosellana*, *Macroglenes penetrans* Kirby (Hymenoptera: Pteromalidae), and *Tetrastichus julis* Walker (Hymenoptera: Eulophidae), a parasitoid of *Oulema melanopus* L. (Coleoptera: Chrysomelidae) are included in this category (De Clerck-Floate and Carcamo 2011). Exotic insect natural enemies are like house cats in the imperfect manner in which pest control is achieved (Medina et al. 2011). Some species, such as *Coccinella septempunctata* L. (Coleoptera: Coccinellidae), have negatively impacted non-target native species (Turnock et al. 2003). Most frequently these negative impacts are direct, stemming from consumption of non-target species by the biological control agent (Howarth 1983; Louda et al. 2003a; Simberloff and Stiling 1996). The *C. septempunctata* situation is probably one of primarily indirect non-target effects, though, in which native lady beetles, particularly those of a size similar to that of *C. septempunctata*, are thought to have been displaced competitively (Turnock et al. 2003).

This chapter is a review of literature relevant to classical biological control of the cabbage maggot in Canadian prairie canola, in three sections. The first is about classical biological control, especially in Canada. It covers the selection of biological control agents and their evaluation in terms of potential for non-target effects and in terms of ensuring only effective agents are released, particularly when other natural enemy species already are present in the area intended for introduction. In the second section, I describe the cabbage maggot and related species in terms of their life cycles and impacts on the prairies, existing management options, and resident natural enemies. The final section covers the biological control agent, what is known about its foraging behaviour, its impact on the pest, and its interactions with other species of natural enemies. This sets the stage for an outline of the research objectives.

Classical biological control

In any classical biological control programme, five phases may be distinguished. In the first phase, the pest is identified as a promising target. Foreign exploration to identify natural enemy species for further study is the second phase. The third phase involves evaluation in quarantine or in the area of origin of those species. The fourth phase consists of seeking the approval of regulatory bodies for introducing the species. The final phase is field colonization of the species in the new area, complete ideally with follow-up research about the natural enemy's establishment and its impact, on the pest and on non-target species. The phases are described below in sequence in greater detail.

If the best use is to be made of available resources, potential target pest species should be evaluated beforehand to ensure a prospect exists for their biological control. If many pests have potential, it is worthwhile to rank them and prioritize some over others

(Barbosa and Segarra-Carmona 1993). It seemed early in the 20th century as though biological control success was much more likely on islands than on large continents, and in any case, that oligophagous natural enemies in the introduced area would adapt to exotic herbivores and control them as well as any introduced species could; however, the observed pest population sizes following release from coevolved natural enemies, as well as the recognition that some natural enemies cause indispensible mortality, or mortality which would not otherwise occur, changed this view in favour of introductions to continents (Sweetman 1936; Thompson 1928). Political pressure, in the form of requests from stakeholders for more complete, or less chemically reliant, control of particular species frequently has a role in which species garner the most attention (Charudattan 2005). It is recommended these political concerns be distinguished from biological and economic criteria in prioritization based on overall ranking, to ensure resources are not exhausted on pressing but biologically intractable problems (Barbosa and Segarra-Carmona 1993).

That DeBach's (1964a) chapter on the principles of foreign exploration follows directly after a chapter on systematics is quite natural, for sound identification is critical to link with previously published information and to select promising regions for natural enemies. In these areas, the associations of the target pest and natural enemy species are examined based on field collections (DeBach 1964a), and in some cases natural enemies of species related to the target species are examined in addition, or instead (Bellows and Legner 1993). Systematics is again critical, to determine the composition of species in the natural enemy complex accurately (Clarke and Walter 1995; Stouthammer 2006). This has been called "inventory research", as it results in a list of species along with relevant

ecological details concerning each species (Zwölfer et al. 1973). Matching climates with the area of intended introduction is a guiding concept in selection of regions to sample (DeBach 1964a), as are considerations of the pest species' centre of evolutionary origin, where the evolutionary history is longest and therefore may contain the most specific and well adapted natural enemies (Gonzalez and Gilstrap 1992). As an alternative to the centre of evolutionary origin, the region within the entire pest's range from which the exotic population originated may contain natural enemies best adapted to those that were introduced (Hoddle 2005). If the history of introduction is not known, identifying the region of origin requires population genetics techniques (Biron et al. 2000; Lozier et al. 2009). These regions matched for climate, evolutionary origin, or origin of introduced genotypes, may become the foci of search, but it is recommended to sample as widely in space and time as possible (Bellows and Legner 1993; DeBach 1964a; Legner and Bellows 1999). The impact of particular natural enemies is usually compared to that due to other sources of mortality as part of the survey (Zwölfer et al. 1973). One or a few species are then selected for further study based on this analysis.

Host specificity testing has long been considered important for biological control (DeBach 1964c; Sweetman 1936; Zwölfer et al. 1973) in that host-specific agents were considered more likely to be successful. Now it is more critical that agents be demonstrably specific, in that the agents will almost certainly be rejected at the regulatory approval stage otherwise (Barratt et al. 2010). Understanding the risk of non-target effects requires testing the agent's response when exposed to the target species and to a carefully selected list of non-target species (McCoy and Frank 2010). Two similar schemes have been proposed. One (Murray et al. 2010; Withers and Browne 2004;

Withers and Mansfield 2005) considers no-choice and choice tests to be indispensible, and seems to be the favoured approach of researchers working in New Zealand and the U.S.A. The other (van Lenteren et al. 2006a; van Lenteren et al. 2006b) employs no-choice tests on a full list of non-target species, and choice tests only on those species attacked in the no-choice tests. This approach is favoured by researchers in Europe and Canada. Both approaches require a list of non-target species be developed for use in the tests.

A procedure to develop this list has been proposed (Kuhlmann et al. 2006). The first step in the procedure is to place species on an initial test list, based on ecological similarity or taxonomic affinity to the target pest, or safeguard considerations where species are included because they are beneficial or rare. Two filters are then applied to the initial list to reduce the number of species. The first filter is to remove species with attributes that do not overlap the target species. The second filter is a practical one, in which those species that are expected to be difficult to find or to produce in culture are removed. Species in more than one category are prioritized. The result is a revised test list, expected in most cases to include some 10–20 species. Species can be added and removed from the revised list as more information becomes available.

In the Euro-Canadian framework of host-range testing (van Lenteren et al. 2006a; van Lenteren et al. 2006b), all species on the revised test list are exposed to the biological control agent in no-choice tests. Positive control tests with the target species are run simultaneously to ensure individuals of the agent are healthy and can forage in the testing environment. Negative controls, of both the target species and non-target species, are included as well. Non-target species attacked in the no-choice tests are next tested in

laboratory choice tests. Non-target species attacked in the choice test can be offered in field choice tests. Species that were not attacked in any of these three testing levels are considered safe. Interpretation of the results generated by following this framework is straightforward whenever a monophagous or polyphagous agent is studied, but is more complicated for species with oligophagous tendencies. This unfortunately is commonly the case, and so additional sources of information frequently are required.

Extensive field surveying of non-target species and their natural enemies is one way to obtain additional information for interpreting data from host range testing. For example, field surveys have been instrumental for assessing the ecological host range of *Peristenus digoneutis* Loan (Hymnenoptera: Miridae), introduced recently to North America for *Lygus* spp. (Heteroptera: Miridae) management. In the laboratory portion of the evaluation, ten species of mirid were compared in both no-choice and choice tests, and all were attacked, but in most cases less frequently than the *Lygus rugulipennis* Poppius controls (Haye et al. 2005). In the field portion, samples of nymphs of 30 mirid species collected in nature were reared until parasitoids emerged; *P. digoneutis* was found in nymphs of nine of these species, but since among the non-target species the highest level of *P. digoneutis* attack was about 5%, while parasitism of *L. rugulipennis* was 14%, the parasitoid does seem specific and safe (Haye et al. 2005). It was possible that attack by hyperparasitoids masked some non-target parasitism by *P. digoneutis*, and so primers were developed to detect its DNA in mirid nymphs by the polymerase chain reaction (Gariepy et al. 2005). These were applied to collections of four mirid species, and results confirmed *P. digoneutis* seldom attacks species other than *L. rugulipennis* in Europe (Gariepy et al. 2008).

Molecular techniques of this sort show great promise for improving biological control practice, including assessment of risk (Mills and Kean 2010). They are based on the polymerase chain reaction, in which copies of gene regions are created by DNA polymerase from thermophilic bacteria when the temperature is cycled from conditions favouring the dissociation of strands of double-stranded DNA, to those favouring binding to dissociated DNA of oligonucleotide primers, to those favouring elongation of a DNA copy from the 3' end of the primers (Sakai et al. 1985). The primers typically are about 20 nucleotides long, and their sequence, along with the reaction conditions used, determine the specificity of the reaction. The technique can be used to detect parasitoid DNA in hosts, and to detect prey DNA in insect predators (Gariepy et al. 2007). When used diagnostically to detect parasitism or predation, it is important to confirm the primers' specificity (Gariepy et al. 2007). In the case of predation, it is important also to determine how long after a meal that meal can still be detected (King et al. 2008).

Molecular techniques can provide some of the information required to interpret results of host range testing, but information must come from other sources as well. Revisiting the case of *Peristenus* species introduced to North America, for example, information about the distribution of susceptible non-target species has been used to suggest risk is greater in the western part of the continent than in the eastern part (Mason et al. 2011). As noted, it will commonly be the case that laboratory choice and no-choice tests reveal the biological control agent to be at least potentially oligophagous. Open-field tests are then recommended (van Lenteren et al. 2006b), but these can only be done in the agent's area of origin, and so non-target species that occur in the area intended for introduction but not in the agent's native range cannot be screened. As an alternative to

cruise ship laboratories in international waters, it may be worthwhile to consider the effects of host species on fitness of the biological control agent. The host species of a natural enemy frequently differ in quality, and in some cases complete development to an adult is possible when feeding on a particular host species but the adult is a "runt", with stunted development of some body parts (Salt 1941). When evaluating the range of species attacked by prospective biological control agents it is recommended that quality of these prey or host species be compared. This can be done by, for instance, comparing the mass of adult predators resulting when larvae developed feeding on different prey species, or for parasitoids the fecundity of adults from different hosts (Van Driesche and Murray 2004b). The unstated assumption seems to be that host species that result in large negative fitness consequences will seldom be attacked in more natural conditions. To understand the evolutionary pressures an agent is likely to experience, and the change evolution is expected to cause to an agent's host range after release, the variation in preference and performance of genotypes and phenotypes should be studied when multiple host species are accepted but some species are less accepted than the target species (Schaffner 2001).

There will always be a degree of uncertainty about risk to non-target species, no matter how thorough host range assessments are. Agents considered for introduction should therefore be assessed with respect to their potential to effect control, to ensure the potential for reward exists to balance the potential risk (Hoelmer and Kirk 2005; McClay and Balciunas 2005). This is conceded to be imprecise and difficult, but nonetheless worthwhile (Zalucki and van Klinken 2006). The criteria used to assess potential of natural enemy may be reductionist, based on particular biological attributes of agents, or

holistic, where emphasis is on the interactions among species and among various sources of pest mortality (Kidd and Jervis 2007). Reductionist criteria include a high search efficiency, as revealed from functional response experiments (Kidd and Jervis 2007). A natural enemy's functional response describes the increase in number or rate of attacks made by an individual enemy as host or prey density increases (Solomon 1949). Reductionist criteria also include whether there is spatial heterogeneity in natural enemy attack, such that some hosts or prey occupy a refuge (Kidd and Jervis 2007). Specificity is included as a reductionist criterion as well (Kidd and Jervis 2007). Holistic criteria include complementary interactions with other natural enemies (Kidd and Jervis 2007).

Complementary interactions among natural enemy species were considered to be the rule early in biological control of insect pests, until Pemberton and Willard (1918) suggested control of the Mediterranean fruit fly in Hawai'i by the introduced braconid, *Opius humilis* Silvestri, was less complete than it might have been, had three other competing braconids not been introduced as well. The fruit fly control programme was re-examined by Smith (1929), who found that although *O. humilis* had indeed been negatively impacted, the total proportion of fly pupae succumbing to parasitism had risen, suggesting improved control. Smith proposed a mechanism as well, based on the niches of the natural enemies, and suggested control will improve to the extent that the species' niches do not overlap, and the niche continues to be a perspective from which multiple species are considered for introduction. Competitive displacement of native natural enemies and of previously introduced species does occur, but in all studied cases of displacement, it has been noted that pest suppression improved (Mills 2006). This can be explained based on a simple model of interacting parasitoids, since the species present

first must have a relatively lower searching efficiency, or else it would not have been excluded (Pedersen and Mills 2004). More complex models show that an additional species with a niche that partially does not overlap the niche of an existing natural enemy is expected to improve host suppression to the extent this non-overlapping niche region succeeds in "breaking the refuge" from attack which previously had driven the system's population dynamics (Pedersen and Mills 2004). If the first species is more efficient in locating hosts, additional, less efficient species can disrupt control (Pedersen and Mills 2004). To judge the merits of additional natural enemies fully in any particular case requires an assessment of niche overlap and aggregation.

One approach to pre-release assessment of multiple- *versus* single-species introductions is the experimental search for emergent multiple predator effects. If multiple species of natural enemy interact, the level of pest mortality may be predictable based on the effects of individual species, or they may instead be higher or lower than expected, and if levels are not predictable an emergent effect of multiple predators is said to have occurred (Sih et al. 1998). When prey mortality increases more than expected, multiple predators are said to have risk-enhancing emergent impacts, and risk-reducing impacts when mortality is less than expected (Sih et al. 1998). Note that multiple predators can cause increased pest mortality without their impacts qualifying as risk enhancement, provided the increase in mortality is the same, or less than is predicted from the impacts of individual natural enemy species.

Risk enhancement usually is associated with prey defences specific to particular predator species that conflict, such that defence against one species leads to greater risk of mortality due to another, whereas risk reduction usually is the result of interference

competition among natural enemy species, or intra-guild predation (Sih et al. 1998). Therefore predictions of pest mortality are required for comparison, and there are two experimental designs available to generate the predictions. In substitutive, or replacement series designs, the total density of natural enemies is held constant across treatments with different combinations of natural enemy species, whereas in additive series designs, the density of each natural enemy species is constant so when multiple natural enemy species appear together, the total natural enemy density is higher than in the single species treatments (Straub and Snyder 2006). Griffen's (2006) study of crab species consuming mussels inside enclosures demonstrated the predictions of the two designs can differ qualitatively, where comparison to the observations can reveal both risk enhancement and risk reduction. This may be partly because substitutive designs confound multiple predator effects with a relaxation of intra-specific competition, whereas in additive designs, natural enemy diversity and total natural enemy diversity are confounded. It may be worth generating both sorts of predictions when possible (Griffen 2006; Straub and Snyder 2006).

If the information collected suggests an agent with high potential for pest suppression and low potential for non-target effects has been identified, a petition for releasing the biological control agent is prepared. In Canada, the procedure was summarized recently (De Clerck-Floate et al. 2006), and it is the Canadian Food Inspection Agency (CFIA) that receives petitions and passes judgement on them. The judgements are based on review by first the Biological Control Review Committee, composed of taxonomists and ecologists employed by the provinces, the federal government, and universities, and sometimes also consultants. Representatives of the

governments of the U.S.A. and Mexico are provided copies as well, and are invited to comment. The committee chairperson makes a recommendation to the regulatory entomologists of CFIA, and this recommendation as well as the recommendation of the regulatory entomologists are reviewed by the director of the Plant Health Division, who decides to permit the release or not, or may instead request additional information. If the petition is approved, the project moves to the fifth phase, colonizing the agent in the new area. Information about the pest on which this thesis is focused is now summarized.

Delia radicum, an exotic pest of prairie canola

The cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae) is a pest of canola on the Canadian prairies and the target of a classical biological control programme. There are at least four other *Delia* species in the Prairie Provinces with larvae that feed on roots of canola (Soroka et al. 2004). These are the turnip maggot, *D. floralis* (Fallén), the seed corn maggot, *D. platura* (Meigen), the bean seed maggot, *D. florilega* (Zetterstedt), and one with no common name, *D. planipalpis* (Stein). Three species are primary pests causing extensive root injury: *D. radicum*, *D. floralis*, and *D. planipalpis* (Brooks 1951). The other two generally are secondary pests, occurring usually with members of the primary pest groups and aggravating the injury (Brooks 1951). A survey in Alberta canola fields has shown *D. radicum* to be relatively more abundant than *D. floralis* in wetter areas with at least 250 mm of precipitation from June to August, and *vice versa* (Griffiths 1986b). *Delia planipalpis* occurs, but rarely (Soroka et al. 2004).

Cabbage maggots winter in diapause, as pupae within puparia buried in soil (Gibson and Treherne 1916; Schoene 1916). Populations of *D. radicum* contain varying proportions of phenotypes that develop at different rates (Finch and Collier 1983), and

whenever populations contain a high proportion of slowly developing phenotypes, emergence in spring is protracted over time. On the prairies, usually about half the adults that will emerge following winter will have done so by the end of May (Andreassen et al. 2010). Adults usually spend their first two days near the location in which immature stages had been spent, then disperse several hundred metres before mating (Finch and Skinner 1975). After mating, females continue to disperse about one kilometre a day for two days before selecting plants on which to lay eggs (Finch and Skinner 1975). Females deposit eggs on the soil, or more usually in the soil, near the plant stem, and larvae from these eggs develop through three larval instars before quitting the root to pupate (Schoene 1916). Diapause is induced facultatively by temperature and photoperiod (Soni 1976), so as few as one or several generations can occur in a year, depending on the location (Finch 1989). In prairie canola crops, there tends to be one generation, in which oviposition is coincident with the bolting stage of the crop in the middle of June, larvae develop through July, and by August most larvae will have pupated (Griffiths 1986a).

Injury to canola roots was first noticed in Winnipeg in the 1950s (Allen 1964). Over twenty years passed before maggots in roots attracted the attention of agronomists and prompted a survey of Alberta, revealing the north central area near the provincial capital, and the north western Peace River area, to be most infested (Liu and Butts 1982). By the 1990s, signs of injury were present in over 95% of fields in each of the three prairie provinces, and the average level of injury per plant had risen substantially as well (Soroka et al. 2004). In general, fields in Alberta were more infested than in the two other provinces, and in all provinces, regions further north tended to be most affected (Soroka

et al. 2004). Root maggots may have cost the canola producers of Alberta $100 million in 1995 (Soroka et al. 2004).

Economically damaging yield losses arise due directly to maggot feeding, and due to fungal pathogens that enter roots damaged by maggots. Canola crops can be of two species, *Brassica napus* L. and *B. rapa* L.; *B. rapa* plants are more attractive to ovipositing females (Dosdall et al. 1994), but this species seldom is grown any longer, and unfortunately much of the research about yield consequences of root injury has been done on this species exclusively. Inoculating *B. rapa* with *D. radicum* eggs or larvae in the laboratory significantly reduces the number of pods, and sometimes the weight of seeds, in addition to making the plant noticeably less stable and liable to lodging (McDonald and Sears 1991). Root injury seems to cause plants to increase allocation to root tissue, as root weight actually rises with level of injury (McDonald and Sears 1992). Cage studies have shown that maggots reduce *B. rapa* seed yield, and *Fusarium* foot rot, when it is present, seems to infect only plants with maggot-caused root injury (Griffiths 1991), causing plants to die prematurely, further decreasing yield (Griffiths 1986a; Griffiths 1991). Root injury does not seem to predispose *B. napus* plants to the *Rhizoctonia* pathogens responsible for brown girdling root rot (Klein-Gebbinck and Woods 2002).

Populations of *D. radicum* have been followed by several researchers to study and compare the various sources of mortality and determine which most influence the proportion that complete their life cycle and the size of future populations. Populations in cabbage fields at Wellesbourne in England (Hughes and Mitchell 1960) seemed consistently to be reduced in size by more than 90% from eggs to first instars establishing

on plants, due to predation and to failure to find and establish on roots. They survived at Wellesbourne with high probability through the three larval instars, and at least 20% were parasitized in the pupal stage, but this percentage did not depend on density. Population size seemed relatively constant to the authors over the three years of investigation. The *D. radicum* populations in Ontario cabbage analyzed by Mukerji (1971) were much smaller than those at Wellesbourne; for example the estimated mean number of pupae per plant in Ontario was just over two, compared to 17–83 per plant in England depending on the generation. In Ontario, mortality from oviposition to establishment on plants was estimated to be much lower as well, at about 19% on average, but pupal mortality was much higher in Ontario, about 70%, and the percentage of pupae parasitized increased with pupal density (Mukerji 1971). In a study of *D. radicum* populations in Holland (Abu Yaman 1960), these seemed, unlike the other two, unaffected by egg predation, and failure to establish on plants after hatching was the major point at which population sizes were reduced. Pupal mortality due to parasitism was 20–35% in Holland. The Wellesbourne site was revisited later, and egg predation then seemed much lower, at about 25% (Finch and Skinner 1988). Research about the relative importance of mortality factors operating throughout *D. radicum*'s life cycle on the prairies has not been conducted, but it is suspected that predation of first instar larvae by carabid and staphylinid beetles can be important (Griffiths 1986a). Based on six prairie canola fields, pupal parasitism is on average about 30% (Hemachandra et al. 2007a).

Species of natural enemies, or groups of species, causing mortality of immature *D. radicum* are not the same everywhere, and no doubt at any particular location the impact of each species changes according to prevailing conditions. Of the predators, red velvet

mites (Trombidiformes: Trombidiidae) seem first to have been identified (Gillette 1888), and Schoene (1916) included trombidiids with ants and some carabid and staphylinid beetle species in a catalogue of noteworthy *D. radicum* predators in New York State. Gibson and Treherne's (1916) analysis of enemies in British Columbia and Ontario echoed the importance of carabid and staphylinid beetles, but was sceptical the mites were of great value. Precipitin tests using *D. radicum* antigens were applied to individuals of 13 carabid and nine staphylinid species, and some members of all species tested positive (Coaker and Williams 1963). Finch (1996) screened 60 carabid species in the laboratory for capacity to consume *D. radicum* eggs, and suggested only those between 2.7 and 10.0 mm long have real potential to consume considerable numbers. The potential may be dampened by the foraging behaviour of the beetles. Epigaeic beetles can eat eggs, but may not be effective predators because *D. radicum* eggs usually are laid just below the soil surface, where adults of several carabid (Finch and Collier 2007) and at least one staphylinid species (Finch et al. 1999) have difficulty in locating them.

Dosdall and Mason (2010) summarized what is known of the parasitoids of *D. radicum*. There are incidental records for some ichneumonid and braconid species, and then there are three species of parasitoid that consistently inflict mortality on immature *D. radicum*. Adult female *Trybliographa rapae* (Westwood) (Hymenoptera: Eucoilidae) oviposit in all three larval instars, and emerge from *D. radicum* puparia. Two staphylinid species, *A. bipustulata* L. and *A. bilineata* Gyllenhal, attack puparia. Eggs are laid in soil, and larvae from these eggs, having located a puparium, chew through the puparium to create a hole, which they enter. Three instars are spent feeding on the fly pupa, then the beetle larva pupates within the puparium, and once the adult stage is reached, it chews a

second, larger hole in the puparium from which it exits. Adults of both species can eat *D. radicum* eggs and larvae, and so may impact pest populations as both predators and as parasitoids.

Integrated pest management, in which multiple tactics are brought to bear on *D. radicum*, is desirable. Soroka and Dosdall (2011) summarized the tactics available, including the point that in canola crops, no insecticides are registered, and so only biological and cultural control methods, and the use of resistant germplasm, are available. Cultural control methods include planting *B. napus* instead of *B. rapa*, using relatively high seeding rates and relatively wider row spacing. Resistant varieties are still being developed. Because these strategies did not seem adequate, it was suggested (Soroka et al. 2002) a search for additional natural enemies be conducted in Europe, with a view to introduce the most safe and effective species to Canada.

The parasitoid communities in Europe were therefore compared with those of the prairies, and *A. bipustulata* was identified as the most promising candidate for introduction and recommended for further study (Hemachandra et al. 2007a). This recommendation required considerable taxonomic research, because *A. bipustulata* had been considered by several authors to be present already in North America. Diagrams necessary for accurate determinations to species became available relatively recently, and so earlier records of *A. bipustulata* may refer to another species (Lohse 1986; Maus 1998; Welch 1997). Dissections of specimens collected across North America for detailed examination of male and female genitalia showed *A. bipustulata* to be undetected from the North American fauna (Hemachandra et al. 2005). The final section of the literature

review concerns this species, its suspected hosts and prey, and its interactions with other species of natural enemy.

Aleochara bipustulata, candidate for introduction to the prairies

Aleochara bipustulata is a member of the beetle family Staphylinidae, which includes over 46,000 described species in 31 subfamilies (Newton et al. 2001). Of these subfamilies, the Aleocharinae is the most speciose, with over 12,000 described species and over 1000 genera, arranged in 52 tribes which are not considered stable, and among which the relationships remain mostly uncertain (Thomas 2009). Many aleocharine lineages are highly specialized, as predators of mites for example, or as inquilines dwelling in nests of ants and termites (Frank and Thomas 2008). The phylogenetic relationships among members of the subtribe Aleocharina remain unclear as well (Maus et al. 2001). The genus *Aleochara* contains at least 300 described species, all of which seem in the larval stage to be parasitoids of Diptera in the suborder Brachycera that form puparia, the Cylorrhapha group (Klimaszewski 1984; Maus et al. 1998; Peschke and Fuldner 1977). There are at least 27 and perhaps as many as 30 species in the subgenus *Coprochara* to which *A. bipustulata* belongs (Maus 1998), and as parasitoids these staphylinid species are associated with host species in 12 families of Diptera (Maus et al. 1998), but host records exist for fewer than half of the *Coprochara* species. Beyond an impression that adult *Aleochara* prey on the egg and larval stages of the Diptera species that host larval development (Klimaszewski 1984), the predacious habits of the adults are not well understood. As a result of the fragmentary nature of this information about host and prey use, understanding the risk posed to non-target species requires the life cycle be examined to determine the species or groups of species which *A. bipustulata* will be

likely to encounter, and also which of these are potentially susceptible to predation or parasitism.

The confusion regarding accurate identification of *A. bipustulata* means the report may be in error, but *A. bipustulata* has been reported to winter as an adult (Heydemann 1956). Fuldner (1960) provided observations in support of *A. bipustulata* spending winter as adults, but unlike Heydemann, indicated these are inside of puparia, and added *A. bipustulata* may winter as first instar larvae or as pupae as well. Soil in a Swiss field devoted the previous year to cabbage, sampled in February, contained adult *Atheta coriaria* Kraatz (Coleoptera: Staphylinidae) but no *A. bipustulata* (N. Holliday, personal communication 2008). A comparison of the seasonal activity of *A. bipustulata* and *A. bilineata* in Sweden does tend to support the notion of *A. bipustulata* wintering as free-living adults, as adults are active in numbers earlier than those of *A. bilineata* (Jonasson 1994), which is known to winter as first instar larvae within host puparia (Fuldner 1960; Whistlecraft et al. 1985a). Completing the life cycle requires adults to mate, but it is not known if mating occurs prior to wintering. Adults also find food for themselves, and find locations in which to lay eggs. It is not known which if any of these three activities take precedence in adults freshly emerged from wintering locations or from host puparia, and it seems likely at least some individuals do each of the activities more than once before dying.

Adults of *A. bilineata* and *A. bipustulata,* when 1–2 d old, seem repulsed by dimethyl disulphide (DMDS), a volatile emitted by decomposing *Brassica*, and so if this compound is used to locate mates, adults early in life seem not to be in search of mates or plants (Du 2013). Adult female *Aleochara curtula* Goeze produce a sex pheromone that

triggers males to attempt copulation (Peschke 1978), and young males produce the same pheromone with at least two consequences: older males in better condition are not aggressive toward the pheromone-producing males, and females almost always repulse these pheromone-producers' attempts to mate (Peschke 1990). If pheromones are emitted and used in the same way by *A. bipustulata*, then mating seldom occurs among young adults. On the other hand, within a day of emerging from host puparia, adult *A. bilineata* require just a few minutes together before they mate (Lizé et al. 2009). Male *A. curtula* produce an aggregation pheromone that attracts adults of both sexes, which acts at a greater range than the sex pheromone of females (Peschke et al. 1999), but whether male *A. bipustulata* produce a homologous compound is not known.

Unless female *A. bilineata* eat, ovaries remain undeveloped and no eggs are laid (Colhoun 1953), and the same is expected to apply to *A. bipustulata*. Female *Aleochara tristis* Gravenhorst eat bovine dung, in which larvae of their hosts develop, but this is not a sufficient diet for egg production, and egg production seems to require animal protein such as from the eggs and larvae of their host, although a diet of dung does enable egg production to commence more rapidly than it would have had only water been available (Heller 1976). Adults of *Aleochara* species that parasitize carrion-breeding flies consume carrion (Peschke et al. 1987). So, adult *A. bipustulata* almost certainly require animal protein to complete their life cycle; it may be they consume exclusively the eggs and larvae of their *D. radicum* hosts; it may be that in addition, adults scavenge for already-deceased animals in this same microhabitat, or it may be that adults forage in this microhabitat for living prey, kill these and consume them. A catalogue of prey consumed by adult staphylinids shows all sorts of insects, and other invertebrates such as molluscs

and oligochaetes, to be consumed by at least some species (Good and Giller 1991), and so the diet of *A. bipustulata* could be quite broad. If foraging is not restricted to the microhabitat of immature *D. radicum*, it could be broader still. The response of adults to a wide range of prey therefore needs to be investigated.

The process of parasitism and host range of *A. bipustulata* are better understood than is what the adults eat. Females are attracted to infested *Brassica* roots (Ferry et al. 2007; Goubert et al. 2013), to DMDS in fields (Ferry et al. 2007) and in the laboratory once a certain age is reached (Du 2013), and adults are attracted to mustard seed meal in the laboratory and in the field (Riley et al. 2007). Female *A. bipustulata* do not seem to be attracted to uninfested plants in the field (Goubert et al. 2013), nor do *A. bilineata* adults seem attracted to uninfested plants (Abu Yaman 1960), although newly emerged and unfed *A. bilineata* adults are more attracted to *Delia*-free rutabaga than they are to clean air (Royer and Boivin 1999). In a laboratory pitfall assay, female *A. bipustulata* were more attracted to traps baited with damaged roots, or with host puparia, than they were to empty traps, and more attracted to damaged roots than to host pupae (Goubert et al. 2013). On a sand substrate, females spend more time where *D. radicum* larval tracks and pupae are present than where the water soluble essence of infested roots had been sprayed, and more time where larvae pupated and may have left frass than where the pupae themselves were (Goubert et al. 2013). In sum, female adults are attracted from a distance by the smell of infested plants, and once in an environment impregnated with these odours, search at a finer scale for locations in which to deposit eggs based on odours given off by host puparia and especially by host larvae (Goubert et al. 2013). As Fuldner (1960) noted, adults in laboratory colonies frequently are observed to create, and spend much time in,

tunnels in the substrate; it may be females move down tap roots, and from there consume *D. radicum* larvae they encounter, or follow tunnels left by *D. radicum* larvae leaving the root to pupate. The carrion-associated *A. curtula* disperses to oviposit prior to most of the host maggots, such that eggs will largely have hatched when the later-dispersing maggots pupate (Peschke et al. 1987). Larvae, which are most likely to survive in the more recently pupariated hosts, select these when given a fair choice, but seem also to accept the first encountered when their placement makes that more likely (Peschke et al. 1987). This serves to illustrate the twin roles discriminating adaptive behaviour and chance seem to play in determining which hosts are selected by *Aleochara* species, such as *A. bipustulata*.

It remains to be determined precisely where females deposit eggs, a question of obvious importance for risk assessment. Adults do not seem to go into forests, even when bait of mustard meal is used (Andreassen et al. 2009), but may well occur in orchards (Balog et al. 2008). The results of Goubert et al. (2013) leave little doubt female *A. bipustulata* in search of oviposition sites base their selection on cues provided by *D. radicum*, but to say they do so is not quite the same as to say they do so always. In particular, adult *A. bipustulata* are associated with carrion and dung in Europe, in addition to rotting *Brassica* and perhaps other vegetation (Andreassen et al. 2007; Welch 1997) They have been trapped in a variety of open habitats, and in these sometimes but not always more frequently in mustard meal-baited traps than others (Andreassen et al. 2009). All calyptrate Diptera species tested whose larvae develop in dung or in carrion were unsuitable as hosts, but several acalyptrate species consistently were suitable hosts

(Andreassen et al. 2009). Additional information about *A. bipustulata*'s use of acalyptrate hosts is therefore required for a more complete assessment of risk.

Risk assessment never will be all encompassing, and so it is desirable also to know what potential *A. bipustulata* has, as a predator and as a parasitoid, prior to petitioning for its release. As egg predators, adult *A. bilineata* and *A. bipustulata* have great potential, consuming on average more than 90 eggs per day when supply is unlimited and eggs are easy to locate on the floor of a Petri dish (Fournet et al. 1999). It has long been clear for *A. bilineata* that this potential is seldom reached (Read 1962), and this may be in part due to the natural placement of eggs being slightly below the soil surface, where they are much less likely to be found (Finch et al. 1999). Other species of epigaeic beetle predators may interfere with foraging adults as well (Prasad and Snyder 2004), widening further the gap between potential predation rates and rates actually achieved. Hartfield and Finch (2003) conducted a field study timed to isolate predation by *A. bilineata* adults to *D. radicum*'s larval stage. If two adults are present per cabbage plant, fewer puparia form and roots are heavier than when no beetles are present. Another finding of interest was that *D. radicum* larval mortality was not increased by applying four, eight, or 16 beetles instead of two per plant, an indication perhaps of intraspecific mutual interference. To evaluate *A. bipustulata*, it should be confirmed to consume immature *D. radicum*, and the outcome of its interaction with other potential predator species should be determined.

Understanding of the potential impact could benefit from clarification as well. In samples of *D. radicum* puparia, more *A. bilineata* adults emerge than do adults of *A. bipustulata* (Brunel et al. 1999; Wishart et al. 1957). This may be because survival of *A.*

bipustulata larvae is more probable in smaller than larger *D. radicum* puparia (Jonasson 1994), as excess pupal material that remains after the *A. bipustulata* larva ceases to feed rots (Fuldner 1960). It may therefore be that the *D. radicum* puparial resource is partitioned partly on the basis of size, but with flexibility as well such that puparia besides the most preferred are accepted as well (Fournet and Brunel 1999). Larvae of *A. bilineata* choose larger *D. radicum* puparia over smaller ones, and *A. bipustulata* larvae do the opposite (Ahlström-Olsson 1994b). In addition, larvae of both species avoid entering puparia that already contain a larva of the same species or of the other species (Royer et al. 1999), a phenomenon expected to reduce the frequency with which larvae compete for hosts, and to cause parasitism overall potentially to rise. It would be worthwhile to offer simultaneously a choice of host sizes and of parasitized and unparasitized puparia to determine the outcome interspecific larval encounter is likely to have for *D. radicum* pupal parasitism rates overall.

Research Objectives

Nine research objectives are identified to build on what already is known concerning *D. radicum* and its natural enemies, and more completely inform the evaluation of *A. bipustulata*. First, to investigate the range of prey consumed by *A. bipustulata*. Second, to confirm immature *D. radicum* are consumed by adult *A. bipustulata* in nature. Third, to determine if the rate of *D. radicum* egg predation when these are exposed to multiple predator species is predictable based on the consumption rates of individual species. Fourth, to compare *A. bipustulata* and *A. bilineata* in their ability to parasitize three host species, including a range of sizes of each host. Fifth, to determine if the host puparium resource is partitioned by the two species with respect to

host mass or to host depth in soil. Sixth, to determine some of the consequences of host size and host species selection on rates of growth and development. Seventh, to bring these together in a matrix model to determine their effects on rate of population increase. Eighth, to study the preferences of *A. bipustulata* larvae between two host species. Ninth, to compare the size of field-collected *A. bipustulata* to these expectations. The fulfillment of these objectives is covered in the next chapter.

Chapter 3 Section 1

Investigations of diet in adult Aleochara bipustulata

Introduction

Farmers of the Canadian prairies have been quick to incorporate canola quality varieties of *Brassica napus* L. and *B. rapa* L. (Brassicaceae) in their rotations, trebling the area seeded over the past 30 years to about 7.5 million ha in 2011 (Anonymous 2011). A five-species complex of root-feeding anthomyiid Diptera (Brooks 1951) has been just as quick to proliferate, with the area infested and severity of infestation rising over the same period (Soroka et al. 2004). *Delia radicum* (L.), introduced accidentally from northwestern Europe (Biron et al. 2000; Gibson and Treherne 1916; Schoene 1916), dominates the complex in regions with greater rainfall (Griffiths 1986b), and may be a promising target for classical biological control by the introduction of another parasitoid species (Turnock et al. 1995), such as *Aleochara bipustulata* (L.) (Coleoptera: Staphylinidae) (Andreassen et al. 2007; Hemachandra et al. 2007a; Soroka et al. 2002). The larva of an *Aleochara* sp. hatches from an egg laid in soil, then develops parasitically within a puparium of Cyclorrhapha (Diptera) species, whereas the adult stage is free-ranging, and predacious (Klimaszewski 1984; Peschke and Fuldner 1977). *Delia radicum* puparia attacked by *A. bipustulata* are found in canola crops across western Europe (Hemachandra et al. 2007a) but not in North America (Hemachandra et al. 2005). Concern about the effect an introduction will have on non-target species (Howarth 1983; Lockwood 1996; Louda et al. 2003b) prompted a study of *A. bipustulata*'s use of habitat, and of the Diptera species suitable for its complete development as a parasitoid (Andreassen et al. 2009). Questions about its habits as an adult predator remain before an introduction can proceed.

Of primary concern, *A. bipustulata* adults may feed on non-target species in Canada. To assesses the risk to non-target species, one must understand the biological control agent's host range (Bigler et al. 2006) This range can be reasonably determined from study of the agent's interaction with non-target species on a carefully crafted list. Information about the range of the biological control agent as it is expressed in natural conditions, garnered from museum holdings, the scientific literature, and surveys in the agent's area of origin, is the foundation upon which this list is constructed (Kuhlmann et al. 2006). Species are included in the list that occur in the same habitat or microhabitat as the pest, that are taxonomically closely related to the pest, or that represent rare or beneficial species (Kuhlmann et al. 2006). Feeding bioassays in which the agent is forced to select a particular non-target species or nothing at all are stressed, either as least likely to mislead (Barratt 2004; Van Driesche and Murray 2004a), or as the first step of a sequence that leads, with correct interpretation, to classification of the agent's host specificity (van Lenteren et al. 2006b), defined on a scale from specialist to generalist (Bigler et al. 2006).

While adult *Aleochara* can be maintained on flesh of various vertebrates (Fuldner 1960; Whistlecraft et al. 1985a) and invertebrates (Andersen 1982; Hertveldt et al. 1984; Nienstedt and Galicia 2001) in the laboratory, little is known about the diet of adults in nature beyond that they feed on the eggs and larvae of their Diptera hosts (Klimaszewski 1984). Starved *Aleochara* adult females produce no eggs (Colhoun 1953; White and Legner 1966), and *Aleochara tristis* Gravenhorst, a parasitoid of dung-feeding *Musca autumnalis* DeGeer (Diptera: Muscidae) puparia, ceases to produce eggs within five days if their *M. autumnalis* food is removed (Heller 1974). Feeding on dung or semi-digested

corn particles in dung by *A. tristis* is observed in the laboratory, and this saprophagy sustains the beetle and permits a more rapid return of the ability to produce eggs once immature *M. autumnalis* are again made available to the beetles (Heller 1974). Species in the family Staphylinidae are believed to have evolved from saprophagous ancestors, and most extant species are either facultative, or highly-adapted and specialized, predators (Frank and Thomas 2008). Prey records for species in the family include all manner of hexapods, as well as oligochaetes, crustaceans, myriapods, nematodes and arachnids (Good and Giller 1991).

A second issue for which additional information is sought is whether adult *A. bipustulata* regularly consume *D. radicum* eggs and larvae in *Brassica* fields. *Delia radicum* mortality during the egg and larval stages, the importance of predation in causing this mortality, and the impact specifically of *A. bipustulata*, have all been subjects of previous research; unfortunately, artifices employed to study this cryptic edaphic interaction leave most conclusions open to alternative interpretations (Finch and Collier 2007). For instance, Abu Yaman (1960) and El Titi (1977) claim mortality from the egg stage to the end of the third and final instar to be above 95%; however, El Titi attributed 45% to predators, whereas only 17% is claimed for predators in Holland by Abu Yaman. Life tables constructed in Canada (Mukerji 1971) and England (Hughes and Mitchell 1960) differ considerably, with 75% and 96% mortality estimated, respectively, from eggs to the end of the larval stages. In Canada, 6% of eggs and 24% of larvae were lost to predation in each generation, whereas in England estimates were about 90% and nil for the same stages. Precipitin tests suggest about 3% of *A. bipustulata* feed on *D. radicum* (Coaker and Williams 1963), but these tests are vulnerable to cross-reactivity

and low repeatability (Symondson 2002), and without information about how long after feeding a meal is detectable, these results are difficult to interpret.

In this study, no-choice laboratory tests using a range of invertebrates as potential prey were conducted to address concerns about non-target effects. To follow up on predation of immature carabid beetles during these tests, and to address feeding by adult *A. bipustulata* on immature *D. radicum*, gut contents of *A. bipustulata* collected in nature were examined. A variety of potential approaches to gut content identification is available (Sunderland et al. 2007). Microscopic examination of gut contents was dismissed, as adult *Aleochara* rely on liquid diet (Colhoun 1953), at least as predators. Immunological analyses were considered, as these are inexpensive and rapid to employ once developed (Fournier et al. 2008), but a polymerase chain reaction (PCR) approach (Agusti et al. 1999; Zaidi et al. 1999) ultimately was favoured as it was expected to be more straightforward to develop. Primers were developed to amplify DNA of *Amara similata* Gyllenhal, *Bembidion quadrimaculatum* (L.) (Coleoptera: Carabidae), and *D. radicum*, and then the specificity of the primers assessed. After characterizing how the probability of detection declines over time since the last meal, these primers were used on gut contents of *A. bipustulata* collected in Switzerland. Samples of *D. radicum* eggs and larvae were taken from plants in the fields from which the *A. bipustulata* were collected, to estimate the proportion of eggs on the soil surface in natural conditions and confirm that the target prey was present at the time of sampling.

Materials and Methods

Petri dish feeding trials

Principles of the recommended procedure for creating a non-target species test list (Kuhlmann et al. 2006) were used. Occurrence in the same or adjacent microhabitat as *D. radicum* was the primary consideration in selecting species. Ten plant-centred samples, 30 by 30 by 5 cm deep, of soil were excavated weekly from 9 June to 19 August, 2008, in a field of organic white cabbage outside Galmiz, Switzerland. These were transported in plastic bags, along with the root and attached soil, to the laboratory where a combination of Berlese-Tullgren funnel extraction, sorting in white plastic trays for larger organisms, and examination of subsamples using dissecting microscopes for the less conspicuous invertebrates, was used to understand at a morpho-species level the diversity of potential prey *A. bipustulata* are likely to encounter, assuming they restrict foraging to the microhabitat of the target pest. The recommendation to add species based on their being related closely to the target was reversed in order to include as wide a taxonomic range of potential prey as possible. Larvae of different Diptera species were also included to explore relatedness as a factor governing vulnerability. Safeguarding rare or beneficial species played a minimal role in choosing species.

Aleochara bipustulata for these tests were from a laboratory colony, started with material from *Brassica* fields around Kerzers, Switzerland. The colony was maintained by feeding adults with immature *D. radicum*, and using *D. radicum* puparia as hosts for larval development (Andreassen et al. 2009). Predators starved as adults but given the opportunity to mate were used in the no-choice mating protocol. Newly-emerged adults were collected the day they emerged from puparia and kept in groups of mixed sex for two days, with access only to water. An individual beetle was transferred to a Petri dish, 5 cm diameter, with a lid that seals (Semadeni AG, Ostermundigen), with moist filter

paper lining the bottom. Individual potential prey organisms were weighed to the nearest 0.1 mg and then introduced to the dish. Immobile items were placed 1–2 mm from the edge of the arena, as *A. bipustulata* were observed to spend most of their time with at least an antenna touching the dish wall. If the organism was not consumed within 10 minutes, the beetle was allowed 24 hours in which to feed on the other species, or not. If not, then the potential prey item was replaced with one *D. radicum* egg. If the egg was consumed, the replicate was considered valid, and the prey item was scored as not consumed. The purpose of the Petri dish trials was to identify taxa that were at high risk of predation, considered to be ones consumed in at least 50% of 20 replicates. Accordingly, testing continued until individuals of a taxon were fed upon 10 times, or until 20 replicates were completed. The origin of potential prey items is listed in Table 1, and most tests used organisms directly collected from the field. Aphids and diamondback moth, were maintained on potted cabbage or pea plants, and colonies of both Collembola species were kept as described by Tomlin (1985). Immature *Amara similata* Gyllenhal (Coleoptera: Carabidae) were the offspring of field-collected adults fed crushed seeds of canola. Colonies of *Piophila casei* (L.) (Diptera: Piophilidae) and *Lucilia silvarum* Meigen (Diptera: Calliphoridae) were maintained with ground meat.

Primer design

To detect prey species' DNA within the guts of *A. bipustulata*, PCR primers were designed, based on recommendations by King et al. (2008) to amplify short (<~300bp) mitochondrial gene sequences with high likelihood of being detected due to their high copy number within cells (Table 2). Cytochrome oxidase I sequences were aligned in Clustal W 2.0 (Larkin et al. 2007), and primer locations selected from regions

of dissimilarity. For *D. radicum*, this involved comparison of the target species (GenBank accession AF325362S1), *Delia platura* (Meigen) (DQ657045), *A. bipustulata* (AJ293083), a julid millipede (JN273777), the carabid *Bembidion quadrimaculatum* (L.) (DQ155801), and the aphids *Acyrthosiphon pisum* (Harris) (AF077776) and *Brevicoryne brassicae* (L.) (EU701547). Alignments for *B. quadrimaculatum* included *A. bipustulata*, *D. radicum*, *Bembidion osculans* Casey (JN171143), *B. proprium* Blackburn (JN171089), and *B. foveolatum* (Dejean) (JN171037); for *A. similata* (DQ155989) they included *A. bipustulata*, *D. radicum*, *Amara ovata* (Fabricius) (DQ156064), and *A. aenea* (DeGeer) (FJ173213). Universal primers for COI, to serve in control reactions confirming the presence of amplifiable DNA and serviceable reagents, were based on C1-J-1751 and C1-N-2191 (Simon et al. 1994), but with degeneracy to accommodate the variety of species used to confirm the other primers' specificity.

DNA handling, extraction & amplification

Aleochara bipustulata, *A. similata*, *B. quadrimaculatum* and *D. radicum* were killed and stored individually in acetone (Bisanti et al. 2009) until DNA was extracted by the cetyltriethylammonium bromide method (Murray and Thompson 1980) and stored at −20 °C in elution buffer. Pinned specimens of the other species, used to assess the specificity of primer pairs, were first crushed in liquid nitrogen before DNA extraction. DNA was amplified in a 15 μl polymerase chain reaction containing EconoTaq PLUS GREEN (Lucigen), molecular grade water, 0.893 μM of each primer, and 5–75 ng of DNA template. Thermal cycling conditions were 94 °C for 3 minutes, 35 cycles of 94 °C for 15 seconds, 15 seconds at the specific annealing temperature for the primer pair (Table 2), and 72 °C for 45 seconds, and then a final elongation step of 72 °C for 5

minutes. Portions (5 μl) of PCR products were resolved by gel electrophoresis on 1.5–2.0% agarose (Sigma) gels in Tris-acetate-EDTA (TAE) buffer using standard conditions. The DNA bands were visualized by staining with Sybr Gold (Invitrogen) in TAE (0.0001% v/v), and viewed and photographed using UV light with a BioRad Gel Documentation Imager.

Primer specificity

To assess the specificity of the *D. radicum* primers, the primers were tested for their ability to detect DNA from *D. radicum* collected from different parts of its range. Nine samples of DNA from individual pupae were tested, three from each of Galmiz (canton Fribourg, Switzerland), Steinegg (canton Thurgau), and Oland island (Sweden), along with a no-template control. To assess whether the *D. radicum* primers were species-specific, the primers were also tested on 26 additional invertebrate species using two samples of each species (Table 3). Control reactions with the universal primer pair were used to ensure quality of template, with *D. radicum* DNA for both pairs of primers to ensure the reagents were functional, as well as no-template reactions for each pair of primers to ensure the reagents were not contaminated by DNA. Similar tests of species specificity were performed for each pair of carabid beetle primers, although with a different group of test species (Table 3).

Primer sensitivity

The sensitivity of the PCR-based assays was assessed to determine how readily they could detect a meal of *D. radicum* eggs in the gut of an *A. bipustulata* over time. Eggs of *D. radicum* were collected by leaving a slice of rutabaga overnight on a bed of moist sand inside the cage of a laboratory colony. Eggs were floated in water, collected in

a coffee filter, and then transferred in groups of 10 to a filter paper lining the bottom of a 5 cm diameter Petri dish. Individual beetles, fed until the previous day only on dry cat food then starved, were released into the dishes and allowed to forage. Beetles observed to have consumed at least five eggs were removed from the dish immediately after they left the cluster of eggs and transferred to a 2 ml microcentrifuge tube. A wad of moist paper towel was added to each tube before it was capped and assigned randomly to be kept at 17 °C in darkness for 0, 4, 8, 12, 16, 20, or 24 h. After the prescribed period for digestion, the paper towel was removed and the beetle killed by addition of acetone. Nine beetles were used for each time period. Test and control reactions were set up as previously described. To determine whether the probability that a meal is detected depends on time since the meal, the number of eggs consumed, and the interaction of these two factors, logistic regression models were compared with the likelihood ratio (L.R.) χ^2 (Quinn and Keough 2002). The prey detectability half life (Greenstone and Hunt 1993) was determined from the final model.

Similar experiments with *B. quadrimaculatum* and *A. similata* eggs were not possible due to lack of sufficient beetles, so the sensitivity of these primers was assessed indirectly. The concentrations of extracted DNA from the three species were measured (Nanovue, General Electric), serially diluted in water, and the lowest concentration of DNA that could produce a visible PCR product on an agarose gel (as described above) was determined for each pair of primers.

Field sampling and analysis

Immature *D. radicum* and adult *A. bipustulata* was sampled in cabbage fields at several locations in Switzerland. Sampling was timed to include an expected peak in egg

density around the middle of May (Freuler 1975). In 2009, sampling was conducted in fields on an organic farm at Galmiz Switzerland. The field measured 9 x 24 m, and was divided in 4.5 x 12 m quarters. Ten plants were randomly selected from each quarter each week to assess *D. radicum*, and four pitfall traps for collecting *A. bipustulata* were placed in each quarter between cabbage plants in a row; each trap was 1.2 m from a long edge and 4 m from a short edge. Plots for weekly samples near Galmiz in 2010 and Sugiez in 2011 were 9 x 48 m, and divided into 48 3 x 3 m areas, to produce a checkerboard of 24 sampled and 24 unsampled areas. Each sampled area had one pitfall trap, nearly in the centre and between two plants in a row, and two plants were randomly sampled each week for *D. radicum*. Additional samples, but only for beetles and pest eggs, were taken in 2010 from 9 x 48 m plots within larger commercial organic fields at Galmiz (*ca.* 3 ha) and Jerisberghof (4 ha), and within an experimental plot at Wädenswil (0.75 ha).

To sample for *D. radicum* eggs near selected plants, the cabbage stem was cut below the leaves, and a 15 cm diameter sheet metal ring with sides 2 cm deep was inserted into the soil so that the stem was in the middle, and the top was flush with the soil surface. The area inside the ring was then carefully searched, and all *D. radicum* eggs visible without disturbance of the soil were removed with a paintbrush and counted. Then the soil within the volume of the ring was excavated into a glass bowl. The bowl was half filled with water, stirred gently, and intact *D. radicum* eggs floating on the water counted (Hughes 1959). The full length of the root and the surrounding soil were then excavated and transported in plastic bags to the laboratory, and examined for *D. radicum* larvae with the aid of a dissecting microscope. Instar determination of larvae was based on the number of slits on the posterior spiracles (Brooks 1951). In all three years in the fields for

regular sampling, sampled plants were replaced by planting with cabbage plants grown in pots from the time the field was planted. These replacement plants would not be subsequently sampled; this system allowed the progression of the *D. radicum* population to be followed without sampling from an empty field.

Each pitfall trap consisted of two disposable plastic cups, 8.5 x 15.5 cm, one inside the other, buried flush with the soil surface. The spout of a funnel was trimmed so that it fit snugly with its top edge 2 cm below the top of the cups, and its 0.8 cm aperture was about 3 cm above the bottom of the cup. This arrangement made the probability of *A. bipustulata* escaping traps by flight negligible, while also excluding some larger carabid beetles that may have eaten the sample. A bait filter paper infused with 20 μl of 10% dimethyl disulphide in paraffin oil, the most attractive concentration to *A. bipustulata* (Ferry et al. 2007), rested at the bottom of the inner cup. As the compound is highly volatile (Ferry et al. 2007), new bait papers were prepared from stock solution immediately prior to each 4 h sampling period. Three sampling periods, from 1600 to 0400 h, were monitored on the first four sampling dates of 2009; afterwards there were six 4 h periods, constituting a full day of sampling by pitfall traps.

Beetles in the traps were immediately stored individually in screw-cap vials in acetone. Vials were kept at -20°C except in the field and during transportation, until DNA extraction and processing as described above. All *A. bipustulata* were screened for the detectable remains of *D. radicum*. Logistic regression was used to determine, for the eight sampling dates in 2009 and three dates in 2011 on which both positives and immature *D. radicum* densities were non-zero (Table 4), whether the probability a beetle would test positive for *D. radicum* DNA changed with either the average density of eggs

per plant, or the average density of *D. radicum* of all instars combined. Whether the proportion of a 4 h catch that tested positive for *D. radicum* changed over the course of a day was analyzed, for the 11 dates on which more than 25 *A. bipustulata* were caught, by log linear modeling of contingency tables. Each date was analyzed separately, using delta=0.5 to correct for low counts in some cells, and a non-significant interaction between period of capture and assay outcome was interpreted to indicate the proportion testing positive was approximately constant over the day. A subset of 45 beetles was selected randomly from the entire collection to test for detectable remains of *B. quadrimaculatum* or *A. similiata*. The probability that feeding on either carabid species was not detected even though it occurred was explored with the statistical theory of repeated zeros in sequential sampling (Kuno 1991; Moon and Wilson 2009).

Results

Petri dish feeding trials

In total, 30 "groups" were screened for vulnerability to *A. bipustulata* predation in these trials; one group turned out to be mixtures of two species, in other cases different stages of the same species represented different groups (Table 1). One third of these prey groups was never fed upon, including *D. radicum* puparia. A further nine were consumed only after 24 h, and not during the 10 min. observation period. Only with third instar *D. radicum* and *A. similata* eggs were at least half of the offered prey consumed during the period of observation. Mass had a complicated influence on predation, as *A. bipustulata* consumed items of the heaviest prey group and some of the inestimably small, but did not consume many items of intermediate size. *Tyrophagus putresentiae* were not consumed, whereas noticeably but immeasurably larger *Sancassania* sp. were eaten sometimes.

Whether members of a group could move or not seemed also to be important; for example, eggs and pupae of *A. similata* were consumed more frequently than larvae. Larvae of both *D. brassicae* and *C. obstrictus* could move, and so started in the centre of the dish, but moved slowly and as a consequence seldom reached the periphery of the dish within 10 minutes and therefore seldom were consumed during the observation period. Larvae of *P. casei* leap, which they frequently did in response to contact, so while all eventually were eaten, few died during the observation period. *Aleochara bipustulata* were not observed to penetrate the cuticle of ants and wireworms, and seldom managed to do so with the full grown calliphorid larvae.

PCR Primer specificity

The selected PCR primers for *D. radicum* successfully amplified the expected size PCR product for all control samples tested (Figure 1). Of the species screened using primers for *D. radicum*, only *D. planipalpis* DNA was amplified (Table 3), and both samples for this species produced bands. All other species' DNA samples produced PCR products only when using the universal primers. Among the *D. radicum* from different areas, primers amplified bands for all nine samples. Pairs of primers for *B. quadrimaculatum* and *A. similata* clearly distinguished samples of these species from controls (Figure 2, Figure 3) and amplified only DNA from the intended species (Table 3).

PCR Primer sensitivity

Remains of *D. radicum* were detected using PCR in all beetles killed immediately after feeding, and since one tested positive after 24 h digestion, the time interval over which predation is detectable is at least one day. The observed proportion of beetles

positive for *D. radicum* DNA declined gradually over this period, and the logistic regression model provided an adequate prediction for change in probability of detection (Figure 4). The prey detectability half life was 15.1 h, with 95% confidence limits of 12.2–18.2 h. Comparison of the three pairs of primers using serial dilutions revealed the lower limit of concentration detectable for *D. radicum* and *A. similata* pairs to be similar, at around 0.2–0.5 ng/μl. Primers for *B. quadrimaculatum* were less sensitive, with lower concentrations around 3.0 ng/μl.

Detection in field-collected samples

The proportion of eggs on the soil surface, of the total present in a field on a sampling date, tended to be less than 0.10, and frequently was 0 (Table 4). Somewhat higher values were observed on 15 June 2011; only eight eggs were counted on this date, inflating the proportion. Averaging over all sampling dates, about 3% of eggs were on the surface.

Both *D. radicum* and *A. bipustulata* were more abundant in 2009 than in the following two years (Table 4). Eggs were present throughout the 2009 sampling period, so it is not possible from these data to determine if *A. bipustulata* feed on both eggs and larvae of *D. radicum*. The proportion of beetles testing positive for *D. radicum* remains in their gut was fairly constant over the sampling period, at around 20%.

The same location in 2010 was nearly bereft of these species. Few *A. bipustulata* were trapped from 26 May–9 June, and none were positive for *D. radicum* (Table 4). No *A. bipustulata* were caught at the site on three subsequent sampling dates. Commercial cabbage and cauliflower crops sampled in July at Jerisberghof and another field near Galmiz were starting to form heads, with 12–16 true leaves, and so although no roots

were sampled in these fields, populations of *D. radicum* probably were predominately large larvae. Eggs were scarce on these occasions, so the predation of *D. radicum* observed may indicate predation on these larvae. A high proportion of beetles from Wädenswil tested positive for *D. radicum*, but this was one positive from two beetles.

Populations were sparse again in 2011. Beetles from the first sampling date tested positive for *D. radicum*, although *D. radicum* populations were undetectable in the field at that time or in the following week (Table 4). Up to 25% of beetles in subsequent collections tested positive, but the most frequently encountered proportion positive was 0.

The proportion of *A. bipustulata* testing positive for *D. radicum* DNA was not related to the density of eggs in the field (L.R. $\chi^2_1 = 2.2$, $P = 0.14$). Neither was it related to the total density of larvae (L.R. $\chi^2_1 = 1.2$, $P=0.28$). The period from midnight to 4:00 contributed little to the daily catch during the first four sampling dates (Figure 5) and subsequent occasions when traps were monitored for the full 24 hours (Figure 6). Few beetles were caught from 4:00 to 8:00 as well (Figure 6). For all 11 dates, removing the interaction from the model of period of capture and counts of test outcomes did not result in a worse fit ($P \geq 0.26$), indicating that the time of capture had no discernible influence on the proportion testing positive (Figures 5 and 6).

None of the 45 *A. bipustulata* tested for remains of carabid beetles were positive for either species. The probability, then, that more than 10% of *A. bipustulata* in the field really eat either species is less than 1%, and higher levels of feeding are even less probable. The possibility remains that the rare predation event does occur, as there is about a 10% chance that levels of predation of 5% of *A. bipustulata* or less have been missed.

Discussion

This study of *A. bipustulata*'s specificity as a predator was motivated by a desire to maintain the Canadian public's supportive attitude toward biological pest control (McNeil et al. 2010) and to fulfill the requirements of regulatory bodies charged with granting or denying permission to introduce beneficial insect species (De Clerck-Floate et al. 2006). Current precepts about predicting specificity from a sequence of tests, beginning with no-choice assays and moving toward tests in the field with non-target species that were attacked at lower levels of the hierarchy (van Lenteren et al. 2006b), were followed. Accompanying the recommended sequence is a framework for interpreting results and translating "host" range into risk assessment and as this framework represents expert consensus, it will be employed here. Before doing so, brief comment regarding replication and statistical analysis is apropos, since these are major themes in the sequence as outlined: statistically valid response is available to nearly all sorts of statistically valid objection (Friston 2012), and so while it is acknowledged that greater replication of the Petri dish feeding trials would be preferred, covering a wider array of potential prey was considered more pressing in the interest of evaluating environmental safety.

Accordingly, there is no risk of direct effects due to predation of adult linyphiid spiders or adult ants, nor to any but the smallest myriapods. The same is asserted for Diptera puparia and wireworms. Mites are only slightly more complicated: the small (*Tyrophagus*) and distasteful (*Platytrombidium*) (Proctor and Garga 2004) species are invulnerable, and the faster moving *Sancassania* nearly so. Mucus seems to discourage *A. bipustulata* feeding on lumbricids more so than on enchytraeids, but both sorts of worm seldom were consumed during the period of observation. Since the three aphid species

were eaten less frequently than *D. radicum* larvae, the risk to them is low to acceptable, and the same can be said of *Ceutorhynchus* larvae and the other species of Diptera. These too, when eaten, nearly always were consumed after 24 h and not during the period of observation. Consumption of Collembola species was infrequent and nothing to trouble about. Ultimately, the only cause for any concern to non-target species to arise from the Petri dish tests was feeding on eggs of *A. similata*, and as conclusively demonstrated by molecular analysis, *A. bipustulata* in the field consume these extremely seldom, if at all. Thus, the currently-accepted process of risk assessment did not identify non-target effects due to adult predation attending the introduction.

In using molecular methods to examine the diet of predators, several methodological issues are important to consider (Gariepy et al. 2007; King et al. 2008; Sint et al. 2011) such as choice of the gene region and size of amplicon, as mentioned above. Preserving the integrity of DNA in the sample between its collection and analysis is also important (King et al. 2008). Acetone was practical in the case of *A. bipustulata*, since DNA was extracted from whole insects, although beetles were observed to become rigid and so it likely will not serve well in studies where dissection of larger insect predators is contemplated. Intra-sample contamination during collection, and artificial inflation thereby of the proportion testing positive, can occur (Greenstone et al. 2011) but does not necessarily accompany the confinement of groups of predators in relatively small volumes (Chapman et al. 2010; Harwood 2008). If pitfall traps with preservative are used, it is conceivable that the preservatives will cause individual predators to void the contents of their guts and contaminate other individuals in the same trap (King et al. 2008). For these reasons *A. bipustulata* were transferred from dry pitfall traps

individually to vials for killing and preservation, and so the risk of contamination is believed to be negligible. Estimates of the proportion of predators in a sample that killed and consumed prey may in theory also be inflated by scavenging on individuals of that prey species that died of other causes (Foltan et al. 2005; Juen and Traugott 2005), but this is believed to have had a minimal contribution in the estimates for *A. bipustulata*. Another possible route of artificial inflation is so-called secondary predation, in which the assayed predator tests positive for prey even though it was another predator, which had first consumed the prey, which was consumed (Hosseini et al. 2008; Sheppard et al. 2005). The extent to which this occurred in this study is not known.

Interpreting the proportion of predators testing positive requires the half-life and its confidence limits be calculated (Greenstone et al. 2010); for *A. bipustulata* and a meal of young *D. radicum* eggs, this was estimated to be 15.1 h (12.2–18.2 h). This is middling compared to estimates derived for other species of predator and prey (Greenstone et al. 2007). These estimates depend on feeding mode, with sit-and-wait predators tending to exhibit longer half-lives than predators more mobile in their strategy of search (Greenstone et al. 2007). These half-life values depend also on the particular species involved, however, as significantly different half-lives are found among coccinellids feeding on coccinellid eggs (Gagnon et al. 2011). Having derived a half-life does not permit direct translation to the situation in the field. Higher temperatures experienced by the predator while digesting a meal tend to decrease the duration of detection by shortening the half-life (Hagler and Naranjo 1997; Hosseini et al. 2008; von Berg et al. 2008). Predators feeding on other prey subsequent to the meal targeted for detection does

not always influence probability of detection (Hosseini et al. 2008), but it can, and its effect may depend on what was eaten (Weber and Lundgren 2009)

Enthusiasm about predation of *Delia* larvae by *Aleochara* adults began with finding adults numerous in tunnels on infested roots (Barnard 1880), and the observation that the adults found there sometimes were consuming maggots (Gibson and Treherne 1916). *Aleochara bilineata* added to field cages with *D. radicum* larvae reduce the number of larvae surviving to pupation, and the more *A. bilineata* there are, the higher the weight of cabbage roots and shoots (Hartfield and Finch 2003), strongly suggesting they move down roots and feed on larvae. Adult *Aleochara* were for some time thought to be habitual predators of *D. radicum* eggs (Hughes 1959; Wishart et al. 1956), but as mentioned above, an important laboratory study showed eggs slightly buried in soil, as the majority are expected to be, are nearly invulnerable to discovery by the predators (Finch et al. 1999). For *A. bilineata*, Read (1962) found adults consume on average 23.8 eggs or 2.6 third instar *D. radicum* each day of their adult lives, demonstrating as long as the quantity of DNA in 10 eggs is about the same as that present in a large larva, that the feeding trial conducted with eggs is relevant also to feeding on larvae. With a detection half-life of 15.1 h, broad limits can be placed on rates of consumption in the more infested field encountered in 2009, when the proportion testing positive was around 0.20. This finding could arise if, out of five beetles: all five had eaten 5 to 10 eggs 20 h ago; four out of five had eaten 19 h ago; three of five had eaten 18 h ago; two of five had eaten 15 h ago; or, which is least probable, one in five had eaten and then immediately been captured and preserved. Therefore, the number of meals consumed by five beetles each day is probably three to six, each of about 10 eggs or a third instar larva. What is very

unlikely, given the results of the Petri dish trials and the results of molecular screening for the two species of carabid beetles, is that these beetles are eating as yet unknown species of prey. Both *B. quadrimaculatum* and *A. similata* have been documented in cabbage fields near the study sites (Freuler et al. 2001), and both are spring breeders (Thiele 1977) and so immature stages would have been present when the *A. bipustulata* were collected.

In summary, I have successfully employed current guidelines for developing a non-target test list and for screening members of that list to determine the risk associated with introducing a classical biological control agent. The potential utility of cutting edge molecular technology (Gariepy et al. 2007; Mills and Kean 2010; Sheppard and Harwood 2005; Symondson 2002) has been proven once again. This study has been the first to demonstrate *A. bipustulata* regularly consume immature stages of *D. radicum* in the field, and this in spite of finding the vast majority of *D. radicum* eggs were slightly below the soil surface, where they had been thought invulnerable to predation. Eggs laid in the soil adjacent to brassica roots may be more detectable and more accessible than those on a card that provides no surface cues or routes to the location of eggs. The specificity of *A. bipustulata* adults as predators was supported, as well as the high frequency with which the adults consume immature *D. radicum*. Canola growers in Canada can look forward to counting *A. bipustulata* among the insect species providing them important ecosystem services in the near future.

Table 1. Taxonomy, origin, and mass of organisms exposed in No-choice tests to determine *Aleochara bipustulata*'s fundamental host range. Total number of replicates performed with each taxon is listed, along with the proportion in which prey were consumed during the 10 minute observation period, over the course of 24 hours, and in total. (Continues on next page.)

				Proportion consumed			
Phylum Subphylum Class Subclass Order Family	Species [stage]	Origin‡	Mass (mg) ± s.e. (N)	in 10 min	in 24 h	Total	Replicates
Annelida							
Clitellata							
Oligochaeta							
Haplotaxida							
Enchytraeidae	sp. [mature]	A	1.67 ± 0.24 (15)	0.20	0.47	0.67	15
Lumbricidae	*Eisenia foetida* (Savigny) [juvenile]	B	17.53 ± 2.76 (25)	0.00	0.15	0.15	20
Arthropoda							
Chelicerata							
Arachnida							
Acari							
Sarcoptiformes							
Acaridae	*Tyrophagus putresentiae* (Schrank) [adult]	A	†	0.00	0.00	0.00	21
	Tyrophagus putresentiae (Schrank) [adult]	C	†	0.00	0.00	0.00	20
	Sancassania sp. [adult]	B	†	0.10	0.40	0.50	20
Prostigmata							
Microtrombidiidae	*Platytrombidium fasciatum* (Koch) [adult]	B	0.76 ± 0.07 (26)	0.00	0.00	0.00	20
Mesostigmata							
Parasitidae	*Parasitus* spp. [deutonymph]	B	†	0.00	0.05	0.05	20
Megoperculata							
Aranae Linyphiidae	*Erigone dentipes* (Wider) & *Meioneta rurestris* (C.L .Koch) [adult]	A	0.91 ± 0.10 (18)	0.00	0.00	0.00	18
Myriapoda							
Chilopoda	sp. 1 [juvenile]	B	0.87 ± 0.44 (3)	0.00	0.00	0.00	3
	sp. 2 [juvenile]	B	†	0.15	0.15	0.30	20
Diplopoda							
Helminthomorpha							
Julida							
Julidae	*Cylindroiulus* sp. [juvenile]	B	3.47 ± 0.36 (21)	0.00	0.00	0.00	20
Polydesmida							
Polydesmidae	*Polydesmus* sp. [subadult]	A	8.74 ± 0.71 (20)	0.00	0.00	0.00	20

Table 1. (continued)

Phylum / Subphylum / Class / Subclass / Order / Family	Species [stage]	Origin‡	Mass (mg) ± s.e. (N)	Proportion consumed in 10 min	Proportion consumed in 24 h	Proportion consumed Total	Replicates
Crustacea							
Malacostraca							
Eumalacostraca							
Isopoda	sp. [mancae]	B	1.6 ± 0.66 (20)	0.00	0.11	0.11	18
Hexapoda							
Entognatha							
Collembola	sp. [adult]	A	†	0.00	0.10	0.10	20
	sp. [adult]	A	†	0.00	0.06	0.06	18
Insecta							
Pterygota							
Hymenoptera							
Formicidae	*Lasius* nr. *brunneus* [adult]	A	1.94 ± 0.07 (28)	0.00	0.00	0.00	20
Heteroptera							
Aphidae	*Myzus persicae* (Sulzer) [adult]	A	0.55 ± 0.04 (26)	0.00	0.05	0.05	21
	Acyrthosiphon pisum Harris [adult]	D	2.89 ± 0.11 (21)	0.00	0.10	0.10	20
	Brevicoryne brassicae (L.) [adult]	A	†	0.00	0.15	0.15	20
Lepidoptera							
Plutellidae	*Plutella xylostella* (L.) [L_3]	A	0.72 ± 0.08 (20)	0.15	0.05	0.20	20
Diptera							
Anthomyiidae	*Delia radicum* (L.) [L_3]	E	3.56 ± 0.43 (15)	0.80	0.20	1.00	15
	D. radicum [puparium]	E	11.17 ± 0.41 (21)	0.00	0.00	0.00	20
Calliphoridae	*Lucilia silvarum* Meigen [L_3]	F	19.9 ± 2.08 (20)	0.00	0.10	0.10	20
Piophilidae	*Piophila casei* (L.) [L_3]	G	3.2 ± 0.27 (10)	0.20	0.80	1.00	10
Cecidomyiidae	*Dasineura brassicae* (Winnertz) [L_3]	H	†	0.18	0.64	0.82	11
Coleoptera							
Carabidae	*Amara similata* (Gyllenhal) [egg]	I	†	0.50	0.50	1.00	10
	A. similata [L_1]	I	0.5 ± 0.03 (19)	0.00	0.33	0.33	18
	A. similata [pupa]	I	27.2 ± 0.69 (21)	0.39	0.28	0.67	18
Elateridae	*Agriotes* sp. [larva]	A	11.4 ± 1.10 (20)	0.00	0.00	0.00	20
Curculionidae	*Ceutorhynchus obstrictus* (Marsham) [L_3]	H	3.5 ± 1.22 (11)	0.18	0.73	0.91	11

‡ A: cabbage field, Galmiz CH; B: compost pile, Delémont CH; C: peat soil, Winnipeg CA; D: laboratory colony, Delémont CH; E: laboratory colony, London CA; F: liver bait, Delémont CH; G: fish bait, Glenlea CA; H: canola pods, Ligniéres CH; I: canola field, Courcelon CH. The abbreviation CH is short for Switzerland, and CA is short for Canada. Delémont and Courcelon are in canton Jura, Lignieres is in canton Neuchatel, and Galmiz is in canton Bern; Winnipeg and Glenlea are in the province of Manitoba.

† too small to measure

Table 2. Oligonucleotide primers employed in this study, with optimized annealing temperatures (Ta) and product size.

Species	Primer	Sequence	T_a(°C)	Product size (bp)
Delia radicum	DrCOIF	5'-CATGCCTCGACGTTATTCAG	59.8	149
	DrCOIR	5'-GAAATAATACTTGTCGTTGTGA		
Bembidion quadrimaculatum	BqCOIF	5'-TTTTTATTTACTGTAGGAGGA	53.0	246
	BqCOIR	5'-AATCCTAAGAAATGTTGAGG		
Amara similata	AsCOIF	5'-TTATTAGACAAGAAAGAGGG	55.8	395
	AsCOIR	5'-ATAATAGCAAATACTGCTCCC		
'Universal'	C1-J-1751d	5'-GGAKCWCCWGAYATAGCWTTYCC	45.0	490
	C1-N-2191d	5'-CCHGGYAAAATTAAAATATAAACTTC		

Table 3. Species used to confirm specificity of primers designed for amplification of *Delia radicum*, *Amara similata*, and *Bembidion quadrimaculatum* cytochrome oxidase I. Readily available species were selected.

Order	Family	Species	Origin[b]	Amplifed with primers for (Y/N):		
				D. radicum	*A. similata*	*B. quadrimaculatum*
Diptera	Anthomyiidae	*Delia radicum*	Galmiz CH	Y	N	–[a]
		D. planipalpis	Winnipeg CA	Y	–	N
		D. platura	Shellbrook CA	N	–	–
		D. antiqua	London CA	N	N	N
	Piophilidae	*Piophila casei*	Glenlea CA	N	N	N
	Drosophilidae	*Drosophila melanogaster*	Bloomington US	N	–	–
Coleoptera	Carabidae	*Agonum cupreum*	Treesbank CA	N	N	N
		A. placidum	Treesbank CA		N	N
		Amara coelatus	Treesbank CA	N	N	N
		A. convexa	Treesbank CA	N	N	N
		A. similata	Courcelon CH	N	Y	N
		Bembidion nitidum	Treesbank CA	N	N	N
		B. quadrimaculatum	Galmiz CH	N	N	Y
		Carabus taedatus agasii	Treesbank CA	N	–	–
		Clivina fossor	Galmiz CH	N	N	N
		Clivina collaris	Galmiz CH	N	–	N
		Stenoplus conjunctus	Treesbank CA	N	–	–
		Synchus impunctatus	Treesbank CA	N	–	–
	Chrysomelidae	*Entomoscelis americana*	Binscarth CA	N	–	–
	Staphylinidae	*Aleochara bipustulata*	Kerzers CH	N	–	–
Lepidoptera	Plutellidae	*Plutella xylostella*	Winnipeg CA	N	N	N
Hemiptera	Aphidae	*Brevicoryne brassicae*	Galmiz CH	N	N	–
		Acyrthosiphon pisum	Delémont CH	N	–	–
		Myzus persicae	Galmiz CH	N	–	–
	Miridae	*Lygus lineolaris*	Landmark CA	N	–	N
Orthoptera	Acrididae	*Melanoplus bivittatus*	Delta CA	N	–	N
Hymenoptera	Formicidae	*Lasius* sp.	Galmiz CH	N	–	–
Araneae	Linyphiidae	*Erigone dentipalpis*	Galmiz CH	N	–	–

[a] not tested

[b] CH is short for the country of Switerland, CA is short for Canada, US is short for the United States of America. Galmiz is in canton Fribourg, Courcelon and Galmiz are in the canton Jura, and Kerzers is in the canton Bern. Shellbrook is in the province of Saskatchewan, London is in the province of Ontario, and Glenlea, Treesbank, Binscarth, Winnipeg, Landmark, and Delta are in Manitoba. The Bloomington *Drosophila* Stock Center at Indiana University is in the state of Indiana.

Table 4. Activity-density of adult *Aleochara bipustulata* in Swiss cabbage fields sampled 2009–11, densities of *Delia radicum* in the same fields, and proportions of *A. bipustulata* that tested positive for *D. radicum* DNA in PCR assays. In 2009, 16 pitfall traps were maintained and 40 plants sampled on each date; in 2010 and 2011, 24 traps and 48 plants were sampled. Activity-densities are means ± standard error. *Delia radicum* densities are averaged over the numbers, indicated in the table, of plants infested with eggs and with any instar of larvae.

					Delia radicum eggs			*Delia radicum* larvae			
									Larvae/plant		
Year	Location	Date	Activity Density 100*Beetles/trap/4 h	Proportion positive (N)	# plants with	Density	Proportion on surface	# plants with	L_1	L_2	L_3
2009											
	Galmiz	3 June[a]	37.5± 9.71	0.17(18)	18	2.8±0.49	0.00	0	0.0±0.00	0.0±0.00	0.0±0.00
		10 June[a]	89.5±26.17	0.26(43)	12	2.9±0.61	0.00	2	2.0±1.00	2.0±2.00	4.0±4.00
		17 June[a]	125.0±25.53	0.23(60)	25	3.2±0.68	0.04	9	0.7±0.17	0.6±0.24	0.1±0.10
		24 June[a]	150.0±31.93	0.17(72)	29	3.2±0.77	0.03	27	0.6±0.18	1.9±0.46	1.0±0.30
		1 July	40.6± 8.45	0.21(39)	33	6.0±1.12	0.07	34	1.4±0.51	1.7±0.27	2.1±0.39
		8 July	66.7±11.91	0.27(64)	34	7.7±1.04	0.14	35	0.9±0.20	2.1±0.44	3.0±0.52
		15 July	47.9± 9.93	0.28(46)	33	5.5±0.80	0.08	31	1.3±0.29	1.0±0.19	2.4±1.04
		22 July	8.3± 3.20	0.25 (8)	14	3.7±0.70	0.00	26	0.4±0.14	0.5±0.26	1.5±0.33
2010											
	Galmiz	26 May	7.6± 2.43	0.00(11)	3	1.0±0.00	0.00	0	0.0±0.00	0.0±0.00	0.0±0.00
		2 June	17.4± 3.73	0.00(25)	1	1.0±0.00	0.00	0	0.0±0.00	0.0±0.00	0.0±0.00
		9 June	10.4± 3.51	0.00(15)	1	1.0±0.00	0.00	5	1.6±0.40	1.0±0.55	0.0±0.00
	Wädenswil	21 July	1.4± 0.98	0.50 (2)	18	2.7±0.36	0.00	–	–	–	–
	Galmiz 2	24 July	17.4± 5.06	0.08(25)	10	1.3±0.21	0.02	–	–	–	–
	Jerisberghof	30 July	11.8± 3.34	0.06(17)	8	1.8±0.31	0.00	–	–	–	–
		6 Aug.	17.4± 5.06	0.19(25)	2	1.0±0.00	0.00	–	–	–	–
2011											
	Sugiez	18 May	7.6± 2.80	0.09(11)	0	0.0±0.00	–	0	0.0±0.00	0.0±0.00	0.0±0.00
		25 May	2.1± 1.19	0.00 (3)	0	0.0±0.00	–	0	0.0±0.00	0.0±0.00	0.0±0.00
		1 June	0.7± 0.69	0.00 (1)	1	2.0±0.00	0.00	1	0.0±0.00	0.0±0.00	1.0±0.00
		8 June	6.9± 2.90	0.10(10)	7	1.9±0.46	0.00	7	0.9±0.34	1.4±0.69	0.1±0.14
		15 June	43.8± 6.81	0.14(63)	6	1.3±0.21	0.25	24	0.3±0.14	0.9±0.21	1.3±0.28
		22 June	5.6± 2.15	0.25 (8)	8	2.6±0.56	0.05	23	0.1±0.06	0.7±0.20	1.3±0.24
		29 June	2.8± 1.37	0.00 (4)	4	4.8±3.42	0.00	16	0.0±0.00	0.6±0.36	1.8±0.51
		6 July	1.4± 0.98	0.00 (2)	2	2.5±0.50	0.00	7	0.0±0.00	0.4±0.30	1.4±0.53

[a] Dates when sampling was for three 4 h periods from 1600 to 400. On other dates there were six 4 h sampling periods

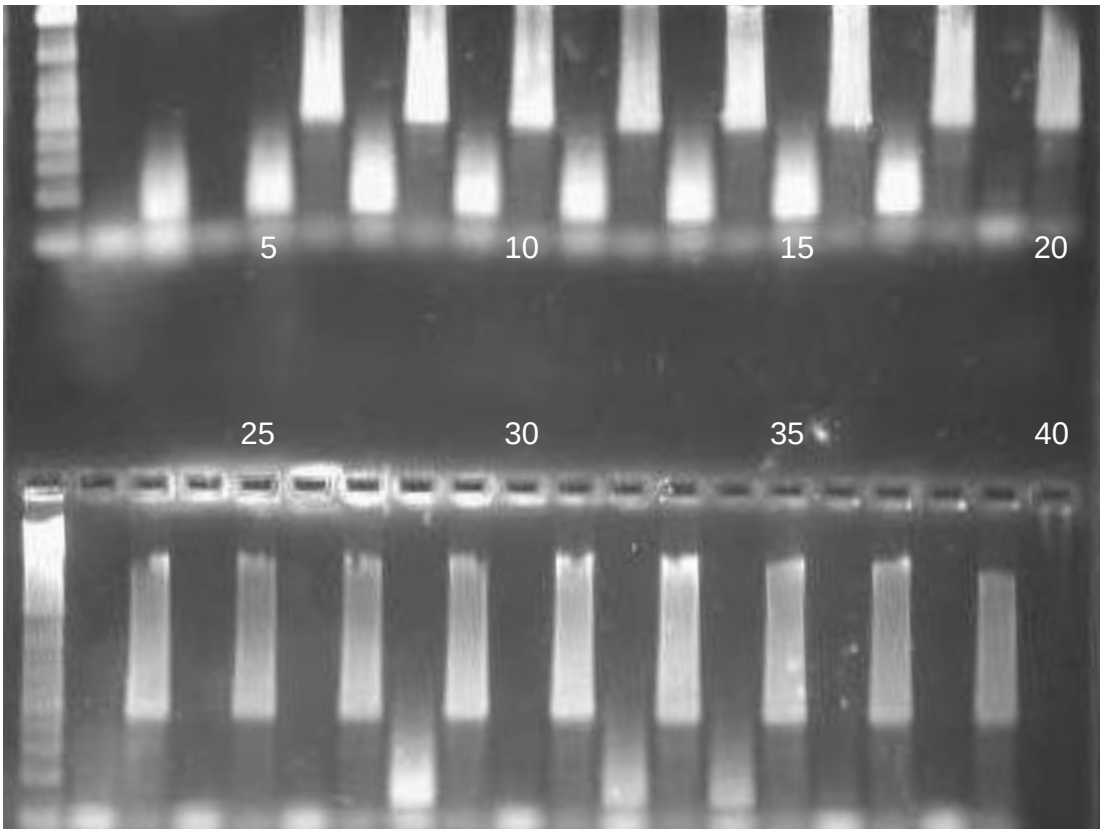

Figure 1. Image of 2% agarose gel run 60 minutes at 100 V to diagnose *Aleochara bipustulata* predation of *Delia radicum*. Lanes 1 and 21 are GeneRuler 1 kb Plus DNA Ladder (Fermentas), with the bottom five bands denoting 75, 200, 300, 400, and 500 bp. Lanes 2 and 4 are no-template controls for *D. radicum* and universal primers, respectively. Lane 3 is a positive control, with *D. radicum* template and primers. Starting at lane 5 are pairs, *A. bipustulata* tested for *D. radicum* and universal primer reactions for each of 17 samples. All control reactions for samples scored positive, and positive reactions for *D. radicum* were scored for samples 1–7 (lanes 5–8), 12 (lanes 28–29), 14 (lanes 32–33), and 15 (lanes 34–35).

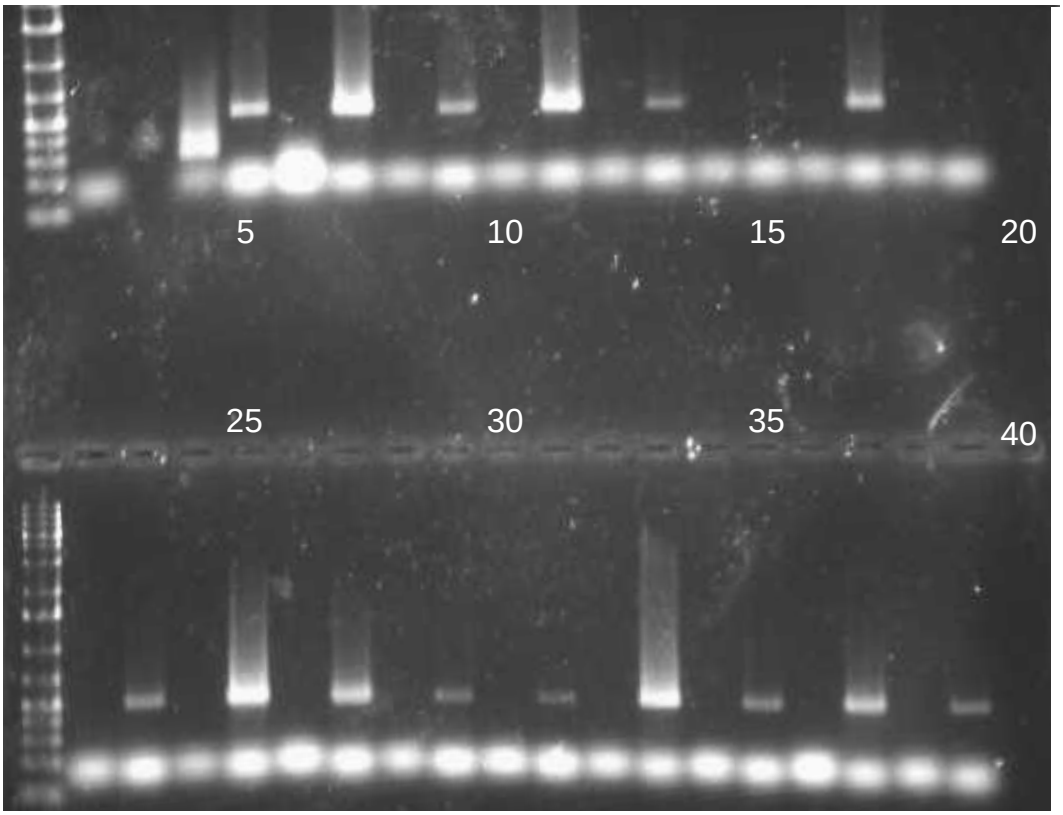

Figure 2. Image of 1.5% agarose gel run 50 minutes at 100 V to diagnose *Aleochara bipustulata* predation of *Bembidion quadrimaculatum*. Lanes 1 and 21 are GeneRuler 1 kb Plus DNA Ladder (Fermentas), with the bottom five bands denoting 75, 200, 300, 400, and 500 bp. Lanes 2 and 3 are no-template controls for *B. quadrimaculatum* and universal primers, respectively. Lanes 4 and 5 are positive controls, with *B. quadrimaculatum* and universal primers, and *B. quadrimaculatum* template. Starting at lane 6 are pairs of *A. bipustulata* tested for *B. quadrimaculatum* and universal primer reactions for each of 16 samples. All control reactions for samples scored positive except samples 5 and 7, which did when tested a second time. No samples scored positive for *B. quadrimaculatum*.

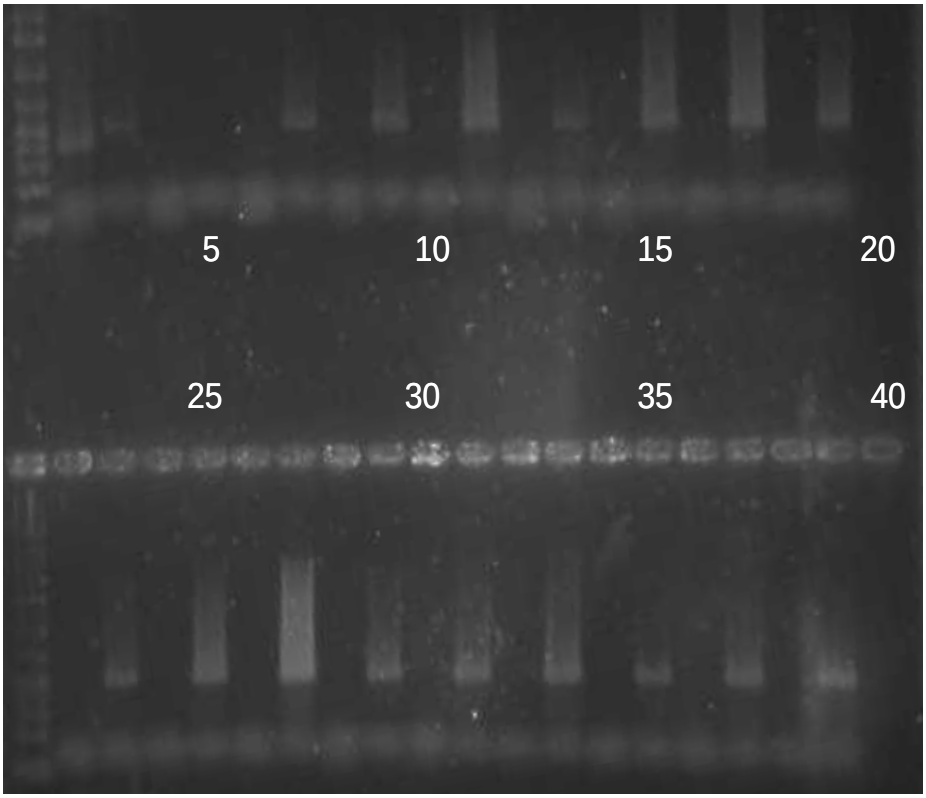

Figure 3. Image of 1.5% agarose gel run 50 minutes at 100 V to diagnose *Aleochara bipustulata* predation of *Amara similata*. Lanes 1 and 21 are GeneRuler 1 kb Plus DNA Ladder (Fermentas), with the bottom five bands denoting 75, 200, 300, 400, and 500 bp. Lanes 2 and 3 are positive control reactions for *A. similata* and universal primers, respectively. Lanes 4 and 5 are negative controls with *A. similata* and universal primers, and water template. Starting at lane 6 are pairs of *A. bipustulata* tested for *A. similata* and universal primer reactions for each of 16 samples. All control reactions for samples scored positive, and no samples scored positive for *A. similata*.

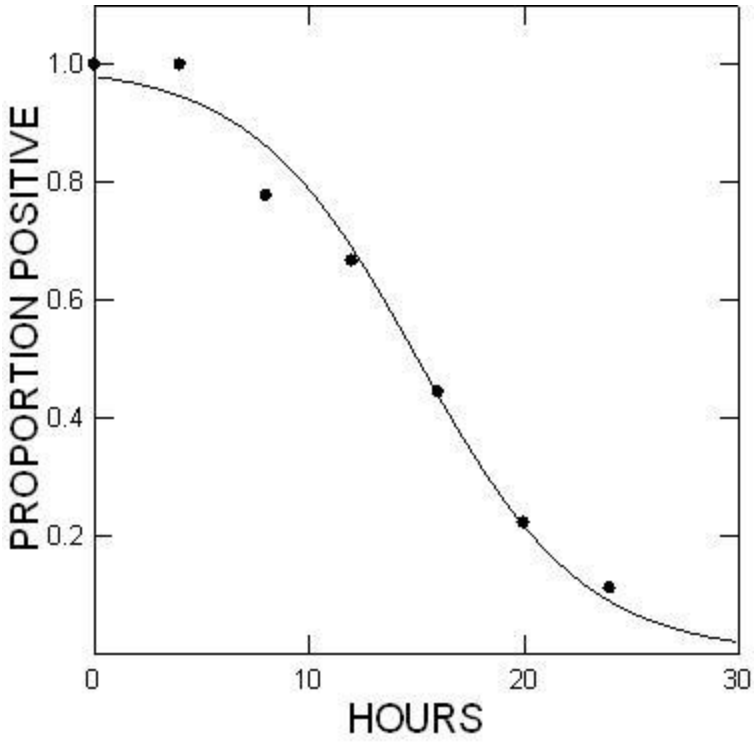

Figure 4. Observed (dots) and modeled (line) proportion of *Aleochara bipustulata* with detectable *Delia radicum* in relation to time since a meal of eggs. Neither the interaction of meal size with time since the meal (L.R. $\chi^2_1 = 1.0$, $P = 0.32$), nor meal size (L.R. $\chi^2_1 = 0.1$, $P = 0.76$) were significant. The term for time since the meal was significant (L.R. $\chi^2_1 = 33.6$, $P<0.001$). The model, with constant = 3.91 and regression coefficient = -0.26, was an adequate fit to the observations (Hosmer-Lemeshow C=1.30, d.f.=5, P=0.93).

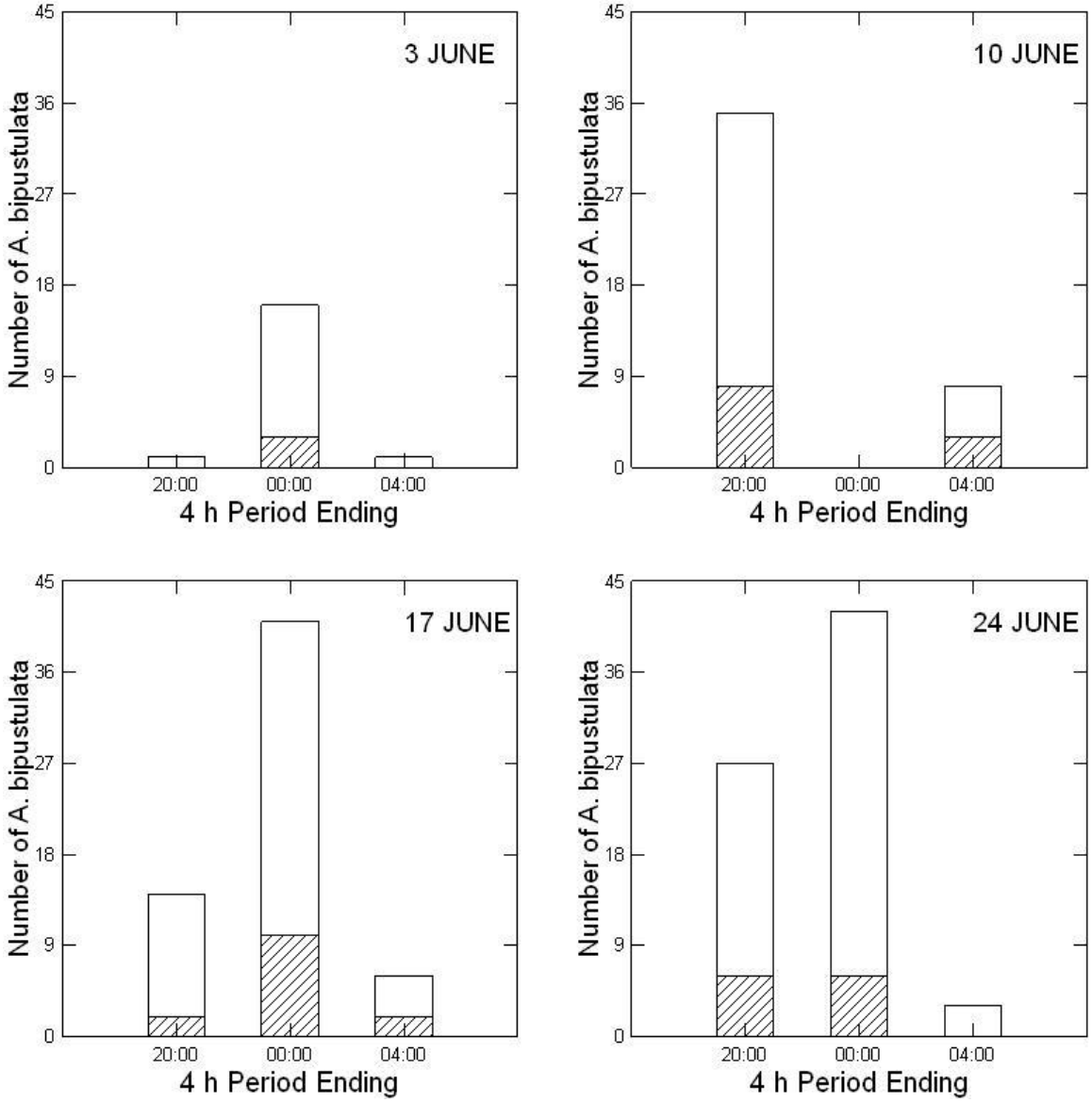

Figure 5. Number of *Aleochara bipustulata* caught in traps on 3, 10, 17, and 24 June 2009 during each of three 4 h periods, and number that tested positive for *Delia radicum* DNA. Bars represent the total capture during the period, and the hatched portion represents the number of beetles testing positive.

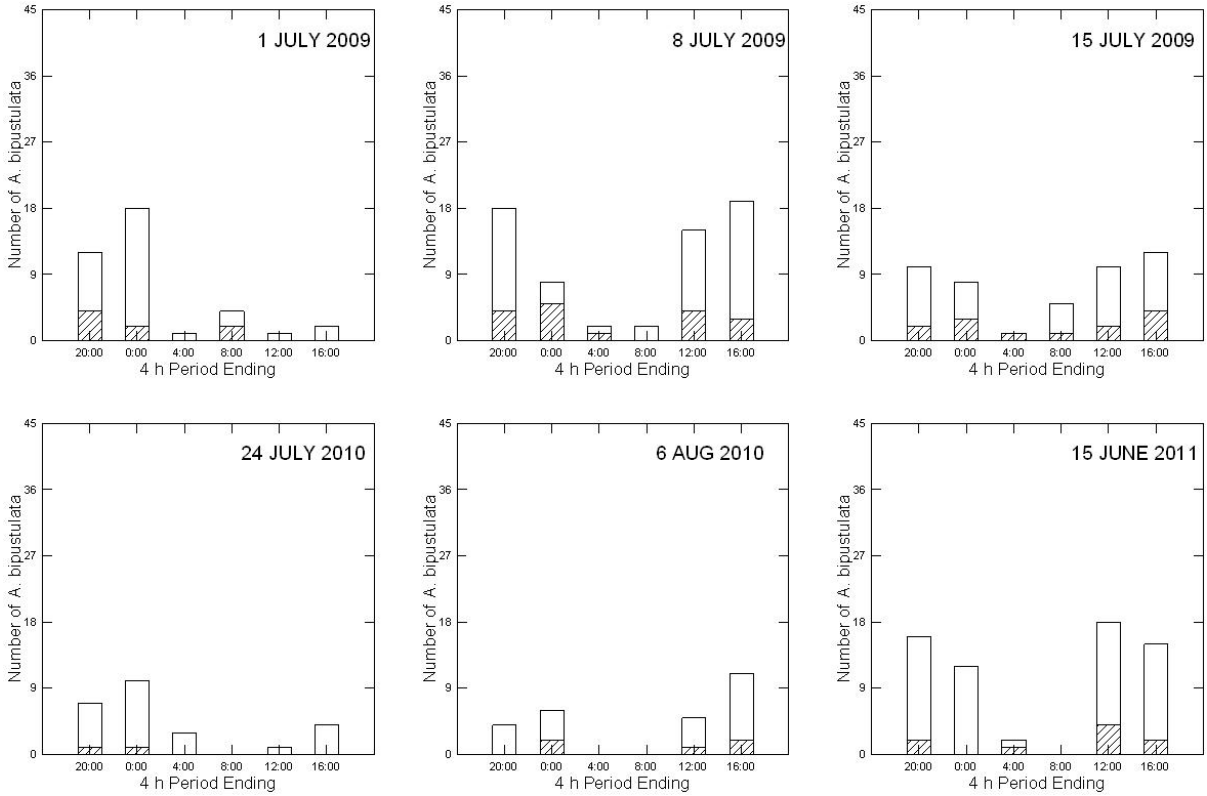

Figure 6. Number of *Aleochara bipustulata* caught in traps on 1, 8, and 15 July 2009; 2 June, 24 July and 6 August 2010; and 15 June 2011, during each of six 4 h periods, and number that tested positive for *Delia radicum* DNA. Bars represent the total capture during the period, and the hatched portion represents the number of beetles testing positive.

Chapter 3 Section 2

Predation of Delia radicum eggs by three epigaeic beetle species

Introduction

The cabbage maggot, *Delia radicum* (L.) (Diptera: Anthomyiidae), was first reported on the Canadian prairies in 1958, infesting roots of *Brassica napus* L. (Cruciferae) plants grown in rapeseed breeding programs (Allen 1964). Introduced from Europe (Biron et al. 2000), *D. radicum* had for decades been recognized as a pest of crucifer vegetables in eastern North America and in British Columbia (Gibson and Treherne 1916; Soroka et al. 2002). Having wintered as pupae, adults disperse several kilometres (Finch and Skinner 1975), then females lay eggs. Eggs are primarily laid below the soil surface, especially in cracks and crevices (Schoene 1916), with one or two eggs left by individual females per deposition, and possibly more than one deposition in a female's visit to a plant (Kostal et al. 2000). Typical peak egg density in vegetables is around 35 per plant (Finch et al. 1975), while eggs in prairie canola tend to be fewer per plant, but the plants are much more dense (Dosdall et al. 1994; Hummel et al. 2009). Infestations have become more severe as more canola is grown (Soroka et al. 2004), and no insecticides are registered for root maggot control in canola (Soroka and Dosdall 2011). Non-native natural enemies are known to be important mortality factors of other alien insect pests in the Canadian prairies (Dosdall et al. 2011), and cabbage maggot may be another promising classical biological control target (Andreassen et al. 2007; Soroka et al. 2002; Turnock et al. 1995).

Before introducing natural enemies of arthropod pests to new areas, competitive interactions among species should be examined to minimize the disruptive influence of competition on control (Mills 2006; Sweetman 1936). Complementary laboratory and field cage assays can be informative for these assessments (Hoelmer and Kirk 2005; Messing et al. 2006). For *D. radicum*, there is but one promising candidate for

introduction, the staphylinid, *Aleochara bipustulata* L. (Coleoptera: Staphylinidae) (Hemachandra et al. 2005; Hemachandra et al. 2007a). Adult *A. bipustulata* consume *D. radicum* eggs and larvae, while *A. bipustulata* larvae develop as parasitoids in *D. radicum* puparia (Fuldner 1960). As a predator, *A. bipustulata* will, if introduced, potentially compete with *A. bilineata* Gyllenhal, habits of which are quite similar (Fuldner 1960), and with adults of small carabid predators such as *Bembidion quadrimaculatum* L. (Wishart et al. 1956). Given the broad European region of sympatry of these three epigaeic predator species, competitive displacement of the other species is unlikely. Attention focused instead on whether mortality due to predation of eggs is likely to be affected.

Individual predators exhibit a functional response when number of prey attacked is influenced by prey density (Solomon 1949). When multiple predators interact, unanticipated impacts may emerge, and the risk to prey may be enhanced or reduced compared to risk predicted from the predators acting alone (Letourneau et al. 2009; Sih et al. 1998; Welch et al. 2012). Additive series experimental designs, in which the density of a given predator species is constant across treatments, and replacement series designs, in which the total predator density is constant across treatments, generate predictions of the impact of multiple predators (Sih et al. 1998; Straub and Snyder 2006), and predictions from the two approaches can differ (Griffen 2006). Complementary use of both approaches is advisable (Griffen 2006; Straub and Snyder 2006).

Before undertaking to predict the outcome of interactions between *A. bipustulata* and similar species it will encounter in North America, a better understanding of the predation process is needed. Encountering unlimited *D. radicum* eggs on the floor of a

Petri dish, adult *Aleochara* and *Bembidion* will consume at least 10 eggs per day (Finch 1996; Langlet and Brunel 1996; Read 1962). Female *Aleochara* can consume more than males (Langlet and Brunel 1996), and female *A. bilineata* and *A. bipustulata* 10–20 d old can consume about 100 per day (Fournet et al. 1999). Eggs naturally will be within more complex backgrounds, and in particular the behaviour of ovipositing *D. radicum* females means the majority of eggs are slightly below the soil surface. Search for these eggs is more challenging, and rates of consumption much lower (Finch and Elliott 1999; Finch et al. 1999).

The two objectives of this study were to investigate further the effect of a soil layer on predation levels by *A. bilineata*, *A. bipustulata*, and *B. quadrimaculatum*, and to look for multiple predator effects. The first objective was addressed by a functional response experiment in the laboratory. Two studies were conducted to address the second objective. In the first, a laboratory study was conducted in which multiple predator treatments were compared to the predictions of both additive and replacement series. The second study was in field cages, with an additive series to compare with multiple predator treatments.

Materials and Methods

Insects for assays

Adult *A. bipustulata* and *A. bilineata* for use in assays were from laboratory colonies, maintained with immature *D. radicum* as hosts for larvae and as food for adults, as described in greater detail elsewhere (Andreassen et al. 2009). The *A. bipustulata* colony was initiated from collections in vegetable *Brassica* fields around Kerzers, Switzerland, and the *A. bilineata* from collections in canola stubble in southern Manitoba,

Canada. The *D. radicum* colony, maintained following Whistlecraft et al. (1985b), originated with puparia from the Agriculture and Agri-Food Canada's Southern Crop Protection and Food Research Centre in London, Ontario, Canada. A supply of fresh *D. radicum* eggs of known age was maintained by floating eggs laid each day in washed sand around a slice of rutabaga in water, and passing the water through a paper filter. The *B. quadrimaculatum* were collected as adults from Swiss *Brassica* fields, and kept for up to two weeks with crushed dry cat food in Petri dishes with moist filter paper lining the bottom before being used in the assays.

Effects of 1°C egg storage on viability and consumption

To reduce the difficulties of synchronizing predator and prey availability, it was desirable to be able to store *D. radicum* eggs. Two experiments were performed on the effects of storing fresh *D. radicum* eggs. The first experiment determined the effect of cold storage up to 4 d on egg viability. Eggs were collected daily and held at 1 °C in darkness on wet filter paper. Ten replicate Petri dishes with wet filter paper lining the bottom were prepared, and on the paper were 15–25 eggs for each storage period of 0, 1, 2, 3, and 4 d. The dishes were subsequently kept 7 d at 20 °C with 16 h light per day. Hatched eggs were counted each day, and the total proportion to hatch was arcsine square root transformed prior to analysis. The 10 d on which replicates were prepared were treated as a blocking factor in an analysis of variance, and mean proportions hatching after 0, 1, 2, 3, or 4 d of cold storage were compared with Tukey's Honestly Significant Difference (HSD) test. In the second experiment, eggs stored 0–4 d at 1 °C were used. Ten eggs of each storage period were transferred to the wet filter paper lining the bottom of a 9 cm diameter Petri dish, so that there were five equidistant clusters of eggs, each

occupying about 1 cm^2, about 3 mm away from the dish's wall. Within groups, eggs were not in contact. One beetle, fed for the previous day on cat food, was released in each dish, and the proportion of eggs consumed in each group after 24 h at 20 °C (16:8 h light:dark) recorded. Ten beetles of each species were tested, and individual beetles used as blocking factors in ANOVA tests of hypotheses that the proportions consumed were independent of period at 1 °C. Again proportions were arcsine square root transformed prior to analysis.

Functional responses—buried vs. *exposed eggs*

The functional responses of adult *A. bipustulata, A. bilineata,* and *B. quadrimaculatum* to density of *D. radicum* eggs were investigated for eggs exposed on the soil surface and for eggs buried slightly below, in assays with potted cabbage seedlings. Seedlings with three or four true leaves were planted individually in the centre of plastic pots, 12 cm tall and 15 cm diameter, in an 8:30 mixture of sand and potting soil to leave a 2 cm rim between the top of the pot and surface of the soil. A 1 x 1 cm peat card (Prasad and Snyder 2004), touching the plant stem and flush with the soil surface, held 1, 3, 5, 9, 13, 17, 21 or 35 eggs on its surface. Cards were soaked overnight in water so eggs would adhere, and eggs had been accumulated for up to 4 d at 1°C. The cards were left exposed, or buried slightly in a dried preparation of the potting mixture from which the buoyant fraction had been removed, by sprinkling a pinch slowly and evenly over the card just to the point at which the eggs were no longer visible (Figure 7). One beetle, which had spent at least the previous 24 h in a dish with cat food, was released on the soil surface. Sexes of beetles were not distinguished. The pot was then sealed in a bag made from white nylon curtain material, held 20 cm above the top of the pot by a U-

shaped wire and secured snugly to the pot's outer surface with elastic bands. The pots were kept in a room with 16 h cool white fluorescent light per day and ambient temperatures (20–27°C) for 24 h, then the eggs remaining were counted. For cards with buried eggs, this involved gently agitating the card in water in a 9 x 1.5 cm glass dish to separate eggs from soil, and picking the eggs from the surface with a paintbrush. Because all eggs were recovered using this technique in a preliminary experiment with five plants at each of six densities and with no predators, all eggs not recovered in trials with predators were assumed to have been eaten. Eight replicate assays were performed for each combination of egg density, egg exposure, and predator species. Assays performed on a particular day were assigned at random.

Analysis followed a recommended two-step procedure, performed in SAS based on code accompanying Juliano (2001). The first step involves establishing the type of functional response from the linear parameter (p_1) estimated by fitting the logit model for proportion surviving as a function of initial density,

$$g(x) = \frac{p_0 + \mathrm{p}_1 + \mathrm{p}_2\mathrm{N}_0^{\,2} + \mathrm{p}_3\mathrm{N}_0^{\,3}}{1 - \mathrm{p}_0 + \mathrm{p}_1\mathrm{N}_0 + \mathrm{p}_2\mathrm{N}_0^{\,2} + \mathrm{p}_3\mathrm{N}_0^{\,3}}$$

A non-significant p_1 indicates a Type I response:

$$N_e = aT\mathrm{N}_0$$

where N_e is the number of eggs eaten during the time period (T = 24 h), a the rate of attack (#/h), and N_0 the initial number of eggs on the card. A significant negative p_1 indicates a Type II response:

$$N_e = \frac{aTN_0}{(1 + aTN_0)}$$

where T_h is the handling time (h), and a significant positive p_1 indicates a Type III response, the attack constant becomes a hyperbolic function of N_0:

$$a = \frac{d + bN_0}{1 + cN_0}$$

and the number eaten is predicted by:

$$N_e = \frac{dN_0T + bTN_0^{\,2}}{(1 + cN_0 + dN_0T_h + bT_hN_0^{\,2})}$$

Nonlinear regression is used in the second step, to estimate the parameters of the appropriate model. Regressions were run on implicit functions, because eggs were not replaced as they were eaten. The parameters *c* and *d* were removed from Type III models whenever the asymptotic 95% confidence intervals included 0 (Juliano 2001). *F* tests for significance of the functional response models, and for their lack of fit, determined whether the regressions accounted for significant proportions of variation in number of eggs eaten among densities, given the variation within densities (Sokal and Rohlf 1981).

Laboratory assays—additive and replacement series competition predictions compared

For this study, the experimental unit was a circular metal pie plate with outward sloping 3 cm tall wall, diameter 30 cm at the top and 27 cm at the bottom. The floor was lined with the soil used in pots above, but passed first through a 5 mm sieve, dried, then moistened with 250 ml water/L soil. This soil layer was about 4 mm thick, so that it was level with four peat cards resting on the floor of the pan. Cards were 10 cm from their nearest neighbours and equidistant between the centre of the pan and the edge. Each card held 5, 10, 15, or 20 eggs; these were buried as described above. Predators were released in the centre, and then a lid of white curtain mesh material glued to a 2 cm cardboard ring was secured with masking tape to the pan. Pans were kept in a room with 16 h light per

day and about 20 to 28°C temperatures for 24 h before counting the number of eggs remaining on each card. Treatments were: 1♂1♀ *A. bipustulata*; 1♂1♀ *A. bilineata*; 1♂1♀ *B. quadrimaculatum*; 2♂2♀ *A. bipustulata*; 2♂2♀ *A. bilineata*; 2♂2♀ *B. quadrimaculatum*; 1♂1♀ *A. bipustulata* + 1♂1♀ *A. bilineata*; 1♂1♀ *A. bipustulata* + 1♂1♀ *B. quadrimaculatum*; 1♂1♀ *A. bilineata* + 1♂1♀ *B. quadrimaculatum*. Sexes were distinguished by comparing the dorsal abdominal segments of beetles, immobilized gingerly between microscope slides and cover slips, with illustrations of genitalia (Drea 1966; Holliday 1977). There were 20 replicate pans for each treatment.

The number of eggs recovered was subject to analysis of variance followed by orthogonal contrasts between high and low densities of the single species treatments. The number of eggs recovered from plates with interspecific predator treatments was compared to numbers expected from both additive and replacement designs to test for multiple predator effects. Comparisons were made based on the whole plates (50 eggs), and on each of the four densities. To stabilize variances for estimates of mean squares within groups, the number of eggs recovered (y) from cards originally holding 15 and 20 eggs was transformed $y^{1.5}$, and for 10-egg cards and the whole plates y^3. Expected values for the additive and replacement designs ($E_{1,2}$) were based on formulae from Griffen (2006); for the additive design:

$$E_{1,2} = N_1 \text{ x } N_2/N_c$$

where N_1 and N_2 are the numbers recovered from the low density intraspecific predator treatments plates, and N_c is the density initially on the card, or in the whole arena (*i.e.* eggs not recovered were enumerated as consumed, and disappearance of eggs in the absence of predators assumed negligible). For the replacement design:

$$E_{1,2} = (N_{1,1} \times N_{2,2})^{0.5}$$

where $N_{1,1}$ and $N_{2,2}$ are the numbers recovered from the high density intraspecific predator treatment plates. Expectations were derived from average values of egg recoveries in single species treatments as the experimental design was completely randomized.

Field cage assays

Predation of eggs by different combinations of the three predator species was studied in field cages in a grassy field outside Delémont, Switzerland. A split plot design was used, with the cages as whole plots receiving predator treatments, and the egg cards placed by individual plants as the split plots. The experiment was repeated 12 times in June –August 2009–2011, and so time was a blocking factor, with eight cages per block. Cages were 60 x 60 x 180 cm tall, of nylon mesh closed on all six sides, and rested inside wooden frames 30 cm tall that prevented cages collapsing from the 20 cm deep layer of sand/soil mixture in their base. Twenty-five canola seedlings were transplanted, 10 cm from one another and from the cage wall, and allowed to grow to the rosette stage, when treatments were applied. The eight treatments comprising a block were three male/female pairs of the three species alone, the three possible pair-wise combinations of the three species with three pairs of each, a cage with three pairs of all three species, and a no predator control. An egg card, with eggs slightly buried, was in contact with each plant stem. Cards held 1, 3, 5, 7, or 9 eggs, each represented once per row and per column of plants in a Latin square arrangement. Predators fed cat food until released into cages were allowed 48 h to forage, then eggs remaining on cards were counted. A clear plastic tarp was suspended above the cages to prevent rain washing eggs from cards. Treatments

were assigned randomly to cages, and a new Latin square for arrangement of egg densities was generated for each cage.

For analysis, the proportion of eggs recovered was arcsine square root transformed prior to ANOVA. Factors were tested for significance by comparison with mean squares of their interactions with the random block effect (Snedecor and Cochran 1980). This involved calculating the variance for a 'plant position' effect, with 24 degrees of freedom, for testing factors applied to subplots. Planned contrasts for whole plot effects compared recovery in control cages with treatment cages, and among the single species treatments between the two *Aleochara* species, and between the average *Aleochara* effect and *B. quadrimaculatum*, with Bonferroni correction to critical *F* values to account for non-orthogonality. Tukey's HSD test was used to compare means of factors applied to subplots. Paired *t*-tests were used to test for differences between recovery in the multi-species cages and the expectations (as calculated above) from the additive design; for the cages with all predators:

$$P_{expected} = (P_{Abip} \times P_{Abil} \times P_{Bq}) / P_{ctrl}$$

Where $P_{expected}$ is the expected proportion of eggs recovered, P_{Abip} is the proportion observed to have been recovered in the cage with *A. bipustulata* alone, P_{Abil} the proportion in the *A. bilineata* alone cage, P_{Bq} the proportion from the *B. quadrimaculatum* cage, and P_{ctr}l the proportion from the control cage.

Results

Effects of 1°C egg storage on viability and consumption

Duration of storage at 1°C affected the proportion to hatch ($F_{4,36} = 13.5$, $P < 0.001$). Compared to unstored eggs, storing reduced the mean proportion to hatch, but the

four durations of storage were equivalent in their effect (Figure 8). The proportion of eggs consumed was independent of cold storage period for all three beetle species (Figure 9).

Functional responses—buried vs. *exposed eggs*

Logistic regression showed the most appropriate functional response model for *A. bipustulata* encountering *D. radicum* eggs on the soil surface to be Type I, and to be Type III for the other combinations of predator species and egg placement (Table 5). For all Type III responses, the parameter b was adequate to describe change with egg density in the rate of attack (Table 5). All six models described significant variation in the number of eggs consumed among densities, and only for *A. bilineata* consuming exposed eggs was there lack of fit (Table 5). The fit of this *A. bilineata* functional response to low densities of eggs is clearly superior to the fit at densities above nine, where egg consumption tends to be overestimated (Figure 10). Neither fitting a model to just the mean values, nor estimating the parameters one at a time while holding the other fixed, were successful strategies to improve this fit. For all three species an asymptote in the number of eggs consumed was more apparent for buried eggs than exposed eggs, where consumption levels appear still to be increasing at the highest densities (Figure 10). Buried eggs at the lower densities seldom were consumed at all.

Laboratory assays—additive and replacement series competition predictions compared

Intraspecific comparisons of egg recovery are illustrated in Figure 11. Fewer *D. radicum* eggs were recovered from plates with high densities of either *Aleochara* species than from plates with low densities. This pattern, although not always significant, was evident at the different densities as well. The carabid consumed neither more nor fewer

eggs in plates, or at any egg density, when more beetles were present. Among the three interspecific treatments, there were no differences between the number of eggs recovered from plates and the numbers predicted based on either experimental design (Figure 12), and so no multiple predator effects were detected overall. A degree of risk reduction was observed, whenever *A. bilineata* and *A. bipustulata* were combined, with more eggs recovered than expected at three of four densities. Risk enhancement was the trend whenever *B. quadrimaculatum* was combined with either *Aleochara* species, although this was significant only for the 10-egg cards encountered by *B. quadrimaculatum* and *A. bilineata* (Figure 12).

Field cage assays

There was nearly complete recovery of eggs from control cages (Figure 13), and so there was little variation in the proportion recovered. To avoid modelling a treatment with essentially no loss, variance due to predator treatment was partitioned into that between the control treatments and the seven treatments with predators (Table 6). Predator treatments significantly affected the proportion of eggs recovered from cages, and control cages differed from cages to which predators had been added (Table 2). Consistent with a non-significant among treatment *F* statistic for treatments with predators added (Table 6), more sensitive contrasts showed that among the single species treatments, as many eggs were recovered from *A. bilineata* cages as from *A. bipustulata* cages, and as many eggs were recovered from the *Aleochara* cages, on average, as from the *B. quadrimaculatum* cages (Figure 13). In all treatments with multiple predator species, more eggs were recovered than expected based on single predator species treatments, indicating risk of predation is reduced (Figure 12).

At the subplot level, egg density, plant row, and plant column all significantly influenced the proportion of eggs recovered from cards (Table 6). The response to egg density was modeled as linear, as higher order terms and their interactions with treatment were not significant. The higher the density of eggs on cards, the greater the proportion of eggs that disappeared (Figure 14). The effects of row and of column were uniform across treatments, even when control cages were excluded from the analysis (Table 6). Among rows, more eggs disappeared in the most southern row than some others, and among columns disappearance was higher in the western row than some others (Figure 14).

Discussion

The functional response study confirmed the risk-reducing impact of burying *D. radicum* eggs (Finch and Elliott 1999; Finch et al. 1999) for all three species of predator tested. A similar study with eggs of the carrot fly also found predation by epigaeic beetle predators to be reduced by a thin covering of sand (Burn 1982). Buried cards frequently were not discovered at all, especially at lower densities. At higher densities the *Aleochara* species consumed more than *B. quadrimaculatum*. Adult *Bembidion*, having encountered prey, modify their foraging behaviour and search the site of encounter more intensively (Grafius and Warner 1989; Mitchell 1963). It is likely that adult *Aleochara* change their behaviour in a similar fashion. The higher levels of consumption at higher egg densities by adults of the *Aleochara* species than adults of *B. quadrimaculatum* suggest either that adult *Aleochara* search intensively in a restricted area for longer than the adult *B. quadrimaculatum*, or that *Aleochara* adults are more likely to rediscover and return to an egg card over the 24 h trial period, or both. Significant models, without evidence of lack of fit, were estimated for all three beetle species encountering buried eggs. Juliano's

(2001) procedure did not perform very well for *A. bilineata* consuming eggs exposed on the surface; perhaps a model in which the distribution of number consumed at each density is other than normal (Fenlon and Faddy 2006) would be better. However, the result may be a consequence of eggs being unevenly distributed within the arena and limited to cards; in these conditions searching may be not very well described by the 'area of discovery'. That the models do not describe very well the process of egg predation is further suggested by the handling times in the four other Type III models, all of which were estimated to be more than one hour. In the laboratory the consumption of an egg requires about one minute before a beetle from any of these species moves again. Attempts to restrict the handling time estimates to near one minute failed; the search for parameters reached the limit, then stopped. Since handling and digesting prey are distinguishable (Jeschke et al. 2002), but are not distinguished in the models estimated here, it is sensible that the estimated handling times should be higher than the time to consume (but not digest) one egg. The difference is quite marked, though, being for instance nearly five hours for *B. quadrimaculatum* foraging for buried eggs. Continuous observation (Jervis 2007) would no doubt have been more informative, except the beetles' tendency to burrow in soil would require some technical accommodation. The Type III model still is the best available to describe the sigmoid shape caused at the lower density end by the probability of any eggs being discovered being quite low, but at any density above 1/card, once one egg is found, more are likely to be found shortly after by restricted area search. This has been described well for beetles searching for carrot fly eggs (Burn 1982).

The *b* parameters, describing the slope of area of discovery as a function of initial density, tended to be higher for a predator foraging for exposed eggs than buried eggs. In other words, predators find eggs more quickly when more eggs are present, and the rate at which eggs are discovered increases more rapidly when the increase is of exposed eggs than when it is of buried eggs. Handling time estimates, conversely, tended to be higher in areas with buried eggs. This is probably an artefact of the estimation technique, since when eggs are exposed more are eaten at a given density, and each consumed egg requires time to eat, so when estimating for buried eggs the technique divides the same total time by a smaller number of eggs consumed, and each therefore is estimated to take longer. It has already been noted the estimates definitely are longer than that required to consume one egg and then start again to move. Adult *Philonthus longicornis* Stephens (Coleoptera: Staphylinidae), for example, consume the contents of six or seven horn fly eggs in a minute, leaving the chewed chorion behind (Hu and Frank 1997). The chorion tends to remain when *D. radicum* eggs are consumed by epigaeic carabid and staphylinid species as well (Hughes 1959). It is unlikely buried eggs really take much longer to consume, and even less likely that buried eggs require more time to digest.

Storing eggs at 1°C reduced their survival, but did not seem to affect their palatability to the three beetle species. A multivariate analysis of variance {Roa, 1992 #4031} might have been used instead to draw this conclusion, except that there were unanalyzed choices in the assays.

In the pie plate assays, the pattern of *Aleochara* consuming more than *B. quadrimaculatum* was repeated in the single species treatments. Increased density led to higher levels of predation for *Aleochara*, but this did not occur for *B. quadrimaculatum*.

This may have been caused by something like territoriality, with beetles disrupting one another so that each *B. quadrimaculatum* is a less efficient forager than when density is lower. In the interspecific plates, different predictions from the two designs would not have been surprising (Griffen 2006), but the observed differences were slight compared to the uncertainty attached to the estimates. The trend was for more predation than expected when an *Aleochara* species and *B. quadrimaculatum* were combined, and for less than expected when both *Aleochara* species are present together. Risk enhancement may happen for the same reason doubling the density of *B. quadrimaculatum* does not increase level of predation, but in this case, the addition of *B. quadrimaculatum*, rather than disrupting *B. quadrimaculatum* already present, instead stimulates the *Aleochara* adults to be more active and spend less time stationary. These multiple predator effects were evident only at the level of particular densities; for the whole plate totals, the three species were functionally substitutable. Risk enhancement intuitively seems less likely in small arenas with little microhabitat heterogeneity and therefore little potential for niche complementarity among predators, but evidence is equivocal in that open field experiments are not more likely to find risk enhancement than experiments in which predators are confined (Letourneau et al. 2009; Tylianakis and Romo 2010). For classical biological control, the whole plate outcome of substitutability strengthens the case for introduction— *A. bipustulata* would appear not to disrupt egg mortality already caused by predators, and would be expected to increase mortality in those portions of the prairies in which it can thrive. At the individual densities, there is evidence that *A. bipustulata* disrupts predation of *A. bilineata*, but not enough for a significant risk reduction overall.

In field cages, the *Aleochara* still outperformed *B. quadrimaculatum*, and more eggs were consumed in cages with *A. bilineata* alone than with *A. bipustulata* alone. Multiple predator effects were observed for all competition treatments, with risk of predation reduced. The significant row and column effects, and absence of interaction of these with treatments, suggest the principal reason risk was reduced was that all predators preferred to forage in the same region of the cage. Epigaeic beetle predators' distribution and abundance are regulated by abiotic factors including microhabitat (Thiele 1977). For example, among tiger beetles on beaches, adults of some species move down the beach over the course of the day whereas others remain in dry areas some distance away (Schultz and Hadley 1989). The beetles studied here seem to have similar microhabitat preferences, or at least to focus foraging in similar areas. Of particular interest is the row effect, where the most eggs were consumed in the most southern row. Because of the cage design, with a 10 cm wooden wall extending above the soil surface, this row would have experienced the most shade. The most western column, where predation also was higher, would have been the first to receive sun in morning, and the first to become shaded later in the day.

Risk reduction was the consistent outcome in field cages, but appeared in the laboratory experiment only with the two *Aleochara* species, and there at some egg densities only. Predator density in the field cages was about 1 predator/600 cm^2 in the single species cages, 1/300 cm^2 when two species were present, and 1/200 cm^2 in the treatment with all three species; in the pie plates there was about 1 predator/300 cm^2 at low density, and about 1/150 cm^2 at high density. Predators in cages, then, were never as crowded as in the pie plates, and so risk reduction is expected to be less in cages than in

plates— if, that is, in cages predators distributed themselves evenly. The significant row and column effects indicate they do not distribute evenly, though, and so are more concentrated in the favourable areas that get less sun. The beetles prefer the same areas, as shown by the non-significant interactions of treatment with row and with column, even when control cages were excluded. By the same token this suggests that apart from the egg densities differing on cards, the pie plate environment was quite uniform. The opposite is likely to be more common, with risk enhancement more likely the larger the arena and the greater the possibilities for segregating the resource. In the case of this study, the three predator species preferred the same portion of the cages, but there are likely scenarios, reproducible in field cages, in which the same predator species enhance the risk of predation.

The implications of risk reduction in experiments like these for biological control are less clear than risk enhancement, or substitutability, would have been. The density of *B. quadrimaculatum* in prairie fields is estimated to be $20/m^2$ (Frank 1971). Typical fields of cabbage may have two adult *Aleochara* per plant (Abu Yaman 1960). If different species are confined to a cage, at densities approximating those in fields, and no evidence emerges of members of the different species interfering with the others' achievement of potential predation levels, the conclusion mortality will be increased by the additional species in unconfined conditions is simple to make. Risk reduction, on the other hand, may presage disruptive interspecific interactions among natural enemy species in the intended area of introduction. It could also be an artefact of confinement, if different species prefer, for example, different microclimates in fields and would encounter one another less frequently in nature than in arenas and cages. Confinement could also inflate

the frequency of interaction, relative to the entire area for introduction, if different species thrive in different geographical areas; results would then indicate outcomes in areas of overlap, but underestimate increases in mortality in areas where the additional species do well, where others had previously not. It may for these reasons be of slight concern, but I have has shown the potential for *A. bipustulata* to disrupt the egg-foraging activities of predators already present in Canada if they are crowded together, as in the field cages.

Table 5. Statistics and model parameters for functional responses of *Aleochara bipustulata*, *A. bilineata*, and *Bembidion quadrimaculatum* to *Delia radicum* eggs, either on the soil surface or slightly buried. The sign and significance of the linear coefficient from logistic regression indicate the type of response. The parameter estimates a, b, and T_h (in hours) are components of these models. (See methods for details.)

Eggs	Predator	p_1±s.e.	$\chi^{2‡}$	Type	a±s.e.	b±s.e.	T_h±s.e	Regression $(F_{2,7})^‡$	Lack of fit $(F_{7,70})^‡$
Surface	*A. bipustulata*	0.042±0.114	0.14	I	0.027±0.002	—	—	699.0**†	0.1
	A. bilineata	0.870±0.131	44.78**	III	—	0.008±0.002	0.648±0.092	43.2**	2.9*
	B. quadrimaculatum	0.2584±0.107	5.8*	III	—	0.147±0.029	2.791±0.076	68.7**	0.8
Buried	*A. bipustulata*	0.014±0.007	4.1*	III	—	0.001±0.000	1.302±0.665	57.0**	0.2
	A. bilineata	0.697±0.153	15.7*	III	—	0.003±0.001	1.738±0.492	19.1**	0.7
	B. quadrimaculatum	0.114±0.040	8.1*	III	—	0.002±0.001	4.756±1.407	8.8*	0.7

‡ Probability: * < 0.05, ** < 0.001

† $F_{1,7}$

Table 6. Analysis of variance table for field cage assays.

Source	d.f.	SS	MS	$F^{\dagger}$
Whole plot				
Treatment T	7	35627.1	5090.0	3.1**
No predators *vs*. predators	1	24468.8	24468.8	14.7***
Among predator treatments	6	11158.2	1859.7	1.1
Block B	11	63410.8	5764.6	3.5***
T x B	77	128397.4	1667.5	
Split plot				
Density D	1	19286.5	19286.5	41.7***
Row R	4	6798.0	1697.7	3.7**
Column C	4	4537.4	1134.3	2.5*
B x Plant position PP	264	122096.8	462.5	
D x T	7	4924.9	703.6	1.6
No predators *vs*. predators	1	2211.0	2211.0	4.9*
Among predator treatments	6	2713.9	452.3	1.0
R x T	28	16355.6	584.1	1.3
No predators *vs*. predators	4	1040.6	260.2	0.6
Among predator treatments	24	15314.0	638.1	1.4
C x T	28	11744.6	419.5	0.9
No predators *vs*. predators	4	929.9	232.5	0.5
Among predator treatments	28	10814.7	450.6	1.0
B x PP x T	1848	835253.3	452.0	
unestimated terms[1]	120			
Total	2399			

† * = $P < 0.05$, ** < 0.01, *** < 0.001

[1] Linear model of density effect

[2] Composed of non-linear density effects and their interactions (never significant) and residual within plant position effects and their interactions

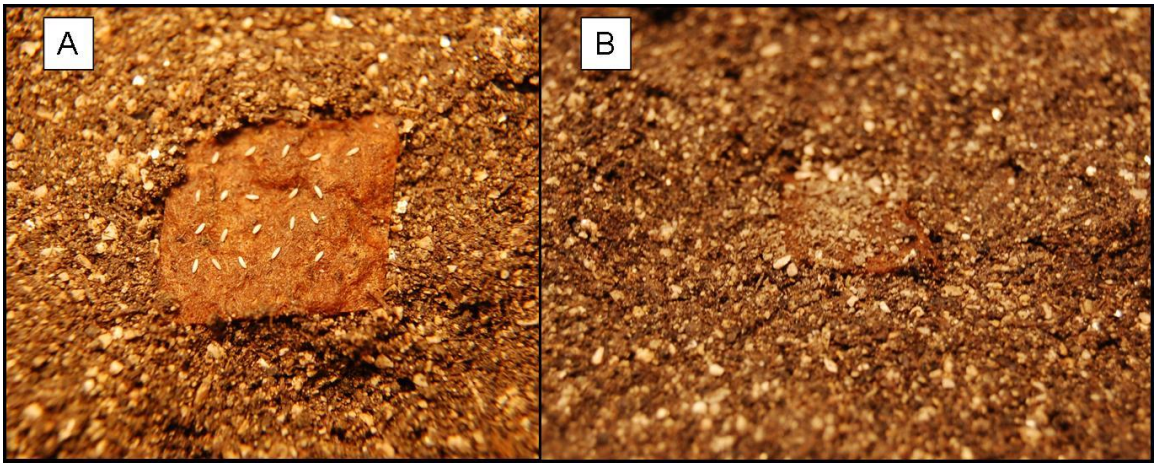

Figure 7. Egg cards **A**: exposed card, with 21 *Delia radicum* eggs; **B**: buried egg card.

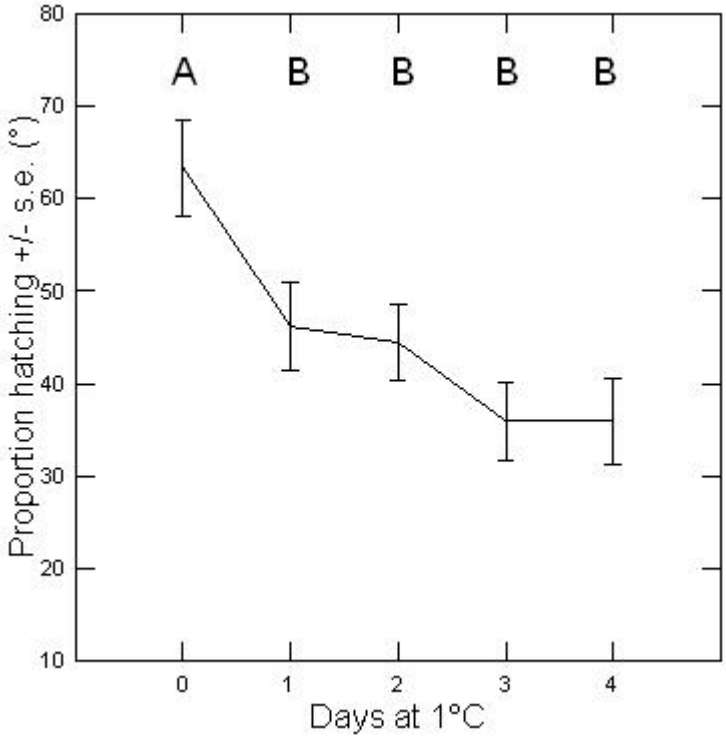

Figure 8. Effect of cold storage on viability of *Delia radicum* eggs, with arcsine square root transformed proportion eclosing expressed in degrees (90° = 1.0; 45° = 0.5; 0° = 0.0). Letters indicate membership in groups whose means differ significantly as indicated by Tukey's HSD test.

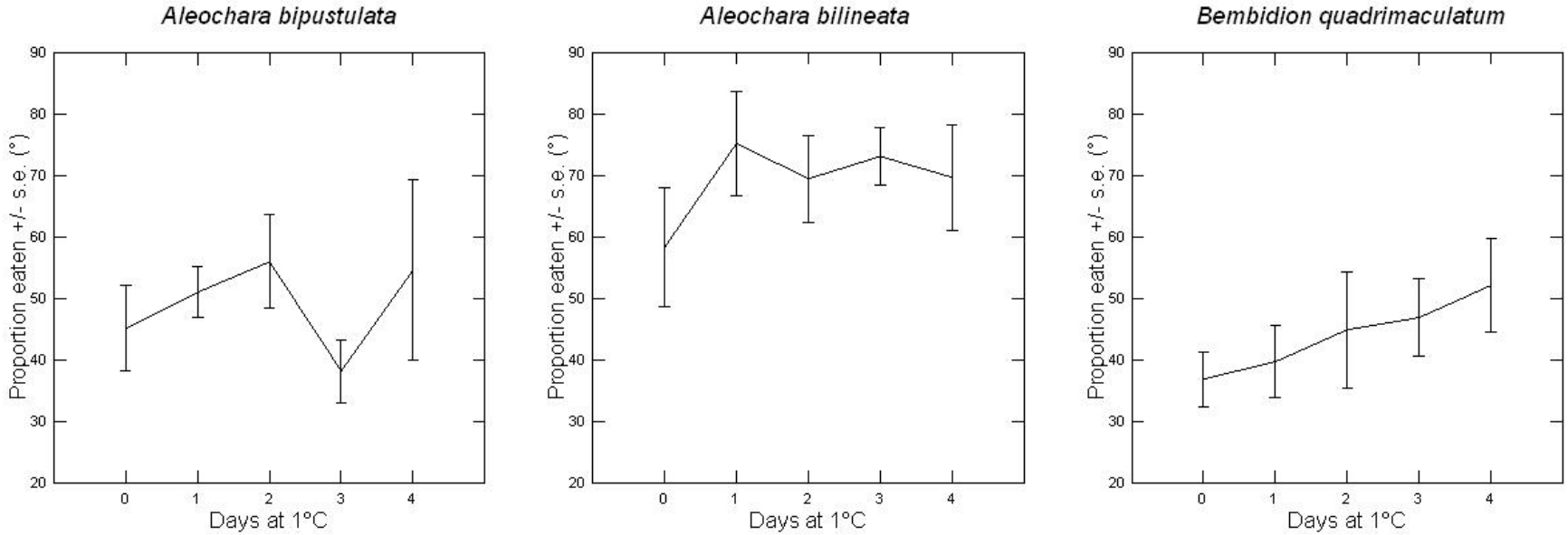

Figure 9. Arcsine square root transformed proportion, in degrees (90° = 1.0; 45° = 0.5; 0° = 0.0), of 10 *Delia radicum* eggs consumed in 24 h by individual *Aleochara bipustulata*, *A. bilineata*, and *Bembidion quadrimaculatum*. The proportion consumed was independent of period of storage for all three species (*A. bipustulata*: $F_{4,36} = 1.6$, $P = 0.21$; *A. bilineata*: $F_{4,36} = 2.1$, $P = 0.11$; *B. quadrimaculatum*: $F_{4,36} = 0.7$, $P = 0.58$).

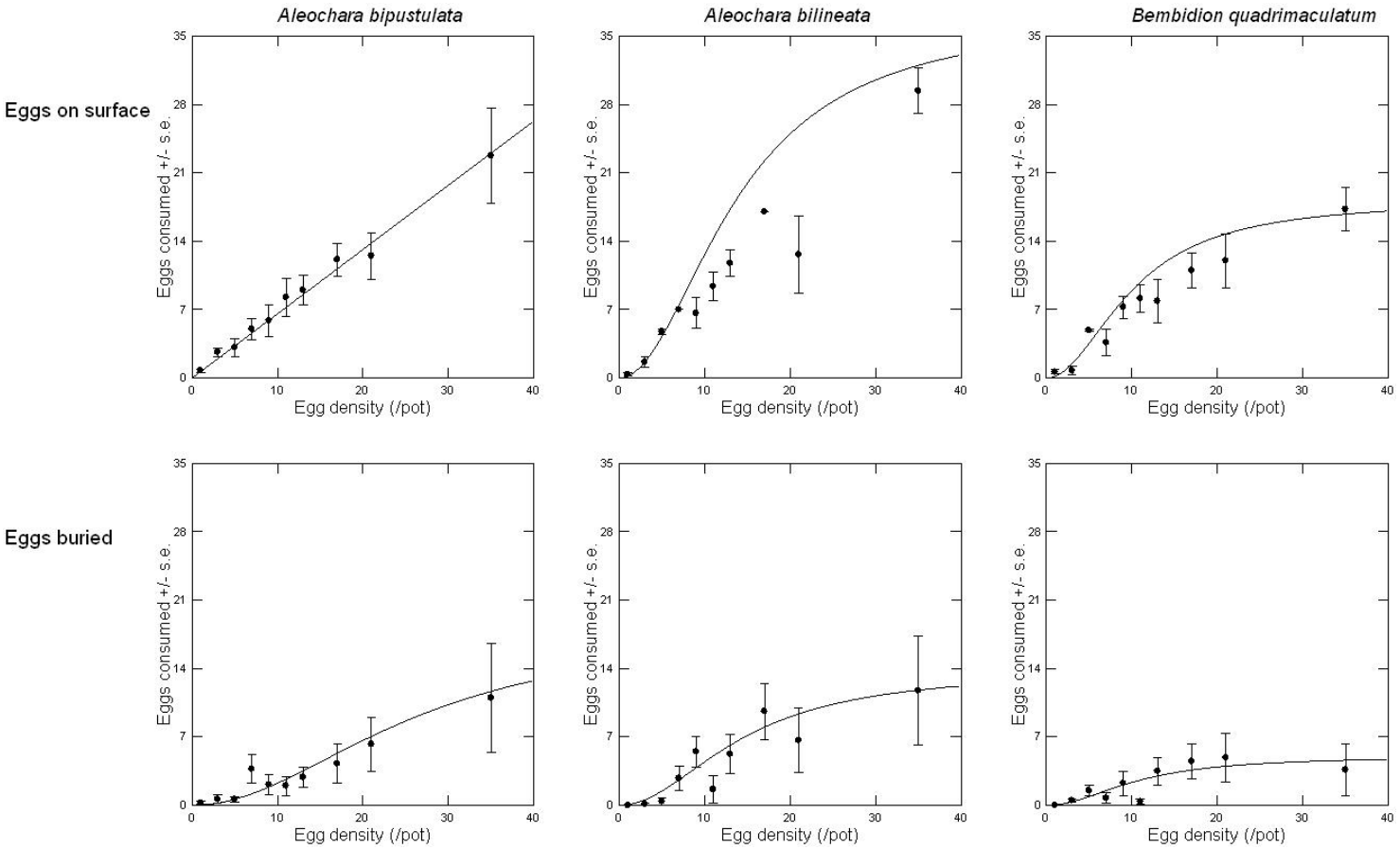

Figure 10. Functional responses of *Aleochara bipustulata*, *A. bilineata*, and *Bembidion quadrimaculatum* to *Delia radicum* eggs on the soil surface, or slightly buried, at densities from 1–35 eggs. Dots are the mean number consumed in eight replicates, and lines show the predicted number eaten based on the functional response model.

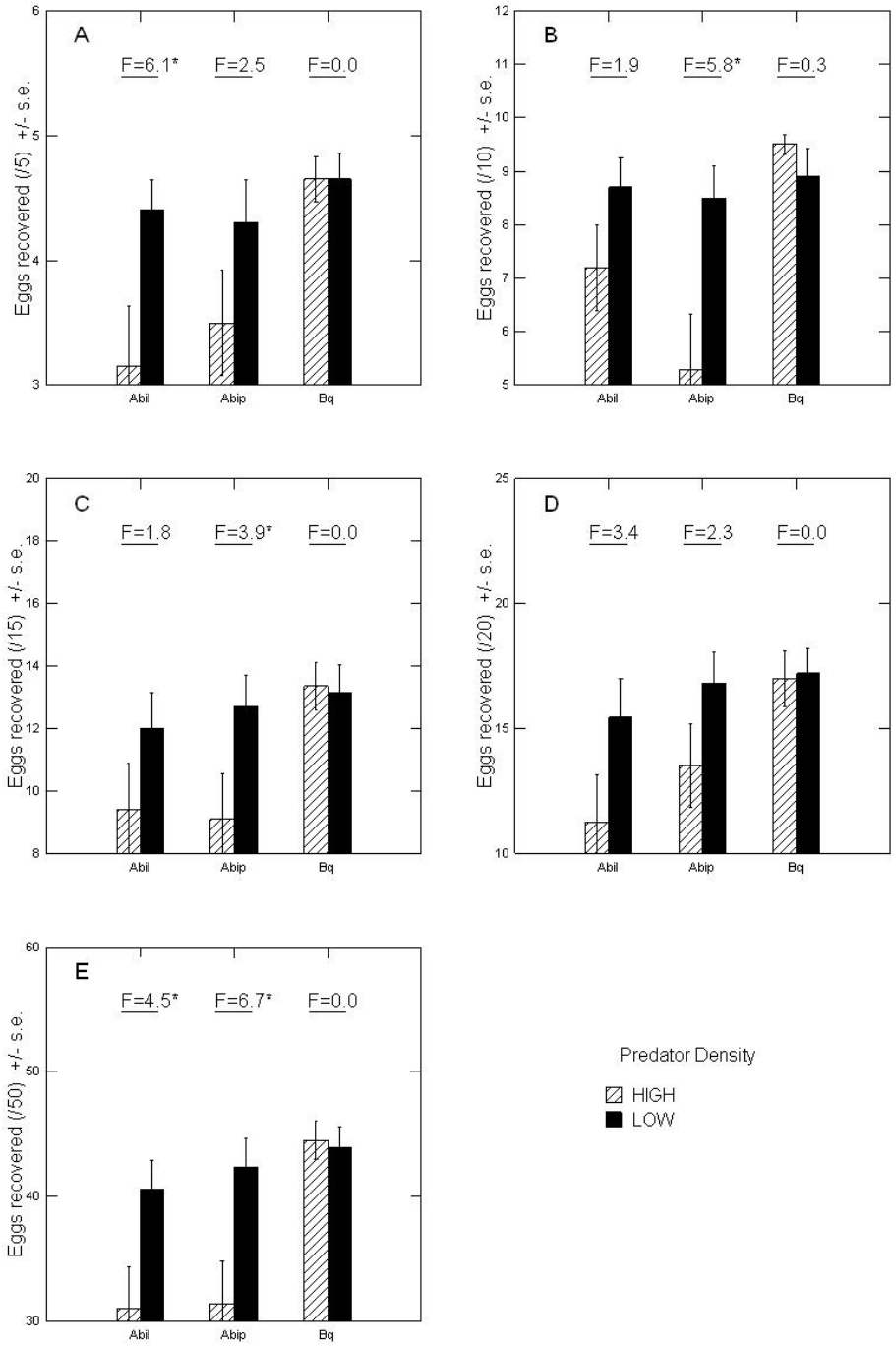

Figure 11. Numbers of *Delia radicum* eggs recovered, from cards with 5 (A), 10 (B), 15 (C), and 20 eggs (D), and from whole plates (E), after exposure to high and low densities of *Aleochara bilineata* (Abil), *A. bipustulata* (Abip), and *Bembidion quadrimaculatum* (Bq). '*' following *F* statistics indicates probability < 0.05 (d.f. = 1, 171). Note that the vertical axes are not rooted at zero, and scales vary among panels.

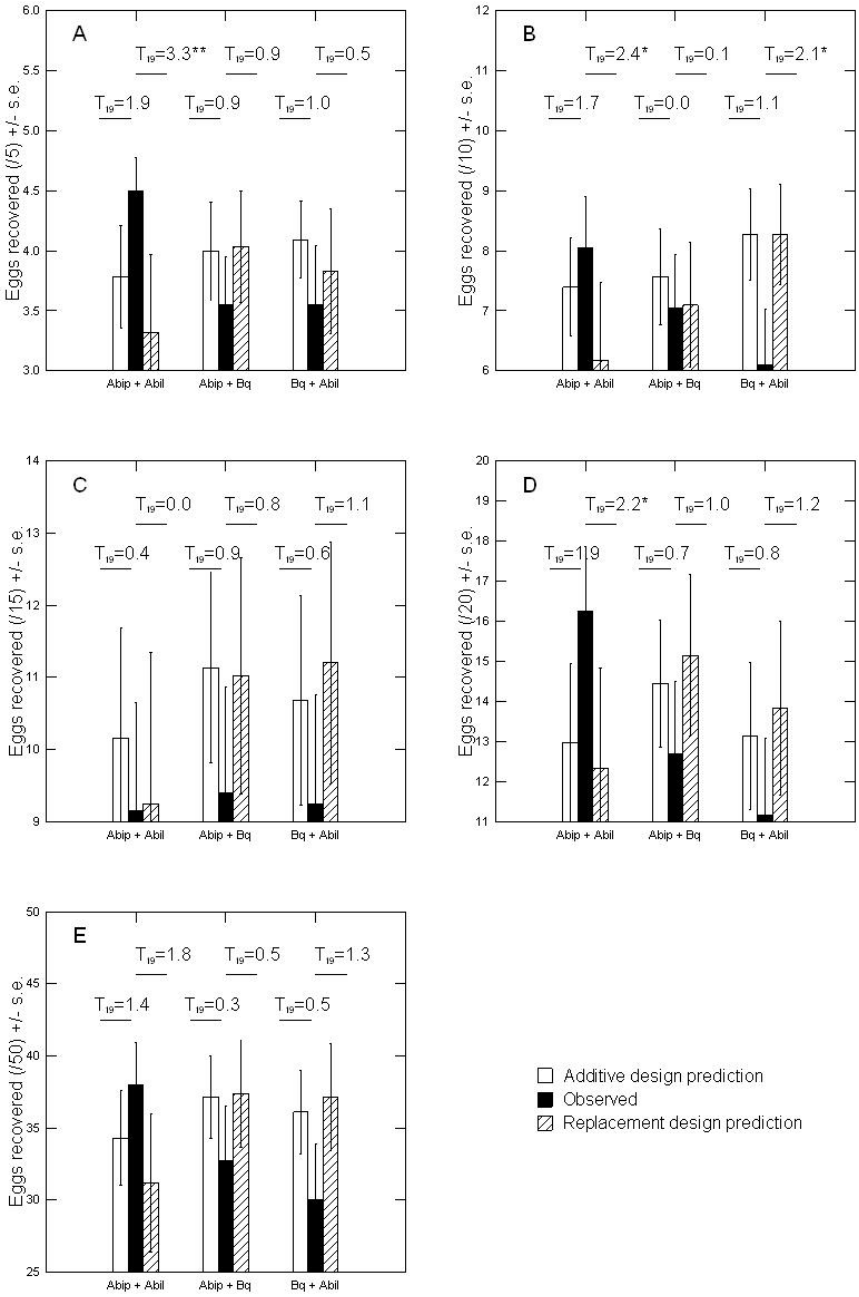

Figure 12. Numbers of *Delia radicum* eggs recovered, from cards with 5 (A), 10 (B), 15 (C), and 20 eggs (D), and from whole plates (E), from interspecific treatments with *Aleochara bilineata* (Abil), *A. bipustulata* (Abip), and *Bembidion quadrimaculatum* (Bq), compared to expectations based on additive and replacement series designs. Following *F* statistics indicates '*' $P < 0.05$, '**' < 0.01 (d.f. = 19). Note that the vertical axes are not rooted at zero, and scales vary among panels.

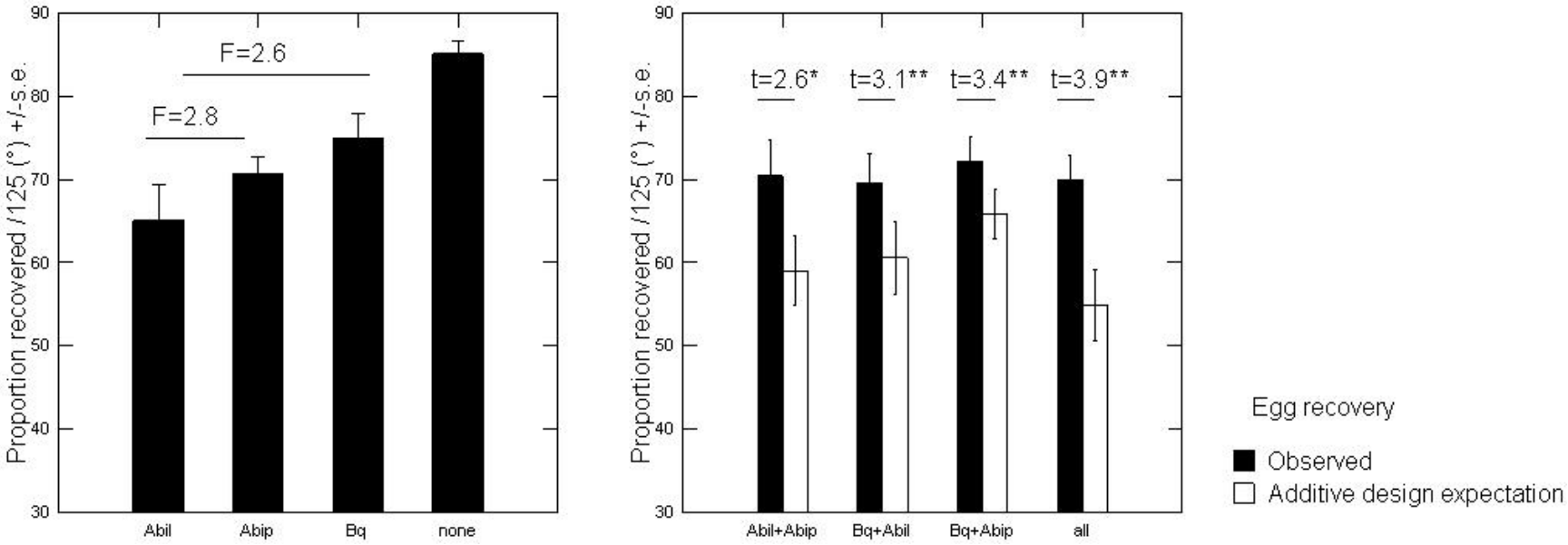

Figure 13. Arcsine square root transformed proportion (°, 90 = 1.0) of *Delia radicum* eggs recovered following exposure in field cages to *Aleochara bilineata* (Abil), *A. bipustulata* (Abip), and *Bembidion quadrimaculatum* (Bq). Left pane shows recovery in control and single species cages. Right pane shows recovery in multi-species cages compared to expectations from additive experimental design. Degrees of freedom for *F* statistics are 1,77; and for *t* statistics 11; * = $P < 0.05$, ** < 0.01..

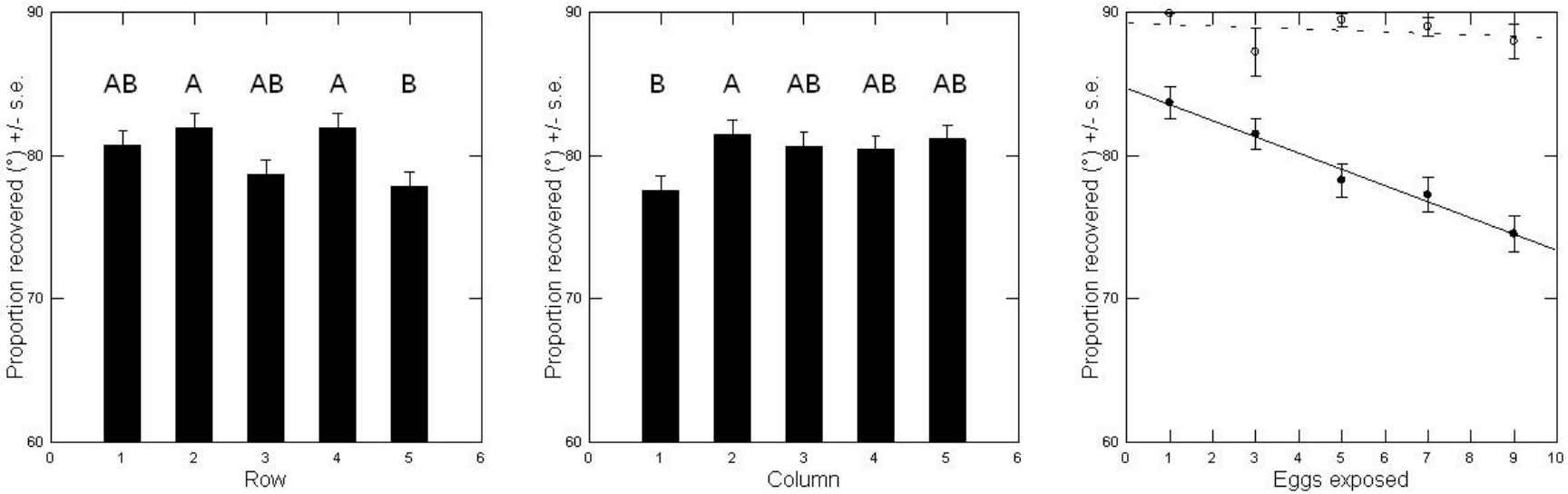

Figure 14. Arcsine square root transformed proportion (°, 90 = 1.0) *Delia radicum* eggs recovered following exposure in field cages, by plant row (1 = North), plant column (1 = West), and number of eggs per card. Values for rows and columns based on the seven treatments with predators, with control cages excluded. For number exposed, solid circles and line are for treatments with predators (y = 84.68 – 1.13(eggs exposed), and open circles with dotted line for control cages (y = 89.22 – 0.10(eggs exposed). Letters indicate membership in groups whose means differ significantly as indicated by Tukey's HSD test.

Chapter 3 Section 3

The resource utilization niche of two rove beetle parasitoids of Diptera: host species, host size, and depth

Introduction

A decision frequently is faced in classical biological control of arthropods about which biological control agents, among the known natural enemies of a pest species, to introduce. Opinion initially tended to favour introduction of multiple species (Fiske 1910; Smith 1929; Thompson 1923). Admonishment, including description of how a relatively more fecund but inferior competitor species may be inhibited from achieving its potential by a superior competitor (Turnbull and Chant 1961), resulted in a small scientific cleft along the 49th parallel wherein Americans tended to favour introducing more species, while Canadians generally preferred to identify a "best" combination, and both claimed the support of theory (Palladino 1990). Reviews of the biological control record indicated the strategy of introducing multiple species may be an important cause of low establishment rates (Ehler and Hall 1982), and even when multiple species are established, one species frequently is credited with successful control (Myers et al. 1989). Today we acknowledge that each additional species introduced will increase the chances of negative non-target effects (Denoth et al. 2002; Hoelmer and Kirk 2005; van Klinken and Raghu 2006), and so species are added reluctantly in creating a combination of natural enemies with optimized control in mind.

Many have modelled populations of a pest and multiple natural enemy species to develop theory that can inform selection, and the models often are cast in terms of the niche, which may engender confusion (McInerny and Etienne 2012). The resource-utilization niche, in which the frequency of consumption is recorded as the resource varies along one or several axes (Schoener 2009), is the main concept in this context. These models predict that if the additional species forages in a niche even slightly more

extensive than the first, a lower host density will result (Pedersen and Mills 2004), but higher pest density will arise from the addition of another parasitoid species if both search the environment as efficiently and in the same way (Kakehashi et al. 1984). In addition, niche overlap is predicted to make coexistence of the two parasitoids impossible, with one species competitively excluded (Murdoch et al. 1996), which has in some cases been observed (DeBach 1966). On the other hand, increasing the diversity of insect natural enemies more often results in higher pest mortality than lower (Stiling and Cornelissen 2005).

The introduction of additional species of parasitoid to Canada is presently being considered for control of *Delia radicum* (L.) (Diptera: Anthomyiidae) in prairie canola (Andreassen et al. 2007; Soroka et al. 2002), a crop in which the pest is increasingly problematic (Soroka et al. 2004). Adult *D. radicum* lay eggs on or just beneath the soil surface near the host plant's stem, and larvae from these eggs go through three instars feeding on root tissue before pupating in the feeding tunnels, or more often in the surrounding soil. Records of the depth at which larvae pupate vary, with the majority of puparia reportedly found in the top 7.5 cm of soil (Schoene 1916), or between 7.5 and 12.5 cm (Gibson and Treherne 1916; Smith 1927). Two important parasitoid species are already associated with *D. radicum* in North America, *Trybliographa rapae* (Westwood) (Hymenoptera: Eucoilidae) and *Aleochara bilineata* Gyllenhal (Coleoptera: Staphylindae) (Hemachandra et al. 2007a; Wishart 1957). Adult female *T. rapae* oviposit in *D. radicum* larvae, and adult parasitoids emerge from host puparia (Wishart and Monteith 1954). The larva of *A. bilineata* hatches from an egg laid in soil and then locates a host puparium, in which a hole is chewed and then the puparium is entered by

the larva (Fuldner 1960; Wadsworth 1915). After feeding briefly, the larva returns to the hole and plugs it with anal secretions, then resumes feeding while passing through three instars; finally it pupates inside the puparium and a new adult emerges from a second, larger hole (Fuldner 1960; Wadsworth 1915).

The parasitoid considered for introduction is *A. bipustulata* (L.), closely related to and possible sister species of *A. bilineata* (Maus et al. 2001), and with a very similar life cycle (Fuldner 1960). So similar are the two species, in fact, that mechanisms that permit their coexistence have been considered. Larvae of both species discriminate among hosts, preferring unparasitized puparia if given a choice (Royer et al. 1999). An incomplete separation in time may be involved, with *A. bipustulata* adults more active earlier in the season (Jonasson 1994). The host resource may also be partitioned based on size, which would result in differences in the sizes of adults of the two species; this has been found in Sweden (Jonasson 1994). In the laboratory, *A. bipustulata* larvae avoid relatively large *D. radicum* if given a choice (Ahlström-Olsson 1994b; Fournet and Brunel 1999; Fournet et al. 1999), whereas *A. bilineata* is as likely to choose puparia in one size class as another (Fournet and Brunel 1999; Fournet et al. 1999) or avoids the smallest puparia (Ahlström-Olsson 1994b). However, host mass did not influence the probability of either species emerging from puparia collected in Germany and Switzerland (Hemachandra 2004). If the species use different host species, in addition to *D. radicum*, this would be expected to reduce the intensity of interspecific competition as well. Until recently it would appear that the host range of *A. bilineata* is narrower than, and circumscribed by, the host range of the less specialized *A. bipustulata* (Maus et al. 1998). Records concerning *A. bipustulata* development on calyptrate Diptera outside the Anthomyiidae now seem

doubtful in light of host-range testing (Andreassen et al. 2009). Whether *A. bilineata* can develop in puparia of acalyptrate Diptera species, and whether *A. bipustulata* can, like *A. bilineata* (Whistlecraft et al. 1985a), exploit onion maggot, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae), as effectively as it can *D. radicum* remain open questions.

There were four parts to this study. In the first part, *D. radicum* puparia were collected from canola fields and weighed to understand better the range of host sizes *A. bilineata* encounters now and *A. bipustulata* will encounter if introduced. In the second section, three species of hosts [*D. radicum*, *D. antiqua*, and the acalyptrate *Piophila casei* (L.) (Diptera: Piophilidae)] were compared for the two parasitoid species in terms of host acceptability and suitability. I also investigated resource utilization as a function of depth of host puparia for *A. bilineata.* In the last section, resource utilization along two axes, host mass and host depth, was studied under intra- and interspecific competition.

Materials and Methods

Insect cultures

Laboratory colonies of *A. bipustulata* and *A. bilineata* were maintained with immature *D. radicum* to feed adults, and *D. radicum* puparia as hosts for larval development, as described in greater detail elsewhere (Andreassen et al. 2009). The *A. bilineata* originated from *D. radicum* hosts collected in fields of canola in southern Manitoba, and the *A. bipustulata* from fields of *Brassica* vegetables around Kerzers in the Swiss canton of Bern. Both species of *Delia* were from colonies at the Agriculture and Agri-Food Canada centre in London, Ontario. *Delia antiqua* was maintained as described by Tolman et al. (1985), and *D. radicum* as described by Whistlecraft et al. (1985b). The *P. casei* colony originated with larvae in a dead fish at the University of Manitoba's

research farm at Glenlea, and was maintained with ground beef or pork for oviposition and larval development, and unrefined sugar and water for adults.

Characterization of Delia radicum *puparia in prairie canola fields*

Three samples of puparia were collected to characterize the *D. radicum* of prairie canola that *A. bipustulata* will encounter if introduced. These were made in Manitoba in late October and early November, near Graysville in 2007 and 2009, and St. Claude in 2009. The shape of the caudal tubercles (Brooks 1951) and number of lobes on the anterior spiracles (Miles 1952) were used to remove individuals that could have been *Delia platura* (Meigen) or *D. florilega* (Zetterstedt) from the samples before puparia were measured. These two species are referred to as "bean seed fly" below, as they are indistinguishable except as adult males (Brooks 1951), and so information regarding them tends to become conflated. Puparia parasitized by *Trybliographa rapae* (Westwood) were readily distinguished based on the overwintering fourth instar larva's habit of occupying just one end of the puparium, and were also excluded from measurement as well, as they are avoided by foraging *Aleochara* larvae (Reader and Jones 1990). The mass of each puparium was measured to the nearest 0.1 mg. For each sample, the distribution of masses was characterized first by the mean, skewness, and kurtosis (Sokal and Rohlf 1981). The masses in each sample were then fit to a two-parameter Weibull distribution:

$$f(x) = (\alpha/\beta)\,(x/\beta)^{\alpha-1} \exp(-x/\beta)^{\alpha}.$$

An Anderson-Darling test was performed to determine whether the Weibull distributions provided significantly good fits.

Acceptability and suitability of fly species for Aleochara *species*

There were nine plastic vials, 4.5 cm tall and 2.2 cm diameter, in each of 108 replicates of this experiment. Vials within a replicate were prepared on the same day. Puparia of *D. radicum*, *D. antiqua*, and *P. casei* were collected daily from the colonies and the mass of each recorded. A range of puparial sizes was produced for each fly species by varying the extent to which larvae were crowded, and moving larvae from their food to sawdust before they would naturally have ceased to feed. One puparium, formed one or two days previously, was buried in each vial, and there were three vials for each fly species. Puparia were on 1 cm of sand tamped by finger to produce a small divot that kept the puparium out of contact with the vial, and under 1.5 cm of loosely packed sand (Wright et al. 1989). The sand consisted of 0.4–2.0 mm diameter particles and was moistened with 100 ml water per dry litre. An *Aleochara* larva that had eclosed up to two days previously was transferred by its pygopodium on a moist camel-hair brush and allowed to run off on to the sand surface. One larva of each of the two *Aleochara* species was transferred to a vial with each fly species, leaving one puparium of each fly species as a control in each replicate. The vials were then held in a 20 °C chamber with 16:8 h light:dark per day. Seven days after the larvae were added, the volume of sand in vials was reduced so puparia rested on a 1 cm layer, making adult insects that emerged easier to see. At this time, puparia were inspected under a dissecting microscope for entrance holes, the presence of larvae inside puparia, and whether larvae having entered had also plugged the hole. Emergence of adult insects was monitored daily, and for *Aleochara* species, sex was recorded based on the terminal abdominal segment's structure in ventral view (Drea 1966). After allowing at least two days for structures to fully harden, the

maximum width of the pronotum of each *Aleochara* adult was measured to the nearest 0.0238 mm with a dissecting microscope.

For each Diptera species, a test was performed to determine whether the probability a fly would emerge was independent of exposure or not to an *Aleochara* larva, based on a likelihood ratio χ^2 (L.R. χ^2) compared to a constant critical value ($\alpha = 0.05$) (Sokal and Rohlf 1981). Host acceptance was viewed as a three step process with a binary response at each step, from the larva creating an entrance hole, to entering the puparium, and finally to sealing the hole. Logit models for each stage were constructed using the predictor variables host mass and dummy coded categorical variables for parasitoid species and host. Models were constructed with host genus as a predictor, and compared with models that distinguished between the two *Delia* species. Likelihood ratio χ^2 tests comparing full and reduced models (Quinn and Keough 2002), using an alpha level of 0.05, were used to select the most appropriate model for each stage. All puparia exposed to parasitoid larvae were considered in modelling the probability of an entrance hole, whereas only puparia with entrance holes were considered in modelling the probability of larvae entering puparia, and only entered puparia considered in modelling the probability of a plugged entrance hole. The probability of complete development, or host suitability, was modelled similarly, by considering only those puparia with a plugged entrance hole. The number of days required for complete beetle development at 20°C was modelled by multiple linear regression, with host mass as a continuous predictor and beetle sex, beetle species, and host species or genus as categorical predictors. Model terms were tested for significance by *F*-ratio tests of error mean squares (Neter et al.

1990). The effect of these predictors on the adult *Aleochara* pronotum width was modeled similarly.

Host location in relation to soil depth

For this study the experimental unit was a 15.0 cm long section of polyvinyl chloride (PVC) pipe, internal diameter 7.5 cm, with a Petri dish lid taped to close one end. The tube was filled up to 12.0 cm with the sand described above. Five *D. radicum* puparia in diapause, 7.0–14.0 mg, were buried at 3.0, 6.0, or 9.0 cm below the sand surface. Five larvae of *A. bilineata* up to one day old were released on the surface, with 12 replicates for each depth of puparia. Tubes were kept seven days at 20°C with 16:8 h light:dark. Puparia were then examined under a dissecting microscope to count those with larvae inside. The proportion (p) entered at each depth was arcsine square root transformed ($y = \sin^{-1}\sqrt{p}$), and the hypothesis that puparium depth had no influence on the proportion parasitized, given the variability at each depth, was tested by linear regression (Sokal and Rohlf 1981).

Host use in relation to competing larvae, host size and host depth

The experimental unit was the same PVC pipe, but now with 30 *D. radicum* puparia in diapause buried in the 12 cm of sand. Ten puparia were buried at 3 cm below the surface, ten at 6 cm, and 10 at 9 cm — a line on the ventral surface (Sharpie®, Oakville, Canada) signifying the depth of each puparium. Five puparia at each depth were 7–9 mg, and five were 12–14 mg. Fifteen replicates of each treatment were done. Treatments were applied by releasing *Aleochara* larvae on the sand suface: 10 or 20 *A. bilineata*, 10 or 20 *A. bipustulata*, or 10 *A. bilineata* and 10 *A. bipustulata*. Tubes were then kept for seven days at 20 °C with 16:8 h l:d. Puparia were examined under a

dissecting microscope to determine which had larvae inside, and these were kept individually for emergence of adult parasitoids.

Data were analyzed by ANOVA, followed by Tukey's HSD test for unplanned pairwise comparisons. For each replicate, the proportion of larvae successful in entering a puparium was transformed as above prior to testing the hypothesis of equal proportions among treatments. Treatments were then analyzed separately, to determine whether the number entering puparia depended on puparium depth, puparium mass, or their interaction.

Results

Characterization of Delia radicum *puparia in prairie canola fields*

The *D. radicum* puparia in the three collections ranged in mass from 3.3–20.6 mg. Distributions exhibited significant skew to the left in both 2009 collections, and kurtosis was never significant (Table 7). Weibull distributions provided adequate fits to the masses in all three collections. Masses differed among samples (Kruskal-Wallis W_2 = 78.7, $P < 0.001$), the 2007 collection from Graysville being the smallest. Masses did not differ between the two samples collected in fall of 2009 (Mann-Whitney $U = 7863.5$, $P = 0.46$). Only nine puparia of *D. platura* or *D. florilega* were recovered in these collections; their average mass was 4.06 mg (±1.48 s.d.).

Acceptability and suitability of fly species for Aleochara *species*

Puparia were selected from cultures to try to cover the possible range of masses for each species, and so the distributions of masses used departed from normality (all *A-D* statistics ≥ 1.02, $P < 0.05$). The 324 *D. radicum* puparia used ranged in mass from 3.4–

16.5 mg, with median 9.4; *D. antiqua* from 3.7–18.8 mg, median 11.1; and *P. casei* 1.4–6.7 mg, median 4.2.

Comparison with the control vials demonstrated that for all three host species, the emergence of adult flies was reduced by exposure to *Aleochara* larvae (Figure 15). Emergence of both *D. radicum* and *P. casei* was independent of the parasitoid species. *Delia antiqua*, on the other hand, were more likely to emerge as adult flies following exposure to larvae of *A. bipustulata* than *A. bilineata*.

The proportion of parasitoid larvae completing each stage of the process toward adult emergence differed between the parasitoid species, depended on the species or genus of host, and in most cases depended also on host mass (Table 8). The odds ratios (Table 8A) illustrate relationships between probabilities and masses, for particular combinations of host species and parasitoid species; they show how the probability changes with each 1 mg change in mass. The logit models (Table 8B) show how the species and mass effects combine to influence the probability of successful outcome for that stage.

Entrance holes were created by *A. bipustulata* equally among *Delia* hosts, and less frequently in *P. casei* puparia; mass had no influence. A significant interaction of parasitoid species with host genus resulted from larvae of *A. bilineata* more frequently creating holes in *Delia* hosts than *A. bipustulata* did, but as for *A. bipustulata*, holes were as likely to be created by *A. bilineata* in puparia of *D. radicum* as of *D. antiqua*. The significant mass term in the logit model reflects the greater probability of *A. bilineata* entrance holes in *P. casei* hosts as mass increased, while the mass x genus interaction

compensates for this mass effect to reflect its absence when hosts were *D. radicum* or *D. antiqua*.

At the stage of larvae entering puparia, mass influenced only the interaction of *A. bilineata* and *D. antiqua*, where the vast majority of puparia were entered, but those not entered were quite small (Table 8). The modelling approach was not sensitive enough for this effect to be included in the final model. Larvae of *A. bipustulata* responded differently to the two *Delia* species, entering puparia of *D. radicum* more frequently, and so host species, as opposed to host genus, appeared in the overall model. Because of this difference, while *A. bilineata* entered puparia of the two *Delia* species equally, a significant positive parasitoid x host species interaction term appears in the overall model, offset for *A. bipustulata* entering *D. antiqua* by the negative term associated with *D. antiqua* hosts. A negative term associated with *P. casei* hosts reflects reduced entry by both parasitoid species in these hosts compared to *D. radicum*.

Most puparia entered by larvae also had a plugged entrance hole (Table 8). The smallest proportion to receive a plug was *D. antiqua* puparia attacked by *A. bipustulata*, and here mass had a significant influence, with probability of plugging reduced as mass increased, which is reflected in the negative mass term in the overall model. As a corollary, host species was included in the overall model instead of host genus, as well as a significant positive parasitoid x host interaction term to account for the equivalence of the two *Delia* species to *A. bilineata*. For both species of parasitoid, a smaller proportion of *P. casei* puparia were plugged than of *D. radicum*, and so a significant negative term associated with *P. casei* puparia appears in the overall model.

For all host and parasitoid species combinations, the proportion to complete development was much reduced compared to the three prior stages (Table 8). Mass influenced the two parasitoid species similarly, being significant only when associated with *D. antiqua* hosts, where the probability of complete development was reduced as mass increased; this effect carries over to the full model as a negative term for mass. The discrepancy between the two parasitoid species developing in *Delia* hosts followed the pattern of the larvae entering and hole plugging stages, with *A. bilineata* performing as well, or even slightly better, in *D. antiqua* puparia as in *D. radicum*, while *A. bipustulata* developed far better in *D. radicum* than in *D. antiqua*. And, as before, the consequence for the overall model was that it included a significant positive parasitoid x host species interaction. Lower order terms were retained by convention, even though the coefficient associated with *A. bilineata* was relatively quite small. Both species fared slightly worse in *P. casei* puparia than in *D. radicum*, and the negative *P. casei* model term reflects this.

The number of days required to complete development depended on the parasitoid species, the host genus, and host mass (Figure 16). The sexes developed at the same rate. Larger adult *Aleochara* emerged from larger hosts, and there were differences between parasitoids and among hosts in the nature of this relationship (Figure 17). For both duration of development and adult size, there was not a significant interaction of host mass and parasitoid species, indicating the two parasitoid species are affected by mass in the same way.

Host location in relation to soil depth

The proportion of puparia attacked by *A. bilineata* larvae did not differ among depths according to ANOVA ($F_{2,33} = 1.4$, $P = 0.27$). Partitioning the variance among

groups, into portions due to linear regression and due to deviations from the regression showed that the proportion of larvae successful in entering puparia is inversely related to depth ($F_{1,1} = 204.2$, $P < 0.05$), as illustrated in Figure 18. Deviations from the linear regression were not significant ($F_{1,33} = 0.0$, $P = 0.91$).

Host use in relation to competing larvae, host size and host depth

The proportion of larvae that entered puparia depended on the treatment ($F_{4,70} = 4.2$, $P < 0.01$). The proportion was higher when 10 *A. bipustulata* were added than when 20 *A. bilineata*, or 10 larvae of each species, were added (Figure 19). Within the five treatments, puparium mass significantly affected the number of puparia attacked only with 20 *A. bilineata* larvae, where more puparia 7–9 mg were selected (Figure 20). The interaction of mass and depth of puparia was never a significant term (Figure 20). Depth alone was the most frequently significant, in three out of the five treatments (Figure 20). With 20 *A. bilineata* larvae, or 10 or 20 *A. bipustulata* larvae, host depth was significant (Figure 20). When 10 *A. bilineata* larvae foraged alone, or when together with 10 *A. bipustulata*, neither depth nor mass influenced the number of puparia attacked.

The proportion of attacked puparia from which adults emerged (Figure 21) did not depend on the treatment ($F_{4,70} = 0.8$, $P = 0.53$). Within treatments, the number of adult beetles to emerge among resource states (Figure 22) is partly an effect of the earlier larval choices illustrated in Figure 20. For *A. bilineata*, the number of adults to emerge did not depend on puparium mass, puparium depth, or their interaction in any of the treatments (Figure 22). At low density, more *A. bipustulata* emerged from puparia 12–14 mg than 7–9 mg, and fewer adults emerged as depth increased (Figure 22). When *A. bipustulata* was present alone at high density, numbers again decreased with increasing depth, but no

longer differed among host masses (Figure 22). Under interspecific competition, both beetle species were as likely to emerge from puparia of one size or depth as from any of the others (Figure 22).

Discussion

Characterization of the mass of field-collected *D. radicum* puparia, from prairie canola stubble, was a vital step in developing this study, and in relating its findings to the previous work of others, given what was known and then further explored here of the importance of intraspecific variation in host mass for the behaviour of *Aleochara*, and for determining the outcome of a parasitic attack. Larvae reach different sizes by the time of pupation on different host plant species, and on different varieties of host plant crops (Hopkins et al. 1999). Two of the three populations measured here had distributions of puparial mass skewed to the left, and the Weibull distribution was found to characterize adequately this dispersion. Hemachandra (2004) also measured pupae of six populations of *D. radicum* from prairie canola, whose means ranged from 10.53–16.06 mg. These measurements agree well with the current study. Attempts to find for comparison data on mass of *D. platura* puparia were not successful, but the few puparia measured here, giving an average mass around 4 mg, was adequate to confirm *D. platura* puparia are much smaller than those of *D. radicum*, in canola.

Previous study of host species use by *A. bipustulata* has shown species to differ in the extent to which exposure to parasitoid larvae, and attack by those larvae, reduces the proportion of pupae to emerge as adult flies (Andreassen et al. 2009). Here, *D. radicum* and *P. casei* were as likely to survive exposure to one parasitoid species as another. Onion maggot pupae, however, were more likely to survive exposure to *A. bipustulata*

than *A. bilineata*. This occurs because of differences in acceptability and suitability among the hosts to the two species of parasitoid.

During the first attempt at classical biological control of *D. radicum* in Canada (Andreassen et al. 2007; Soroka et al. 2002), comparative study of host use by *A. bilineata* and *A. bipustulata* arose when it was noted: *A. bipustulata* was more prevalent in puparia of bean seed fly than of *D. radicum*, and among *D. radicum* puparia to be more prevalent in smaller puparia (Wilkes and Wishart 1953). Adult *A. bilineata* emerged at least as frequently from bean seed fly as from *D. radicum* in that survey as well, and no mention was made of *A. bilineata* being more likely to emerge from certain sizes of puparia than others (Wilkes and Wishart 1953). Fuldner (1960) and later Jonasson (1994) measured field-collected beetles and concluded *A. bipustulata* to be on average the smaller species. Both also divided field-collected *D. radicum* puparia into small, medium, and large classes and recorded the *Aleochara* to emerge; *A. bipustulata* were less likely to complete development in large puparia in the first case, a sample of 134 puparia (Fuldner 1960), and emerged only from small and medium classes in a sample of 212 puparia, whereas *A. bilineata* emerged equally from the three classes (Jonasson 1994). Among seven size classes in the largest such sample of 327 *D. radicum* puparia, puparia in one size class were as likely to be parasitized by *A. bilineata*, parasitized by *A. bipustulata*, or unparasitized as puparia in any other size class (Hemachandra 2004).

The observations regarding size in nature provoked several laboratory investigations of host size and host species selection and suitability. Fuldner (1960) noted *A. bipustulata* larvae do not avoid entering large *D. radicum* puparia, and feed normally within them, but tend to die in the third instar after they cease to feed and the remaining

host pupal material rots. This explained *A. bipustulata*'s infrequent success in puparia of *D. floralis* (Fallén) (Diptera: Anthomyiidae), which are large compared to *D. radicum* (Andersen 1982). However, *Aleochara bisolata* Casey (Coleoptera: Staphylinidae) does not initiate feeding on pupae of some species that nonetheless are entered, and in choice conditions may create a hole in and explore one puparium before selecting another, indicating an additional level beyond puparial penetration at which hosts must be acceptable before size-based suitability components of the interaction come to bear on the outcome (Wright et al. 1989). Palatibility does influence *A. bipustulata*'s host relations as well, as puparia of *Ophyra aenescens* (Wiedemann) (Diptera: Muscidae) are entered but the hole seldom is plugged, and the fly usually survives (Andreassen et al. 2009). Among potential host species, acceptance becomes less likely as size increases, but acceptability seems more closely related to the particular species and its puparial structure than to size; *D. radicum* for example are accepted much more frequently than expected from the generalized size relationship, and *Fannia scalaris* (F.) (Diptera: Fanniidae) less frequently (Andreassen et al. 2009). Suitability is more closely related to mass, and generally becomes less as mass increases (Andreassen et al. 2009). Size does work, then, as a generalization but is not as informative as studying the particular combination of host and parasitoid species. Intraspecific variation in host mass was not part of that study. Palatability has been mentioned, to which one may add the defensive reaction of pupae to *Aleochara* attack described by Drea (1966), the effectiveness of which is expected to depend at least on the particular combination of species involved, if not also on intraspecific size variation, as well as puparium thickness, which varies within species and influences susceptibility to at least some hymenopteran parasitoid species (Hagley et

al. 1993). Whether puparium thickness varies in a predictable way with puparium mass is not known. Another aspect of the acceptance interaction of particular *Aleochara* species with a particular host species, tied closely to puparium structure, is orientation of the larvae. The puparium of *Calliphora erythrocephala* Meigen (Diptera: Calliphoridae) has regions of preference where an attacking *A. curtula* Goeze larva will in all likelihood create an entrance hole; attacking *A. bilineata* larvae have preferred regions as well, distinct from those of *A. curtula* (Fuldner 1968), at the mid point of the dorsal surface (Royer et al. 1998). Regions are found by exploring the puparium surface (Fuldner 1968), and so some potential host species, or size, may not provide the cues for orientation to proceed to the creation of a hole.

Others have compared the acceptability, and in some cases suitability, of hosts to the two *Aleochara* species studies here. Brunel and Langlet (1994) determined in no-choice tests of *A. bilineata* that 80 % of *D. radicum* and 78 % of *D. antiqua* puparia are accepted, on average, and that temperature, humidity, and pupal age can modulate these percentages. Among small, medium, and large *D. radicum* puparia, *A. bipustulata* accept the three classes equally, but do not complete development in large puparia as often as in the other two classes, while *A. bilineata* accepts small and medium puparia more often than large ones, but once accepted, is as likely to complete development in puparia in one class as any other (Fournet et al. 1999). *Aleochara bipustulata* larvae are as likely to accept a *D. radicum* puparium as one of *D. antiqua* when given a choice, and more likely to accept *D. radicum* than the acalyptrate *Lonchaea* sp. (Diptera: Lonchaeidae) (Ahlström-Olsson 1994b), but since larvae were provided a choice of puparia in that study, predictions about the relative acceptability of *Delia* and acalyptrate hosts in no-

choice situations might not be the same. Larvae of *A. bilineata* entered puparia of the *Lonchaea* species but did not complete development in 70 trials (Ahlström-Olsson 1994b), although larvae can develop to adults within puparia of *Lonchaea corticis* Taylor (personal observation). If Brunel and Langlet's criterion for acceptance was a plugged entrance hole, the 83–84 % acceptance of *D. radicum* and *D. antiqua* observed in this study agrees well with their estimates. Larger *D. radicum* were not less accepted by *A. bilineata*, contrary to the results of Fournet et al. (1999), but as in that study complete development in *D. radicum* did not depend on host mass. It may be the 'large' *D. radicum* size class of Fournet et al. (1999) included larger puparia than were used here, induced to diapause for instance, and so avoidance of the largest puparia was not evident in my study. Like Fournet et al. (1999), I found *A. bipustulata* accepted all sizes of *D. radicum* equally, but unlike that study, I did not find them less likely to complete development in the largest puparia, which may again be due to underrepresentation of extremely large puparia. Within *D. antiqua*, the probability of complete development was inversely related to mass; this likely was due primarily to rotting of remaining host material in the largest puparia, although at least for *A. bipustulata*, may have also been related to failure to seal the hole in larger hosts, and so a greater risk of contamination throughout development, and not just following the cessation of feeding. Entrance hole data show that for either *Aleochara* species, the two *Delia* hosts are equivalent in the overall effect puparia thickness and orientation may have had, but that *A. bilineata* seems either to be more stimulated, or to be better able, to penetrate. Larvae of *A. bilineata* may have had difficulty orienting on the surface of *P. casei* puparia, especially the smallest ones, and *A. bipustulata* seems to have as well, albeit less severely. Palatability of pupae

and their defensive reactions become important at the stages of larvae entering and plugging holes, where *A. bilineata* frequently could or would not continue attack of *P. casei* but seems to accept more readily the larger *D. antiqua*. For *A. bipustulata*, although the *D. antiqua* puparium seemed to provide cues sufficient for orientation, and was seldom insurmountable as a barrier, the pupa within was less attractive to larvae than those of the other species, and frequently larvae that did enter died of starvation or defensive reaction before they could plug the hole. Seldom was an *A. bipustulata* able to develop within *D. antiqua*, likely due to too much host material, whereas in *P. casei*, *A. bilineata* seemed seldom able to consume enough.

The time required to complete development differed between the parasitoids and among hosts, and within hosts, increased with mass. Although *A. bilineata* has sometimes been found to require more time at room temperature than *A. bipustulata* (Fuldner 1960), generally it is the more rapidly developing species (Ahlström-Olsson 1994a; Fournet et al. 2000), as in this study. Within a host species, more time generally is needed to complete development as host mass increases (Ahlström-Olsson 1994a), which agrees with what was observed here. That the relationship with mass differed among host species, being relative to *D. radicum* quicker in *D. antiqua* and slower in *P. casei*, could arise if the proportion of total mass contributed by pupal and by non-comestible puparial materials differ among host species. Adult size increased with host size as expected (Langlet et al. 1998; Wright et al. 1989), and the relationship now can be used to work backward from field-collected beetles and estimate the size of host from which each was most likely to have emerged. Because host species differ in the proportion of total mass that is puparium, this could have contributed to difference in pronotum width among hosts of a

given mass. So too would puparium shape, those of *P. casei* being relatively more elongate than the others, and so a unit increase in mass is accompanied by a greater increase in puparium width within *Delia* hosts than within puparia of *P. casei*. As a result, the regression of pronotum width on mass has steeper slope for *P. casei* hosts. It is sensible as well, based on the pronotum of *A. bilineata* naturally widening toward its posterior end while that of *A. bipustulata* becomes less broad at the posterior and is widest in the middle (Maus 1996), that for a given host species and mass, *A. bilineata* pronota are slightly wider at the widest point.

Comparing attack by *A. bilineata* larvae on puparia at different depths was undertaken to determine whether along this axis of pupal resource distribution a refuge from parasitism exists. Refuges arise when parasitism, or more inclusively mortality due to natural enemies, is confined to one part of the host population, the other escaping (Hassell 2000). Species with minimal refuges have high mortality due to biotic factors (Myers et al. 1994), and top-down control can result (Costamagna and Landis 2011). Refuges based on depth in soil are known to protect *Musca domestica* L. from some of its pupal parasitoid species (Geden 2002), and to protect *D. radicum* larvae from *T. rapae* parasitism (Hemachandra et al. 2007b). *Aleochara* larvae avoid light (Fuldner 1960), and so unburied puparia are seldom attacked (Wright and Muller 1989), a sort of refuge from pupal parasitism, unlikely to occur often due to dipteran larval behaviour as they seek a spot to pupate.

The proportion of *A. bilineata* larvae successfully entering puparia did in fact decrease with depth, and so there may be a partial refuge from pupal parasitism based on depth in soil. Female *A. curtula* migrate from carcasses at the same time as larvae of their

host (Peschke et al. 1987), and *A. bipustulata* seem to do the same (Goubert et al. 2013). They may, in so doing, oviposit in the tunnels of migrating host larvae, rather than near the surface. Adults in laboratory colonies do spend much of their time below the surface in tunnels (Fuldner 1960). Larvae of *A. bisolata* follow tracks left by larvae of their host to locate the puparia that form (Wright and Muller 1989), and the *Aleochara* species studied here likely do so as well whenever possible, and so it is not known precisely how closely the scenario studied imitates that usually encountered by *Aleochara* larvae. In particular, females may lay eggs in larval tunnels of their host, rather than near the soil surface. *Aleochara* larvae may be adapted to move primarily horizontally, through tunnels for instance, rather than vertically. Fuldner (1960) has shown how, in the absence of host larval tracks, foraging *Aleochara* larvae seem to find puparia by moving randomly through the substrate. Foraging larvae are arrested by dimthyl disulphide (Du 2013), a by-product of *Brassica* decomposition (Ferry et al. 2007). If larvae forage randomly until arrested, more time would be needed in the tubes to find the deepest puparia, and in the meantime some *A. bilineata* larvae may have starved. Determining that puparia at 9 cm can be found was a prerequisite to the final experiment, where it was determined if either species has a preferred depth, how this is manifested in combination with an expected preference for large or small puparia, and finally if these preferences were displayed at low density whether they would become relaxed as density of larvae increased due to discrimination against previously parasitized hosts.

When at least one *D. radicum* puparium is available for each foraging larva, *A. bilineata* prefer those 11–19 mg to those 4–8 mg, whereas *A. bipustulata* prefer the smaller hosts (Ahlström-Olsson 1994b). Among small, medium, and large *D. radicum*, *A.*

bilineata display no preference, but select medium and larger hosts preferentially when *A. bipustulata* larvae are present, while *A. bipustulata* prefers small and medium puparia when foraging alone, and chooses more small than medium or large puparia when in competition (Fournet et al. 1999). *Aleochara* larvae detect whether a prospective host already has been parasitized based on the plug in the entrance hole (Fuldner and Wolf 1971; Lizé et al. 2010; Royer et al. 1999), and both species avoid parasitized hosts when unparasitized hosts are available (Royer et al. 1999). As larval density increases, more than one larva per puparium becomes increasingly likely (Royer et al. 1999), and only one can survive, although frequently both die (Fuldner 1960). It was expected therefore that in the low density treatments, nearly all puparia of the preferred size, small for *A. bipustulata* and larger for *A. bilineata*, would be attacked, but it was not known whether remaining larvae would accept more often the less-preferred size, or keep moving downward. Contrary to expectations, there was no preference for host mass for either species at low density; *A. bipustulata* were more likely to accept shallow puparia, and *A. bilineata* appeared to select at random from the six resource states available. Moving to high density, one expects to see more superparasitism, and the proportion of larvae successfully entering puparia did fall, but not significantly within species. At high density, *A. bilineata* did exhibit preference, but unexpectedly this was for smaller puparia, in addition to shallow puparia, while *A. bipustulata* continued to show no preference based on size. It was expected under interspecific competition that preferences for size and avoidance of parasitized puparia would result in the six states, or at least the four shallowest, being used equally, and equal attack of the six states was observed.

Aleochara bipustulata and *A. bilineata* seem to exploit the same resource in the same way, to a first approximation, and since this will lead to competition whenever the resource is limiting, continuing potentially to the competitive displacement of one or the other (Reitz and Trumble 2002), mechanisms by which niches separate and competition is reduced have been explored to explain their observed coexistence. In Jonasson's (1994) analysis, niches are separated by host size and species, with *A. bipustulata* preferring and performing better in puparia of bean seed fly and small *D. radicum*, and separation in time due to *A. bipustulata* wintering as adults as opposed to *A. bilineata* wintering as larvae and widely different times required for development. A slightly different tack was taken by Fournet and Brunel (1999), who attributed coexistence largely to the larvae's habit of avoiding puparia already attacked, and suggested preferences for host size may exist but seldom act in natural environments where rejecting a host likely means starvation. Neither is a superior intrinsic competitor, although *A. bilineata* is more aggressive in that it will more often attack an occupied puparium (Fournet and Brunel 1999). If this means interspecific competition is stronger in its effect on one species than on the other, then the Lotka-Volterra competition model would permit coexistence if the impact of competition for one species is greater interspecifically than intraspecifically, and greater intraspecifically than interspecifically for the other (Tokeshi 1999). It is possible also for competition to occur without its being strong enough to affect coexistence (Tokeshi 1999). Here, both species developed well in *D. radicum* puparia, and *A. bilineata* developed well in *D. antiqua* but not *P. casei*, while for *A. bipustulata*, the puparia of *D. antiqua* were very poor hosts. *Piophila casei* puparia for *A. bipustulata* were inferior to *D. radicum*, but better for *A. bipustulata* than for *A.*

bilineata. *Aleochara bipustulata* have apparently not been reared from field-collected *D. antiqua* (Andreassen et al. 2009), and so *A. bilineata* does not seem to compete with *A. bipustulata* for this resource. Whether *A. bipustulata* parasitized acalyptrate hosts in nature is unknown, but they are undeniably smaller than *A. bilineata* on average (Fuldner 1960; Jonasson 1994); perhaps adult *A. bipustulata* in *Brassica* fields observed in connection with *D. radicum* developed primarily within puparia of *D. platura*. There was no evidence of preferences for *D. radicum* puparium size for either parasitoid species in the tubes, however. The niche breadth and crowding estimates do tend to support the notion (Fournet and Brunel 1999) that when competing for *D. radicum* hosts, avoidance of already parasitized hosts, as opposed to preference for size, is the overriding influence.

This study arose as part of a classical biological control project. Causing competitive displacement of *A. bilineata* would be an unintended non-target effect (Messing et al. 2006), although perhaps less serious than normal (van Lenteren et al. 2003), given *A. bilineata* is now in North America only accidentally (Klimaszewski 1984). Competitive displacement is not likely, given their coexistence in the Palaearctic. Additional species, such as *A. bipustulata*, have the potential theoretically to disrupt through competition the natural enemies already present and lead to a more severe pest problem (Watt 1965). This seems more probable than displacement of *A. bilineata*. By the same token, one may expect the introduction to increase mortality and improve control to the extent that *D. radicum* exist in places and times in Canada that make them invulnerable to *A. bilineata* predation, and to the extent *A. bipustulata* can cause mortality during these times and in these places. On a geographical scale, it does seem adults of *A. bipustulata* are more abundant than those of *A. bilineata* in Brittany in France (Fournet

and Brunel 1999) and southern Sweden (Jonasson 1994), while in Germany (Fuldner 1960) and at times Switzerland (Riley et al. 2007), *A. bilineata* is more common; canola in northern parts of the Canadian prairies tends to be more affected by root maggots (Soroka et al. 2004), and so it is vague but reasonable to suppose northern prairie root maggot problems will be improved to the extent the region resembles Brittany and southern Sweden as habitat. This sort of regional difference affects the relative importance of different parasitoid species of red scale in California, for instance (DeBach 1965). Both parasitoid species accept and do well within *D. radicum* puparia, but have potentially other hosts besides, which differ; both avoid parasitized puparia, which will reduce competition wherever both species' other requirements are well met and so both are abundant.

Table 7. Statistics calculated to characterize distributions of *Delia radicum* puparia in Manitoba canola fields. Negative skewness (*g1*) is to the left; negative kurtosis (*g2*) indicates platykurtosis, with more items in the distribution's tail than occurs in a normal distribution. Statistics α and β describe Weilbull distributions, the adequacy of whose fit is shown by Anderson-Darling statistics.

Location	Year	N	$\bar{x}$ (mg)	g_1	s.e.$_{g1}$	g_2	s.e.$_{g2}$	α	β	A-D	*P*
Graysvillle	2007	126	10.68	-0.295	0.216	-0.134	0.428	11.74	4.32	0.3	0.94
	2009	134	14.07	-0.478	0.209	0.814	0.416	15.18	5.76	0.4	0.84
St. Claude	2009	124	13.59	-0.449	0.217	-0.193	0.431	14.88	4.67	0.8	0.51

Table 8. Acceptance and suitability of hosts (*Delia radicum*, *D. antiqua*, *P. casei*) for *Aleochara bilineata* and *A. bipustulata*. **A:** For each combination of parasitoid and host, number (N) entering stage (creation of entrance hole, larva entering puparium, plugging entrance hole, development of adult) and proportion that complete that stage, and odds ratio (O.R.) and likelihood ratio χ^2 (L.R. χ^2) for that combination in logistic regression against host mass. **B:** Parameters of logit models [$g(x)=\ln[\pi(x)/(1-\pi(x))]$] selected to describe best the probability of completing each stage for all combinations of parasitoid and host species, and likelihood ratio χ^2 and McFadden's ρ^2 for that model. For χ^2, * indicates $P < 0.05$, ** < 0.01, *** < 0.001.

A

		Hole				Larva				Plug				Adult			
		N	Prop.	O.R.	χ^2_1	N	Prop.	O.R.	χ^2_1	N	Prop.	O.R.	χ^2_1	N	Prop.	O.R.	χ^2_1
A. bilineata	*D. radicum*	108	0.93	1.09	0.3	100	0.94	1.05	0.1	94	0.95	0.96	0.1	89	0.44	0.91	1.1
	D. antiqua	108	0.88	0.87	2.4	95	0.96	1.74	5.7*	91	0.99	0.52	2.1	90	0.54	0.76	14.9***
	P. casei	108	0.47	1.79	7.9**	51	0.76	1.54	1.7	39	0.79	1.03	0.0	31	0.35	1.47	0.9
A. bipustulata	*D. radicum*	108	0.77	0.87	2.7	83	0.95	1.09	0.2	79	0.97	0.81	0.5	77	0.43	0.92	0.7
	D. antiqua	108	0.76	0.99	0.0	82	0.78	0.86	3.0	64	0.63	0.84	3.9*	40	0.08	0.47	6.4*
	P. casei	108	0.69	1.00	0.0	75	0.83	1.07	0.0	62	0.89	1.41	0.8	55	0.38	1.10	0.1

B

Stage	$\pi(x) =$	χ^2	ρ^2
Hole	$-0.47 - 0.95$**A. bilineata* + 0.30*mass + 2.44**Delia* + 1.99**Delia***A. bilineata* − 0.37**Delia**mass	79.7***	0.11
Larva	$3.05 - 1.78$**D. antiqua* − 1.51**P. casei* -0.33**A. bilineata* + 2.19**D. antiqua***A. bilineata*	29.6***	0.08
Plug	$5.00 - 2.80$**D. antiqua* − 2.28**P. casei* − 0.70**A. bilineata* − 0.15*mass + 4.78**D. antiqua***A. bilineata*	63.9***	0.22
Adult	$1.36 - 0.18$*mass − 0.00**A. bilineata* − 2.04**D. antiqua* − 1.09**P. casei* + 2.90**D. antiqua***A. bilineata*	45.1***	0.09

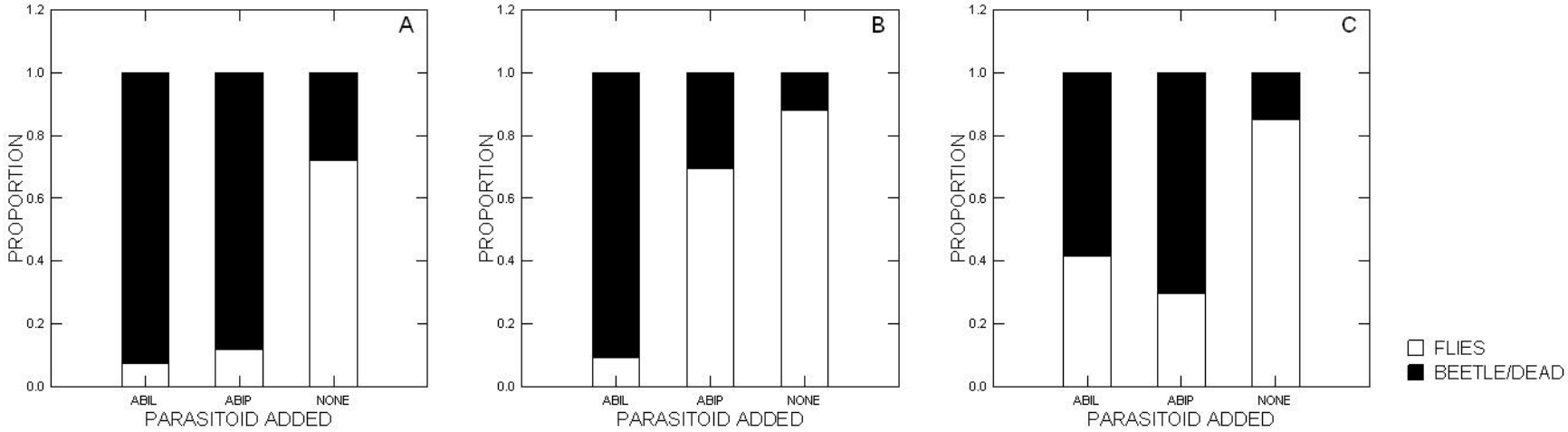

Figure 15. Emergence of adult flies from puparia of *Delia radicum* (A), *D. antiqua* (B), and *Piophila casei* (C) after exposure to parasitoids *Aleochara bilineata* (ABIL) or *A. bipustulata* (ABIP), or to no parasitoid larva. For *D. radicum*, the proportion emerging as flies depended on treatment (L.R. $\chi^2_2 = 134.8$, $P < 0.001$), but not on the species of *Aleochara* (L.R. $\chi^2_1 = 1.3$, $P = 0.25$). For *D. antiqua*, the proportion depended on treatment (L.R. $\chi^2_2 = 166.2$, $P < 0.001$), and was reduced by exposure to *A. bipustulata* relative to controls (L.R. $\chi^2_1 = 11.4$, $P < 0.001$). For *P. casei*, the proportion depended on treatment (L.R. $\chi^2_2 = 80.0$, $P < 0.001$), but not on the species of *Aleochara* (L.R. $\chi^2_1 = 3.4$, $P = 0.06$).

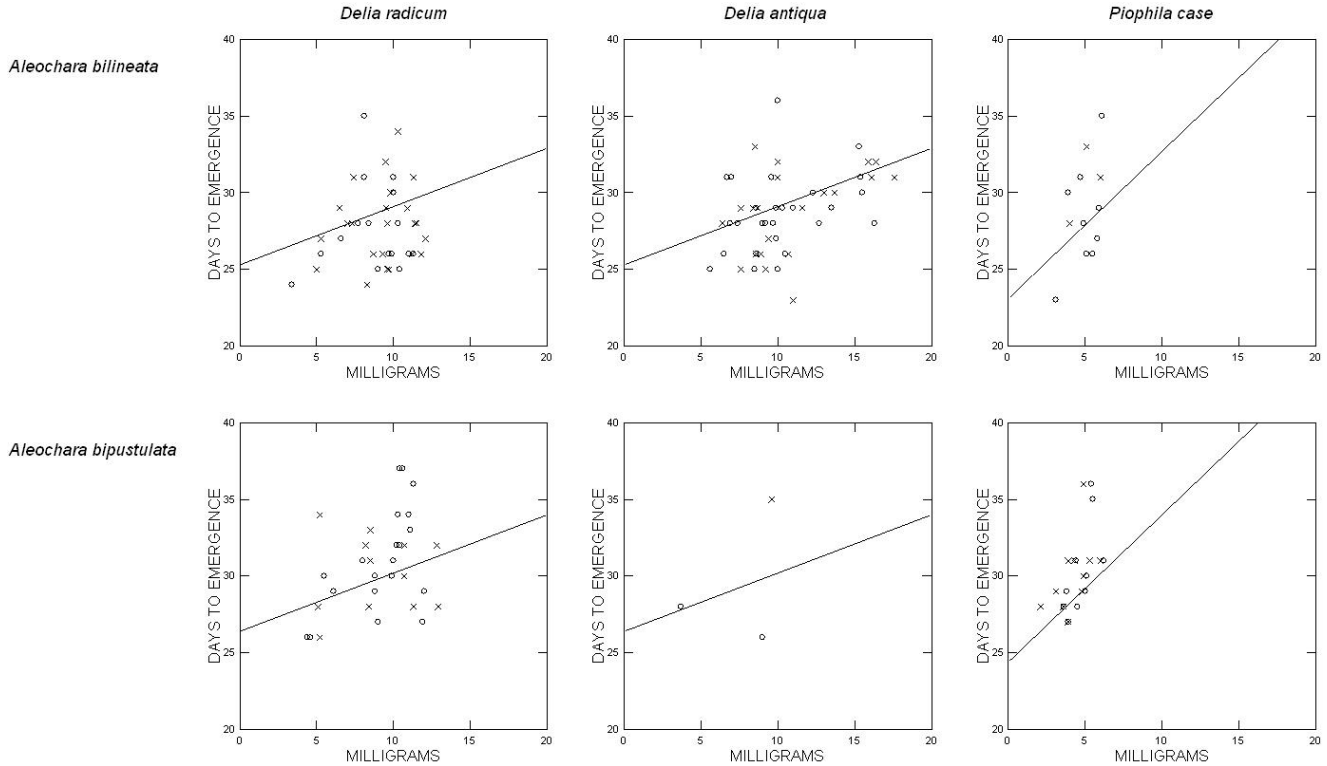

Figure 16. Observed (points) and predicted (lines) days required for emergence of adult parasitioid *Aleochara bilineata* and *A. bipustulata* from the hosts *Delia radicum*, *D. antiqua*, and *Piophila casei*. Circles are females and crosses are males. The general linear model found best to predict time for development was: days = 26.4 + 0.39 (mass) – 0.85 (host sp. = *D. antiqua*) + 1.2 (host sp. = *P. casei*) – 0.94 (parasitoid = *A. bilineata*). The model was selected based on significant reduction in error variance by: i) adding to a model with host mass a term for host species ($F_{2,157} = 5.2$, $P < 0.01$), which explained significantly more variance than including a term for just host genus ($F_{1,157} = 4.0$, $P < 0.05$); and ii) adding a term for parasitoid species to a model already containing mass and host species ($F_{1,156} = 10.2$, $P < 0.01$), but not from adding a term for the interaction parasitoid x mass ($F_{1,155} = 1.1$, $P = 0.30$), nor for parasitoid x host ($F_{2,154} = 0.1$, $P = 0.90$). Including a term for beetle sex in a model already containing parasitoid species, host species, and host mass did not increase significantly the amount of variance explained ($F_{1,155} = 0.4$, $P = 0.55$).

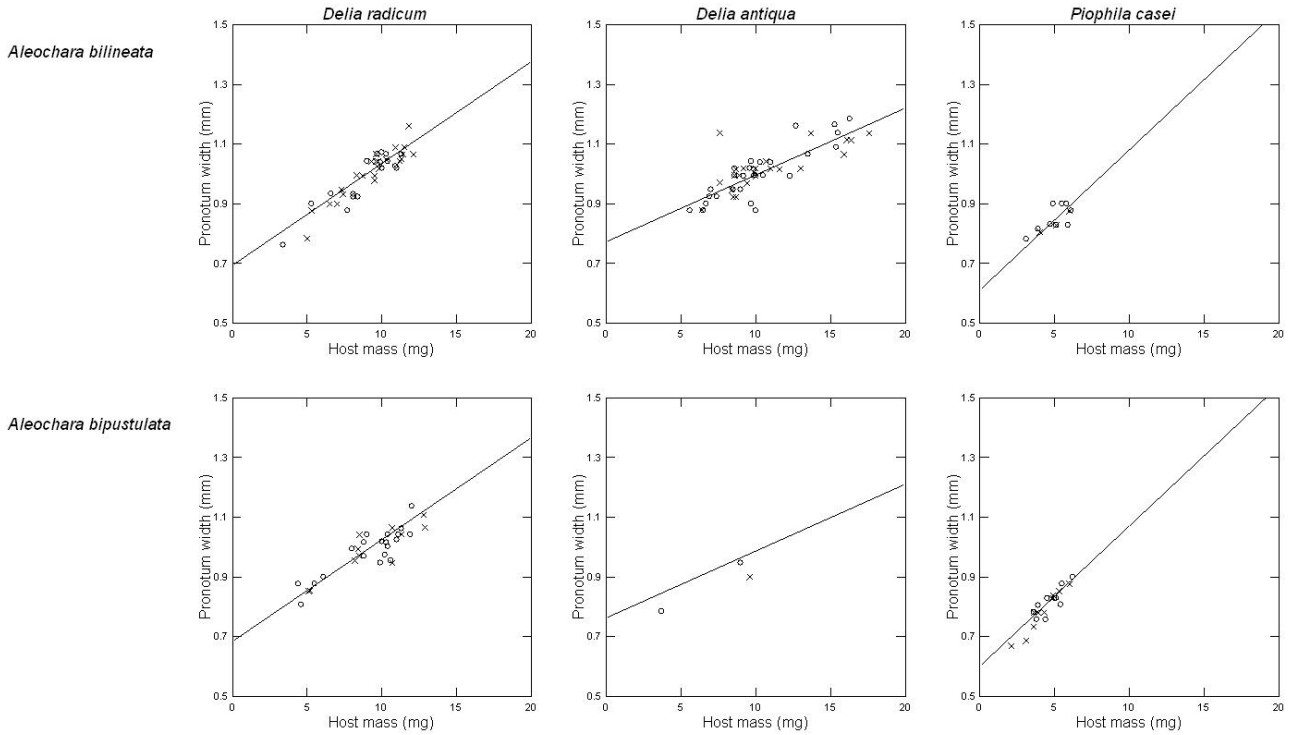

Figure 17. Observed (points) and predicted (lines) pronotum width of adult parasitioid *Aleochara bilineata* and *A. bipustulata* from the hosts *Delia radicum*, *D. antiqua*, and *Piophila casei*. Circles are females and crosses are males. The general linear model found best to predict size was: width = 0.6848 + 0.0341 (mass) − 0.0788 (host = *D. antiqua*) − 0.0827 (host = *P. casei*) + 0.0097 (parasitoid = *A. bilineata*) − 0.0118 (*D. antiqua**mass) + 0.0128 (*P. casei**mass). The model was selected based on significant reduction in error variance by: i) adding to a model with host mass a term for host species ($F_{2,157} = 11.7$, $P < 0.001$), which was better than including only host genus ($F_{1,157} = 6.9$, $P < 0.01$); ii) adding to a model already including terms for host mass and host species an additional term for the interaction of these factors ($F_{2,155} = 11.5$, $P < 0.001$); and iii) adding to a model with host species, host mass, and their interaction a term for parasitoid species ($F_{1,154} = 6.6$, $P < 0.05$), but not from including terms for parasitoid x host interaction ($F_{2,152} = 2.3$, $P = 0.11$), nor for parasitoid x mass interaction ($F_{1,153} = 0.5$, $P = 0.05$). No reduction in error variance resulted from adding a term for beetle sex to a model already containing host species, host mass, parasitoid species, and a host x mass interaction ($F_{1,153} = 0.1$, $P = 0.81$).

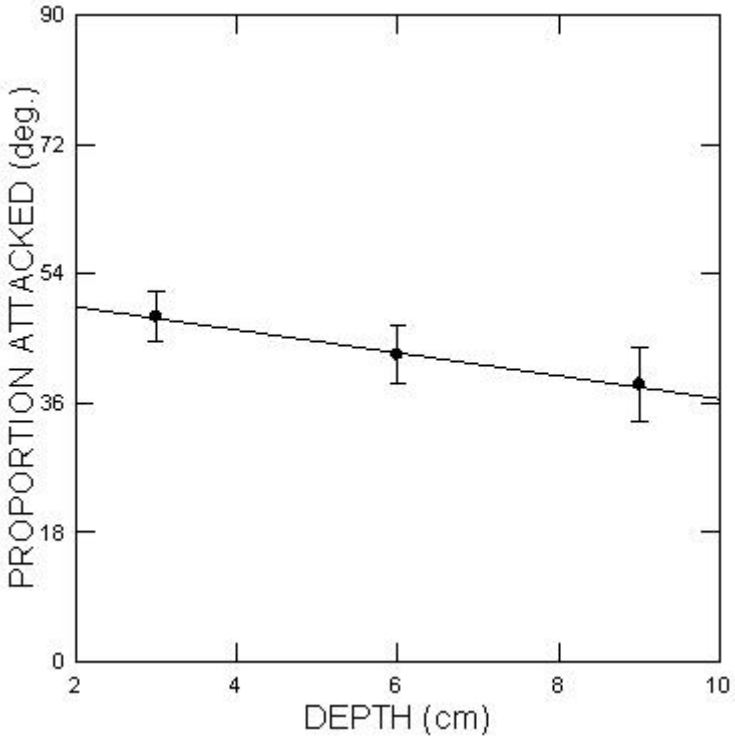

Figure 18. Arcsine transformed proportion (in degrees, 90° = 1.0) of *Delia radicum* puparia attacked at 3, 6, or 9 cm below the surface by larvae of *A. bilineata*. The line describing the relationship is: y = 52.52 − 1.57*depth.

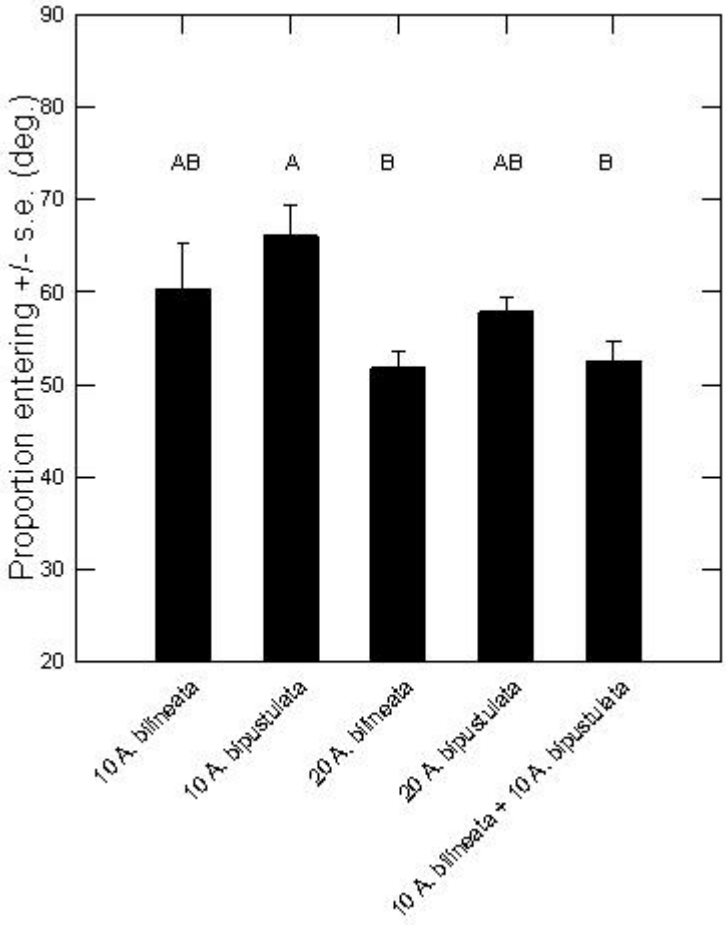

Figure 19. Arcsine transformed proportion (in degrees, 90° = 1.0) of *Aleochara* larvae placed on the soil surface that successfully entered puparia. Letters indicate membership in groups whose means differ significantly as indicated by Tukey's HSD test.

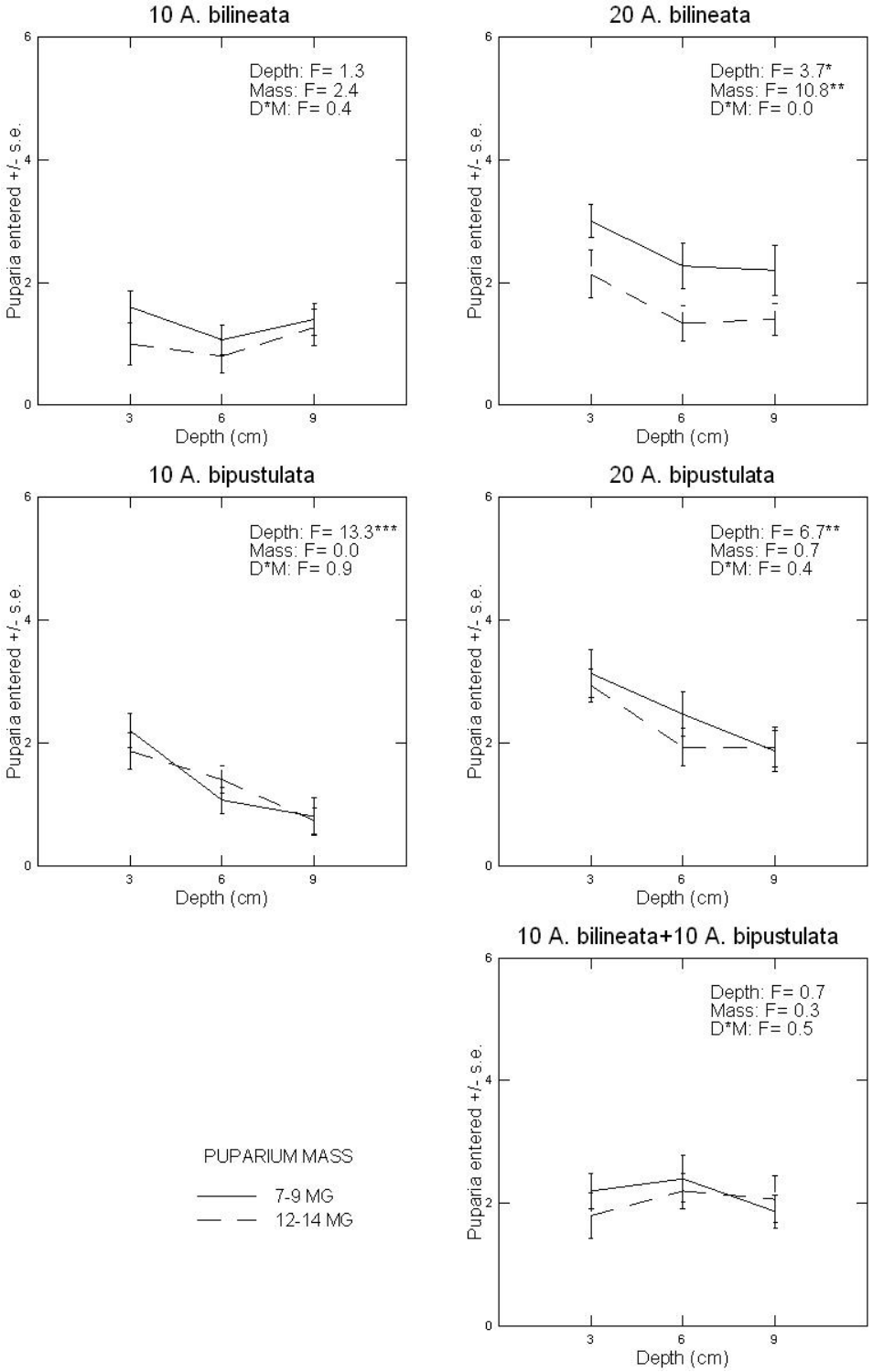

Figure 20. Mean number of puparia selected (/5 per 'resource state') by larvae of *Aleochara bilineata* and *A. bipustulata* along two resource axes, depth and puparium mass, in five treatment combinations of larvae added**.** Degrees of freedom are 2, 84 for depth and for the interaction, and 1, 84 for the effect of mass; asterices following *F* statistics indicate probability: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

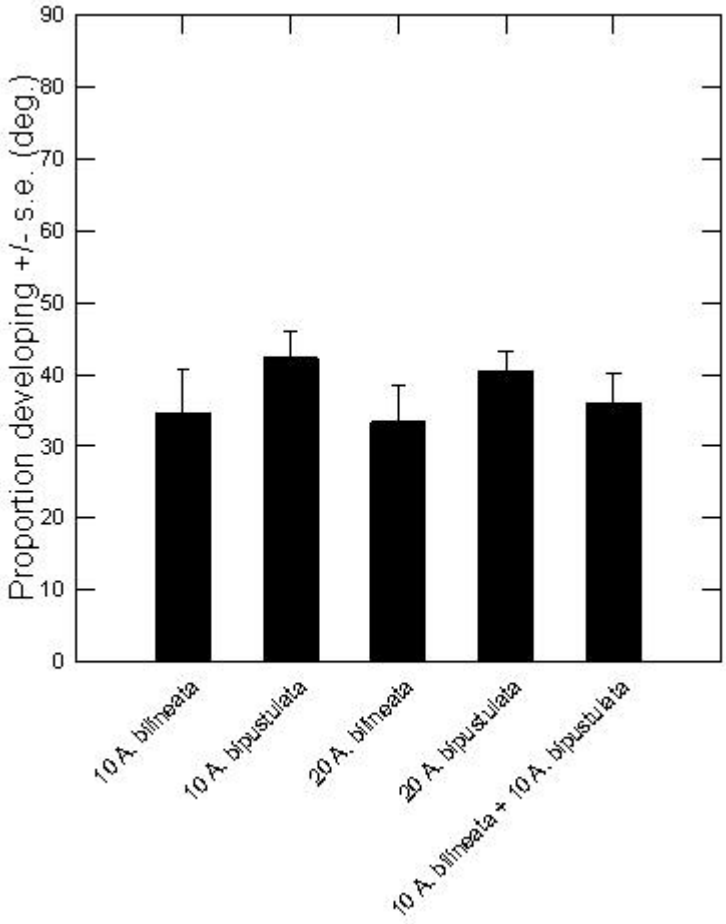

Figure 21. Arcsine transformed proportion (°, 90 = 1.0) of larvae inside puparia that completed development to adult *A. bilineata* or *A. bipustulata* in the different treatments.

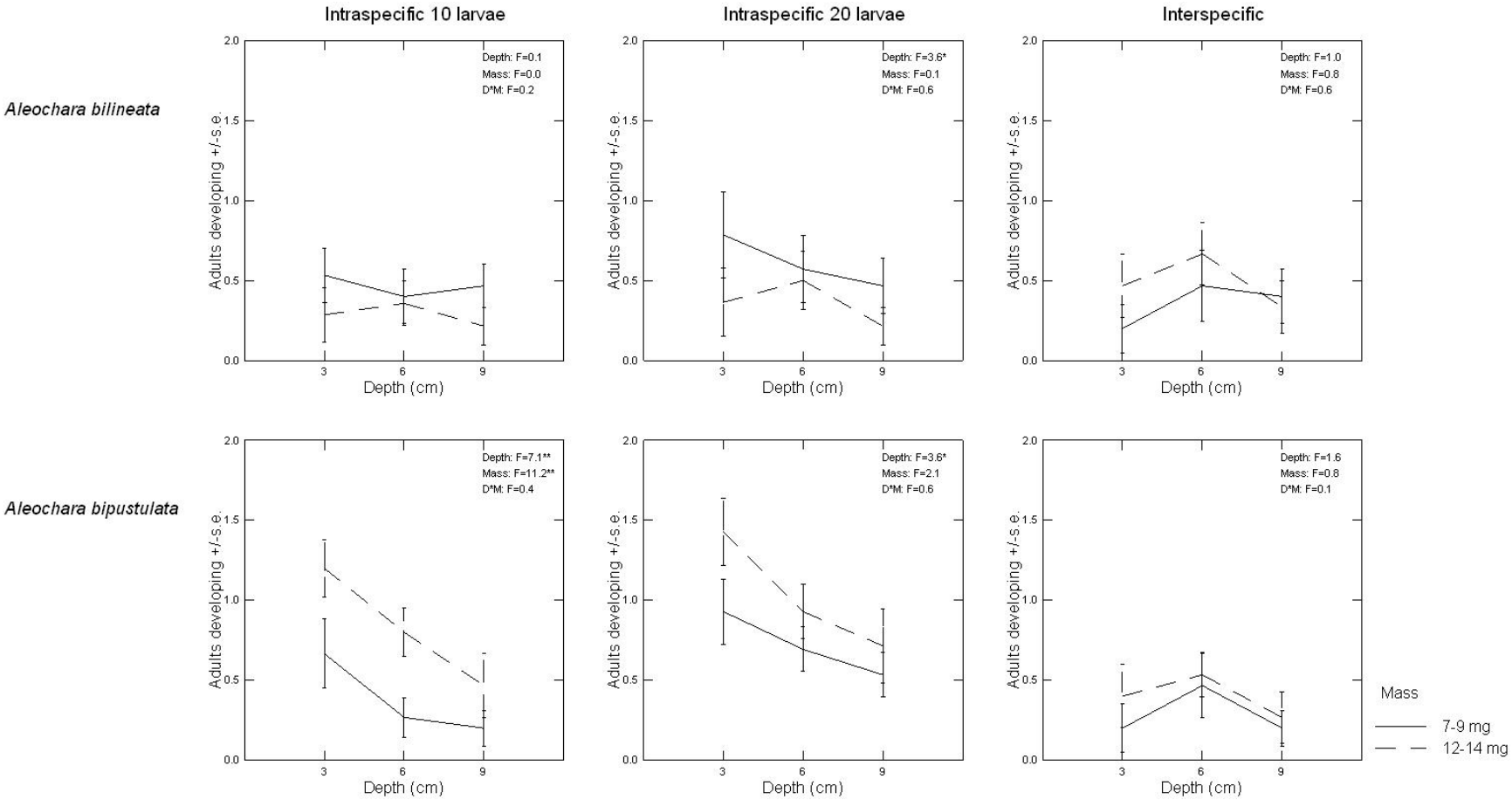

Figure 22. Mean number of *A. bilineata* and *A. bipustulata* that emerged from *D. radicum* puparia of two size classes at three soil depths. Degrees of freedom are 2, 84 for depth and for the interaction, and 1, 84 for host mass; astericies following *F* statistics indicate probability: * $P < 0.05$, ** $P < 0.01$.

Chapter 3 Section 4

Host selection preferences and some of their consequences in Aleochara bipustulata

Introduction

The difficulty in assessing the hosts used by natural enemies considered for classical biological control has long been recognized (Thompson and Parker 1927). If undesirable effects on non-target species are to be forestalled, such an assessment must nonetheless be made. In the majority of cases, this assessment will involve host specificity testing, in which a range of species are offered to the prospective agent, perhaps testing a comparatively long list of species under conditions designed to permit maximum expression of host range, and testing a subset under more natural conditions (Van Driesche and Murray 2004a; van Lenteren et al. 2006b). These tests will ideally reveal the fundamental host range, defined as all host species the natural enemy is capable of utilizing, but the field host range is expected to differ, not least because species within the fundamental host range will differ in their relative acceptability, suitability, and quality (van Klinken 1999). The quality of hosts in terms of fitness components such as offspring size and fecundity can be compared (Van Driesche and Murray 2004b). Host selection behaviour is expected to correspond to these fitness consequences (Godfray 1994), but reliance on single fitness proxies to predict behaviour is likely to mislead (Roitberg et al. 2001). Field work is indispensible to complement fundamental host range testing (Haye et al. 2005; Manners et al. 2011) and to understand how fitness consequences govern behaviour in nature (Janssen 1989; Visser 1994).

Canada soon will consider whether to introduce *Aleochara bipustulata* (L.) (Coleoptera: Staphylinidae) for management of *Delia radicum* (L.) (Diptera: Anthomyiidae) in prairie canola. Larvae of *Aleochara* species are parasitoids of Cylorrhapha (Diptera) pupae, in which they hatch from eggs laid in soil to locate and

enter a dipteran host puparium, then seal the hole through which they entered before consuming the pupa (Klimaszewski 1984; Peschke and Fuldner 1977). The field host range of *A. bipustulata* depends therefore on where eggs are deposited, and then on which Diptera species among those encountered by larvae hatching from the eggs are accepted and suitable for complete development. Many species are acceptable to *A. bipustulata* larvae, when they have no choice, but frequently species that are unsuitable for development are accepted less often than *D. radicum* (Andreassen et al. 2009). Accepted host species consistently suitable for *A. bipustulata* larval development appear to be of two sorts: other species in the family Anthomyiidae, and relatively small, distantly related acalyptrate species, such as *Lonchaea corticis* (Taylor) (Diptera: Lonchaeidae), or the carrot fly *Psila rosae* (F.) (Diptera: Psilidae). All Anthomyiidae species are not equally suitable; the onion maggot, *D. antiqua* (Meigen), seldom supports *A. bipustulata* development, even though it is consistently suitable for the closely related *A. bilineata* Gyllenhal (Chapter 3 Section 3). *Aleochara bipustulata* from acalyptrate hosts are relatively small. Larger puparia produce larger adults (Fuldner 1960; Langlet et al. 1998), but there exists a chance the pupae will spoil and the developing *Aleochara* larva will die before it can completely consume its host, and spoilage becomes more likely as host size increases (Fuldner 1960).

There are several components to the role of size in determining the rate at which *Aleochara* genotypes increase. For instance, whereas the number of ovarioles is fixed in most staphylinid species, in *Aleochara* species, the number varies, and is positively correlated with adult size (Welch 1993). *Aleochara* eggs absorb water starting 30 h after they are laid, and increase in size (Gauvin et al. 2001), but vary considerably in size when

laid as well (Boivin and Gauvin 2009). Larger larvae, from larger eggs, are more active and more likely to locate hosts than smaller larvae (Boivin and Gauvin 2009). Egg development rate and proportion hatching are independent of size (Boivin and Gauvin 2009). Whether there is any relation between the sizes of eggs and the sizes of mothers is not known. Host size influences the time required to complete development, being longer in larger hosts (Ahlström-Olsson 1994a; Chapter 3 Section 3). Langlet et al. (1998) found no relationship between egg production over 60 d and size of adult *A. bilineata*, but the majority of adults in that study would likely have emerged from pupae 9–12 mg, and since females were isolated after one mating, sperm depletion over the 60 d may have confounded whatever effect size had on egg production through time. One copulation results on average in 80 spermatozoids being stored in the female *A. bilineata*'s spermatheca (Lizé et al. 2009). In carrion-associated *A. curtula* Goeze, small females lay about one egg per day, while larger *A. curtula* are capable of producing about 16 eggs per day; small males are unable to defend preferred mating spots on carcasses and are usually forced to contend with "satellite" locations nearby, where receptive females are less likely to be (Peschke 1988).

Aleochara bilineata and *A. bipustulata* both are associated in Europe with the *Delia* host species whose larvae develop feeding on roots of crucifer crops: *D. radicum*, *D. floralis* (Fallén), *D. florilega* (Zetterstedt), and *D. platura* (Meigen) (Maus et al. 1998). Puparia of *D. florilega* and *D. platura* are similar in size and difficult to distinguish and therefore frequently are lumped as "bean seed fly"; they usually are somewhat smaller than those of *D. radicum*, which in turn tend to be smaller than *D. floralis* (Wishart 1957). It may be that the pool of puparial host resources is partitioned between the two

Aleochara species, minimizing competition (Fournet and Brunel 1999; Jonasson 1994). Whether as a result of selection to reduce interspecific competition or not, larvae are known to discriminate among species and sizes of potential hosts, and female choice of oviposition site may additionally make certain encounters of potential hosts and foraging larvae more common; behaviour of larvae and females is expected to be adaptive. In surveys across Europe, for example, *A. bipustulata* did not emerge from thousands of *D. floralis* puparia, and was more frequently a parasite of bean seed fly than *D. radicum* (Wishart et al. 1957). Voucher material associated with the research of Wishart's group was lost in 1972 when the Canada Department of Agriculture institute in Belleville closed (Hemachandra et al. 2005), and so cannot now be examined to confirm specimens were *A. bipustulata* and not another species with similar external structure; the description of genitalia necessary to make this distinction has been available only since the 1980s (Lohse 1986). In southern Sweden, *A. bipustulata* adults emerged from puparia of bean seed fly, and from small and medium *D. radicum*, but not from large *D. radicum*, whereas *A. bilineata* emerged equally from all host classes (Jonasson 1994). Additionally, the distributions of sizes of *A. bipustulata* and *A. bilineata* in pitfall traps overlapped, but *A. bilineata* were more frequently larger and *A. bipustulata* smaller (Jonasson 1994). In contrast, in northern Germany, *A. bipustulata* was as likely to emerge from *D. radicum* in one size class as it was from any of six other classes (Hemachandra 2004). In choice assays, *A. bipustulata* larvae prefer small *D. radicum* to large *D. radicum* hosts, and prefer *D. radicum* to relatively small, acalyptrate *Lonchaea* sp. (Lonchaeidae), but show no preference between *D. radicum* and *D. platura*, or *D. antiqua* (Ahlström-Olsson 1994b).

There were three objectives to this study. The first was to examine the consequences of host size and species selection for rates of *A. bipustulata* growth, survival, and reproduction. A matrix population model (Caswell 2001) was constructed to bring these elements together in a single estimated finite rate of increase. The second objective was to determine whether larvae prefer hosts on which finite rate of increase is highest, and if this preference is altered by proximity to, or the relative abundance of, host types. Also, size of field-collected *A. bipustulata* was examined to determine which sizes of host contribute most to natural populations of *A. bipustulata* adults.

Material and Methods

Insects for laboratory assays

The *D. radicum* used in the experiments were maintained as described by Whistlecraft et al. (1985b), and originated with stock from the Agriculture and Agri-Food Canada centre in London, Ontario. The *Piophila casei* (L.) (Diptera: Piophilidae) were maintained on ground meat, and the colony originated from maggots in dead fish set out at the University of Manitoba research farm at Glenlea, Manitoba. The *A. bipustulata* originated from cabbage and cauliflower fields around Kerzers, Switzerland, and were kept in the laboratory with *D. radicum* as hosts (Andreassen et al. 2009).

Host acceptance & suitability, and development time

Puparia were collected from the fly colonies, and the mass of each puparium recorded to the nearest 0.1 mg. Puparia of *P. casei* ('PIO'), which on average weighed 3.1 mg (±0.98 s.d.), were used directly. Puparia of *D. radicum* were divided into three size classes: the largest to correspond to puparia typical of Canadian canola fields (10.5–11.5 mg, 'LDR'), a small class to be comparable with the weight of *P. casei* puparia

(4.5–5.5 mg, ‘SDR’), and an intermediate class (7.5–8.5 mg, ‘MDR’). So that beetles would emerge from a class of puparia at once, to form couples from beetles of the same age, the order in which classes were parasitized was randomized, and on a day 15–30 puparia of a class would be exposed to *A. bipustulata* larvae. Cold storage of non-diapausing *D. radicum* puparia of up to eight weeks having no known adverse effects (Whistlecraft et al. 1985b), puparia often were kept up to three weeks at 1°C after the first two days at 20 °C, to assemble a group of a class. Puparia of *P. casei* were two days old when exposed to larvae. In each exposure, one puparium was exposed to one *A. bipustulata* larva in a no-choice test. Puparia were buried in sand moistened with 100 ml water per dry litre, in plastic vials 4.5 cm tall and 2.2 cm diameter. Puparia rested on a 1 cm layer of sand, packed by finger to form an indentation that kept puparia away from the vial’s wall, and under 1.5 cm sand packed loosely (Wright and Muller 1989). An *A. bipustulata* larva, 24–48 h old, was transferred to the sand surface with a moist paintbrush, then vials were kept 7 d at 20 °C with 16 h light daily. These conditions of light and temperature were used for all laboratory aspects of this study. Puparia, immersed briefly in water to make them more transparent, were inspected under a microscope for host acceptance, defined as the presence of a larva inside a puparium. Accepted puparia were reburied in vials, and kept a further 7 d before reducing the depth of substrate in vials to about 0.5 cm. Puparia on this reduced layer of sand were inspected daily for host suitability, defined as the emergence of adult parasitoids. *G* statistics were calculated to test the hypotheses that the frequencies with which hosts are accepted, and with which accepted hosts are suitable, are independent of host class. ANOVA was used to test the hypothesis that the number of days between the addition of an *A. bipustulata*

larva to vials and the emergence of an adult beetle is independent of host class, and the Tukey-Kramer method (Sokal and Rohlf 1981) was used to compare among classes.

Longevity and fecundity

Pairs of female and male *A. bipustulata* were created from beetles that emerged within a day of one another. These couples were kept in 4.8 cm tall, 4.8 cm diameter polystyrene jars, with lids secure but not tightened to allow air flow. A moist filter paper lined the bottom, and two *D. radicum* pupae removed from the puparium were provided as food; paper and pupae were replaced three times each week. Eggs of *A. bipustulata* were removed from jars each day and counted. All beetles were monitored daily until they died, and so there were no censored observations. Twenty-five LDR couples, 26 MDR, 28 SDR, and 15 PIO couples were followed in this way.

To model survival over time, the fit of several distributions from the generalized gamma family were compared with probability plots (Lee 1992), before selecting the Weibull distribution of survivorship over time (t):

$$S(t) = e^{-(\tau t)^{\wedge}\gamma}$$

Distributions were fitted using the FITDIST module in Systat 13.1 (Systat 2009). Likelihood ratio tests were used to determine if covariates for beetle sex and host class improved the fit using Systat's Survival Analysis procedure (Lee 1992).

The average number of eggs laid each day for *A. bipustulata* from the different host classes was used to estimate parameters of a four-parameter model originally for poultry egg production (Yang et al. 1989):

$$y(t) = a \bullet e^{-xt} / 1 + e^{-c(t-d)}$$

In this model, y(t) is the average number of eggs laid on day t, *a* is a scale parameter, *x* the rate of decrease in egg production after the peak, *c* a reciprocal indicator of variation in sexual maturity, and *d* the mean age at which egg production starts. The daily values were weighted by the number of females contributing to each in estimating parameters by nonlinear least squares regression. One model was fit to adults in each of the four host classes. The day of peak egg production (t_{max}) was calculated for beetles in each host class using the relation:

$$t_{max} = d + [\ln(c\text{-}x - \ln(x)\ ]/c$$

and then Yang's model was solved at t_{max} to determine the modelled maximum daily production for beetles in each class.

Effects of host on offspring

To compare among beetles that emerged from different host classes, egg size, egg viability, and egg development rate, all eggs laid by the female of each couple on the 10^{th} day after her first oviposition were retained. The length and width of each egg were measured with an ocular micrometre to the nearest 0.0238 mm on the same day the eggs were laid, and egg volume calculated as an ellipsoid with equal minor axes (Burington 1973):

$$V = 4/3 \bullet \pi \bullet \text{width}^2 \bullet \text{length}$$

Eggs were then kept on moist filter paper and observed daily for hatching for 12 days. The proportion of eggs from each couple to hatch was arcsin square root transformed prior to testing for differences among groups by ANOVA. Mixed model nested ANOVA (Sokal and Rohlf 1981) was used to determine if the volume of eggs, or days required to hatch, differed significantly among host classes, given the variation among beetles.

The ability of larvae from females that emerged from the different classes to find and attack hosts was compared by interrupting the parasitism process after different intervals. Puparia of *D. radicum*, 1–3 d old and 7–12 mg, were buried in vermiculite moistened with 150 ml water per litre, in 1.5 ml microcentrifuge tubes with a puncture for ventilation in the lid. Eighty larvae of each class were released on the vermiculite surface, one per tube, then lids were closed and tubes held upright until the randomly assigned number of days (1, 2, 3, or 4) had elapsed. Puparia then were examined to determine if larvae had entered, and if so, whether the entrance hole had been plugged. Results were analysed with logistic regression, with beetle class as a dummy coded variable.

Matrix model

Matrix population models (Caswell 2001) were constructed for *A. bipustulata* from each of the four host classes, to bring together the information gathered above and to determine whether the population growth rate (λ) differed among classes. These models project population size and structure from one time period to the next, each time period in this case representing a day. The matrices were square, with three sections of columns corresponding to the egg stage, larval and pupal development, and adult life.

Eggs having been found to be as viable and to develop at the same rate regardless of host class (see Results), columns for the egg stage were the same in all four matrices. To model survival and development, without assuming a stable age distribution within each stage (Caswell 2001), the negative binomial distribution was used. The egg stage was divided into six ($= \alpha$) pseudo-stages, six being approximately the mean duration (T) squared and divided by the sum of mean duration and variance of duration [V(T)]. Each

pseudo-stage had its own column, and in each pseudo-stage column was a probability of remaining in that pseudo-stage (P) along the matrix diagonal, and a probability of graduating from that pseudo-stage to the next one (G) on the sub-diagonal. Caswell's recommendation to multiply the pseudo-stage graduation probability (γ) by stage-specific survival (δ) to obtain G surely overestimates mortality, and so a pseudo-stage specific rate of survival (ζ) was calculated. To illustrate for the egg stage:

$$\gamma = T /(V(T) + T) = 6.7982 / (0.6702 + 8.7982) = 0.9103$$

$$\zeta = \sqrt[\alpha]{\delta} = \sqrt[6]{0.767} = 0.9567$$

Then:

$$P_{Egg} = (1- \gamma)\ \zeta = 0.08569$$

$$G_{Egg} = \gamma\ \zeta = 0.87088$$

The same approach was applied for the larval/pupal stage. The probability of complete development and the average time required for development differed among host classes (see Results), and so each of the four matrices had different numbers of larval/pupal pseudo-stages, and different matrix elements P and G corresponding to these pseudo-stages. These appear in Table 9, along with the results on which they were based.

For each of the host classes, the matrix section concerning the adult stage had 90 columns, to keep them comparable, as PIO beetles older than 90 days did not lay eggs (see Results). The dimensions of the PIO matrix therefore was 6 egg pseudo-stages + 27 larval/pupal pseudo-stages + 90 = 123 rows and columns; dimensions of the matrix for SDR were 122, for MDR 123, and for LDR 126. This section had non-zero elements on the sub-diagonal, representing the probability of a female age x at time t to be still alive at time t+1; in the first row, representing the number of female eggs laid per day by females

aged x. Because beetles from *Delia* host classes had the same rates of survival, which differed from that of beetles from PIO hosts (see results), parameters were the same for all *Delia* host classes, and distinct ones were used for the PIO matrix. The survival probability element a_{ij} was based on the hazard function of the Weibull survivor distribution (Lee 1992):

$$a_{ij} = 1 - h(x)$$

$$h(t) = \gamma/\tau\ (x/\tau)^{\gamma-1}$$

for age x = 1 to 90. The parameters τ and γ are provided in the caption to Figure 24. Element a_{ij} is in the i^{th} row and j^{th} column, so for instance the element corresponding to survival of PIO adults aged 20 d surviving to day 21 is $a_{54,53}$ since 6 egg pseudo-stages + 27 larval/pupal pseudo-stages + 20 = 53; the value of this element is:

$$1\text{-}h(20) = 1 - 1.8093/64.7202 \bullet (20/64.7202)^{1.8093-1} = 0.989193$$

For age-specific fecundity appearing in the matrices' first rows, the elements a_{1j}, j = (6 egg pseudo-stages) + (# larval/pupal pseudostages in host class) + x, and x from 1 to 90, were $0.5y(x)$ for the Yang model described above, with parameters as in the caption to Figure 25. The multiplication by 0.5 indicates the assumption of an equal sex ratio.

The dominant eigenvalue of each matrix was calculated as an estimate of the finite rate of increase (λ) (Caswell 2001) for *A. bipustulata* in that host class. These calculations were done in Excel with the PopTools add-in (Hood 2009). There are procedures to attach confidence intervals to estimates of λ (Caswell 2001), but these were not straightforward to implement on the projection matrices, constructed as they had been separately for the egg, larval/pupal, and adult periods based on different individuals in each case. Therefore, for each of the four matrices, λ was calculated again at the lower

and at the upper 95% confidence interval of: proportion surviving the egg stage; proportion surviving the larval/pupal stage; each of the two parameters (τ, γ) of the hazard function describing survival over time of the adult stage; each of the four parameters (*a, x, c, d*) of the daily egg production function.

Laboratory choice tests

Two experiments about the preference of *A. bipustulata* larvae were performed in 1 cm tall, 5 cm diameter Petri dishes with moist sand. Puparia were buried with their anterior end perpendicular to the dish wall, with 2 mm between wall and puparium. In the first experiment, one *D. radicum* puparium (10–12 mg) was buried opposite a puparium of *P. casei*. An *A. bipustulata* larva was released on the surface either in the centre of the dish, or directly above the *P. casei*, or directly above the *D. radicum*. Thirty replications were performed at each release point. After 7 d, both puparia were then inspected for entrance holes, for the presence of larvae inside, and for plugged entrance holes. Puparia with larvae inside were considered accepted, and a likelihood ratio χ^2 statistic calculated to test the hypothesis that a constant proportion of the two species will be attacked regardless of larval placement location.

Four puparia were buried in each replicate of the second experiment, two *D. radicum* and two *P. casei*, or one of one species and three of the other. Thirty replicates were again performed for each of the three treatment combinations. A larva was released in the middle of the dish and left 7 d as above. For each treatment, a likelihood ratio χ^2 was calculated to test whether host species would be accepted in proportion to their abundance.

Aleochara bipustulata *size in nature*

Beetles were collected at various locations in Switzerland between 2005 and 2011. The width of the pronotum of these beetles was measured to the nearest 0.0238 mm with a micrometer on a dissecting microscope. The median width, as well as the range in width, were calculated for each collection.

Results

Host acceptance & suitability, and development time

The proportion of puparia accepted, proportion of accepted puparia suitable for development, and the number of days required for development all differed among the four classes of hosts (Figure 23). The proportion of SDR and MDR puparia accepted was the same, and higher than the proportion of LDR puparia (L.R. $\chi^2_3 = 11.0$). More LDR were accepted than PIO, but the difference was not significant (L.R. $\chi^2 = 6.6$). The same pattern was evident in host suitability, with SDR and MDR indistinguishable (L.R. $\chi^2 = 0.4$), a greater proportion of SDR and MDR suitable than LDR (L.R. $\chi^2 = 7.9$), and no statistical difference between LDR and PIO (L.R. $\chi^2 = 0.2$). The days required for development differed among classes ($F_{3,235} = 34.8$), increasing among *D. radicum* with the size of host, and for PIO falling between SDR and MDR.

Longevity and fecundity

The Weibull distribution fit the survival durations well, particularly for *A. bipustulata* from *D. radicum* hosts, although tending at later times to overestimate the probability of survival, especially for PIO *A. bipustulata* (Figure 24). Distinguishing among beetles from different classes of *D. radicum* did not improve model fit (L.R. $\chi^2_2 = 1.4$, $P = 0.50$), nor did distinguishing among sexes (L.R. $\chi^2_1 = 0.6$, $P = 0.43$). Including a

covariate for host species did improve fit, as *A. bipustulata* from *D. radicum* did tend to live longer (L.R. $\chi^2_1 = 19.1$, $P < 0.001$).

Average egg production over time rose to a peak, the tapered gradually for *A. bipustulata* from all four classes of hosts (Figure 25). The fitted egg production models appeared to overestimate production very early, and after egg production was observed to cease for *A. bipustulata* from all four host classes. The adult age at which egg production peaked was for PIO beetles 29.6 days, for SDR 49.6, for MDR 23.2, and for LDR 29.7. The number of eggs laid on this peak day was modelled to be 6.7 for PIO beetles, 7.7 for SDR, 9.9 for MDR, and 15.5 for LDR.

Effects of host on offspring

The 991 *A. bipustulata* eggs laid on the tenth day after oviposition commenced by beetles in the four host classes were equivalent statistically in all respects measured (Figure 26). Eggs were about 0.104 mm^3. Approximately three quarters of eggs hatched, and these tended to require 6–7 d at 20 °C to do so. In the host exploitation experiment, both the proportion of total puparia with larvae inside and the proportion of total puparia with plugged entrance holes increased with time elapsed before interruption (Figure 27). Neither for larvae entering puparia ($Z < 1.6$, $P > 0.10$), nor for plugging the hole ($Z < 0.8$, $P > 0.37$) did host class influence this relationship. About 70 % of puparia had larvae inside by the fourth day, at which time about 60% of entrance holes had been plugged.

Matrix model

The finite rate of increase for beetles in the four host classes are in Table 10. Values for PIO beetles tended to be lower than those for beetles in the three *D. radicum* classes, while values for the *D. radicum* classes tended to be quite similar. The values did

not seem greatly affected by parameterization based on confidence limits instead of mean values; PIO beetles evaluated at the means had a λ of 1.052465, for example, a minimum estimate of 1.038297 at the lower confidence limit of the fecundity function parameter *a*, and a maximum at the upper confidence limit of the same parameter of 1.060718. The fecundity parameters seemed to have a greater impact on λ than did parameters for survival. Evaluated at the mean values, SDR and MDR beetles had slightly higher rates of increase than LDR beetles, but reading across the different confidence limits to the fecudity parameters, one sees SDR or LDR beetles have the lowest rates, and MDR are never predicted to increase the slowest among *D. radicum* classes. Rates of increase of the PIO and SDR classes seemed more affected by changes to the fecundity parameters than did MDR or LDR beetles, and rates were more sensitive to changes in the parameters *a* and *d* than to changes to *x* or *c*. The confidence limits for parameters of PIO beetles never overlap those for MDR or LDR beetles, and only one confidence limit for SDR falls within the range of confidence limits for PIO. The rate of increase of beetles attacking PIO hosts is lower than for those attacking *D. radicum* hosts.

Laboratory choice tests

In both experiments, a strong preference for puparia of *D. radicum* was evident across all treatments (Figure 28). In the first experiment, all 30 larvae placed over *P. casei* puparia selected a host, 29 placed over *D. radicum* did the same, of larvae placed in the centre 27 chose a host; there were therefore too few larvae which did not choose hosts to determine whether placement affects frequency of any acceptance. The proportion of larvae choosing either host species did not depend on where the larva was placed (L.R. $\chi^2_1 = 1.4$, $P = 0.49$). In the second experiment, 28 larvae made a choice when host

species were equally abundant, 27 made a choice in dishes with more *D. radicum* than *P. casei*, and 25 larvae chose hosts when *P. casei* puparia were more common; again too few failed to make a choice to determine formally if the frequency of acceptance depends on host ratio. More *D. radicum* were accepted in the second experiment than expected based on the relative abundance of the host species (3 *D. radicum*: 1 *P. casei*: L.R. χ^2_1 = 4.1, P = 0.04; 2 *D. radicum*: 2 *P. casei*: L.R. χ^2_1 = 14.9, $P < 0.001$; 1 *P. casei*: 3 *D. radicum*: L.R. χ^2_1 = 35.0, $P < 0.001$).

Aleochara bipustulata *size in nature*

A total of 54 pitfall trap collections, ranging in size from one to 71 adults, are summarized in Table 11. Only five collections contained beetles large enough to have likely emerged from *D. radicum* in the LDR class of this study. About half of the collections included beetles large enough to have emerged from MDR hosts, but the median size of collections was within the range for MDR hosts only once. In fact, the median was less than expected for *A. bipustulata* from the SDR size class in 78% of the collections.

Discussion

The class of host attacked by an *A. bipustulata* larva was shown to have many consequences. At the first stage of the process, that of host acceptance, small and medium *D. radicum* were more accepted than the other two classes. Similar size-based intra-host species differences in acceptance have been reported in other *Aleochara* species. For example, *A. bisolata* Casey, accepts small *Musca domestica* L. (Diptera: Muscidae) more frequently than large ones (Wright et al. 1989). Just as *A. bisolata* survives better in the small *M. domestica* puparia it is more likely to accept (Wright et al. 1989), *A. bipustulata*

survived best in small and medium *D. radicum* puparia. There was a host species effect, beyond that of host size in my study, as shown by the higher acceptance and suitability in small *D. radicum* hosts than in *P. casei*. Given the technical difficulty of studying undisturbed an interaction taking place naturally under soil, it is not surprising we do not understand completely whether differences in observed acceptance have more to do with the larva's levels of excitation and inhibition in the environment of that host on the one hand, or, on the other hand, differences among classes of host in ability to withstand such an attack, such as thickness of the puparium wall. Both likely are important, since *Aleochara curtula* Goeze larvae are known to be differentially excited by different suitable species of host (Pfenning and Fuldner 1976), but with *A. bipustulata* attacking *D. radicum*, and *A. bisolata* attacking *M. domestica*, large hosts are less frequently accepted even though larger puparia are likely to emit at least as much lipid-soluble stimulant of the sort Pfenning and Fuldner (1976) described as the smaller puparia. I have observed puparia with an entrance hole and first instar *Aleochara* larva inside, but not feeding and presumed dead, and with melanised spots on the otherwise off-white pupal surface. This may be a defensive reaction, and larger pupae may be more successful in mounting it. It is suspected also that larger pupae are surrounded by a thicker puparium than their smaller conspecifics, and as a direct consequence, *A. bipustulata* are more likely to be able to enter small puparia before they exhaust their reserves and expire. Puparia of *D. radicum* are most frequently entered by larvae of *A. bilineata* on the dorsal side in regions where transverse ridges on the puparium surface are shortest, suggesting higher-ridged areas are avoided (Royer et al. 1998), but it is not known if larger puparia have higher ridges than their smaller conspecifics, nor whether a ridge height or puparium thickness

exists that *Aleochara* larvae refuse to attempt or cannot manage in an absolute sense. Puparia of *D. antiqua* collected in Michigan onion fields are occasionally observed with dead *A. bilineata* larvae half outside (G. H. Whitfield, personal communication 2008), and *A. bipustulata* in the same condition can be observed in the laboratory.

The time required for complete development could also be influenced by puparium thickness, if thickness differences exist, since more time likely is required to penetrate thicker puparia. The greater volume to be consumed in larger puparia is likely more important in determining differences in development time among *D. radicum* hosts than thickness of the puparium wall, though: such an effect of intraspecific host size variation has been demonstrated in the carabid parasitoid, *Brachinus explodens* Duftschmid (Saska and Honek 2012). Although some coleopteran parasitoids develop at the same rate regardless of host (Weber et al. 2006), other parasitoids do not (Salt 1941), and the greater time required for *A. bipustulata* development in *P. casei* than in SDR hosts indicates that the host species differ in nutritional quality, or the speed with which they are consumed, or both.

Survival rates of adults from different host species differed as well, providing further support for the notion that *D. radicum* and *P. casei* differ in nutritional value, in addition to differences in adult size. Similar host species effects on rates of development and survival occur in the egg parasitoid, *Telenomus remus* Nixon (Hymenoptera: Platygastridae) (Pomari et al. 2012). In *T. remus*, however, wasps develop most rapidly on host species that permit shorter lifespans, suggesting a trade-off, whereas in *A. bipustulata*, PIO hosts are associated with both an extended immature period and a shorter adult lifespan. It may be that certain essential lipids are in relatively short supply

in PIO hosts, since *A. bilineata* is, in common with many other species of parasitoid, unable to synthesize lipids (Visser et al. 2010), a trait likely found also in *A. bipustulata*. If lipid shortage it was, lipid deficiency appears also to have influenced egg production. Beetles from SDR hosts had a higher maximum daily oviposition rate, and were able to sustain production near the peak longer, than beetles from *P. casei*. Among *A. bipustulata* from *D. radicum* hosts, the rate of oviposition followed the predicted pattern (Honek 1993) of positive correlation with size. The regressions fit to average daily egg production do not fit very well beyond about 100 days, and might have fit better if unweighted regression had been used instead since fewer females contribute to the average as time increases and females die.

It is interesting, then, that these apparent deficiencies were not manifest in the offspring of beetles from different hosts. Size, viability, developmental rate, and ability to locate and attack hosts all were the same across host classes. Concerning *A. bilineata*, the possibilities were raised that egg size is adjusted in females in response to the environment, or that females are physically unable to produce eggs of an exactly similar and optimal size (Boivin and Gauvin 2009). Another possibility, that egg size is constrained by adult size, or at least that larger females produce larger eggs, is frequently observed in other species (Fox and Czesak 2000), but was not here. That eggs within clutches differed in size tends to support the notion that eggs of uniform size cannot be produced, but does not eliminate the possibility of size adjustment in response to the environment. Whether larvae from eggs laid by PIO beetles perish of starvation earlier than those from beetles that developed in *D. radicum* puparia may not be evident in the host exploitation experiment conducted here, given the relatively small volume of

substrate in the vials. This would be worthwhile to pursue, given the survival rate differences in survival rates of adults. Female *A. bipustulata*, faced with suboptimal nutrition related in all probability to lipids, allocate resources to producing healthy offspring more than to boosting number of offspring, and at the expense of adult life expectancy.

To bring together the various consequences of developing on different host classes, and estimate their combined effect on rate of increase, a population projection matrix was constructed. This was important for interpreting the choice test results and measurements of field-collected *A. bipustulata*, since beetles are expected to favour hosts on which the rate of increase is highest. Because even slight shifts toward earlier reproduction can cause changes in rate of increase equivalent to large increases in fecundity (Lewontin 1965), and development time differed in the different host classes, an age-within-stage model (Caswell 2001) with a one day projection interval was appropriate. Matrices of this sort have been used successfully to model dynamics of prairie grasshopper populations in response to weather conditions, for example (Hardman and Mukerji 1982). For *A. bipustulata* attacking *D. radicum*, small and medium hosts appear to compensate for reductions in fecundity relative to beetles from large hosts, such that rates of increase are maintained around 1.07–1.08. Rate of increase may actually be slightly greater in the small and medium hosts. These estimates are slightly greater than the 1.06 calculated previously for *A. bipustulata* developing on *D. radicum* (Fournet et al. 2000). The estimate for development in PIO hosts, 1.05, is lowest of all, reflecting the influence of reduced probability of survival to adult, reduced fecundity, and probably least significant the reduced adult life expectancy.

Results of both choice tests, that *A. bipustulata* prefer *D. radicum* to acalyptrate hosts, agree with previous choice tests (Ahlström-Olsson 1994b), and with predictions based on rate of increase. That this preference is maintained without regard to the relative abundance of hosts, or a longer distance to travel to the preferred host, speaks to the ability of *Aleochara* larvae to discriminate among hosts; larvae are noted already for their ability to avoid interspecific (Reader and Jones 1990; Royer et al. 1999) and intraspecific competition (Royer et al. 1999), particularly among close relatives (Lizé et al. 2006). The scenario created in the tests is contrived and does not consider *Aleochara* larvae following the trail left by maggots emigrating from food to pupate (Goubert et al. 2013; Wright and Muller 1989), or with the adult female's choice of oviposition site (Fournet et al. 2001), both of which could reduce the frequency with which larvae face choices very similar to those offered. Female *A. bipustulata* are attracted to *D. radicum*-infested plants and to *D. radicum* pupae, and given the choice are more attracted to damaged plants than to pupae, unless the substrate with the pupae also contains larval tracks and probably frass, suggesting females are attracted at a distance by infested plants, then at close range move near larvae dispersing or dispersed from host plants to pupate in soil (Goubert et al. 2013). That preference of larvae is still evident, in spite of these simplifications and in spite of conditions of density and proximity which make encounters with *P. casei* puparia very likely, suggests selection has favoured adaptations in *A. bipustulata* that lead it to maximizing rate of increase.

The measurement of field-collected beetles was meant to determine if these preferences also tend to operate in nature, and are of particular importance to the ongoing evaluation of *A. bipustulata* for introduction, to address whether the full spectrum of *D.*

radicum puparial sizes is exploited by *A. bipustulata*. In a sample from Sweden of 25 *A. bipustulata* adults and the *D. radicum* hosts from which they emerged, 56% came from hosts 4–9 mg, 44% from hosts 10–14 mg, and none from hosts 15–20 mg (Jonasson 1994). In a similar sample of 44 adults and their hosts, from northern Germany, however, *A. bipustulata* emerged from seven host size classes in proportion to the number of hosts in those classes (Hemachandra 2004). Jonasson's study included also measurements taken on about 700 adult *A. bipustulata* in pitfall traps, greater than 75% of which had pronota 0.88 mm wide or less, 0.88 mm being the estimated upper limit of *A. bipustulata* from the SDR class of this study. This agrees with the results from Switzerland. As noted, the large *D. radicum* class was based on the size of *D. radicum* puparia in canola stubble at Graysville MB, a sample of 127 puparia with mean 10.7 mg and standard deviation 2.9; puparia 4.5–5.5 mg contributed 3% or less to the *D. radicum* population in this field. Average puparium mass can differ among canola fields, but the Graysville collection is the second smallest of eight populations from prairie canola stubble collected 2000–2009 (Hemachandra 2004; Chapter 3 Section 3). A similar comparison of host and parasitoid sizes was used in assessing *A. bisolata* for introduction to Australia (Wright et al. 1989). There the size of field-collected beetles agreed well with the distribution of the target species size, and on this basis, *A. bisolata* was judged capable of considerable impact.

Evaluating *A. bipustulata* for introduction to Canada is the background against which this study was conducted. It arose from testing the host range of *A. bipustulata* larvae, which showed other species in the family Anthomyiidae, and several relatively small acalyptrate species, can support development consistently (Andreassen et al. 2009). It was thought female *A. bipustulata* may restrict oviposition to the zone around roots of

crucifer plants, in which case pest species would be encountered by larvae primarily, and susceptible non-target species only seldom (Andreassen et al. 2009). This study has shown development in at least some acalyptrate species has negative fitness consequences, and so theoretically should not be favoured. However, *A. bipustulata* is reportedly a common visitor to dung and carrion, as well as rotting vegetable matter (Welch 1997), even though all species tested from these habitats except a different specie of piophilid were not suitable for complete development (Andreassen et al. 2009). Adults are found in a variety of open areas, but were never found within closed forests (Andreassen et al. 2009). The attraction of female adults first to infested *Brassica* plants and then to dispersing larvae (Goubert et al. 2013) does tend to suggest a highly evolved host-finding mechanism that leads *A. bipustulata* larvae to encounter *D. radicum* puparia when they are susceptible to parasitism. It cannot necessarily be discounted, though, that the same or a similar process leads larvae to encounter other species of host. There are at least three possible interpretations of the observed range of adult sizes. The first is that *D. radicum* is the exclusive host species; *D. radicum* can develop on a variety of cruciferous weed species in addition to crops, and some of these do result on average in relatively small puparia (Finch and Ackley 1977). However, larvae tend not to survive very well on these same plant species (Finch and Ackley 1977), and so in a landscape with much *Brassica* agriculture, these *D. radicum* would be a very small proportion of the total population, even if weedy crucifers also were fairly abundant. Exclusive development in *D. radicum* puparia may have led to the observed pronotum widths, but probably did not. The second possibility is *A. bipustulata* develop occasionally in *D. radicum* puparia, and most commonly in puparia of the bean seed fly. This is largely the scenario envisaged by

Jonasson (1994), and may, if it occurs, involve foraging for bean seed fly puparia exclusively around roots of crucifer plants, where they are secondary herbivores that invade root tissue already damaged, or in any of their other plants that host bean seed fly on a primary basis. The third possibility is that hosts in all sorts of relatively small species are attacked, including acalyptrate Diptera, in habitats that may include carrion and dung. There are only the larval choice tests conducted previously (Ahlström-Olsson 1994b) and as part of this study to recommend favouring the second possibility over the third. As noted, what is known of the behaviour of foraging females (Goubert et al. 2013) is illuminating but cannot inform a choice between these two possibilities.

Table 9. Elements P and G, and number of pseudostages, corresponding to the larval/pupal stage within population projection matrices for *Aleochara bipustulata* from four host classes (small *Delia radicum* SDR, medium MDR, large LDR, and *Piophila casei* PIO.)

Class	Survival	Mean duration (d)	Variance	P	G	Pseudostages
LDR	0.384	31.9	2.44	0.068724	0.896449	30
MDR	0.570	30.7	2.84	0.082816	0.896580	28
SDR	0.607	28.8	3.43	0.104585	0.877095	26
PIO	0.307	29.8	3.45	0.096683	0.860522	27

Table 10. Finite rate of increase (λ) of *A. bipustulata* developing on four classes of hosts, calculated from population projection matrices based on mean parameters for each host class, and at the lower and upper 95% confidence limits to parameters.

		λ for Host Class			
Evaluated for:		PIO	SDR	MDR	LDR
mean values		1.052465	1.076309	1.077017	1.071142
	95% c.l.				
egg survival	lower	1.045842	1.071156	1.071856	1.066254
	upper	1.057579	1.080599	1.081261	1.075149
larval/pupal survival	lower	1.047456	1.073075	1.073249	1.066243
	upper	1.056302	1.080190	1.080515	1.076701
adult survival τ	lower	1.051268	1.076052	1.076757	1.070861
	upper	1.053224	1.076492	1.077197	1.071343
adult survival γ	lower	1.051111	1.076173	1.076894	1.070946
	upper	1.053263	1.076419	1.077115	1.071260
fecundity *a*	lower	1.038297	1.051128	1.074477	1.068835
	upper	1.060718	1.087991	1.079290	1.073212
fecundity *x*	lower	1.056351	1.078785	1.077724	1.071793
	upper	1.048737	1.073936	1.076316	1.070497
fecundity *c*	lower	1.054755	1.079659	1.077545	1.072114
	upper	1.050556	1.073149	1.076512	1.070451
fecundity *d*	lower	1.060077	1.089743	1.079200	1.073211
	upper	1.045030	1.062928	1.074775	1.069048

Table 11. Median and range of pronotum widths for *Aleochara bipustulata* caught in Swiss fields 2005–2011**.** *Delia radicum* puparia 4.5–5.5 mg are expected to yield adults with pronota 0.852–0.881 mm, 7.5–8.5 mg to yield 0.938–0.966 mm, and 10.5–11.5 mg to yield 1.024–1.052 mm. Expected widths from *D. radicum* hosts based on regression model: width (mm) = 0.772892 + 0.028659•mass (mg) ($F_{1,31}$ = 110.5) (*cf.* Section 3). The smallest *P. casei* to support *A. bipustulata* development weighed 2.1 mg and produced a beetle with pronotum 0.669 mm wide, and the largest weighed 6.0 mg, producing a beetle with pronotum 0.876 mm.

			Pronotum width (mm)		
Year	Location	Date	N	Median	Range
2005	Courtemaiche	27 June	1	0.857	
		4 July	1	0.762	
	Galmiz	21 June	1	0.857	
		28 June	9	0.833	0.643–0.904
		5 July	6	0.845	0.785–0.952
		12 July	50	0.785	0.524–0.952
		19 July	8	0.821	0.666–0.904
		26 July	19	0.762	0.595–1.000
		2 Aug.	21	0.762	0.571–0.952
		9 Aug.	5	0.809	0.619–0.904
		16 Aug.	8	0.702	0.500–0.785
		23 Aug.	3	0.762	0.762–0.976
		26 Aug.	1	0.904	
		30 Aug.	2	0.809	0.762–0.857
	Jerisberghof	21 June	2	0.881	0.809–0.952
		28 June	2	0.833	0.809–0.857
		26 July	3	0.809	0.738–0.928
		2 Aug.	2	0.714	0.571–0.857
		9 Aug.	12	0.738	0.619–0.833
		16 Aug.	2	0.678	0.666–0.690
		30 Aug.	1	0.666	
	Lordel	28 June	1	0.738	
		5 July	3	0.833	0.762–0.952
		12 July	1	0.809	
		26 July	3	0.809	0.714–0.928
		2 Aug.	1	0.666	
		30 Aug.	1	0.714	
2008	Galmiz	4 June	1	0.714	
		8 July	15	0.833	
		22 July	4	0.857	0.738–0.952
		5 Aug.	2	0.869	0.738–1.000
		4 June	18	0.893	0.738–1.071
2009	Galmiz	10 June	42	0.833	0.547–1.023
		17 June	59	0.833	0.690–1.000
		24 June	71	0.809	0.571–0.976
		1 July	35	0.809	0.619–0.952
		8 July	62	0.809	0.619–1.000

Year	Location	Date	Pronotum width (mm)		
			N	Median	Range
		15 July	45	0.809	0.595–0.928
		22 July	6	0.845	0.762–0.952
2010	Galmiz	27 May	12	0.881	0.809–1.047
		3 June	25	0.833	0.666–0.986
		10 June	15	0.833	0.690–1.000
	Jerisberghof	24 July	25	0.857	0.587–0.952
		30 July	17	0.833	0.595–0.904
		6 Aug.	26	0.821	0.643–1.095
	Wädenswil	21 July	2	0.785	0.738–0.833
2011	Sugiez	18 May	14	0.797	0.547–0.976
		2 June	1	1.047	
		8 June	10	0.833	0.690–0.928
		15 June	63	0.833	0.609–1.023
		23 June	8	0.857	0.714–1.071
		30 June	4	0.821	0.762–0.952
		6 July	2	0.916	0.881–0.952

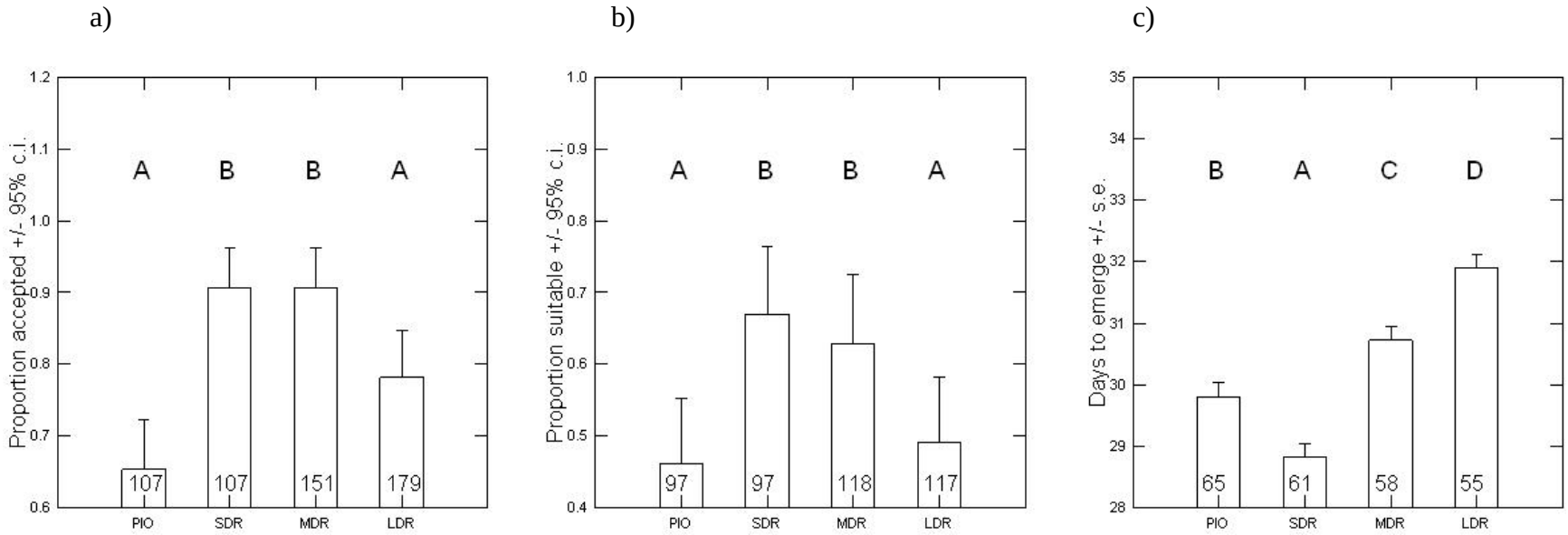

Figure 23. Proportion of puparia in classes accepted by *Aleochara bipustulata* larvae (a), proportion of accepted hosts suitable for complete development (b), and days required for development (c)**.** Classes are *Piophila casei* (PIO), and small (SDR), medium (MDR), and large (LDR) *Delia radicum*. Numbers on bars are the number of individuals contributing to that bar.

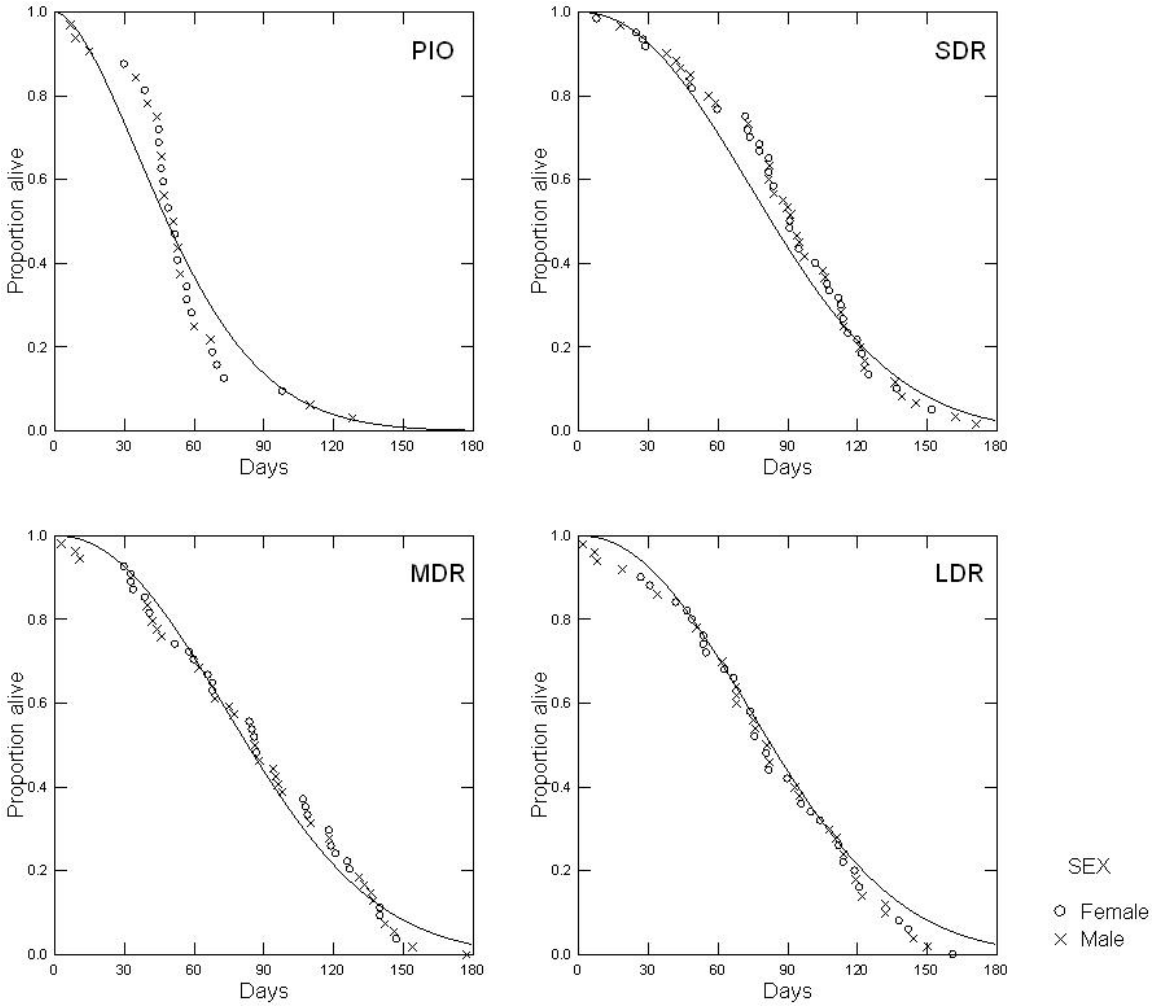

Figure 24. Survival of adult *Aleochara bipustulata*, that emerged from *Piophila casei* (PIO), or small, medium, or large *Delia radicum* puparia. Points are individual beetles, and lines are Weibull distributions of survival $S(t) = e^{-(t/\tau)^{\gamma}}$; for *Piophila* $\tau = 64.72$ (6.681 s.e.) and $\gamma = 1.809$ (0.228 s.e.), and for *Delia* $\tau = 98.298$ (3.700 s.e.) and $\gamma = 2.167$ (0.136 s.e.).

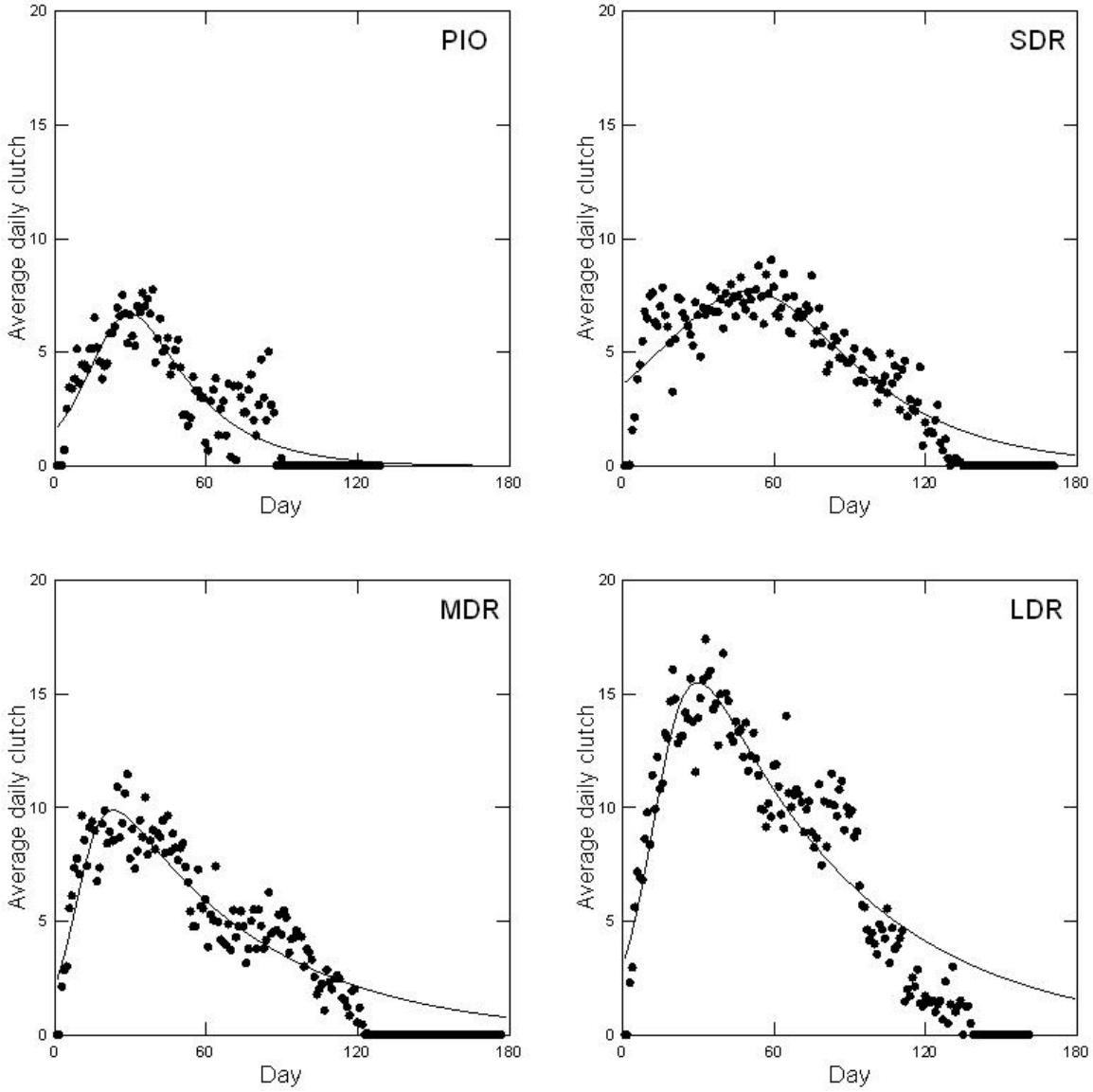

Figure 25. Average number of eggs laid per female *Aleochara bipustulata* per day, with lines modelling the function $y(t) = a \bullet e^{-xt} / 1 + e^{-c(t-d)}$. For beetles from *Piophila casei* hosts, a = 33.162217, x = 0.040856, c = 0.125934, d = 23.730737, $F_{4,125}$ = 552.5, observed *vs.* predicted R^2 = 0.78; from small *Delia radium* a = 54.274551, x = 0.026291, c = 0.055135, d = 47.918185, $F_{4,167}$ = 858.9, R^2 = 0.71; from medium *D. radicum* a = 16.139307, x = 0.016812, c = 0.177030, d = 10.481235, $F_{4,173}$ = 899.4, R^2 = 0.81; from large *D. radicum* a = 28.116720, x = 0.015979, c = 0.139201, d = 14.995952, $F_{4,157}$ = 1503.4, R^2 = 0.86.

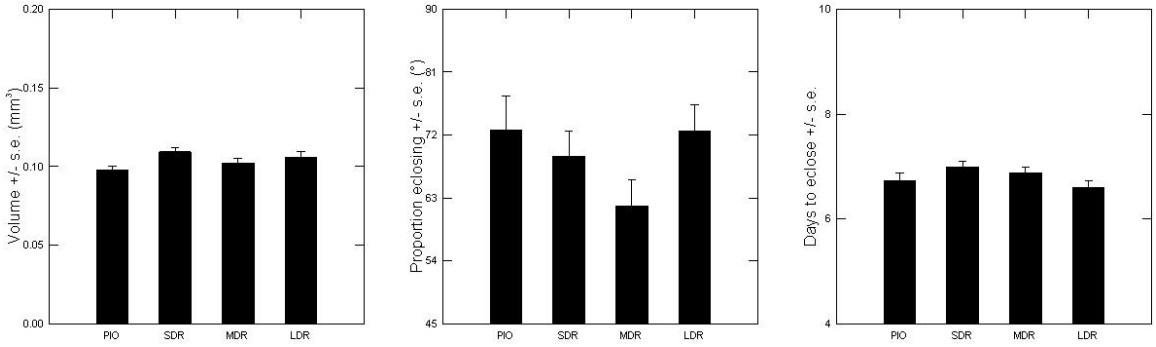

Figure 26. *Aleochara bipustulata* egg volume, arcsine square root transformed proportion (90° = 1.0, 45° = 0.5) of eggs to eclose, and days required to eclose, of eggs laid by beetles that developed in four classes of hosts. Neither egg volume ($F_{3,90} = 1.2$, $P = 0.30$), nor proportion to eclose ($F_{3,90} = 0.4$, $P = 0.76$), nor days to eclose ($F_{3,89} = 1.5$, $P = 0.22$) differed significantly among host classes.

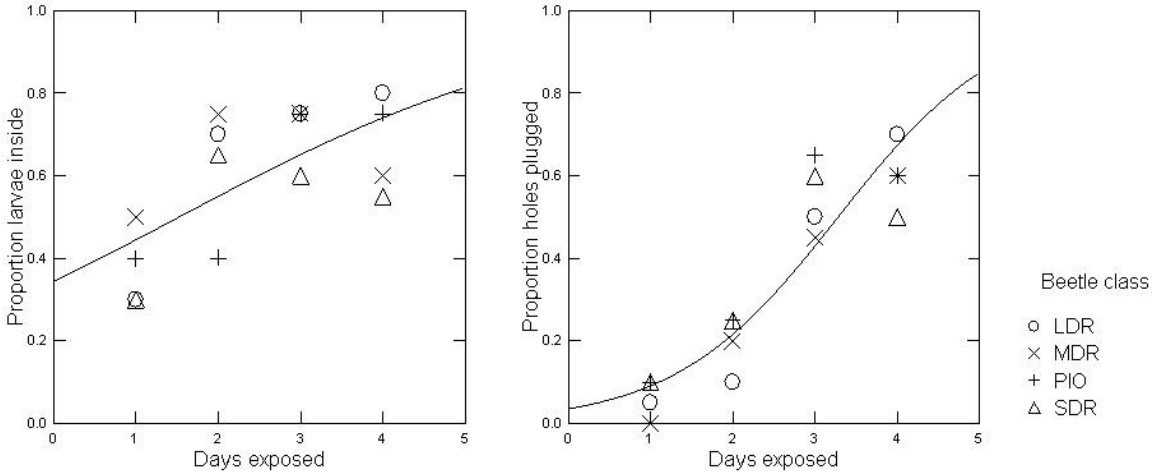

Figure 27. Host exploitation by *Aleochara bipustulata* larvae from four classes over time. Points are the proportion of 20 replicate vials. Lines are cumulative logit distributions, described for larvae entering puparia by estimated parameters for the constant of -0.6439 and slope 0.4231, and for plugging entrance holes by constant -3.3029 and slope 1.0076.

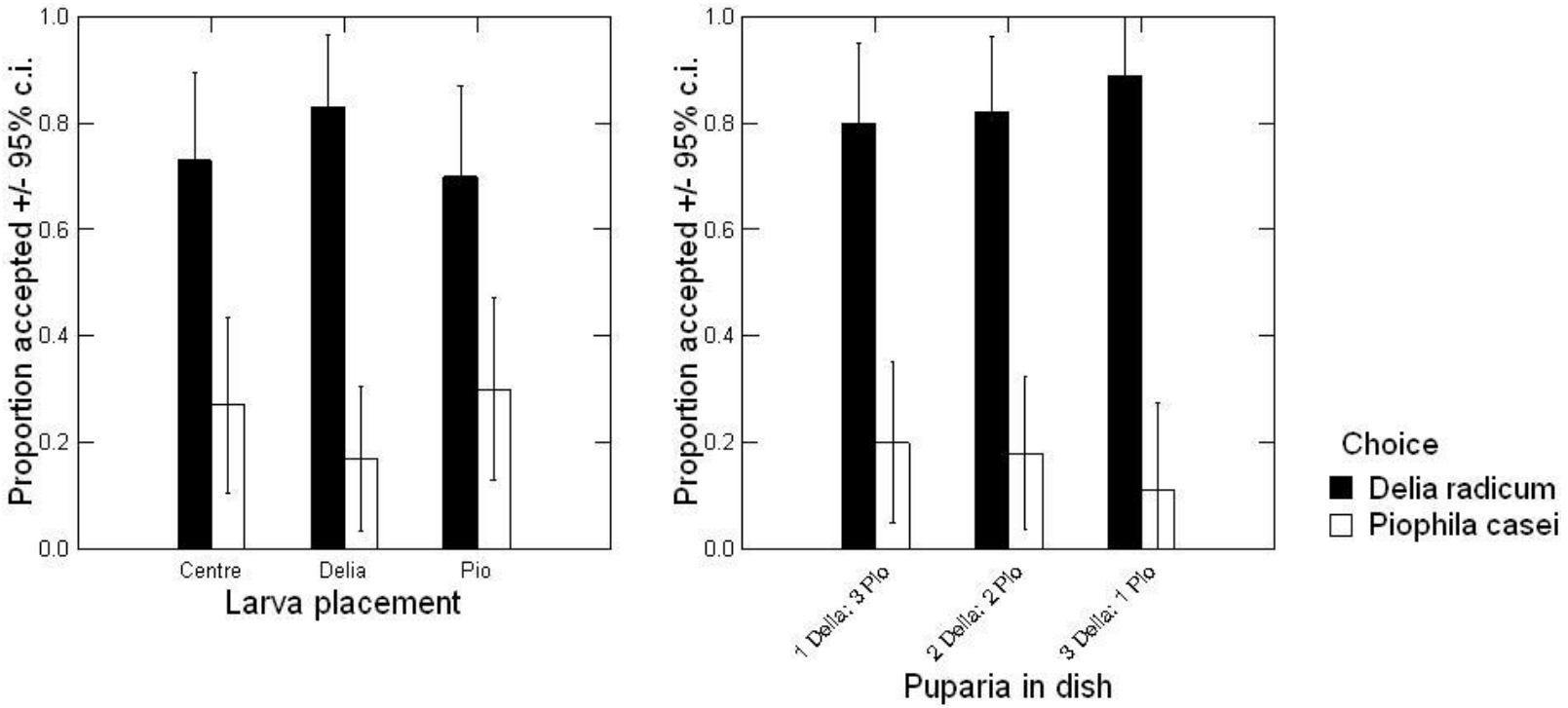

Figure 28. Proportion of *Aleochara bipustulata* choosing as hosts *Delia radicum* and *Piophila casei* puparia in different circumstances. In the left pane, larvae were added to the centre of a Petri dish, directly above the *D. radicum* puparium, or directly above the *P. casei*. Right pane illustrates larvae added to the centre of dishes with different ratios of the two host species.

Chapter 4

General Discussion

Much was learned about *A. bipustulata*. Flight activity occurs mostly during certain periods of the day (Koskela 1979), and from pitfall traps it seems movement on the ground does as well, being uncommon during the night and early morning, and probably most common in the evening. However, there was no evident pattern of changes in feeding activity through the day, and beetles were as likely to test positive for *D. radicum* DNA whenever they were caught. From the measurements of adults, they seem to find hosts primarily in locations other than the root zone of *Brassica* crops. This had been the general impression of *A. bipustulata* researchers, until I found the calyptrate fly species associated with dung and with carrion to be unsuitable hosts (Andreassen et al. 2009), after which it seemed its association with these habitats in previous literature may have been the result of misidentification. It does now seem relatively small species are the main hosts, and these may well be acalyptrate species whose larvae develop in dung or carrion.

It had also been quite uncertain that immature *D. radicum* are consumed by adults, because adults are expected to have difficulty finding eggs (Finch et al. 1999), and because adults whose potential otherwise might be high suffer from interference caused by other beetle species in the same habitat (Prasad and Snyder 2004; Prasad and Snyder 2006). The functional response study confirmed buried eggs are eaten less often, but levels of predation were higher than 0, even in field cages where there was evidence of competition. Screening for *D. radicum* DNA confirmed immature stages of *D. radicum*

are consumed by *A. bipustulata* under field conditions. The main coleopteran culprit blamed for causing predation of *D. radicum* eggs to be less than expected is *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) (Prasad and Snyder 2004; Prasad and Snyder 2006). This species may not eat many eggs, as was known for some time already, and may in fact, when present, cause smaller beetles foraging for things such as eggs to be less active, but they are known to be important predators of *D. radicum* larvae (Finch and Elliott 1994) and potential predators of pupae (Raworth et al. 2004). Since the research about interference seems to be influencing agricultural policy in North America, in particular concerning the value of trying to conserve ground beetles for pest management, it does seem worthwhile to point out where previous research may have misled.

Finally, the comparative study of niches was undertaken with a view to define for the two *Aleochara* species which sizes of *D. radicum* hosts are most suitable and most preferred, as this has been frequently discussed in the literature. Neither species seemed to have much preference, and, in fact, within *D. radicum* hosts, for both *Aleochara* species, host size had no influence on any stage of host acceptance or on host suitability. That these species coexist in Europe, and it may be possible only because the two species partition the available resource of host puparia, but it does not seem to be exclusively *D. radicum* that is partitioned.

The risk and the potential associated with *A. bipustulata*, as both a predator and as a parasitoid, can be evaluated based on previous information and in light of the research in the previous chapter. This may assist in the preparation of a petition for its release.

The risk of predation to non-target species in Canada depends in part on whether adults restrict foraging activity to the zone around *Brassica* roots. The method used to

select species for testing assumed that they do, or at least that risk there is as great as it is anywhere else. It cannot actually be stated from either the results here or from other sources that this is the case. If they forage only on and in soil near *Brassica* roots, then it seems from the laboratory assays that animals with no or limited mobility, and that are also in this zone, could be at risk. At the same time, it seems if foraging is restricted to this area, then one of the most frequently encountered susceptible prey will be the larvae of *D. radicum*. The molecular assays indicate many adult beetles in *Brassica* fields recently consumed immature *D. radicum*. Immature *B. quadrimaculatum* and *A. similata* were seldom if ever consumed.

The risk of parasitism to non-target species, thought to be small based on Andreassen et al. (2009), must be re-evaluated now that field-collected *A. bipustulata* have been measured. The population growth rate estimates suggest acalyptrate hosts are not expected to be favoured whenever larvae have a choice, and this was confirmed in the assay. Extrapolation would lead to the prediction *A. bipustulata* females should seldom leave eggs near these small hosts as well. Stopping there, and combined with the observations of long-range attraction to infested *Brassica* plants and short range attraction to *D. radicum* larvae (Goubert et al. 2013), all indications would have been that *D. radicum* is the primary host and the only species at risk of regular parasitism, including perhaps to other *Brassica*-associated *Delia* species. The measurements showed, however, that the majority of *A. bipustulata* adults in Swiss fields have most likely developed in a host other than *D. radicum*. This is the case in Sweden as well, although there *D. platura* are as heavily parasitized by *A. bilineata* as they are by *A. bipustulata*, but the distributions of sizes of the two beetle species are quite different (Jonasson 1994).

If the *D. radicum* problem is pressing, it may be worthwhile to assume *A. bipustulata* develop mainly within puparia of *D. platura* and *D. florilega*, which are pests, and consider these slightly off target effects not to be entirely non-target either.

Whether *A. bipustulata* has potential as a control agent will also inform the acceptance or not of the host species use assumption. As a predator, adults were able to find eggs around plants even when they were slightly buried, and so claims about their lack of ability to do so (Finch et al. 1999) may have been overzealous to a degree. It was confirmed, however, that many fewer eggs are consumed when these are buried than when left exposed on the soil surface. The results of screening field-collected *A. bipustulata* for *D. radicum* DNA do confirm these beetles have potential as predators of *D. radicum*, and this potential may be principally as predators of larvae. In field cages, and at some prey densities in the laboratory, *A. bipustulata* did seem to disrupt foraging by adult *A. bilineata*. Results from the field cages indicate this likely occurs as a result of the two species tending to favour the same areas for foraging. On the other hand, the two species, although they are long-lived, do seem to be most active in fields at different times of year (Jonasson 1994), which may serve to reduce the frequency with which the species compete for eggs for food, if the resource ever is really limited.

As a parasitoid, *A. bipustulata* does seem to have potential as a complement to *A. bilineata*. There seemed to be a partial refuge from parasitism based on depth of pupation, with the deepest puparia less likely to be attacked. When larvae of both species were present, there was difference among host depths or host sizes in the probability of attack. Larvae of *A. bilineata* seemed to focus on smaller puparia, a surprise in light of previous research (Ahlström-Olsson 1994b); larvae of *A. bipustulata* preferred the shallowest

puparia, and with individuals of both species avoiding puparia already containing larvae, the result was that the host resource was more evenly used. As in the cage study, species were forced to interact by releasing them in the same tube, something which may not happen if adults are free to find for themselves locations to occupy and to lay eggs.

Producers of *Brassica* crops in Europe benefit from the ecosystem services provided by *A. bipustulata*, as a predator and as a parasitoid. Might not the producers of canola in Canada do the same? It does seem introducing *A. bipustulata* would benefit producers in the same way, and it is likely that effects on non-target species will not cause widespread ecological disaster or effects on human health. There certainly are risks to non-target species, and in a risk-averse society, I have the impression these risks are too vaguely defined at the moment for regulatory agencies to grant permission to release here. My impression may be mistaken, since agents that are not strictly monophagous continue to be introduced around the world (Jenner and Kuhlmann 2009). There are risks and benefits associated with proceeding to introduce *A. bipustulata*, and there are risks and benefits associated with not doing so. The risks and benefits for both potential courses of action do seem relatively mild, and so a final decision may be difficult to make.

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