DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS **CSG 15**

Research and Development

Final Project Report

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Project title	Biocontrol of the cabbage root fly by the release of predators
DEFRA project code	**HH1830SFV**
Contractor organisation and location	Horticulture Research International Wellesbourne Warwick CV35 9EF
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Executive summary (maximum 2 sides A4)

The use of pesticides in the production of fresh produce is of major concern to the public, both for environmental reasons and to avoid the possibility of pesticide residues in food. DEFRA/PSD is currently reviewing all compounds with anti-cholinesterase activity, and as a consequence, some insecticides have been withdrawn, and others may be withdrawn later as a result of this and the EU pesticide review. There is therefore an urgent need to develop alternative means of controlling pests. Whilst the development of biological control techniques for protected crops has been extremely successful, only one 'biocontrol' agent (*Bacillus thuringiensis*) is used regularly in annual crops grown outdoors in the UK, and use of this biocide is limited. Thus the development of effective biological control techniques for annual field crops presents a major scientific and technical challenge. **The main aim of this project was to determine whether biological control of a pest insect, the cabbage root fly (*Delia radicum)* could be achieved under field conditions by releasing a predatory insect, the staphylinid (rove) beetle *Aleochara bilineata.*** This is in line with DEFRA's current policy objective to provide robust strategies and methods of pest control suitable for integrated crop and farm management, and in line with the Government's policy of pesticide minimisation. DEFRA's scientific aim is to provide growers with the tools to enable them to minimise the use of pesticides, while maintaining a viable UK horticulture industry.

Aleochara bilineata was chosen as the vanguard species, as it develops as a parasitoid within the pupal stage of the cabbage root fly, and so has a finely-tuned system for seeking out cabbage root fly larvae under field conditions. However, under natural conditions, these predatory beetles become active too late in the life-cycle of the fly to prevent crop damage. The aim, therefore, was to mass-rear the beetles so that they could be released to destroy the eggs and larvae of the fly and hence reduce crop damage. Tests showed that, contrary to popular belief, *Aleochara* is not an effective predator of the egg stage of the cabbage root fly. Similarly, the beetles were not effective predators of the first-instar fly larvae, mainly because the root damage done by first-instar larvae did not produce sufficient plant breakdown products to attract and arrest the searching beetles. However, the beetles were effective predators of the second-instar stage of the fly larvae, the stage that starts to do noticeable crop damage.

A major stumbling block in rearing *Aleochara* is that an artificial diet has not yet been developed for its host insect, the cabbage root fly. However, *Aleochara* can be reared in pupae of the closely-related onion fly (*Delia antiqua*), a species which can be mass-produced on an artificial diet. Tests were done to determine whether *Aleochara* reared in onion fly pupae would kill as many cabbage root fly larvae as *Aleochara* reared in cabbage root fly pupae. The results of a field plot experiment done in 1999 showed that *Aleochara* reared in onion fly pupae were as effective at killing larvae of the cabbage root fly as beetles reared in cabbage root fly pupae. Therefore, the commercial production of *Aleochara* could be done most effectively using onion fly.

Using a range of plant types, an experiment was done in 2000 to determine the influence of plant type on the effectiveness of *Aleochara.* The effectiveness of the beetles was influenced greatly by the host plant on which the fly larvae were feeding. Levels of “control” considered acceptable (producing a reduction in pupal numbers of > 75%) were achieved only for the Chinese cabbage (77%) and turnip (80%) plants. Such levels of "control", however, equate only to a relatively ineffective insecticide treatment. In another experiment in 2000, to determine the most effective timing for release of *Aleochara*, the numbers of eggs recovered from plots in three experimental fields averaged 120, 100 and 85/plant, respectively. These are much higher than the 40 eggs/plant maximum recorded in commercial fields in which insecticides are applied on a regular basis. In two of the fields, a combination of high egg numbers and rapid plant growth meant that the released beetles reduced pupal numbers only around the roots of cabbage and Kohl rabi plants. In the third field, in which the plants were slower to establish, so that the larvae on the fly roots were less dispersed, the data were consistent and reasonably high reductions in pupal numbers (45-70%) were recorded from five of the six plant types tested. In this field, pupal numbers were lowered to the greatest extent when the *Aleochara* were released to coincide with timing of 70% egg lay by the cabbage root fly population. However, the numbers of fly pupae recovered still ranged from 3-9 pupae/plant, much higher than the 1/plant generally obtained when conventional soil insecticides are used. The results obtained from a large field experiment done in 2001, to develop methods of scheduling the release of *Aleochara,* were disappointing. It was difficult to draw any firm conclusions, as there was no overall trend that could be applied to the complete set of data. The problem with field experiments of this type appears to be that there are too many variables that cannot be controlled directly by the researcher.

While *Aleochara* under no-choice situations feeds on the larvae of its host-fly to accumulate the protein required to mature its own eggs, under field conditions *Aleochara* feeds also on other small invertebrates. Hence, releasing *Aleochara* earlier in the life-cycle of the pest than it would occur naturally, has an adverse effect on some of the other invertebrates thought to be important in the natural control of the cabbage root fly. The behaviour of *Aleochara* is such that it largely avoids the adverse effects of insecticides, but it cannot avoid the parasitic wasp *Trybliographa rapae*, against which it compete extremely poorly when fly populations are high. Hence, the idea that releasing *Aleochara* would help also to build-up the numbers of this beetle, and so help to increase the overall levels of parasitism in field *Brassica* crops, now seems untenable.

The data collected during 1999 and 2000 indicated that each released beetle could kill between two to five fly larvae prior to pupation. Hence, the beetles are unlikely to “control” high fly populations unless the beetles themselves are released in high numbers. Thus the difficulty of predicting the numbers of cabbage root flies that will enter a particular field is a major constraint. There would be no way of knowing the numbers of beetles to release in each field unless the fly population was monitored on a regular basis. In addition, to be effective, the beetles may have to be released at regular intervals over a protracted period of time and the logistics involved in the production of beetles to cope with such an approach would be both extremely difficult and expensive. The situation is exacerbated even further, as the current research has shown clearly that the relative effectiveness of the beetles also changes from crop to crop, from field to field and from year to year.

There is considerable interest in the use of *Aleochara* to control the cabbage root fly and throughout the project, contact has been maintained with biocontrol companies interested in developing this technique. The results of the work have been presented at a number of meetings for scientists and growers in the UK and overseas.

Scientific report (maximum 20 sides A4)

1. INTRODUCTION

The main aim of this project was to determine whether biological control of a pest insect could be achieved under field conditions by releasing a predatory insect. During the last 80 years, many researchers have suggested that field populations of the cabbage root fly (*Delia radicum* L.) could be controlled by releasing the staphylinid (rove) beetle *Aleochara bilineata*. Therefore, this beetle was chosen as the vanguard species, primarily because the beetle develops as an obligate parasitoid within the pupae of species of *Delia*, such as the cabbage root fly (Fig. 1a), and so has a finely-tuned system for locating cabbage root fly larvae under field conditions. However, under natural conditions, the beetles emerge only shortly before the fly larvae pupate, and so become active too late in the life-cycle of the fly to prevent crop damage. Despite this fact, many researchers believe that crop damage could be prevented if the beetles were reared and released earlier in the life-cycle of the pest, as, apart from being a parasitoid, *Aleochara* will also eat the eggs and larvae (Fig. 1b) of the fly. Before time is spent trying to convince biotechnology companies to mass-rear this predator, however, further data are required to show that the release of *Aleochara* could be effective against the cabbage root fly under open field conditions.

Fig. 1. The beetle *Aleochara bilineata;* a) emerging from a pupa of the cabbage root fly, and b) feeding on cabbage root fly larvae

Results collected during 1998 at HRI Wellesbourne (Project HH1815SFV) indicated that under semi-field conditions (covered crops), releasing low (2/plant) numbers of *Aleochara* gave good control of fly larvae on the roots of artificially-infested *Brassica* plants. The current project has concentrated on trying to take the research to the next stage, to determine whether control of fly larvae could be achieved in the open field when the dispersal of the predators is not restricted and when the pest infestation is not distributed regularly through the crop, but varies widely from plant to plant even within the same crop. The work has concentrated on field-cage and field experiments, as these reflect more closely the highly variable types of conditions that *Aleochara* will have to contend with if it is to become a successful biological control agent in commercial field situations.

2. OBJECTIVES

The overall objective was to show whether releases of the predatory beetle, *Aleochara bilineata*, were capable of controlling natural infestations of the cabbage root fly. The five main objectives were-

1) To rear populations of *Aleochara bilineata* in pupae of both the cabbage root fly and the onion fly and to compare the effectiveness of the two *Aleochara* "biotypes" in controlling the cabbage root fly.

2) To determine how the different plant types and plant spacings used in cabbage and swede crops affected the distribution of the cabbage root fly and *Aleochara bilineata*.

3) To modify the cabbage root fly pest forecasting system so that it could be used to schedule accurately the timing of all *Aleochara* releases. To determine the numbers of *Aleochara* required and where and when to release them to achieve acceptable levels of cabbage root fly control in cabbage crops.

4) To quantify how the released *Aleochara* interact with the complex of non-target invertebrates found above and below the surface of the soil in brassica crops.

5) To develop methods for scheduling the release of *Aleochara* to avoid them being affected adversely by insecticides applied to control other pest-insect species.

1. THE RELATIVE EFFECTIVENESS OF TWO "BIOTYPES" OF *ALEOCHARA* (Objective 1)

A major stumbling block for anyone attempting to mass-rear *Aleochara* is that an artificial diet has not yet been developed for its host insect, the cabbage root fly. Therefore, the host insects still have to be reared on swedes (*Brassica napus* var. *napobrassica*), a process which is both labour-intensive and physically-demanding. In MAFF Project HH1815SFV some of the *Aleochara* were reared in pupae of the closely-related onion fly (*Delia antiqua*), as such pupae can be mass-produced using an artificial diet. However, before onion fly pupae are used as the host insects for rearing the *Aleochara*, we needed to determine whether *Aleochara* reared in onion fly pupae killed as many cabbage root fly larvae as *Aleochara* reared in cabbage root fly pupae. To do this, populations of the two *Aleochara* "biotypes" were reared in the Insect Rearing Unit at Wellesbourne.

Experimental. On 14 April 2000, nine plots were each planted with 15 x 35 plants of cabbage (cv. Derby Day), spaced 0.6m apart both within and between the rows. The experiment was set up as a 3 (treatments) x 3 (replicates) Latin square. The treatments involved releasing *Aleochara* that had been reared in cabbage root fly pupae into 3 plots, releasing *Aleochara* that had been reared in onion fly pupae into 3 plots, and releasing "no beetles" (the "check" treatment) into the other 3 plots (see Fig. 2). As soon as the plots were planted, they were covered with cages made by pushing the ends of 2m lengths of plastic cold-water piping into the soil to make semicircular hoops and then covering the hoops with pieces of Envirofleece, the edges of which were buried into the soil. This was done to keep off the natural infestation of flies (see later).

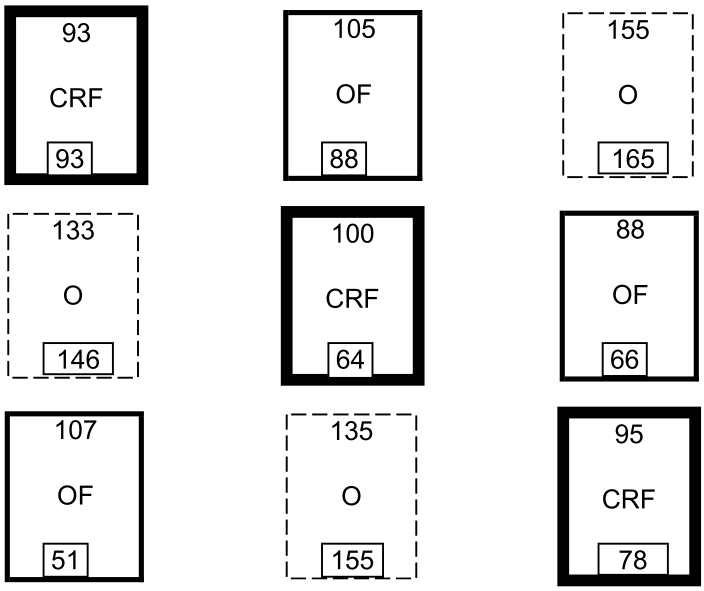
On 5 June, the Envirofleece was removed and nine plants towards the ends of the central bed of plants in each plot were each inoculated with 80 cabbage root fly eggs. The inoculation required a total of 12,960 fly eggs, which were obtained from the culture of flies maintained in the Insect Rearing Unit at HRI Wellesbourne. Once the eggs had been washed onto the plants, the plants were again covered with Envirofleece. The test plants were then left undisturbed for a further 90 day-degrees (in this case 11 days) to allow the fly larvae to accumulate sufficient heat units to hatch from the eggs and develop as far as the second instar, the larval stage that arrests the predatory *Aleochara* beetles.

On 16 June, three *Aleochara* from the two different "biotypes" were released onto each test plant. At the time the beetles were released, the foliage of the test plants weighed 730±58g and the roots 23±2g. On each of the nine plots, the southern sub-plots were left covered and the northern plots were left open (Fig. 2). Provided the beetles are arrested by this level of fly infestation, similar data should be obtained from both the covered and the uncovered sub-plots (see Fig. 2).

The cabbage root fly has to accumulate 250 day-degrees above 6^{o}C from egg hatch to 50% pupation and a further 200 day-degrees to complete pupal development. The current test plants were sampled after an accumulation of 350 day-degrees (13 July), about half-way through the pupal development period. This was done to make sure that all of the fly pupae extracted from the soil samples were intact. When the sampling of pupae is delayed beyond this point, some of the insects accumulate sufficient heart units to emerge as flies. Once this occurs, the samples contain both full and empty fly puparia and this makes the extraction of the pupae much more time-consuming. To prevent the insects continuing their development and emerging as flies once the samples had been collected, the samples were maintained in a cold store (4^{o}C) from the day of collection until they were washed out.

Results & discussion
The layout of the experiment and the total numbers of pupae recovered are shown in Fig. 2.

Fig. 2. Layout of the nine experimental plots and the numbers of cabbage root fly pupae recovered from the plots in which beetles reared in cabbage root fly (CRF) pupae and onion fly (OF) pupae and no beetles were released. The [] indicate the test plant that were kept covered throughout the entire experiment.



Origin of released *Aleochara*	Plot	No. of pupae/9 plants Replicate 1	2	3	Pupae/plot Total	Mean	Mean
From cabbage root	Covered	78	64	93	**235**		
fly (CRF) pupae	Open	95	100	93	**288**	87± 5	9.7
From onion fly	Covered	51	66	88	**205**		
(OF) pupae	Open	107	88	105	**300**	84 ± 8	9.4
No beetles (O)	Covered	155	146	165	**466**		
("check")	Open	135	133	155	**423**	148 ± 5	16.4

Although slightly more pupae were collected from the six open sub-plots in which beetles were released, the differences recorded, 220 pupae from the covered plants and 294 pupae from the open plants, were relatively small in terms of biological control. Therefore, many of the beetles must have stayed in the open sub-plots, as the mean for the "check" treatment was 450 pupae. The mean numbers of pupae recovered/plant from the two *Aleochara* "biotypes" and the "no beetles" treatment were 9.7 (CRF), 9.4 (OF) and 16.4 (no beetles), respectively. Hence, the two biotypes appeared to be equally effective as predators of cabbage root fly larvae, as each biotype destroyed about 40% of the available fly larvae. Therefore, in this experiment each *Aleochara* appeared to kill about 2-3 fly larvae. This rate of kill will need to be increased considerably, if *Aleochara* is to be considered a serious contender for controlling the cabbage root fly in commercial *Brassica* crops.

Conclusions

***Aleochara* beetles reared inside onion fly pupae were as effective at killing larvae of the cabbage root fly as beetles reared in cabbage root fly pupae. There was no evidence that the beetle biotypes searched for the specific fly species in which they had completed their earlier development. Hence, any commercial production of *Aleochara* would be done most effectively within onion fly pupae, as these can be mass-produced using an artificial diet, whereas cabbage root fly pupae cannot.**

2. INFLUENCE OF PLANT TYPE ON THE EFFECTIVENESS OF *ALEOCHARA* (Objective 2)

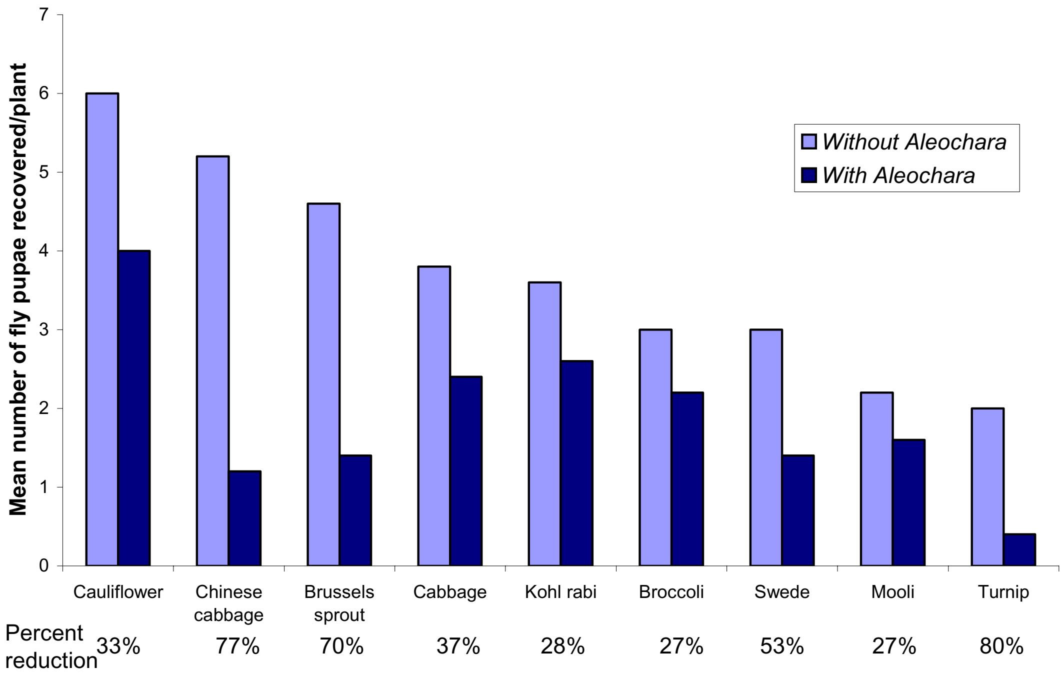
Experimental. In June 2000, ten small field-cages were each planted with nine plant types, one of each of broccoli, Brussels sprouts, cabbage, cauliflower, Chinese cabbage, Kohl rabi, Mooli (Japanese radish), swede and turnip. The positions of the plants within the cages were allocated at random.

On 29 June, 40 cabbage root fly eggs were washed around each of the stems of the 90 test plants and 11 days later (10 July) two beetles were released/plant. Beetles were released around the plants in five of the cages, the other five cages were used for the “check” (no beetles) treatment. As described earlier, the plots were harvested and the soil samples collected 350 day-degrees after egg-inoculation.

Results & discussion

The data (Fig. 3) have been arranged in descending order of the numbers of pupae recovered from the “check” plants on which *Aleochara* were not released. Most ($P = 0.05$) fly pupae (6/plant) were recovered from around the cauliflower and fewest (2/plant) from around the turnip plants. The degree of predation also appeared to be affected by plant type. The reduction in pupal numbers from the nine plant types ranged from 27% to 80% (mean 48%). Beetles seemed to be more effective predators around the roots of Chinese cabbage (77% reduction) and turnip (80%) than around those of broccoli (27%) and Mooli (27%).

Fig. 3. The numbers of cabbage root fly pupae recovered from the soil from around the roots of nine different cruciferous vegetable plants, after inoculation of 40 fly eggs/plant and the subsequent release of *Aleochara* onto half of the plants.



This experiment shows the relative survival rates of the fly larvae when similar numbers of cabbage root fly eggs were placed around nine different types of cruciferous vegetable plants. Under normal field conditions, however, the situation is far more complex, as the flies lay more eggs on certain plants than on others. This preference is not fixed, however, and there are also critical times in the growth stages of most plant types when they become highly preferred for egg-laying by the flies. Hence the situation in the field is extremely dynamic and can change radically, often over extremely short periods of time.

Conclusions
The effectiveness of the released beetles as predators of cabbage root fly larvae was influenced greatly by the type of host plant on which the fly larvae were feeding. Levels of "control" considered acceptable, that is those producing a reduction in pupal numbers of 75% and upwards, were achieved only for the Chinese cabbage (77%) and turnip (80%) plants. Such levels of "control", however, equate only to what most people would consider a relatively ineffective insecticide treatment.

3. MOST EFFECTIVE TIMING FOR THE RELEASE OF *ALEOCHARA* (Objective 3)

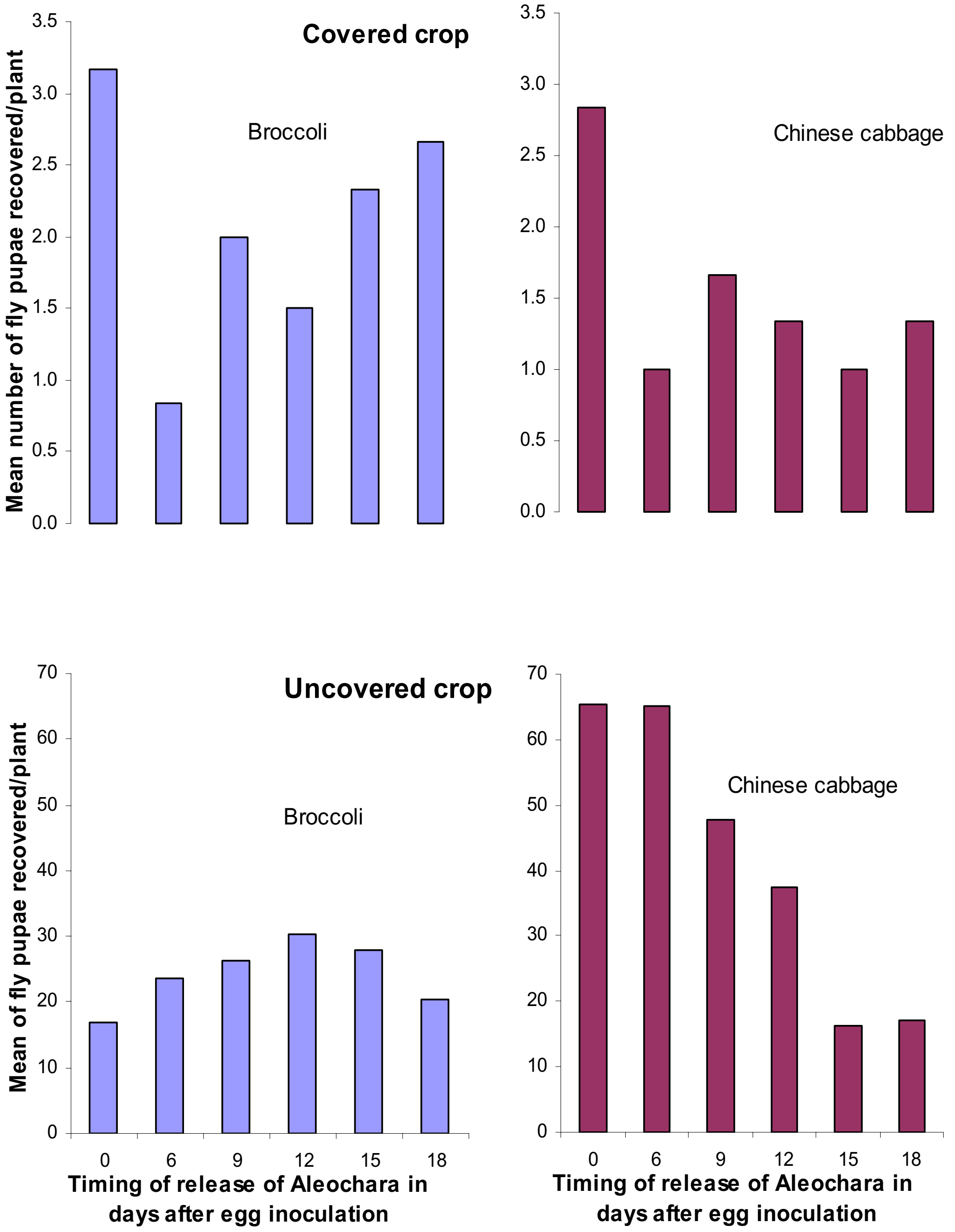
Detailed experiment. On 16 June 2000, thirty-six small plots were each planted with 2 plants of broccoli and 2 plants of Chinese cabbage. The experiment was planted as a 6 x 6 Latin square and was then covered with one large piece of Envirofleece.

On 27 June, the fleece was removed and 40 fly eggs (total of 5,760 fly eggs) were washed onto the soil at the base of each plant. To do this, the lower leaves of the plants had to be moved aside. When this was done, large numbers of ground beetles were disturbed from under the Chinese cabbage plants, where the bases of the outer leaves touched the soil, and few under the broccoli plants, where the pronounced stem kept the bases of all leaves well clear of the soil surface. The 36 plots were then covered with individual cages. Beetles were released onto the six replicates of each treatment on 3, 6, 9, 12 and 15 July, respectively, which corresponded to days 6, 9, 12, 15 and 18 after the eggs had been inoculated.

On the day the beetles were released, half of the plots were covered again with fleece. The other three plots were left open, but were covered with wide-mesh (50mm square) netting to prevent damage by pigeons.

Results & discussion
The data (Fig. 4) show from the "check" treatments that similar numbers of insects (about 3/plant) were recovered from both the broccoli and Chinese cabbage plants. For the covered broccoli plants, the beetles were most effective as predators when released on day 6 (0.75 pupae recovered/plant), presumably because at this stage the fly larvae were still highly aggregated. On the other plants, pupal numbers averaged about 2/plant and so were reduced by only about 30% by the released beetles. In contrast, the numbers of pupae recovered from the covered plots of Chinese cabbage were reduced by about 50%, irrespective of when the beetles were released. The root system of Chinese cabbage plants is such that the fly larvae stay aggregated close together throughout the whole period of their development.

Fig. 4. The numbers of cabbage root fly pupae recovered from around the roots of broccoli and Chinese cabbage plants when the plants were each inoculated with 40 cabbage root fly eggs and *Aleochara* was released 6 to 18 days later. The lower histograms show the impact of the natural infestation of flies during the course of this experiment.



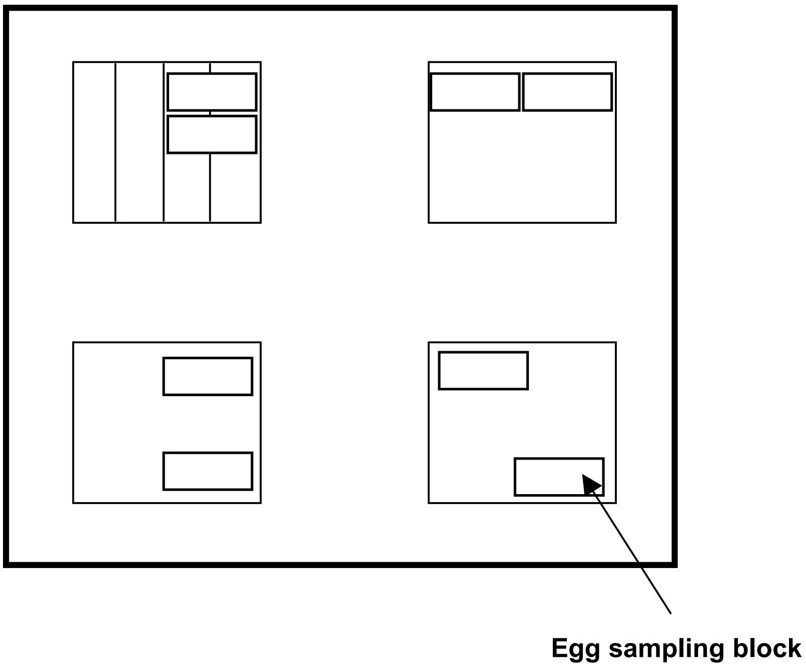
The two lower histograms illustrate the inherent difficulties in trying to do experiments on arthropod predation under “open field” conditions. Once these plots were opened, large numbers of eggs were laid on the test plants by the population of wild flies. For both plant types, about 20 times as many fly pupae were recovered/plant from the uncovered than from the covered plants. The differences between the maximum of 30 pupae/plant from the broccoli and the 65 pupae/plant from the Chinese cabbage probably occurred because Chinese cabbage 1) was preferred for egg-laying, was 2) more nutritive for larval development, or 3) from a combination of the two. Fly numbers as high as this destroy most plants. However, Chinese cabbage is rarely killed, mainly because it rapidly produces new lateral roots to replace those eaten by the fly larvae. Chinese cabbage is helped also by its leaf bases being so close to the soil surface, as this helps to conserve moisture around its roots, which helps to alleviate the water stress caused by the loss of roots destroyed by the feeding fly larvae. The decline in the numbers of pupae recovered from the uncovered Chinese cabbage plants is not a treatment effect, but reflects simply the fact that the earlier the plots were uncovered the more eggs they received from the wild population of flies.

Conclusions The original intention was to use detailed experiments of this kind to pinpoint accurately the critical timing for releasing beetles. The data collected, however, failed to indicate that there was an optimum timing. As in Objective 3 (see Fig. 4), plant type appears to have a major effect in this experiment, as again predation was higher around the Chinese cabbage (50% reduction) than around the broccoli (30% reduction) plants.

Main experiment. Four plots of cabbage, each containing 225 plants were transplanted into three different fields of the Research Station (Cottage Field, Sheep Pens, Pump Ground West) on 24 May 2000. The four plots in each field were grouped in the way shown in Fig. 5. Beds of carrots were drilled to provide the flies with local shelter. The area between the plots was sown with cereal to produce a green ground cover, which was kept low (10cm high) by mowing. On 7 June, as all plants were infested with flea beetles and some plants were infested also with cabbage aphid, all 12 plots were sprayed with a mixture of Aphox and Decis. The plots were then all covered with Envirofleece to help the plants recover from earlier pigeon damage. These cabbage plants were used to arrest flies in the areas being used for the subsequent trials.

On 13 June, thirty six cabbage plants from each plot were replaced by six plants of each of cabbage, Spanish radish, cauliflower, Kohl rabi, swede and Brussels sprouts (see Fig. 5). Further plants, three of each of the six types, were transplanted into the plots in which *Aleochara* would not be released. These plants were used to monitor the numbers of eggs laid on the various crop types.

Fig. 5. Layout of the four sub-plots used in each of the three fields (Cottage Field, Sheep Pens, Pump Ground West) to test the impact of releasing *Aleochara* at the timing of 10%, 50% and 70% egg-lay by the cabbge root fly. The fourth plot in each field was the "no beetle" (check) treatment.



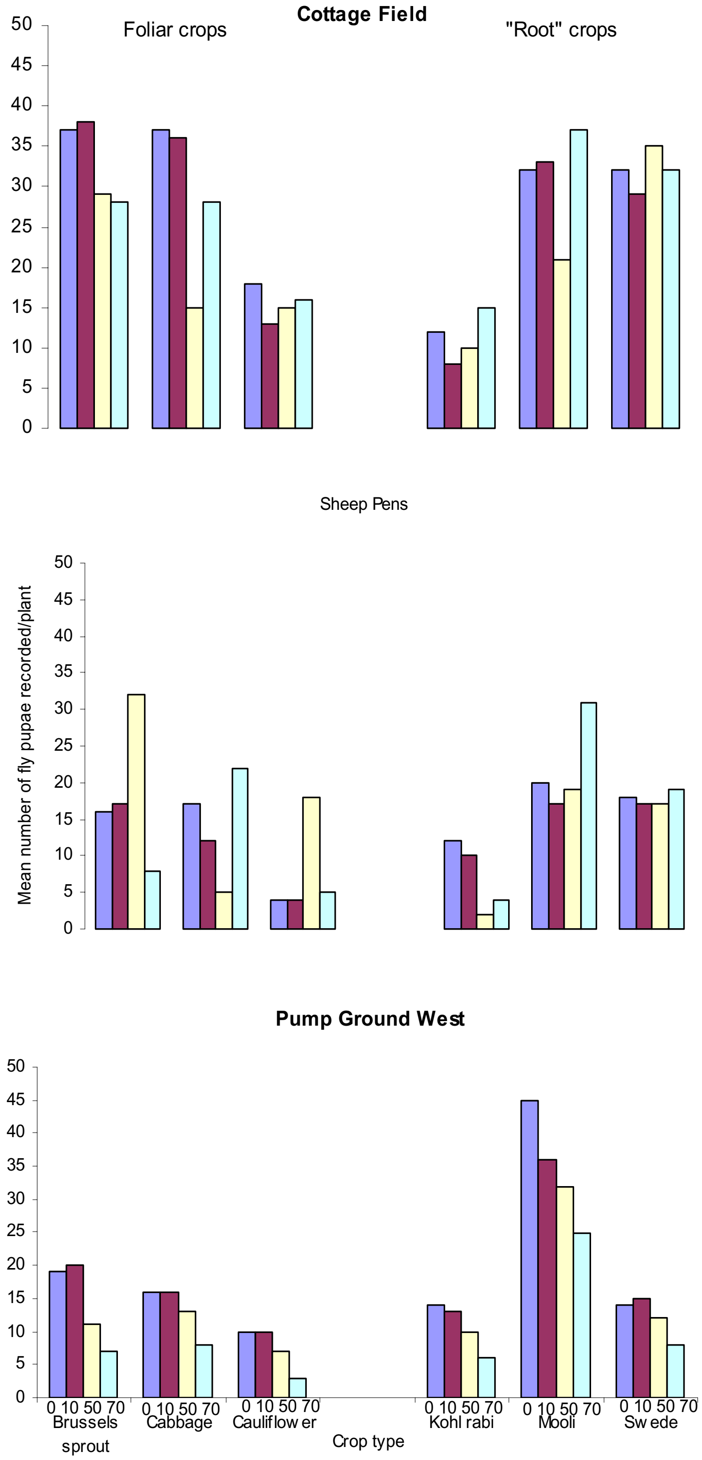
The four experimental treatments in each field involved releasing beetles at the time of 10%, 50% and 70% egg-laying by the fly and using the fourth plot (no beetles release) for the "check" treatment. The original long-range cabbage root fly forecast was that 10% egg-lay should occur on 3 July. However, following a spell of hot weather, the revised estimate was for 6 days earlier, 27 June. This highlights the inherent difficulties in trying to schedule beetle releases to coincide accurately with specific stages in the life-cycle of the fly.

The Envirofleece was taken off the experimental plants on 20 June, as egg-laying by the second generation of flies was predicted to start in the last week of June. The *Aleochara* were released at the rate of 3 beetle/plant on 28 June (10% egg-lay), 12 July (50% egg-lay) and 25 July (70% egg-lay). Egg samples were collected twice each week and the numbers of fly larvae that managed to pupate were recorded by extracting the pupae from soil samples at the end of the experiment.

Results

The original intention was to use the three fields as replicates, so that the data collected could be combined to produce more robust conclusions. However, as Fig. 6 shows, the results varied considerably from field to field, because the crops grew at different rates in the three fields and this affected both the numbers of flies arrested and subsequent plant damage.

Fig. 6. The numbers of cabbage root fly pupae recovered from around the roots of the six different cruciferous crop plants when *Aleochara* was released in the three different fields (Cottage Field, Sheep Pens, Pump Ground West) to coincide with the timing of 10%. 50% and 70% egg-lay by the natural infestation of flies.



Percent reduction

	Brussels sprout	Cabbage	Cauliflower	Kohl rabi	Mooli	Swede
0 - 50%	42%	19%	30%	29%	29%	14%
0 - 70%	63%	50%	70%	57%	44%	43%

Sheep Pens. In this field, the numbers of pupae recovered were lowered by the 10% and 50% egg-lay treatments for the cabbage plants, by the 50% and 70% treatments for the Kohl rabi plants and by the 70% treatment for the Brussels sprout plants. The four unexpectedly high values, recorded from the releases made at the timing of 70% egg-lay around the cabbage and Mooli plants and the timing of 50% egg-lay around the cauliflower and Brussels sprouts plants, suggest that the released *Aleochara* were having an adverse effect on some other naturally-occurring biological control agent. The most likely agent could be the larvae of the fly *Phaonia trimaculata*, as the larvae of this fly, which destroys the larvae of the cabbage root fly, were also found in the soil samples taken from this field. A second alternative is that the *Aleochara* were destroying the adults or larvae of some of the predatory ground beetles found commonly in cultivated fields (see Objective 4).
Pump Ground West. This was the only field in which the data showed a definite trend. The release of *Aleochara* to coincide with 10% egg-lay failed to reduce the numbers of fly pupae recovered on all plants except Mooli. However, for all six plant types, pupal numbers were reduced (mean reduction = 27%) by the beetles released to coincide with 50% egg-lay and even further (mean reduction = 55%) by the beetles released to coincide with 70% egg-lay.

Conclusions. The results from the only field (Pump Ground West) in which the data were consistent between plant types, indicated that pupal numbers were lowered to the greatest extent when the *Aleochara* were released to coincide with the timing of 70% egg-lay by the pest fly population. Even with this treatment, however, the numbers of fly pupae recovered at harvest ranged from a 43% (swede) to a 70% (cauliflower) reduction. The greatest concern from both a scientific and a practical point of view is how to account for the huge variation that occurs from field to field, even in the same locality.

4. INFLUENCE OF RELEASED *ALEOCHARA* ON BIODIVERSITY (Objective 4)

Soil samples, 15cm diameter x 15cm deep, were taken from around the roots of fifty plants in plots in which *Aleochara* had been released at the rate of two beetles/plant and in plots in which *Aleochara* had not been released. The overall aim was to determine whether *Aleochara* had any effect on the naturally occurring beetles found commonly in field brassica crops.

Results and Discussion. Slightly more *Aleochara* (32) were recovered from the plots in which the beetles were released than in the control plots (25). Of the ground beetles that were recovered in reasonable numbers, the numbers of the carabid beetles *Bembidion lampros*, *B. quadrimaculatum* and *Notiophilus biguttatus* were not affected by the released *Aleochara.* These beetles are normally active by day and feed on other small invertebrates that they encounter on the open soil surface between the plant rows. In contrast to these species, the two predatory beetles, *Bembidion obtusum* and *Trechus quadriastriatus,* that normally aggregate in the soil immediately around the roots of brassica plants were affected adversely by the presence of *Aleochara.* The data indicated that releasing *Aleochara* reduced the numbers of *Bembidion obtusum* present to about half (22:10) and the numbers of *Trechus quadriastriatus* to about one-third (31:10), when compared to the plots in which *Aleochara* was not released. *Aleochara* also appeared to reduce the numbers of *Oxytelus rugosus* and *Xantholinus linearis*, two other naturally-occurring rove beetles found also around the roots of brassica plants. These two beetles feed normally on dead and decaying plant material. It appears that these beetles were also being killed directly by the *Aleochara* as considerable amounts of decaying root material, the food normally consumed by these two saprophagous species of rove beetle, were always found when the soil/root samples from around the plants on which *Aleochara* had been released were washed onto the sieves.

Conclusions. While *Aleochara* under no-choice situations feeds on the larvae of its host-fly to accumulate the protein required to mature its own eggs, under field conditions *Aleochara* feeds also on other small invertebrates. The current results show that releasing *Aleochara* earlier in the life-cycle of the pest than it would occur naturally, has an adverse effect on some of the other invertebrates thought to be important in the natural control of the cabbage root fly.

5. SCHEDULING *ALEOCHARA* RELEASES TO AVOID INSECTICIDES (Objective 5)

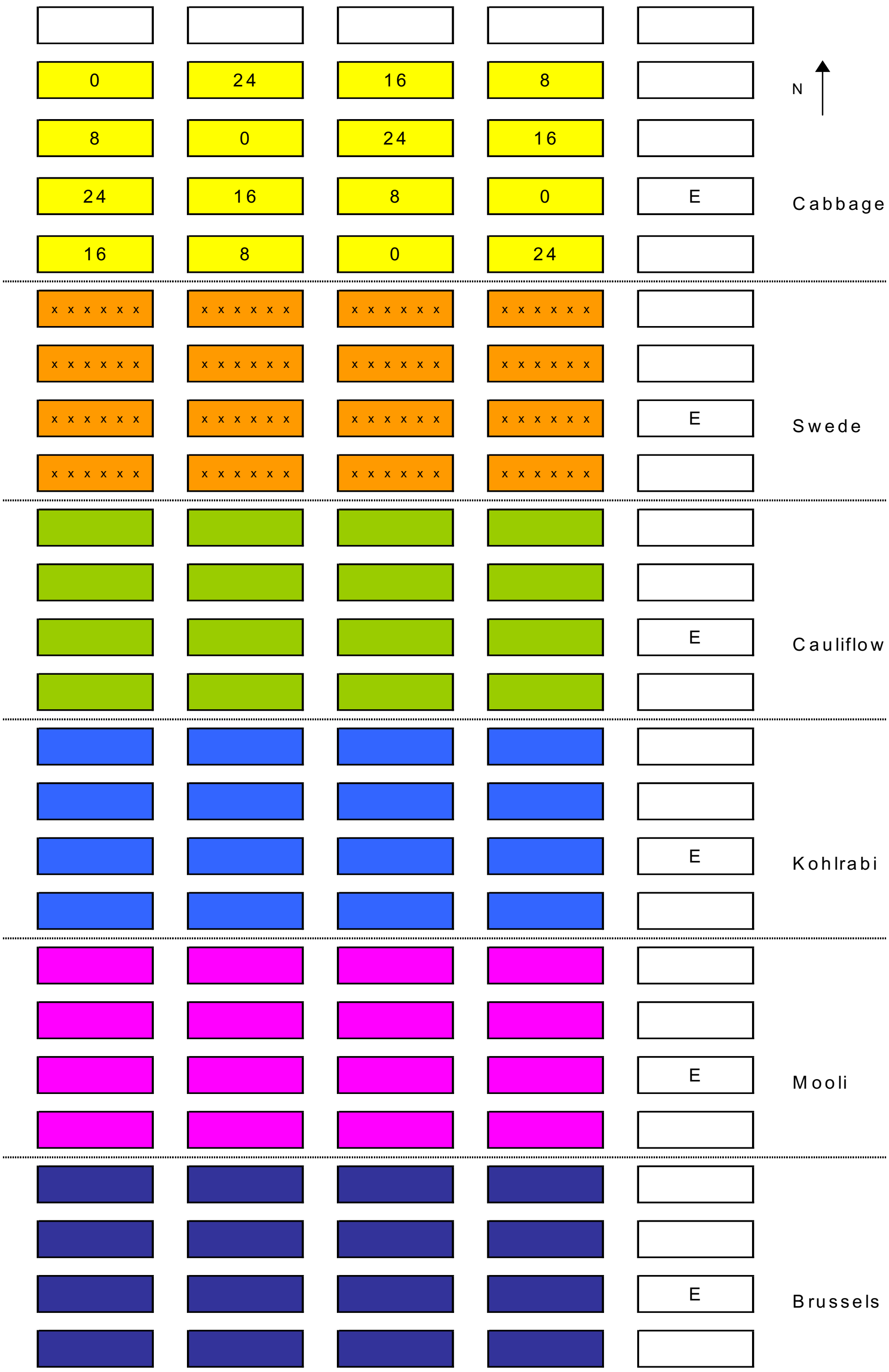
Insecticides, per se, now appear to pose less of a threat to predatory arthropods, as the insecticides that can be used in cruciferous vegetable crops are now extremely limited and are also usually applied at only low doses.

In addition, the behaviour of *Aleochara* is such that, in effect, it avoids contact with insecticide. The beetle spends most of its time below the soil surface and so is not affected greatly by residues from insecticide sprays applied to the foliage of plants, particularly as many of the newer sprays lose their insecticidal properties once they come into contact with soil. Similarly, the beetles only search in the soil around plants with well-established fly infestations, which occur only on untreated plants or plants around which any soil-applied insecticide is no longer effective.

As it is easy to schedule the release of beetles to avoid the times when insecticides are applied to control other pest insect species, this experiment concentrated on how the beetles dispersed to neighbouring plants when they were released on a central plant. Six types of cruciferous crop plants, three foliage crops (cabbage, cauliflower, Brussels sprouts) and three "root" crops (Kohl rabi, Mooli, swede), sown into 308 Hassy trays on 2 May 2001, were transplanted into the field on 14 June. The plants were grown across a field as one large contiguous block, so that the two crops (cabbage, Brussels sprouts) at each end were planted as 6 x 5 plots (Fig. 7) and the other four crops as 6 x 4 plots. The inner 16 plots, each containing 25 (5 x 5) plants, of each plant type were used as the treatment plots, so that a 4 x 4 Latin square could be used as the experimental design. The outer blocks of plants around all four edges of the complete plot were used as the "guard" plants. This prevents the higher numbers of insects found on the extreme edges of most crop plantings, the so-called "boundary effect", from adding to the variance in the experimental plots.

Throughout the season, the numbers of fly eggs laid each week on the six crop types was monitored by sampling the soil from around ten guard plants (E- Fig. 7), five on each side of each crop. The four experimental treatments involved the release of 0, 8 (1st release date), 16 (1st & 2nd release dates) and 24 (all 3 release dates) beetles on the central plot in each 25-plant test plot. For clarity, the randomisation of the four replicates of the four treatments is shown in Figure 7 only for the cabbage crop. The beetles were released on 19, 25 and 31 July.

Fig. 7. Layout of the 2001 field experiment to show the 4 x 4 Latin square design (numbers shown for the cabbage plot only), the row of plants sampled (xxxxx - shown only in the swede plot) for pupae and the positions (E) where the fly eggs were monitored in each of the six crop types.



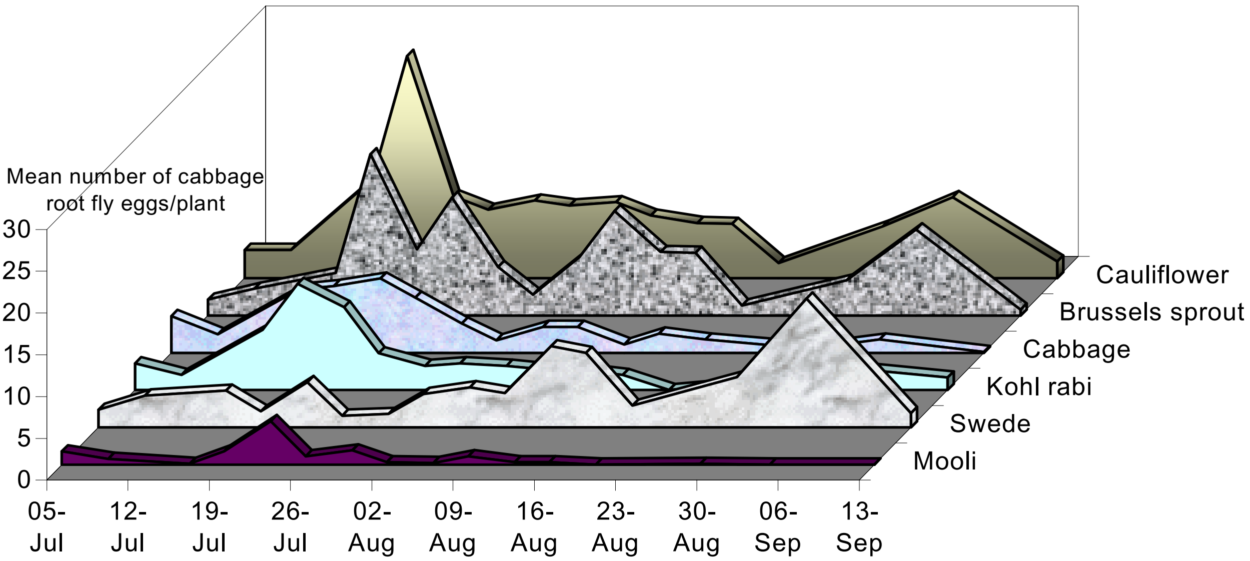
At the end of the experiment (20 September), the aerial parts were weighed from 5 plants (xxxxx - Fig. 7) in each plot and 15cm wide x 15cm deep soil samples, which included the plant root, were taken from the same plants and stored in numbered polythene bags in a 4^{o}C cold store. The roots of the plants were weighed at the time the fly pupae were extracted from the soil samples. The five plants (1-5) sampled were taken from the middle row of each plot and represented the plant (3) on which the *Aleochara* were released, the two adjacent plants (2 & 4), and the two plants (1 & 5) one row further away.

The fly pupae were extracted from the soil samples by flotation, counted, placed into tubes numbered 1-480 and then stored in a domestic refrigerator. This was done to allow the insects to complete their diapause (overwintering) development, which for the cabbage root fly and its parasitoids requires 100 days of cold treatment. Once this had been done, the tubes of pupae were brought back to normal laboratory temperatures and the numbers of flies and parasitoids that emerged were recorded.

Results & Discussion

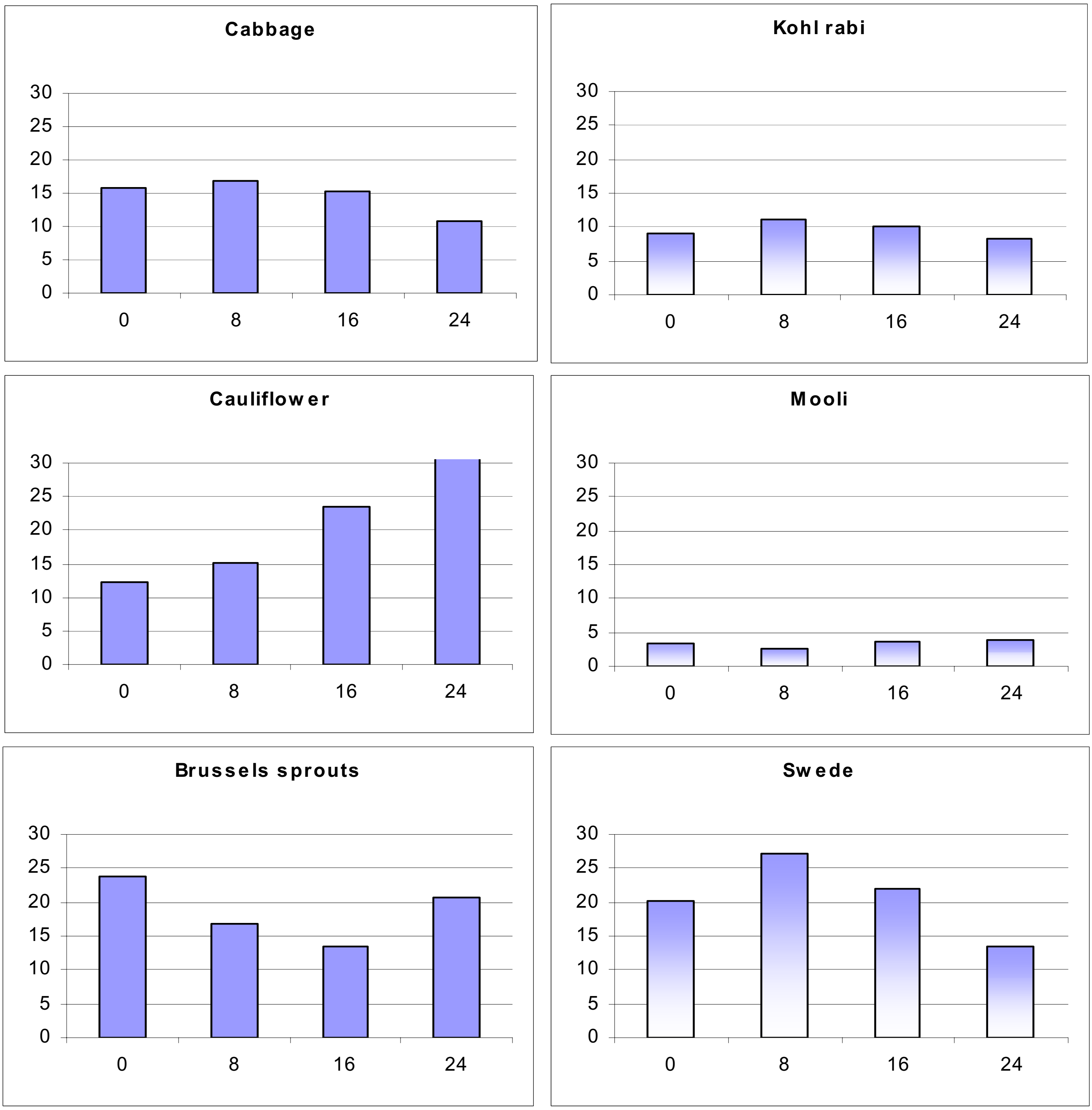
The numbers of fly eggs recovered from each crop type varied considerably (Fig. 8) and ranged from 130/plant for cauliflower to 15/plant for Mooli. This again highlights the over-riding influence of plant type, as Mooli was the most preferred plant in 2000 and the least preferred (Fig. 8) in 2001. The results show also that egg-laying is no longer confined to discrete generations and that following the peak of egg-laying by the second generation of flies on 26 July, the flies continued to lay high numbers of eggs on the cauliflower and Brussels sprouts plants throughout most of August.

Fig. 8. The mean numbers (eggs/plant) of cabbage root fly eggs collected each week from around the six plant types used in the 2001 field experiment.



The direct effects of the released predatory beetles, in eating cabbage root fly larvae and hence reducing the eventual numbers of fly pupae, were far from obvious. The overall aim of this experiment was to record how beetles released onto the central plant would disperse to the neighbouring plants. It was hoped that fewest pupae would be recovered from the central plants, on which the beetles were released, and that pupal numbers would increase the further the sampled plant was from the release point. In addition, differences were also expected between the three treatments in which 8, 16 and 24 beetles were released onto individual plants. However, irrespective of how the data were analyzed, the numbers of pupae recovered/plant were similar for all plants within a given crop, even on the plants on which 8 *Aleochara* beetles had been released on three separate occasions (the 24 treatment). There seemed little point, therefore, in taking more samples to try to make the data more robust, as the 480 samples (which can be processed at about 8 samples/hour) that were taken represented a relatively high proportion (20%) of the total plants available. Therefore, the data have been presented simply as the means for the 20 plants sampled (5/plot x 4 replicates) from each of the six plant types (Fig. 9).

Fig. 9. The mean numbers of cabbage root fly pupae collected from the soil around the six plant types used in the 2001 field experiment.



2001 Foliar crops Root crops

The numbers of pupae recovered varied from crop to crop and appeared to reflect simply the numbers of eggs laid on four of the crops. In this experiment, the insects appeared to survive in relatively high numbers on swede and cabbage (Fig. 10). However, there was no indication that pupal numbers were reduced to a greater extent on those crop plants, such as Mooli, on which relatively few fly eggs were laid.

Fig. 10. The relative numbers of cabbage root fly eggs and pupae recovered from around the six plant types during the 2001 field experiment.

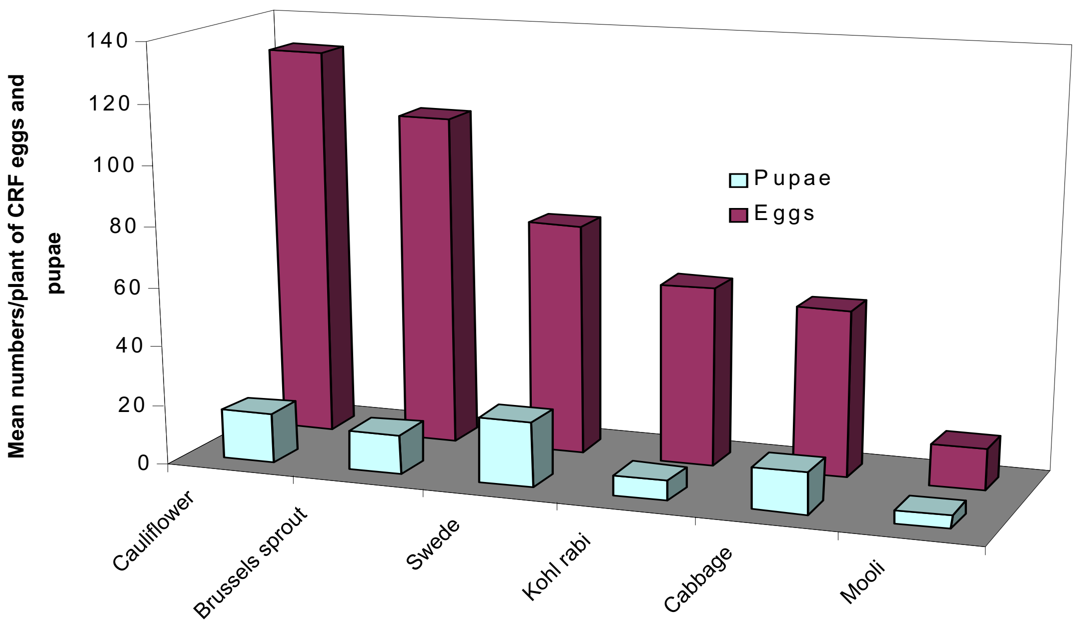
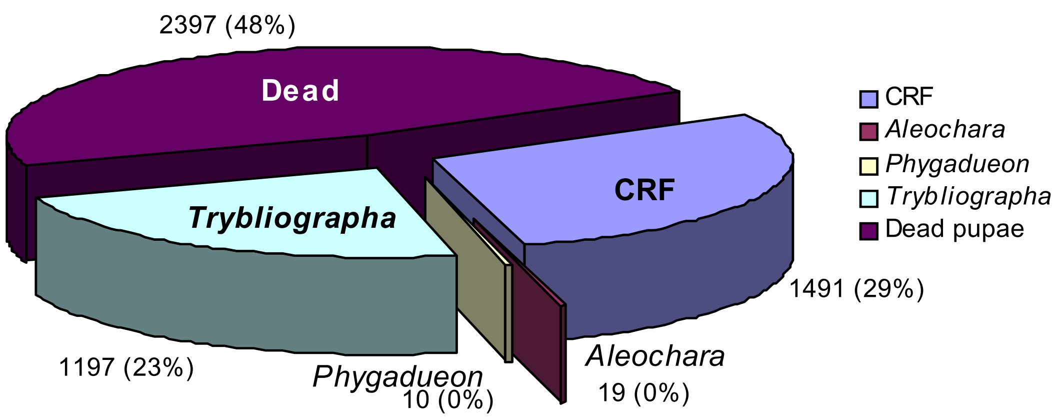


Fig. 11. The numbers and types of insects that emerged from the cabbage root fly pupae extracted from the 480 soil samples taken from the six plant types used in the 2001 field experiment.



The proportions of the various insects that emerged from the fly pupae, following the cold treatment (Fig. 11), were unexpected. As can be seen (Fig. 11), the numbers of the parasitoid wasp (23%) were similar to those of the fly (29%). The unexpected results were that the numbers of *Aleochara* and the pupal parasitoid *Phygadueon trichops* were so low that they were effectively zero. The most unexpected result was that no insects emerged from nearly half (48%) of the pupae collected. It is more usual for the numbers of dead pupae to account for somewhere between 5-15% of the total. The current value is a clear indication of multiparasitism. This occurs when parasitoid numbers are high and as a result more than one parasitoid larva hatches within (the wasp *Trybliographa*) or chews its way into (the beetle *Aleochara*) a fly pupa. When this occurs, neither parasitoid, whether of the same or the different species, usually survives. Obviously, when the parasitoids are searching through the soil to find cabbage root fly larvae/pupae, the most frequent encounters will be with the fly larvae/pupae that are easiest to find. Hence, these are the ones that are usually parasitized more than once. The extremely low numbers of *Aleochara* that emerged from the samples, even in crops in which *Aleochara* was released, indicates that *Aleochara* does not compete well with *Trybliographa.* Hence, the idea that releasing *Aleochara* would help also to build-up the numbers of this beetle, and so help to increase the overall levels of parasitism in field *Brassica* crops, now seems untenable.

Conclusions

The results obtained during the last year of the project were disappointing. It was difficult to draw any firm conclusions from the data collected, as there were no overall trend that could be applied to the complete set of data. By selecting certain data sets, it was possible to show small trends in a particular direction. However, by selecting other data sets, it was quite easy to show a "trend" in the opposite direction. The last time data as variable as these were collected by the author was in 1983, when field trials were done to try to show whether predatory insects destroyed more fly larvae when fly numbers were high. As in the current experiments, the 1983 data could not be interpreted in any meaningful way. The problem with this type of field experiment appears to be that there are too many variables that cannot be controlled directly by the researcher.

Technology Transfer

I had discussions on a regular basis with Drs Phil Walker and John Dale of Biological Crop protection Ltd (UK) about the progress of the research and the difficulties they were likely to encounter if they attempted to mass-rear this predatory beetle. We also kept a close joint interest in the work being done by De Groene Vliege of The Netherlands, as this company is still trying to find a cost-effective way of mass-rearing this beetle.

At the 53rd International Symposium on Crop Protection in Belgium, I was approached by an employee of Koppert Biological Control Systems concerning an update on our progress and to obtain my opinions on another predatory staphylinid beetle, a species of *Atheta*, which Koppert hope might prove useful in field crops. At the time (May 2000) I was still relatively optimistic about showing that biological control using living arthropods could be effective under field conditions. However, following the 2001 field trial, I am much less convinced. I would also be wary of testing a genus like *Atheta*, as such beetles are much smaller than *Aleochara* and so would need to be released in even higher numbers to have any possible impact as predators.

Papers and posters [All included some aspects of the *Aleochara* work]

Finch, S., Elliott, M.S. & Torrance, M.T. (1999). Is the parasitoid staphylinid beetle *Aleochara bilineata* an effective predator of the egg stage of its natural host, the cabbage root fly? *IOBC/WPRS Bulletin* **22(5):** 109-112.

Hartfield C.M., Nethercleft M. & Finch, S. (1999). The effect of undersowing brassica crops with clover on host finding by *Trybliographa rapae* and *Aleochara bilineata*, two parasitoids of the cabbage root fly, *Delia radicum*. *IOBC/WPRS Bulletin* **22(5):** 117-124.

Finch, S. & Collier, R.H. (2000). Integrated pest management in field vegetable crops in northern Europe - with focus on two key pests. *Crop Protection*, 19, 817-824.

Finch, S. & Collier, R.H. (2000). Host-plant selection by insects – a theory based on “appropriate/inappropriate landings” by pest insects of cruciferous plants. *Entomologia Experimentalis et Applicata,* 96, 91-102.

Hartfield C.M. & Finch, S. (2002).Releasing the rove beetle *Aleochara bilineata* in the field as a biological agent for controlling the immature stages of the cabbage root fly, *Delia radicum. IOBC/WPRS Bulletin* (in press).

Presentations [All included some aspects of the *Aleochara* work]

Finch, S. Invited speaker for the 51st International Symposium on Crop Protection. Spoke on “The mechanism by which undersowing reduces pest insect damage in brassica crops”. Gent, Belgium, 4 May 1999.

Finch, S. Invited speaker to the XIVTH International Plant Protection Congress. Spoke on “IPM in field vegetable crops”. Jerusalem, Israel, 25-30 July 1999.

Finch, S. Invited Guest speaker at Summer Vegetable Walk. Spoke on “Everything you wanted to know about Cabbage Root Fly and Flea Beetles but were too scared to ask”. HRI Kirton, 3 August 1999.

Finch, S. Spoke on “Insecticidal and non-insecticidal methods for controlling the cabbage root fly”. Sou’westers Club, Hayle, Cornwall 22 November 1999.

Finch, S. Spoke on “Current research on the cabbage root fly” at a Technical forum on swede, turnip and kohlrabi. NFU Building, Stoneleigh, Warwickshire 6 December 1999.

Hartfield C.M. & Finch, S. Releasing the rove beetle *Aleochara bilineata* in the field as a possible biological agent for controlling the cabbage root fly, *Delia radicum.* IOBC/WPRS –Working Group Meeting “Integrated Control in Field Vegetable Crops”, Godollo, Hungary. 1-3 November 1999.

Finch, S. Integrated pest management in field vegetable crops – the possible impact of using a parasitoid rove beetle to control the cabbage root fly in brassica crops. Seminar presented to students on MSc Course in Integrated Crop Management. Joint University of Bristol/Bath. Long Ashton 20 March 2000.

Finch, S. Invited Chairman for the 52nd International Symposium on Crop Protection. Spoke and chaired session on “Integrated Pest Management of Insects". Gent, Belgium, 9 May 2000.

Finch, S. Spoke on " How intercropping and undersowing with clover affect host finding by pest and beneficial insects." XXI International Congress of Entomology. Symposium 6. Insect Management with Physical Methods. Iguassu Falls, Brazil, 24 August, 2000.

Collier, R.H. Spoke on " Novel methods of pest management". British Association Festival of Science. Imperial College, London, 8 September 2000.

Finch, S. Spoke on "The effect of undersowing with clover on populations of pest and beneficial insects". Welsh Pest Management Forum meeting on "*Pest & Weed Control for the Organic Grower*". Cardiff, 11 October, 2000.

Finch, S. Spoke on "Controlling the cabbage root fly with predatory beetles. HRIA/HDC Discussion Forum, HRI-Kirton, 11 January 2001.

Finch, S. Integrated pest management in field vegetable crops – the possible impact of using a parasitoid rove beetle to control the cabbage root fly in Brassica crops. Seminar presented to students on MSc Course in Integrated Crop Management. Joint University of Bristol/Bath. Wellesbourne, 20 February 2001.

Finch, S. The problems associated with trying to control the cabbage root fly by releasing the predatory rove beetle *Aleochara bilineata.* Institute for Horticultural Development, Knoxfield, Australia. 4 December 2001.

Finch, S. Releasing *Aleochara bilineata* as a biological control agent. Seminar presented to MSc Students in Integrated Crop Management. Joint University of Bristol/Bath. Wellesbourne, 19 February 2002.

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