

Organic farming research project report submitted to:

Organic Farming Research Foundation
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Date: January 29, 2002

Project title: **Biological Control of *Delia* sp. in Cole Crops with Rove Beetles,
Aleochara sp. (Part 2)**

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Project budget: \$20,000 (2 years)

Funding provided by OFRF: \$5,600 (yr 2)

Project period: 2000-2001

Additional support provided by:

Lower Mainland Horticultural Association, Abbotsford, BC
Fraserland Farms Ltd, Delta, BC
Snow Farms

OFRF project number: 00-69, awarded Spring 2000

Note: OFRF provided a previous grant award for the first year of this project, which is reported under project No. 00-25.

1. Project Summary

The objective of this study was to examine the use of rove beetles, *Aleochara* spp., for the biological control of root maggots, *Delia* spp. in organic cole crops. We were interested in manipulating *Aleochara* numbers by either conservation strategies or by augmentative releases of mass-reared *Aleochara bilineata*. The results to date indicate the following

1. **Mulching** with straw and composted yard waste significantly reduced the root maggot population infesting individual broccoli plants. *Aleochara* spp. parasitism activity was higher in the mulched plots than control. Parasitism of root maggot pupae by *Aleochara* beetles was 37.5% (or 6 out of 16 pupae) in mulched plots and only 9.5% (or 2 out of 21 pupae) in the unmulched control plots; this increase in percent parasitism was statistically significant. Also, carabid and staphylinid beetle abundance was higher in straw mulched plots than control plots, during the early part of July. For the rest of the sampling period (May to August) there appeared to be no difference in natural enemy activity between mulched and control plots. Mulching may have also interfered with the oviposition activity of female *Delia* spp. flies. However, an experiment involving the introduction of *D. radicum* eggs to straw mulch and control plots demonstrated that *D. radicum* mortality (egg to larvae) was higher in mulch plots. These results further suggest that natural enemy activity, in this case predation, was higher in the mulched plots than in the control plots. From this one year of data, it appears that mulches may be an effective tool for suppressing root maggot populations, increasing parasitism by *Aleochara* and enhancing predator activity in general.
2. Data collected as part of the mulch survey through out the season, indicated that natural enemy levels within the field were generally low in the beginning of the spring and did not build-up until July. Our study of natural enemy populations in field margins, also conducted in the 2001 growing season, demonstrated higher densities of carabid and staphylinid beetles in grassy field margins than in weedy or bare ground field margins. These findings support the idea that natural enemies do not overwinter in the field and provides strong support for a tactic such as a beetle bank, which provides natural enemies with a grassy overwintering habitat within the field. A **beetle bank** was constructed on a commercial organic farm in the fall of 2001. The beetle bank, built on Snow Farms, Ladner BC, will be monitored in 2002 as part of a study that will continue to examine the impact of refuges for conserving natural enemies and enhancing biological control in agricultural fields. This study will be conducted at Washington State University and will include several organic farms in the state.
3. **Augmentation** trials with *A. bilineata* in 2001 were inconclusive. No significant reduction in root maggot populations resulted, however, these trials were conducted in the summer in dry weather and it is expected that in the cooler wetter spring conditions, results may differ. Augmentation field trials should be repeated in the spring and in irrigated fields in the summer. Mass-production of *A. bilineata* was also a major limiting factor for this portion of the study. A study to develop *A. bilineata* production protocols will be conducted in 2002.

4. Releases of marked *A. bilineata* to measure **dispersal** in the field was unsuccessful as none of the 250 individuals released were recovered in the 20 pitfall traps placed in a grid pattern in the field. It is unclear if this lack of recapture is due to rapid dispersal of beetles from field or the lack of dispersal of beetles within the field. Also dispersal of beetles may differ depending on the amount of plant cover in the field. For example, this study was conducted in the summer when plant cover was very high. Dispersal activity may be greater in the spring when plant cover is lower. Dispersal has been shown to be a major factor in limiting the efficacy of introduced biocontrol agents, therefore, it will be important to conduct a dispersal study in order to develop release protocols for *A. bilineata*. A more effective dispersal study design should utilize a denser grid of pitfall traps and include other types of traps, e.g. sticky traps, to capture individuals dispersing via flight.

2. Introduction

Growers of field vegetables are challenged to produce a crop economically but with minimal pest damage. Organic production of cole crops is particularly challenging, because these crops are attacked by a wide variety of pests including aphids, various lepidoptera (imported cabbage worm, diamondback moth, loopers, and cutworms), thrips, flea beetles and root maggots. Root maggots (*Delia* spp.) are especially problematic for the seedling stage of broccoli, cauliflower, and cabbage crops and throughout the season for rutabagas and radishes.

For organic field vegetable production there are few tools that are effective under a broad range of field conditions. For example, relatively small plantings of cole crops (e.g. market gardens) can be kept pest free with floating row covers. Unfortunately, this control strategy is not feasible on larger acreages. Another commonly cited non-chemical control strategy for root maggot control is timing of planting to avoid peaks in the adult stage of the root maggot population. This approach is problematic for growers with season long and/or fresh market commitments (e.g. CSA and home delivery services).

Despite these challenges in controlling root maggot pests, biocontrol options do exist. There are many entomophagous arthropods that can play an important role in the management of pest populations, when they are not destroyed by (or 'lost to') insecticides. For the control of root maggots two such natural enemies are the staphylinid beetles, *Aleochara bilineata* and *A. bipulsulata*. These beetles are predators of root maggot eggs and larvae as adults and ectoparasitoids of the pests' pupal stage as larvae (Klimaszewski 1984, Royer and Boivin 1999). Adults of *A. bilineata* and *A. bipulsulata* are potentially useful for biological control because they are capable of consuming large numbers of *Delia* eggs and young larvae- the larvae of *D. radicum* are the injurious stage (Royer and Boivin 1999).

In field crops natural enemies can be used for biological control either by introduction of a particular species to the agroecosystem or by conservation of the naturally occurring fauna of beneficial organisms (Landis and Orr 1996). Both types of biological control (introduction and conservation) could potentially be used to optimize the management of

root maggot populations. The overall objective of this study was to explore tactics for improving biological control of root maggots by either conservation of natural enemies or by augmentative releases of *Aleochara bilineata*.

Natural enemies can be conserved either by reducing deleterious inputs (e.g. pesticides) or by increasing resources required for survival (e.g. overwintering sites, pollen and nectar sources) (Barbosa 1998). Some conservation strategies include creating semi-permanent habitat or refugia either at the field margin (Dyer and Landis 1997) or within the field, e.g. beetle banks (Thomas *et al.* 1991, 1992). From these areas natural enemies are able to move into the field and feed on pests (Wissinger 1997, Wiedenmann and Smith 1997). Mulching has also been shown to be an effective tactic for enhancing natural enemy populations (Brust 1994, Riechart and Bishop 1990).

In this study we explored mulching and beetle banks as tools for natural enemy conservation. Although our original focus was on *Aleochara* spp. conservation, we expanded the scope of the study to include also carabid (ground beetles) and spiders, as many studies indicate that these arthropods are effective against wide variety of pests (Riechart and Lockley 1984, Thiele 1977, Sunderland 1999, Snyder and Wise 2001, Symondson *et al.* 2002). Also, Finch (1996) has shown that many carabids are effective natural enemies of root maggots, at least in the lab.

Augmentative releases of *A. bilineata* have been suggested as potentially useful tool for the biological control of *D. radicum* (Royer and Boivin 1999, Whistlecraft *et al.* 1985). Although *A. bilineata* is a widespread natural enemy (Klimaszewski 1984) native populations of *A. bilineata* are not necessarily synchronized with the injurious stage of the pest, i.e. overwintering *Aleochara* adults emerge after the first generation of root maggot larvae have damaged seedlings (Royer and Boivin 1999). The goal of an *A. bilineata* release would be to reduce the size of the larval population of *D. radicum* to below economic injury levels. At present *A. bilineata* is only available for biological control of *D. radicum* in France (G. Boivin personal communication). Previous studies have demonstrated that *A. bilineata* is present in the Pacific North West region and is a good candidate for some form of augmentative or inoculative biocontrol release.

3. Objectives

The original (as stated in our proposal) objectives of this study were the following

1. To determine the in-field vegetation management techniques (e.g. beetle banks, mulching) that enhance native rove beetle populations
2. To compare the efficacy of inoculative releases of mass-reared *A. bilineata* in controlling *D. radicum* in early season cole crop production.

Due to problems with mass-production of *A. bilineata* and the late start to the 2001 due to heavy rains in the spring, the original objectives were modified as follows

1. Compare the efficacy of two different kinds of mulch on enhancing natural enemy activity, including *Aleochara* spp., minimizing root maggot populations, and minimizing weed densities.

2. Establish a demonstration beetle bank on a producing organic farm as a site to monitor the impact of beetle banks on natural enemy and pest populations over time.
3. Document predation of *D. radicum* eggs by *A. bilineata* in field plots and dispersal patterns of released *A. bilineata* in an organic broccoli field.
4. Develop a rearing protocol for *A. bilineata* that is simple and easily implemented.

These modifications dealt with the specific problems of not being able to conduct field trials with *A. bilineata* in the spring as originally planned, due to the insufficient number of beetles available for field trials. Our beetle supply was limited due to some unexpected problems with the mass production system. Also the scope of the dispersal study was more limited again because mass production did not meet the expected goal of 1000 individuals. Finally, the wet spring weather put our co-operating growers behind schedule and beetle banks were not built in the spring as originally anticipated (see Appendix 1).

4. Materials and Methods

We investigated two methods for increasing in-field diversity of natural enemies: mulches and beetle banks. Also we evaluated the impact *A. bilineata* on root maggot populations and the dispersal activity of the beetles in the field. All of the studies were conducted on producing organic fields in Ladner and on Westham Island, BC.

In-field diversity: Mulches

Mulch plots were established in three organic broccoli fields (Fraserland Organics, Ladner, BC) approximately 1 to 3 weeks after transplant (from seed bed). Two different kinds of mulch were evaluated in this study: barley straw and composted yard waste (from Delta Municipal Landfill). A control treatment of no mulch was also included. Mulch was applied in a 5-cm layer entirely covering the ground around and between individual plants (Fig. 1). There were a total of three different sets of mulch plots corresponding to three different planting dates. The location of plots in each field is summarized in Figure 2.

The first set of mulch plots were established on May 18, 2001 (“field 1”). Plots were 2.5 meters long and 3 rows wide (approximately 1 meter). The plots were set up in 2 groups of four and treatments were randomly assigned (Fig. 2). Plots were marked out with flagging tape in order to prevent interference with farm operations, especially cultivation. Three additional control plots were designated in adjacent rows in the same field; these plots were not marked out with flagging tape and consisted of a pitfall trap (see Fig. 2) placed between two plants in the same row.

We established the second set of plots on June 11, 2001 (“field 2”). These eight plots were similar to the first set and included three control treatments. An additional 12 plots were also established in this field (Fig. 2). These plots were larger (5 meters long and 3 rows wide) with the straw mulch and control treatment being adjacent to each other. The yard waste mulch treatment was not included as part of the larger plots’ design, because equipment for spreading yard-waste mulch over the wider plot area was not available

(straw bales were carried into the field and spread by hand). The larger plots were spread out along the entire length of the field, in two rows at opposite ends (East and West) of the field (Fig. 2).

We established the third set of plots on June 15, 2001 (“field 3”). Three control and three straw mulch plots were set-up in this field in a manner similar to the large plots described for the second set, i.e. 5 meters long with straw mulch and control treatments being adjacent to each other.

All of the smaller plots had been cultivated twice prior to mulch applications. The larger plots (10 meters) differed in the number of mechanical cultivations prior to mulch application. The third set of plots had no prior cultivation; the plots on the west side of field 2 (2 West) had been cultivated once prior to mulch application, and the plots on the east side of field 2 (2 East) had been cultivated twice prior to mulch application. Thus, for the larger plots there were three straw mulch plots for each of the three levels of cultivation (0, 1 and 2 prior cultivations). There were also three control plots for the treatments of one and two prior cultivations. However, the grower was concerned about the amount of weeds that would occur in plots without cultivation, therefore there were no replicates of the no prior cultivation and control.

The effect of mulching on pest and natural enemy activity was assessed in the following ways, in the following fields:

1. Natural enemy collection using pitfall traps (field 1 and 2)
2. Root maggot survey using soil samples (field 1 and 2 small plots)
3. Plant growth effects comparing fresh and dry weight ratios (field 1 and 2 small plots)
4. Weed survey on larger plots (field 2 and 3)

In addition we conducted an experiment involving the addition of a known number of *D. radicum* eggs to the larger plots in fields 2 and 3. The objective of this experiment was to indirectly assess natural enemy predation of *D. radicum* eggs in mulch and control plots by comparing the number of *D. radicum* larvae.

Pitfall Trap Survey of Natural Enemies

A pitfall trap (1 L mason jar) was placed in the centre of the plot. Each trap was covered with a wooden lid and the lids were covered with mulch (straw or composted yard waste). Pitfall traps were filled with a soap and water solution and were changed on a weekly basis until the field was cultivated, following harvest. Pitfall samples were initially processed by recording the number of arthropods, specifically carabid and staphylinid beetles, spiders, collembola and others. Carabids and staphylinids were preserved in alcohol for further identification and size classification.

Soil Surveys for Root Maggots

Soil samples were collected from the 16 smaller plots during the time of harvest, approximately 6 weeks after transplant. A 3-L sample of soil was collected from around the root of three plants in the middle of each plot. Samples were taken from

approximately 8 cm radius around the root and below to a depth of 20 cm. The soil samples were initially sorted by hand and then by floatation. The number of *Delia* spp. pupae and larvae, and the number of beetle adults were counted. We held all pupae until either adult flies or beetle parasitoids emerged.

Plant Growth Surveys

At the same time that soil samples were taken, root samples were also taken so that we could assess the effect of mulch on plant growth. Roots were excised from three plants per plot. The vegetative portion was separated from the root portion immediately below the lowest leaf node on the broccoli stalk.

We then washed roots for 10 minutes in running water to remove all soil and debris. Any *Delia* spp. larvae or pupae emerging from roots were included in the corresponding samples for the soil survey (see above). Roots were dried between layers of paper towel for 5 minutes and then weighed on an electronic balance. Roots were placed in paper bags and dried in drying ovens at the University of British Columbia (Vancouver, BC) for four days at 60°C. Dried roots were weighed and the ratio of root dry to fresh weight was calculated for each sample. The ratios of root dry to fresh weight were compared among the three different treatments.

Weed Survey

A weed survey was conducted on July 26, 2001 on the larger (5 m long) plots in fields 2 and 3. These plots received one of three different prior cultivation treatments (i.e. cultivated twice, once or not at all prior to mulch application-see above for details regarding level of replication). Mulch was applied to all plots, between June 11 and June 15, 2001. The weed survey consisted of counting all of the weeds along a 2 m long and 20 cm wide transect through the middle of each plot. The mean numbers of weeds per plot were compared between control and straw-mulch plots and between the different levels of mechanical cultivation.

Experiment: Prey Enrichment

This experiment was initiated on July 26, 2001. *Delia radicum* eggs were obtained from a colony at Simon Fraser University (Burnaby, BC). Twenty eggs were placed on the soil surface, immediately around the base of 2 plants in the center of each of the 18 larger plots (9 straw and 9 control) in fields 2 and 3. Eggs were approximately 48 hours old. Two weeks later (August 16, 2001) the roots and soil of the treated plants were removed and the presence of *D. radicum* larvae, in roots or soil, and root feeding damage were assessed. The number of maggots per plant was compared between the straw mulch plots and the control plots.

In-field diversity: Beetle Banks

A beetle bank was established at Snow Farms, Ladner BC, in October 2001. This bank was initially to be established in the early spring of 2001. Delays in spring planting schedules, due to the extremely wet spring weather, resulted in postponement of beetle bank establishment.

The beetle bank was established following the protocols outlined by Thomas et al. (1991) and Marshall (1998). The bank is approximately 40 cm high, runs the entire North-South length of the field and is approximately 1 meter wide (Fig. 3). Banking was accomplished by two directional ploughing (Thomas et al. 1991). In the spring of 2002 the bank will be sown with a mixture of cock's-foot (*Dactylis glomerata*) and Yorkshire fog (*Holcus lanatus*) at a rate slightly higher than the recommended three grams per square meter (Alec McElrich personal communication).

We monitored natural enemy activity along the field margins, of the field the beetle bank was established in, during the late spring and summer of 2001 using pit fall traps. Pitfall traps were setup as described above for the mulching experiment and were checked on a weekly basis. This data will be used for comparison of subsequent years' data and of the natural enemy activity levels in the beetle bank compared to the field margin.

Aleochara bilineata

Production

Delia spp. pupae were collected in the fall and winter of 2000 from fields planted with cole crops the previous growing season. A total of 17 *Aleochara* individuals were collected from these pupae. Adult beetles were contained in 500 ml plastic tubs with screened lids and sand substrate. A piece of rutabaga was also placed in the tub (Royer and Boivin 1999). Beetles were feed a diet of *D. radicum* eggs and small pieces of ground beef (Hertveldt et al. 1984). In addition to these 17 beetles approximately 40 *Aleochara bilineata* individuals were obtained from Agriculture and Agri-Food Canada (Saint Jean, PQ). These individuals were held in a similar manner as described above. All beetles were maintained at 17 to 25°C and 16:8 photo period however when *D. radicum* availability was low (see below) beetles were held at 10°C and 24 hr dark to prolong lifespan. The rearing protocol for the beetles was based on the protocol described Whistlecraft et al. 1985 and Herveldt 1984 but was modified in order to minimize the amount of equipment and handling required. Beetles were provisioned with *D. radicum* pupae as the host. Pupae were placed in tubs containing beetles and after 96 hours the beetles were aspirated out and placed in fresh tubs. Pupae remained in tubs until beetles or flies (unparasitized pupae) emerged. Newly emerged beetles were transferred to fresh tubs and handled as described above.

The *D. radicum* colony used for production of *Aleochara* beetles was initiated from approximately 1000 freshly laid eggs, obtained from the insectary at Simon Fraser University (SFU), Burnaby, BC. *Delia radicum* were reared following the SFU protocol that is similar to that described by other authors (Royer and Boivin 1999). Eggs were sprinkled around a rutabaga planted in a pot with sand. Pupae were collected approximately 10 to 14 days later and either used for *Aleochara* spp. production or for continued *D. radicum* production. Adult *D. radicum* flies were held in a small plexi-glass cage and provisioned with a moist dental wick for water. Flies were also provisioned with a food source that consisted of a mixture of skim milk powder, brewers yeast and sugar. A piece of rutabaga was placed in a petri dish filled with sand, for oviposition. Cages for adult flies and pots with *D. radicum* immatures were all held at 17 to 25°C and 16:8 photoperiod.

Dispersal Study

Two hundred and fifty (250) *A. bilineata* adults were marked with a small spot of nail enamel on the elytra. The mark consisted of a small white spot on either the left or right elytra. Beetles were released at 8:30 pm on July 20, 2001 in field 2 of the mulch study (see above). Beetles were released at the approximate midpoint between the two sets of mulch plots in field 2. Between sixty and seventy beetles were released at each of four points along this central row (Fig. 4). Field 2 was chosen as it already had a network of 12 pitfall traps at the west and east ends of the field, as part of the mulch study. An additional four traps were placed five rows over from the release row, on both the east and west sides (Fig. 4). These twenty pitfall traps were monitored for two weeks following the release of the beetles.

Cage Study

A manipulative experiment involving both prey (*D. radicum* eggs) and predator (*A. bilineata* adults) enrichment was conducted in a 3 week old (from transplant) organic broccoli field on Westham Island, BC. This field was not used for any of the other experiments or releases described for this study. The experiment was conducted in cages built around a 1.5 m long by 1-m wide (3 rows) plot of plants. Cages consisted of wooden stakes drilled into the corners of the plots. Window screen fabric was suspended between the stakes with staples. The cage was 30 cm high, the window screen fabric was buried 3 cm below the soil surface. The tops of the cages were open. The total number of plants per cage ranged from 9 to 16.

Twenty 48-hr old *D. radicum* eggs (Simon Fraser University, Burnaby, BC) were placed around the base of plant in each screened cage. (Our open cage design did not control for additional egg laying by wild *Delia* spp. females, however population levels were quite low during the time of the study, based on sticky card data from surrounding fields.) Five *A. bilineata* adults were also added to each cage. Both *D. radicum* eggs and *A. bilineata* adults were added to cages in the evening, approximately 8 pm, to minimize the chance of *A. bilineata* dispersal from cage. There were 16 replicates. A control treatment consisted of adding 20 *D. radicum* eggs around the base of 16 randomly selected (uncaged) plants on the opposite end of the field. Two pitfall traps were placed among these 16 plants to monitor predator density in the vicinity.

Two weeks later, roots from all 16 treatment and 16 control plants were removed and the number of maggots per plant and in surrounding soil were counted. Also the amount of plant damage on plant roots was qualitatively assessed. Thus predation of *D. radicum* eggs was assessed indirectly by comparing the number of maggots infesting control and treatment plants.

5. Results

In-field diversity: Mulches

Natural enemy survey: Table 1 and Figure 5 summarize pitfall trap data (staphylinid and carabid) for the two types of mulch and control plots in fields 1 and 2. Maintaining a constant level of replication throughout the study was difficult because growers accidentally tilled many of the pitfall traps up during the course of normal farming operations. Pitfall traps placed in unmulched portions of the field were especially vulnerable to damage. Nevertheless, an analysis of the survey data summarized in Table 1 and Figure 5 do reveals the following trends:

- 1) In-field abundance of carabids and staphylinids was highest during July for all three treatments.
- 2) Some mulch plots had greatly increased predator densities, while others did not differ from the control, leading to a great deal of variability. This variability was not associated with the location of the different mulch plots in the field.
- 3) Figure 5 demonstrates the proportion of staphylinids, carabids or spiders caught in each type of treatment. From May 24 to June 21 (field 1) carabids were the most abundant arthropods in control and yard waste mulch plots, while staphylinids and carabids were approximately equal in abundance in the straw mulch plot.
- 4) In Figure 5b the data summarize the proportion of staphylinids, carabids and spiders caught in the larger plots in field 2 from June 28 to July 26. The majority of beneficial arthropods caught in both plots were staphylinids. Interestingly the proportion of carabids in the mulched plot was very low compared to the data from field 1 and the control plot from field 2. Overall twice as many arthropods were caught in the straw mulch plot (482) than in the control plots (236).

Table 1. Combined mean (\pm s.e.) of both carabid and staphylinid beetles caught in pitfall traps. N=number of pitfall traps for each treatment. The number of pitfall traps also equals the number of plots for each treatment since there was one trap per plot.

Date	Field	Straw mulch	Composted Yard Waste	Control
May 24	1	2 (n=2)	6.5 \pm 2.12 (n=2)	6 (n=1)
May 31	1	8.75 \pm 2.5 (4)	1.33 \pm 2.31 (3)	2 (1)
June 7	1	5.75 \pm 2.06 (4)	2.67 \pm 1.15 (3)	2 (1)
June 14	1	12.25 \pm 4.65 (4)	4.67 \pm 2.08 (3)	1 (1)
June 21	1	8.5 \pm 3.79 (4)	8.67 \pm 2.52 (3)	0 (1)
June 28	1	6.75 \pm 4.65 (4)	5 \pm 2 (3)	2.33 \pm 1.15 (3)
June 28	2	5.75 \pm 3.95 (4)	5 \pm 4.24 (2)	8.75 \pm 3.77 (4)
July 5	1	17.5 \pm 4.65 (4)	19.33 \pm 12.09 (3)	25 \pm 11.53 (3)
July 5	2	34.25 \pm 27.13 (4)	23 \pm 16.97 (2)	13.75 \pm 7.41 (4)
July 12	2	39.75 \pm 53.21 (4)	33 \pm 32.53 (2)	14.53 \pm 3.87 (4)
July 19	2	21.33 \pm 16.65 (3)	N/A	10.5 \pm 4.2 (4)
July 26	2	14.33 \pm 14.46 (3)	N/A	14 \pm 8.83 (4)
August 2	2	4 (3)	N/A	9 \pm 4.58 (3)
August 9	2	9.67 \pm 4.51 (3)	N/A	7.33 \pm 5.51 (3)

Due to the uneven replication statistical analysis was possible on only a subset of the data. The analysis performed covered the data from the period of June 28, 2001 to August 9, 2001. The three control and three straw mulch plots that had data for each of these six weeks were used for the analysis. These plots were all the larger plots in Field 2. There was no significant difference in the overall number of beetles caught in the straw mulch versus control plots ($t=-1.62$ $p=0.114$). There was however a marginally significant time X treatment interaction in repeated measures ANOVA ($f=2.249$, $p=0.073$). This result is perhaps the most important since it shows that although carabid and staphylinid densities were increasing overall in the field, in July, the increased activity was initially greater in the mulched plots, as can be seen in Figure 6. The level of natural enemy activity decreased after the initial rise and was similar again to the control.

Soil surveys: Mulch treatments had significantly fewer pupae and maggots than the control treatments (Fig 7). This trend that was consistent for both field 1 ($F=4.74$, $p=0.05$) and field 2 ($F=15.52$, $p=0.0043$). Specifically there were significantly more *Delia* immatures recovered in the control plot compared to both types of mulch plots. There was no difference between either type of mulch treatment, however straw mulching resulted in the fewest pupae or maggots. There was no significant difference in the number of staphylinid beetles recovered in soil samples between the three treatments ($F=0.4055$, $p=0.6814$) in field 1, this data was not recorded for soil samples in field 2. However, significantly more pupae were parasitized by *Aleochara* spp. in the mulched plots than in the control (Pearson Chi-square=4.19, $p=0.04$). Approximately 30% pupae collected from the mulched plots were but only 9.5% or less of the pupae collected from the control plots were parasitized (Table 2).

Table 2. Effect of mulch treatments on the percentage parasitism of *Delia* spp. pupae by *Aleochara* spp.

Field/Treatment	# parasitized pupae (<i>Aleochara</i> spp.)	Total Pupae (all replicates per field)	% Parasitism
1/Control	2	21	9.5%
1/Straw mulch	3	9	33%
1/Yard waste mulch	3	10	30%
2/Control	1	30	3.3%
2/Straw mulch	2	6	33%
2/Yard waste mulch	3	11	27%

Plant performance: Mulching did not have a significant effect on the dry weight to fresh weight ratio of broccoli roots in field 1 ($F=3.28$, $p=0.10$) or field 2 ($F=0.325$, $p=0.734$) (Fig. 8). Visual inspection of broccoli heads prior to harvest did not reveal any observable difference in head size of plants in mulch treatments compared to control. None of the plants, in any of the treatments, wilted or died during the course of the study.

Weed Survey: There were significantly fewer weeds in straw mulched plots than in control plots ($t=4.489$, $p=0.006$). Table 3 summarizes the results of the weed comparisons. The number of prior cultivations did not affect the efficacy of straw mulch in suppressing weed densities ($F=0.3858$, $p=0.696$).

Table 3. Mean (\pm standard error) number of weeds per 2m transect in mulch and control plots (n=3).

Number of prior cultivations (mechanical)	Control	Straw mulch
0	N/a	12.33 \pm 4.3
1	44.33 \pm 6.98	14.67 \pm 3.28
2	32.33 \pm 8.67	17.33 \pm 4.37

Experiment Prey Enrichment: Predation of *D. radicum* eggs in straw mulch plots compared to un-mulched control plots was assessed indirectly by measuring the number of maggots in soil samples taken from plants where eggs were released. Maggot densities were lower in the mulched plots, although this effect is only marginally significant (t=1.974, p=0.057; Fig. 9).

In-field diversity: Beetle Banks

A beetle bank has been established on one farm in Ladner, BC. This bank will be planted with orchard grass in the spring of 2002 and we will begin monitoring the response of natural enemies to this structure. The number of beetles and spiders caught in pitfall traps in the margins of this field, during 2001, are summarized in Table 4. This data provides a baseline for assessing impact of beetle banks on natural enemy diversity and abundance. For example field margin data collected in subsequent years can be compared to data collected from the beetle bank, to assess if overwintering (early May data) densities of beetles are higher in beetle banks or same as the field margin.

Table 4. Natural enemy abundance in field margins as measured by pit fall trap catches. Data were collected from margins of the field where a beetle bank was established in October 2001.

Date	# traps	Staphylinids	Carabids	Spiders
May 11	6	6	4	4
May 18	6	1	3	3
May 24	6	9	47	18
May 31	6	14	34	33
June 7	5	15	27	14
June 14	5	5	30	22
June 21	5	8	33	27
June 28	5	6	23	13
July 5	5	14	44	6
July 12	5	26	21	10
July 19	5	20	13	13
July 26	5	18	56	4
Aug 2	5	45	56	23
Aug 9	5	29	70	14
Aug 16	5	31	80	13
Aug 23	5	10	27	14
Aug 30	5	12	47	19

***Aleochara bilineata*: Production and Releases**

Production: From the initial colony of 57 *Aleochara* spp. individuals, we produced a total of nearly 400 offspring. The field collected individuals were most likely a combination of *A. bilineata* and *A. bipulsulata*. Assuming that half of the individuals in the initial colony were females (28 females) then our rearing protocol produced 13.6 adults per female during the course of 5 months of production. The main constraint to production was the uneven supply of fresh *D. radicum* pupae. *Delia radicum* production was especially hampered by poor longevity and oviposition activity of adult flies. Upon examination many of the dead adult flies were covered with fungi, suggesting a possible fungal contamination (e.g. *Metarhizium* spp. or *Beauveria* spp.) of the *Delia* colony.

To prolong *Aleochara* lifespan beetles were placed in cooler conditions until *D. radicum* pupae were available. Repeated chilling and warming of *Aleochara* may have reduced their fecundity. Also *Aleochara* spp. are known to be cannibalistic on eggs and first instar larvae. Cannibalism may have also been a factor that reduced production output. Many of the pupae that were placed in the tubs with *Aleochara* for parasitism were not parasitized (fly emergence instead of beetle). This suggests that timing of pupal availability with first instar *Aleochara* activity needs to be more precise. Also many of the pupae had no adult emergence. Upon dissection these pupae were found to be desiccated, which may have also been in part due to fungal infection.

Cage Studies: There was no significant difference in the number of maggots recovered around the plants in cages with *A. bilineata* compared to the maggots collected around the control plants ($t=1.274$, $p=0.2124$; Fig. 10). Recovery of maggots in both the *A. bilineata* and control treatments was less than 20% of the number of eggs released. A sample of eggs used in this study were kept at 21°C and 75% RH in the lab and had over 90% emergence suggesting that the low maggot recovery levels in the field plots was due to some issue of egg mortality in the field and not egg quality. Pitfall trap catches from the vicinity of the control plots were 4 staphylinid beetles and 1 carabid beetle, indicating that natural enemies were present in the field during the 14 days of the study.

Dispersal Study: None of the 250 *A. bilineata* adults that we released were recovered in the pitfall traps placed at various distances from the release points. Prior to this experiment nail enamel was placed on the elytra of 10 beetles in the lab. These beetles were allowed to live in the sand substrate of rearing tubs for one week and then were placed in a soap and water solution similar to the pitfall traps for 14 days. Enamel remained intact on all 10 of the test beetles therefore it is unlikely that enamel wore off on marked beetles in the field.

6. Discussion

In field diversity: Mulching

Mulching appears to be a successful way to increase the activity of *Aleochara* spp. in the field, as parasitism of *Delia* spp. pupae was higher in both types of mulch plots than in the controls. Our findings contradict those of Hellqvist (1996) who found that parasitism by *A. bilineata* was reduced when cauliflower was mulched with grass clippings compared to the unmulched and no insecticide control. The difference in the studies may indicate that *Aleochara* spp. are sensitive to microenvironment, responding differently in

a grass-cauliflower compared to a barley straw-broccoli system. Also, the Hellqvist (1996) study did not specify the time of year when the study was conducted; our results showed that staphylinid abundance increases from May to July (see Table 1). Further our results showed that parasitism rates were higher in mulched plots in both the May and June transplants. Parasitism of pupae by *Aleochara* spp. will reduce the size of the adult population of *Delia* spp. that would lay eggs on subsequent plantings of cole crops in the same or surrounding fields. Since both *Aleochara* spp. have at least two generations per growing season, increased parasitism by beetles in the early part of the growing season also increases the beetle population later in the summer. *Aleochara* spp. are also effective predators of *Delia* spp. consuming up to 24 eggs per individual per day (in the laboratory) (Read 1962). Thus, enhancing the parasitism activity of beetles by mulching cole crops at the beginning of the season will also provide a source of *Delia* egg predators for subsequent plantings later in the season.

Both types of mulch examined in this study reduced the number of maggots and pupae per plant when compared to the bare ground control. Also, there were fewer maggots recovered in straw-mulched plots, two weeks after *D. radicum* eggs had been added. These two results in combination suggest that *Delia* spp. mortality is higher in mulched plots than in unmulched plots. These results, in particular the experiments adding *D. radicum* eggs, cannot tell us about the direct causes of maggot reduction in mulch plots. Some of the mortality factors may have included predation of eggs or young *D. radicum* larvae, by various arthropod natural enemies, increased mortality from microbial agents, i.e. fungi, viruses and nematodes, or some other factor. A more effective assessment of predation of *D. radicum* eggs in mulched versus control plots should involve, a timed removal of introduced eggs, e.g. measurement of egg predation on a 24-hour basis.

In addition to the two *Aleochara* species there are many other natural enemies that have been identified as predators of *Delia radicum* eggs. These include many species of carabid beetles in the genera *Amara*, *Bembidion* and *Agonum* (Finch 1996). In our study the commonly found carabids were from the following genera *Carabus* spp., *Pterostichus* spp., *Amara* spp., *Agonum* spp., and *Bembidion* spp. Humphries and Mowat (1993) conducted an extensive three year study on the effect of straw mulch and cattle manure on root maggot predators. These authors found that while carabid numbers increased in the cattle manure treated plots, carabid catches were 26% lower in the straw mulched plots compared to the control. Our findings are consistent with the Humphries and Mowat study. In particular, Figure 5 demonstrates that carabids make up a smaller proportion of the total natural enemy catch in the straw plots than in the control and composted yard waste treatment. Later in the summer, Figure 5b, carabids are an even smaller proportion of the total pit fall trap catch in the straw mulch plots. The results of the Humphries and Mowat study and our study suggest that carabids and staphylinids may respond differently to the microenvironment changes that arise from straw mulch application. Ramert (1995) trapped more staphylinids in grass-mulched carrot plots than unmulched. Very few studies appear to focus on staphylinid beetles as predators, except for studies focused on *Aleochara* spp., which is unfortunate since our study and the few others conducted show evidence that staphylinids may have an important role in mulched systems.

Our results indicated that while natural enemy abundance was greater overall in the field in July, the increase was significantly higher in the straw mulch plots during the first part of July. For the remainder of the season natural enemy levels were the same in straw and control plots. These results are important because they indicate that the effects of mulching on natural enemy abundance are time dependent. A similar finding was observed by Purvis and Curry (1984) who found that carabid abundance went up initially after application of manure mulch but then returned to levels similar to the control. The variation in resources available upon mulching should be examined further, since the impact of mulches for natural enemy conservation could be enhanced by perhaps applying smaller layers of mulch at several different times in the growing season.

Besides natural enemy induced mortality another possible explanation to the reduction in maggots and pupae per plant in the mulched plots is reduced egg-laying activity by adult flies. It has been suggested that mulches act as a physical barrier to oviposition sites around the base of individual brassicae plants (see Hellqvist 1996). But grass mulch did not reduce oviposition by *D. floralis* (Hellqvist 1996). We did not specifically measure egg-laying by adult flies, but it may be possible that reduction in *Delia* spp. in mulched plots may be the result of the combined increase in natural enemy activity and reduction in oviposition by adult flies. Also it is important to keep in mind that despite having higher numbers of maggots and pupae per plant than the mulched plant, control plants did not suffer any reduction in growth as a consequence of maggots. Overall plant loss to root maggots was very low in fields 1 and 2 (H. Niven personal communication) suggesting that this may have been a low year for root maggots. The efficacy of mulches should be examined under conditions of high (i.e. economic injury levels) root maggot pressure.

A final advantage of mulch treatment, observed during this study, was decreased weed density. Currently, growers of large-scale organic field crops in British Columbia, mechanically cultivate fields for weed control followed by hand hoeing. This represents a significant input of labour, energy and disturbance of soil. Mulching, in contrast, was applied only once during this study, suppressed weeds and pest populations, enhanced natural enemies, and also likely helped to build soil tilth. The cost of straw for mulch may be prohibitive for some growers in areas, such as British Columbia. However, composted yard waste obtained from the municipal waste yard was relatively cheap and as effective as straw mulch in the suppression of pest populations, although we have not yet measured its effectiveness in weed suppression. The yard waste mulch may be easier to distribute mechanically than the straw mulch.

In the region around Ladner, BC, *Delia radicum* damage is especially pronounced in the beginning of the season, (April to May). One reason for this may be that natural enemy levels, in the field, do not build up to levels that impact pest populations until later in the season. Our mulch study results demonstrated a dramatic increase in natural enemy abundance in the field between May and July. Mulching, however, may not increase the density of natural enemies in the field in the beginning of the season. This was difficult to determine from our study, since our pit fall traps placed in unmulched control plots

were repeatedly run over by farm machinery, reducing the number of control replicates to one, during the critical May portion of the study. Nevertheless, most beetles are unlikely to overwinter in the field, since the requirements for overwintering would include some form of vegetative shelter that minimizes temperature fluctuations (Thomas et al. 1992).

In field diversity: Beetle banks

Conservation of natural enemies between seasons, i.e. from year to year can be accomplished by increasing the habitat for the overwintering population of beneficial insects. Habitat for overwintering populations has been studied in relation to the non-crop fauna around the field margin (Varchola and Dunn 2001, Lee et al. 2001, Frampton et al. 1995, Thomas et al. 1992). However, as stated above for biological control of root maggots, natural enemies need to be abundant in the field in the early part of the growing season. Predators surviving at the field margins colonize the field over the course of the growing season, as demonstrated by our mulch study results. Thus providing overwintering habitat for natural enemies, in the field, is a reasonable solution to the problem of conservation and timing of pest control. British studies have demonstrated the efficacy of in-field refuges or beetle banks for increasing the abundance of natural enemies that overwinter in the field (Thomas et al. 1991). For example, Thomas et al. (1991) found a higher density of overwintering predators within the first year of beetle bank establishment than compared to the field or bare ground treatments. Some species, including the aphid predator *Tachyporus hypnorum* (staphylinid), were shown to disperse from the beetle bank into the field relatively early in the season (Thomas et al. 1991). Unfortunately, this study did not report on *T. hypnorum* dispersal in fields without beetle banks. In general, studies indicate that beetle banks require two to three years to become established. (A picture of an established beetle bank can be viewed at the following web site: www.game-conservancy.org.uk/research/beetlebanks/frameset.html)

The growers that we approached about beetle banks expressed an interest in the idea of a beetle bank, but only one grower was willing to make a commitment. A beetle bank and other forms of conservation biological control represent very different ways of thinking about an agricultural field, because not all parts of the field are for production and management must be modified to work around non-tilled refuges inside of fields. Also most growers expressed some concern about the requirement for soil to be raised 40 cm high in order to construct the bank. This appeared to be the biggest obstacle, as it requires extra effort, in terms of cultivation. At present a beetle bank has been successfully built in one field, following the British guidelines. This bank will be monitored over the course of the next three years- we will monitor natural enemies, including *Aleochara*. Overwintering densities and in field densities of natural enemies will be compared to control fields, which do not have beetle banks. This beetle bank and the information collected from it should serve as a model for other growers and researchers.

Augmentation of *A. bilineata*: Production

Based on the rearing protocol and results of Whistlecraft et al. (1985) Finch (1989) concludes that production of a sufficient number of *A. bilineata* for a biological control programme for *D. radicum* would be too “daunting”. However, Finch (1989) presumably

based his assessment on the requirements in a conventional field, where pesticide applications would repeatedly kill any beetles released. More recent work indicates that early season releases may be sufficient for reducing *D. radicum* pressure, especially if there is an endemic population of natural enemies later in the season (Royer and Boivin 1999, Turnock et al. 1995). Furthermore, in an organic system where other strategies are being used, *A. bilineata* releases have the potential to be the most effective. Our goal was, firstly, to develop a rearing protocol that was easily initiated and maintained thus addressing the issue of production costs (Finch 1989). Secondly, we wanted to demonstrate activity of *A. bilineata* in the field with a cage study to document predation. Thirdly, we wanted to implement a large-scale release to document dispersal. Difficulties in production hampered the extent of field trials.

The biggest methodological constraint for demonstrating field efficacy of augmentative *A. bilineata* releases was production of a sufficient number of beetles for the trials. Whistlecraft et al. (1985) were able to produce 10,000 *A. bilineata* per week with five hours of labour (weekly) with an average production of 6 eggs per adult female per day. This is far more productive than our results of 13.6 offspring per female over the course of 5 months. Based on an examination of some of the shortcomings of our production protocol the following requirements for effective mass production of *A. bilineata* are outlined below.

- 1) Segregation of *A. bilineata* production and *D. radicum* production. The tubs used for *A. bilineata* production and the pots required for *D. radicum* production required a lot of space and should also be kept separately to minimize the spread of fungus and other pathogens.
- 2) Adequate lighting, temperature and air flow control. Temperature control was an especially significant factor that delayed the production of *D. radicum* and also inhibited the establishment of a continuously running colony. Also lack of sufficient airflow resulted in increased humidity, which exacerbated the fungal problems. Cooling and warming *A. bilineata* adults was not an optimal treatment either
- 3) Synchrony of *D. radicum* and *A. bilineata* production. Again not having a sufficient supply of fresh *D. radicum* pupae delayed *A. bilineata* production.

Since the most difficult aspect of production was rearing and maintaining a constant supply of *D. radicum* eggs and pupae it may be worthwhile to consider alternative hosts. Other rearing protocols use *D. floralis* and *Musca domestica* either as eggs and young larvae for food or pupae for parasitism (Whistlecraft et al. 1985, Hertveldt et al. 1984). *Musca domestica* is commercially available (Rincon Vitova Insectaries, Ventura CA) and is fairly easy to rear for egg and pupal production. This may be a more feasible host for producing *A. bilineata*, as there would be no need to maintain a host colony over several generations since a new cohort could be purchased inexpensively. As with all mass-rearing protocols it is important to ensure that rearing natural enemies in an alternate host does not reduce their preference for the target host/pest in the field (Bigler 1994).

Augmentation of *Aleochara bilineata*: Releases

Results of field trials suggest that augmentative releases of *A. bilineata* may have a limited role in the biological control of *D. radicum* during the later parts of the growing season, i.e. mid-July and later. Cage trials demonstrated that mortality of released *D. radicum* eggs was quite high around the control plants. The low number of maggots recovered in the control plots suggests additional mortality factors for eggs, such as desiccation and predation. Desiccation is highly likely since *D. radicum* eggs are known to easily desiccate (Finch 1989). Given the timing of the study (mid-summer) it is also likely that natural enemies were active in the field and thus the released eggs would have been eaten by predators in the control plots. However since our study was conducted in fields that were not irrigated it is important to consider repeating it in irrigated fields where *D. radicum* egg survival may be higher.

The dispersal study did not recover any of the 250 marked individuals. Although mark-recapture studies have generally low recovery rates (Jervis and Kidd 1996) the lack of recapture does facilitate speculation. In particular, it has been shown that *A. bilineata* responds quite strongly to chemical odours produced by root maggot frass and damaged brassicae plants (Royer and Boivin 1999). Since beetles were released into a mature broccoli field three weeks prior to harvest, it is possible that chemical odours from plants were strong. Dispersal of the released beetles may have been quite localized as a result of these odours. If this is the case then releases in more mature fields should consist of many release points with a few individuals. Tomlin et al. (1992) conducted season long releases and recaptures of *A. bilineata*. Interestingly, their recaptures were lowest from mid-July to mid-August. This result, similar to our findings and conducted at the same time of the year, may indicate again the sensitivity of *A. bilineata* to slight changes in environment. e.g. mean daily temperatures. Tomlin et al. (1992) suggested that lack of recapture may be correlated to plant cover, which is at its peak in the mid-summer. A mark-recapture study should be conducted in the spring and in newly planted fields throughout the growing season when plant cover is lower. Temperature and other abiotic factors should also be recorded in order to determine, via correlation, the factors that affect *A. bilineata* activity upon release.

Recommendations

1. Increasing in-field diversity: Mulching-Our results demonstrated that mulching had a positive impact in reducing the size of the root maggot population infesting individual roots. There was no consistent trend that indicated that mulching increased the abundance of natural enemies in the field, in a sustained manner. Nevertheless, results from additional experiments did suggest, indirectly, that *D. radicum* eggs in mulched plots were subject to additional mortality factors in the field, perhaps predation by natural enemies. It is recommended that growers consider mulching further as a method for reducing root maggot pressure on cole crops. Issues that should be explored further include timing of mulch application, effect of different types of mulch application, and the impact of mulch applications on other cole crop pest populations, specifically slugs and cutworms. Additionally, mulching may have a positive impact on reducing other types of soil dwelling pests. However, growers should also be cautious as excess mulching can have negative consequences for plant performance (Relf 1998).

2. Increasing in-field diversity: Beetle Banks-The experience from speaking to a number of growers in both British Columbia and Washington state about beetle banks indicates the following:

- 1) Growers are interested and supportive of the idea of conservation biological control using tools such as beetle banks
- 2) Beetle banks require additional labour input for construction and maintenance, as well as seeds. The initial investment of time for beetle bank construction is the biggest hurdle in getting banks established on farms
- 3) The beetle bank constructed at Snow Farms, Ladner, BC will be monitored over the next three years. Additionally two more banks are planned for organic farms in Washington State and will also be monitored. These three banks should provide the model that will determine the utility of banks for natural enemy conservation AND pest control for the Pacific Northwest.

3. Augmentation with *Aleochara bilineata*- Releases of predatory beetles for biological control of *D. radicum* may not be necessary in the later parts of the summer as our results showed that *D. radicum* egg mortality was very high in plots without *A. bilineata* release. *Delia radicum* egg mortality was probably due to warm and dry conditions that occurred in August. Also, in organic fields natural enemies were more active in the field by the end of July, probably contributing to high predation pressure on *D. radicum* eggs. However, the efficacy of *A. bilineata* for early season control of *D. radicum* should be investigated further.

7. Outreach

March 2001 Presentation to the Fraser Valley Vegetable Growers Association.

September 2001 Meet with organic growers in Washington State to discuss finding from previous research, including this study, and plans for upcoming research.

November 2001. Contacted editor of Washington Tilth grower newsletter regarding an article about natural enemies of cole crop pests. This will probably be done sometime in the spring of 2002.

December 2001 Presented poster summarizing mulching study results at the Entomological Society of America Meeting, in San Diego Ca. (see Appendix 2).

January 2002. Copy of this report submitted to Lower Mainland Horticultural Improvement Association.

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Figure 1a. Mulch applications. Foreground plot is composted yard waste mulch treatment followed by bare ground control, composted yard waste and finally straw mulch treatments. These plots are the small plots used in fields 1 and 2.



Figure 1b. Close up of straw mulch treatment.

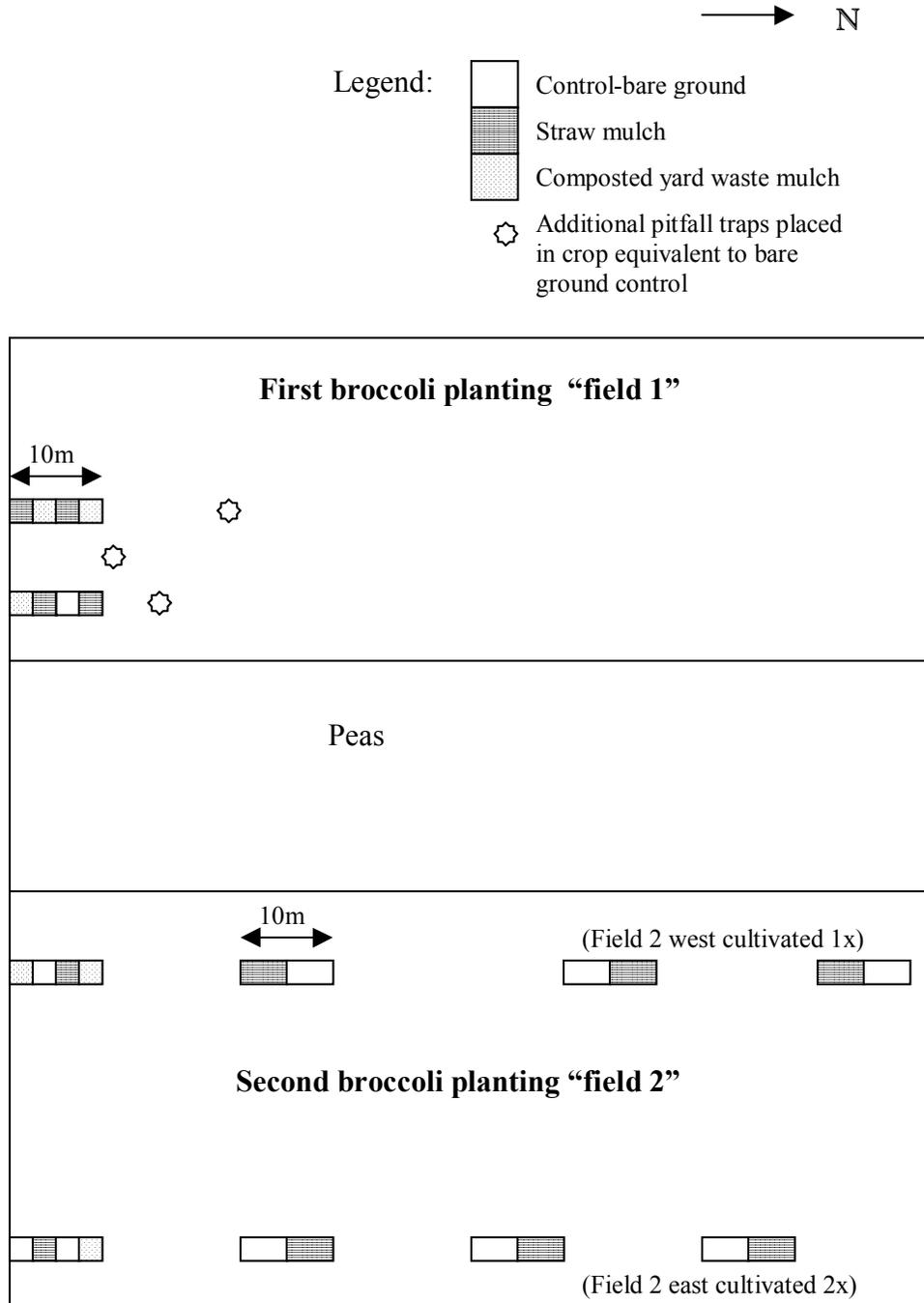


Figure 2. Layout of plots and location of pitfall traps in fields 1 and 2. Rows ran in a north south direction. The width of all plots was approximately 1 meter or 3 rows. These plots were used to assess the effects of mulches on natural enemy abundance. The larger straw and control plots were also used to examine impact of mulching and cultivation on weeds and also predation of introduced *D. radicum* eggs.



Figure 3. The beetle bank constructed at Snow Farms, Ladner, BC. The bank is approximately 40 cm high, 1 meter wide and runs the entire North-South length of the field. The area filled with water is cultivated and planted with crops and/or beneficial insect plants (flowering) in the spring. The beetle bank will be planted with grasses in the spring as well.

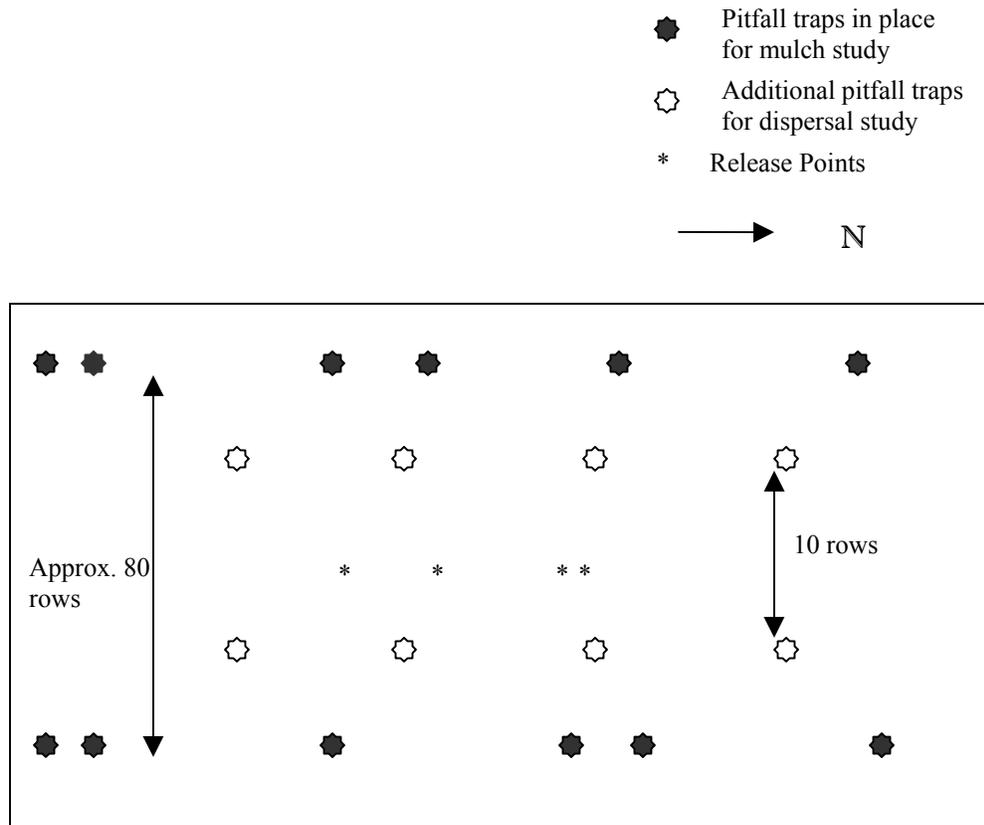


Figure 4. Approximate layout of pitfall traps and location of release point for the study of dispersal patterns of introduced *Aleochara bilineata*. Location of black pitfall traps corresponds to location of mulch plots in field 2 (see Figure 2).

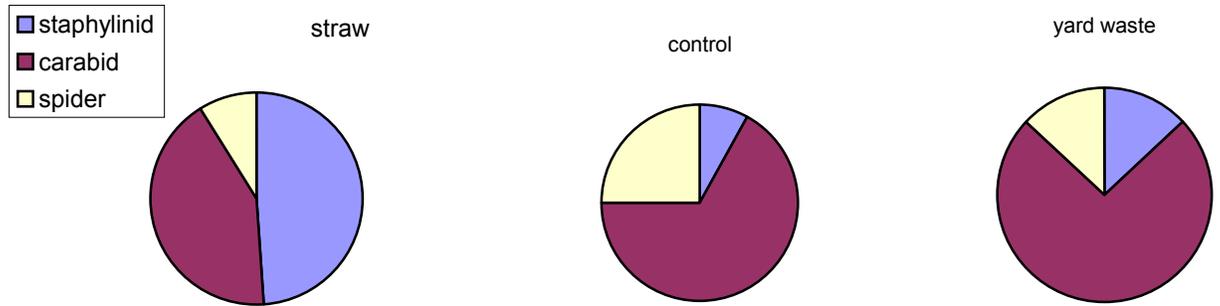


Figure 5. Proportion of staphylinids, carabids and spiders caught in pitfall traps of mulched and control plots, in field 1. Data represent catches from May 24 to June 21, 2001

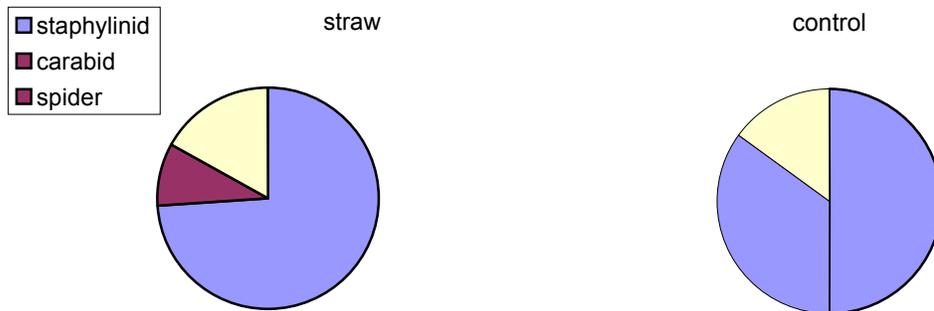


Figure 5b. Proportion of staphylinids, carabids and spiders caught in pitfall traps from straw mulched and control plots in field 2. Data represent catches from June 28 to July 26, 2001.

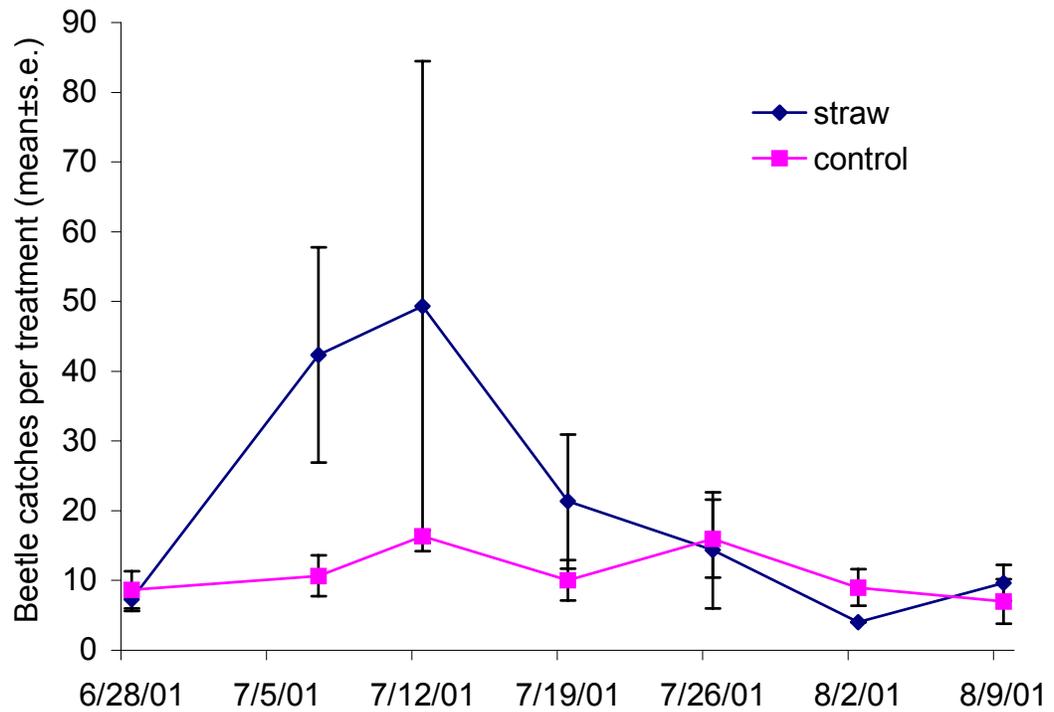


Figure 6. Staphylinid and carabid catches in straw mulch and control plots. Each point represents the mean ± s.e. for three replicates. Data are from large plots in field 2.

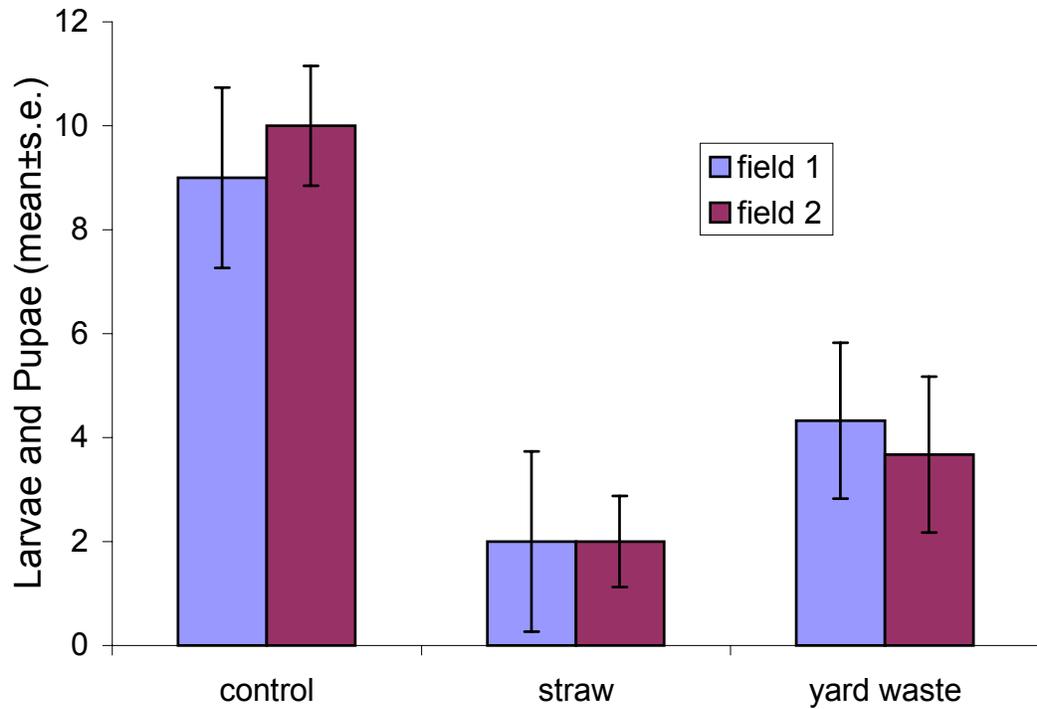


Figure 7. Effect of mulching on number of *Delia* larvae and pupae recovered in soil samples. Control treatments are significantly different from mulches for both fields (Tukey-Kramer HSD).

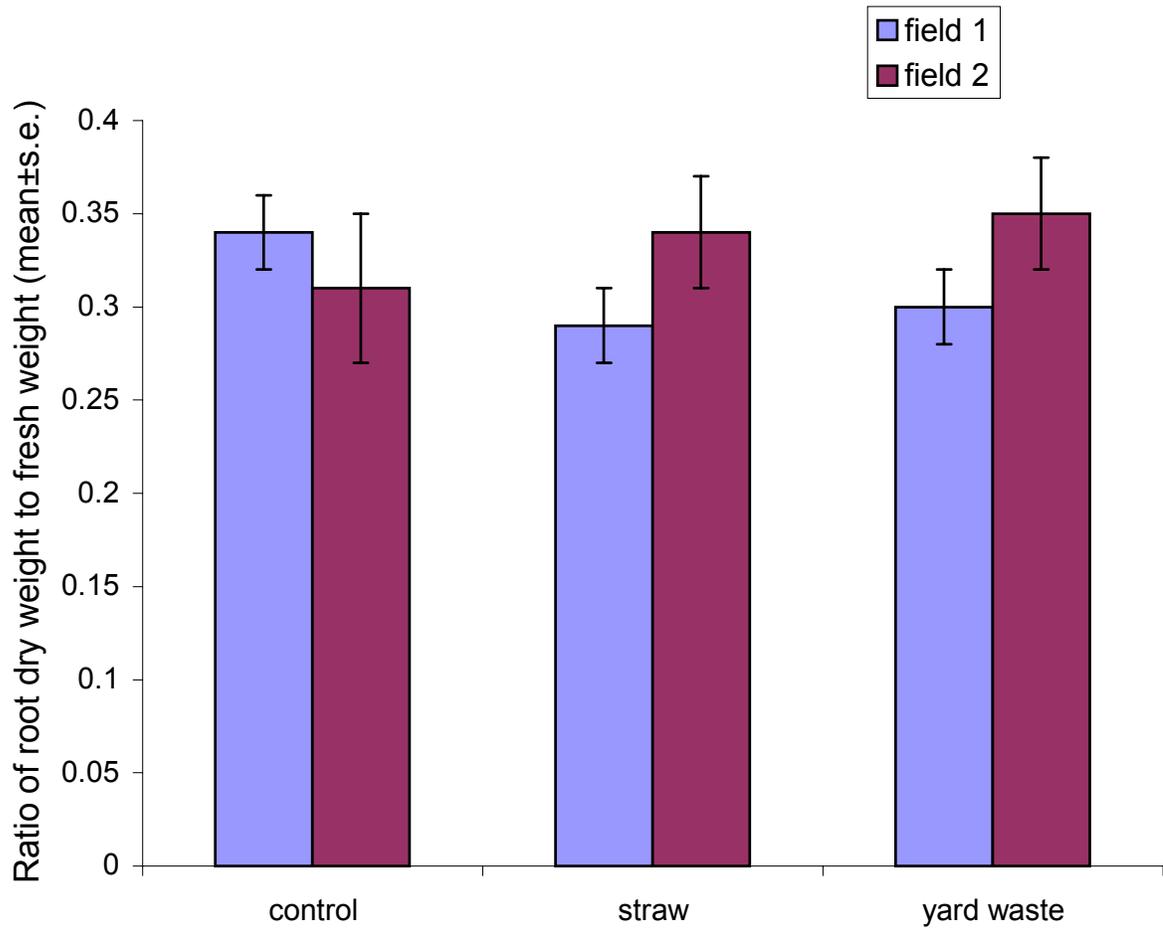


Figure 8. Effect of mulching on the root dry to fresh weight ratio.

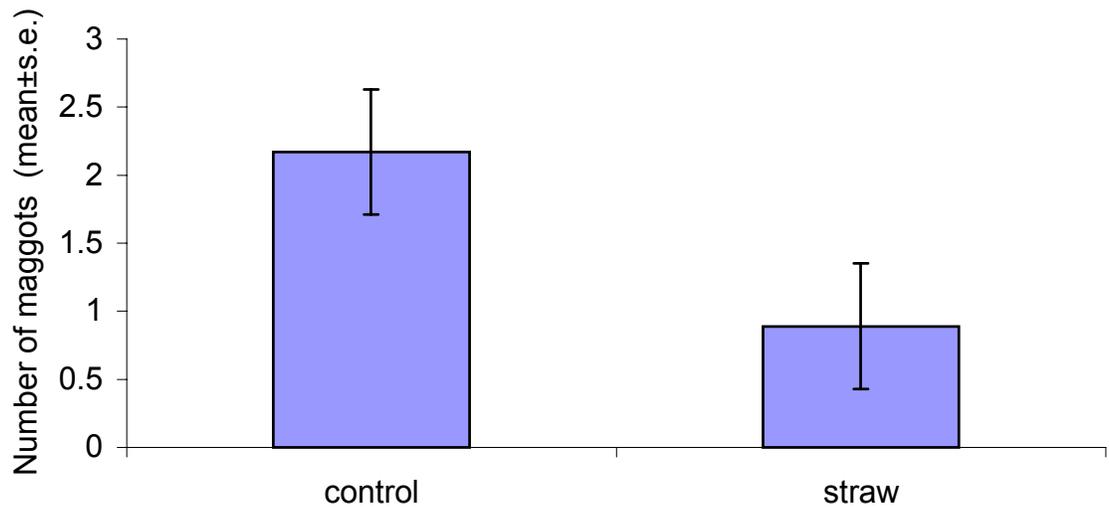


Figure 9. Effect of mulch treatment on survival of *D. radicum* eggs, as inferred from maggot numbers. (N=18)

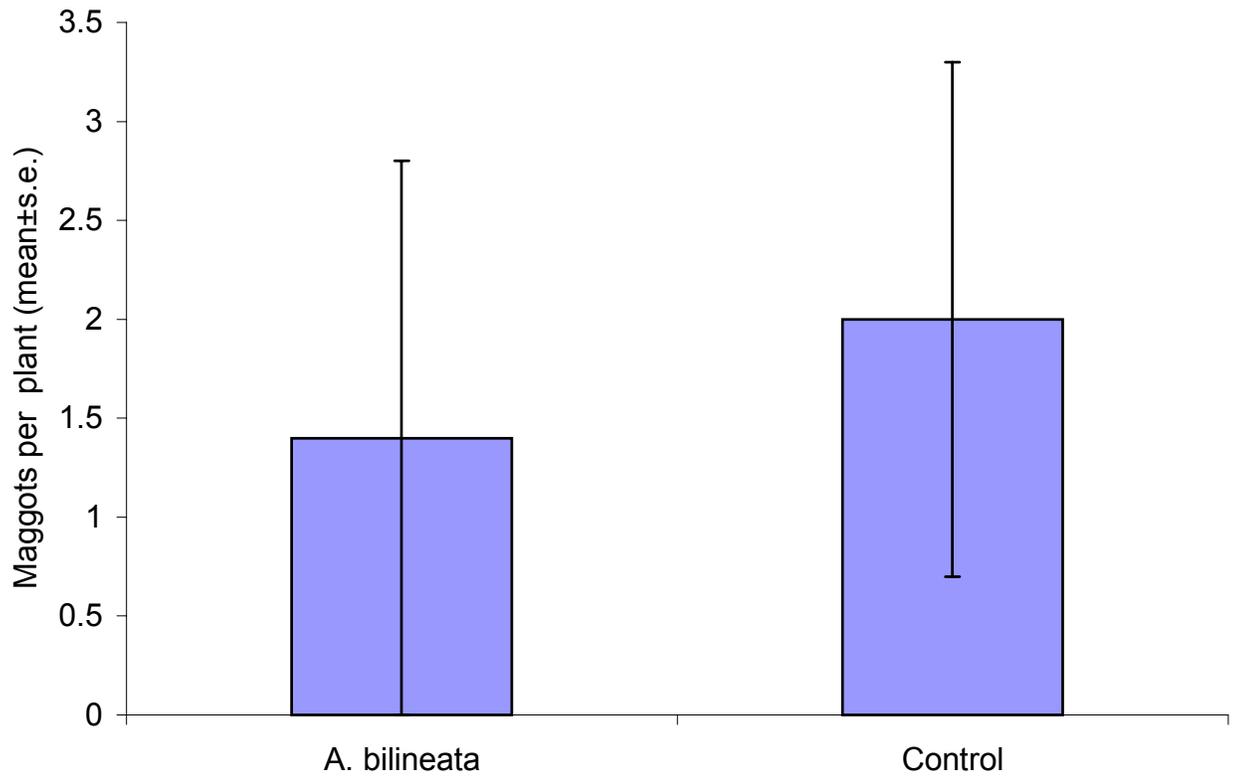


Figure 10. Effect of *A. bilineata* on the survival of *D. radicum* eggs, measured by the number of maggots surviving two weeks after release of *D. radicum* eggs. (N=16)

