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Managing biodiversity in field margins to enhance integrated pest control in arable crops ('3-D Farming' Project)

by

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ABSTRACT

There is considerable potential to manage field margins to increase pest control by natural control agents and, in addition, to enhance biodiversity. This project aimed to develop management strategies for enhancing biological control of aphid pests in field crops, allowing farmers to fulfil their environmental commitments without jeopardising profitable crop production.

Strategies for the manipulation of aphid parasitoids, using aphid pheromones, and of hoverflies, by establishing wild flowers in field margins, were developed and tested on commercial cereal fields at four sites, with pilot trials in several vegetable crops in the final year. Data from cereal trials clearly demonstrated the importance of early parasitoid activity for summer aphid control. Use of an aphid pheromone stimulated rapid spread of parasitoids into cereal crops in spring to coincide with aphid invasion, significantly reducing aphid numbers. Flower-rich margins also significantly reduced cereal aphid numbers in many site/years, providing essential food for female aphidophagous hoverflies, especially *Episyrphus balteatus*, which then layed their eggs in the crop near aphid colonies. Hoverflies played an important role in maintaining control of pest aphid numbers, the effect being greatest after the impact of parasitoids (an early season control agent) began to wane in mid-summer. Thus, the effects of parasitoids and hoverflies were comlementary and together significantly reduced aphid population growth rates. Pitfall trap catches of the carabid beetle *Harpalus rufipes* appeared to be increased by the aphid pheromone in some site/years. There was no apparent effect of the pheromone on parasitoid activity or aphid populations in any of the vegetable crops investigated, although parasitoid numbers were very low in some of these trials. Further trials using pheromones more closely matched to those produced by the main vegetable aphid species are recommended. Flower-rich margins appeared to increase parasitoid impact on aphids on organic broccoli.

The foraging and oviposition behaviour of the hoverfly *Episyrphus balteatus* was also studied in the laboratory. The attractiveness of flowering plants to hoverflies was positively associated with the number of eggs that females subsequently produced. A range of UK native plant species were found to be equally or more attractive to hoverflies when compared to the non-native *Phacelia tanacetifolia* that is widely quoted in the literature as promoting hoverfly populations near arable crops. In particular, a range of umbellifer species, yarrow and white campion were highly attractive to *E. balteatus*. Provision of these species in managed field margins would provide a plentiful supply of high quality pollen and nectar at the critical point in hoverfly life cycles. *E. balteatus* females were attracted to aphid-infested wheat plants for oviposition, their searching behaviour resulting in a preference for larger plants, similar to those on which damaging aphid populations periodically occur in the summer.

Large-scale, spatio-temporal dynamics and movement of beneficial insects was investigated, including the influence of some biotic factors. Beneficial invertebrates were sampled using pitfall traps, in conjunction

with measurements of plant cover and soil moisture, to investigate within-year and between-year changes in spatial distribution. The spatial distribution of most ground-dwelling predators was significantly clustered into patches and for some species these extended across field boundaries. For most species the location of patches and gaps remained consistent within the same year but was less consistent between years. Numbers of predatory invertebrates peaked in early July and then started to decline, but in July were more abundant in peas than in cereal crops. Many species of ground-dwelling predators were positively associated with weed cover but there was an optimum level of weed cover beyond which predator numbers declined. Soil moisture strongly influenced the survival of beetle larvae overwintering within fields and an optimum level was found. Measurements of beetle emergence highlighted the importance of arable soils as an overwintering site. Within one field the average density was 157 predatory beetles m^{-2}.

Large scale mark-release-recapture experiments with several carabid beetles showed that although they could move between fields the majority remained within the field where they emerged. Field margins/boundaries containing tussocky grasses encouraged predatory beetle species that overwinter as adults, and their early spread into the crop complemented the initial impact of parasitoids on colonising aphid populations. Set-aside margin strips, although not sown with a plant mixture designed to encourage beneficial invertebrates, reduced the abundance of cereal aphids in one of two years. They had almost no effect on the invertebrates within the crop, but for some groups their numbers varied with distance from the field edge. There is potential to develop plant mixes for set-aside that will improve biocontrol. A margin cost calculator was developed that will allow farmers to calculate the cost of establishing different types of margins on their farms based upon income foregone and agri-environment payments. The distribution of pea aphids was highly ephemeral but predatory beetles contributed to their control.

A molecular PCR test was developed to detect aphid remains in the guts of polyphagous predators. Aphid-specific bands were still detectable in spiders 8 hours after they had fed on an aphid. Analysis of field-collected spiders revealed that they fed on aphids with equal efficiency up to 100m into the crop. Around 15-25% of money spiders collected in cereal crops had fed on aphids, whilst as much as 88% of those collected from a pea crop had fed on pea aphids. 21% of large carabid beetles (*Pterostichus* spp.) collected in cereal fields had consumed aphids; 23% collected from fields with a set-aside strip and 18% from fields without a set aside strip. The proportion of beetles that had consumed aphids was not significantly affected by distance from the margin, at least up to 100m, regardless of the presence of a set-aside strip.

SUMMARY

Agriculture is undergoing important changes as a result of CAP reform and continuing pressure to improve its environmental profile. Restrictions on pesticide use and the withdrawal of increasing numbers of compounds from the crop protection armoury mean it is essential to develop new, sustainable approaches to pest control. Research is required to further promote the development of such methods and to improve our understanding of, and ability to manage, farmland ecosystems to ensure agriculture retains profitability whilst addressing environmental concerns.

Non-crop habitats constitute one of the most important sources of biodiversity within farmland but their beneficial influence on adjacent crops has not been properly taken into account. In many arable areas, field margins are the only major non-crop habitat, acting as the main source of beneficial species, and it has been recognised for some time that field margins can play an important role in the development of novel manipulation techniques to enhance insect predators and parasitoids. Hoverflies, many of which are important aphid predators, can be increased by encouraging wild flowers in field margins, whilst aphid sex pheromones can be used to increase parasitization rates in the field by encouraging movement of parasitoids between margins and the crop at critical times. It is essential to develop these approaches in a unified way and test them on a commercial field scale. The diversification of field margins through agri-environment schemes, primarily designed to increase farmland biodiversity, offers an ideal opportunity to do this. Field margins are also important habitats for other major predator groups, such as carabid beetles and spiders, and the diversification of margin habitats on farms will also affect these groups. Insect interactions between field margin habitats and the crop and the overall density, diversity and distribution of both pests and beneficials are influenced not only by margin management but also by the crop husbandry practices employed in the field. Recent developments in the statistical analysis of intensive spatial data allow these interactions to be investigated more closely.

The overall aim of the project was to use field margin management techniques to increase the abundance and diversity of beneficial insects and spiders and manipulate their distribution and dispersal on farmland for the control of aphid pests.

Specific objectives were:

1. To provide farmers with advice on field margin management to optimise integrated pest management whilst maintaining biodiversity benefits and profitability.
2. To test and further develop a novel aphid control strategy involving the manipulation of parasitoids using aphid sex pheromones in field margins.
3. To develop and evaluate the use of specific native flowering plants in field margins to enhance the abundance and diversity of aphid-eating hoverflies in adjacent crops.

4. To measure the effects of margin and crop management on aphid and beneficial insect abundance, dispersal and spatial distribution in both the margin and adjacent crops.
5. To measure the spatial and temporal distribution of cereal aphids and the extent to which these are controlled by predatory and parasitic species.
6. To measure the impact of recently introduced field margin management options on the biodiversity of aphids and their natural enemies.

MANIPULATION OF APHID PARASITOID AND HOVERFLY ABUNDANCE AND DISTRIBUTION

Methods

In 2000, 2001 and 2002, field trials were done on cereal crops at four sites in England and southern Scotland. In 2003, a further cereal trial was done, whilst trials were also done on vining peas, organic broccoli and organic lettuce. For all the cereal trials, three fields were selected each year at each site:

1. A field with a tussocky grass margin, along which pheromone lures were deployed in autumn, followed by pheromone deployment in the adjacent crop in spring to manipulate aphid parasitoids.
2. A field with a flower-rich field margin to encourage hoverflies.
3. A field with neither pheromones nor a flower-rich margin to act as a control.

In 2003, treatments had to be modified to accommodate the available conditions. At the organic lettuce site and one of the pea sites, a single large field bordered by a flower-rich margin was used. The pheromones were deployed at one end of the field and the opposite end was used as an untreated control area. At the other pea site, a single very large field was used, with each treatment on a different side, one of which had a flower-rich margin. At the organic broccoli site, opposite sides of a large field were used for the pheromone and control treatments, whilst the flower margin treatment was in a second field.

Insects were sampled weekly along four 100m transects, one in the margin and three in the crop at 10m, 30m and 100m. Aphids were counted *in situ*, whilst adult parasitoids, adult hoverflies and carabid beetles were sampled using suction net samplers (Vortis/D-vac), water traps and pitfall traps, respectively. The aphid sex pheromone, (4*aS*,7*S*,7a*R*)-nepetalactone, formulated into 4cm strips of PVC polymer, was deployed in the margin in autumn and in the crop in spring. The timing of deployment of the pheromone in the crop was determined by the timing of aphid immigration in the spring.

Key Results

Cereal aphid population development patterns varied from year to year. In 2000 and 2002, aphid populations remained at low levels throughout the summer and showed no signs of exponential growth. In contrast, in 2001 typical exponential growth began in mid-June followed by a population crash in early July. In 2000 and 2002, there was a significant parasitoid presence in the crop during the early stages of aphid colonisation, whereas in 2001 parasitoids were virtually absent at this time, providing strong evidence that **early parasitoid activity hinders aphid population development sufficiently to prevent exponential growth**.

Two factors prevented a damaging aphid outbreak in 2001; firstly the cold, wet, weather conditions at the beginning of the season caused significant aphid mortality and hindered delayed exponential population growth and, secondly, large numbers of hoverflies bred on the aphids in the crop during the summer, curtailing the outbreak. **This emphasises the importance of maintaining a diverse natural enemy community in agricultural ecosystems, as this provides stability for natural biocontrol in the face of environmental variability, particularly variability in climatic conditions.**

Five parasitoid species were recorded attacking cereal aphids, but ***Aphidius rhopalosiphi* was always the most abundant early in the season and so can be regarded as the most important species for cereal aphid control. Habitats that include a high proportion of grasses, such as pasture and grass-rich field margins, are valuable reservoirs of cereal aphid parasitoids**. Early in the season, parasitoid sex ratios within the crop were consistently female-biased, whilst during the aphid population crash at the end of the season they were male-biased. **Because males are much more sedentary than females, this suggests that a significant proportion of the population of parasitoids foraging within the crop early in the season had immigrated from surrounding semi-natural habitats, which had acted as overwintering sites, and that females rapidly leave the crop when aphid populations decline.**

No effects of the pheromone were evident in 2001 due to the virtual absence of parasitoid activity in early summer, as a result of the cool, wet, weather conditions prevailing at that time. However, conditions in 2002 were much more conducive to both aphid and parasitoid activity, allowing good data on the effects of the pheromone to be obtained. **At the Yorkshire and Scottish sites, where aphid numbers were greatest, twice as many were counted in the control fields than in the pheromone-treated fields. The pheromone did not appear to cause an increase in the number of parasitoids present, but it stimulated rapid spread of parasitoids through the crop at the critical time when aphids were beginning to invade.**

Pitfall trap catches of the carabid beetle *Harpalus rufipes* appeared to be increased by the aphid pheromone in some site-years. The reasons for this are unknown. Analysis of data from the 2003 cereal trial revealed a significantly greater proportion of males in the pheromone-treated field than in the other two fields, suggesting that males were responding more than females. However, until a behavioural response has been definitely confirmed, the field results, even though they are statistically significant, should be treated with caution, as there still remains a possibility that these results are simply due to chance.

Very large numbers of adult hoverflies were caught during 2001 and this was partly due to an abundance of the marmalade hoverfly, *Episyrphus balteatus*. This species is known to be migratory and the UK population in 2001 may have been boosted by migratory individuals from continental Europe. A sudden increase in catches of adult hoverflies within cereal crops in mid summer in most site-years was almost certainly due to the emergence of the second generation, which had developed as larvae feeding on the abundant aphids in the crop that year. **There was a highly significant trend of increasing numbers caught with distance into the crop, suggesting that these highly mobile insects disperse from the margins, where they feed on nectar and pollen, and distribute their eggs throughout the crop.**

The most common hoverflies trapped at all sites were the two species normally associated with arable land, *E. balteatus* and *Metasyrphus corollae*. As *E. balteatus* is a migratory species, arriving into cereal crops in June and July, natural predation from hoverflies in May and early June must rely on other species. **The provision of early flowering plants in the margin to enhance the potential of other species, such as *M. corollae,* will improve the temporal spread of the natural control of aphids by hoverflies. In addition, they will provide high quality/abundant nectar and pollen sources that will enable the females of all species of interest, including *E. balteatus*, to increase their egg load and therefore the number of aphidophagous larvae in adjacent crops.** Other aphidophagous species are also important natural predators and so a range of flower types should be encouraged in field margins to ensure that there is a suitable selection of flower types for hoverflies with different flower preferences. **There was strong evidence that the presence of a flower-rich margin along at least one side of the field can have a significant impact on aphid numbers in cereal crops.** There were significantly fewer aphids present on the crop in fields with such margins than in control fields for seven out of twelve site-years.

The trials in the final year of the project were designed to highlight problems specifically associated with high value vegetable crops and identify areas that would need to be addressed in further work in order to adapt the approach developed for cereal aphid control. Field vegetable crops present a far greater challenge for biological control of aphids than do cereals, principally because of the very low tolerance levels for aphid contamination and crop damage. **Data from the pea trials did not reveal any obvious effects of the aphid sex pheromone, nepetalactone, on pea aphid populations.** There was also no evidence that the pheromone significantly affected aphid parasitoid numbers or spatial distribution at either site. **The most striking result from the broccoli trial was the large numbers of aphid parasitoids in the crop alongside the flower-rich margin.** Before the grower treated the crop with soap solution, the density of aphids on the crop near the flower margin was almost half that in the control plot and it is possible that the high parasitoid activity would have prevented significant aphid damage if the soap treatment had not been applied. However, very few parasitoids were present in the field containing the pheromone-treated and control plots and so it was not possible to assess the potential of the pheromone for manipulating the main brassica aphid parasitoid *Diaeretiella rapae*. The organic lettuce trials were done in August 2003 when the weather was very hot and dry. Consequently, very few aphids and natural enemies were present in the crop and it was not possible to assess treatment effects.

IDENTIFICATION OF THE FACTORS INFLUENCING APHID AND BENEFICIAL INSECT ABUNDANCE, DISPERSAL AND SPATIAL DISTRIBUTION ACROSS FIELDS

Methods

The study area for this part of the project covered 66 ha in Dorset, comprising six arable fields separated by mature hedgerows or grassy banks, and included both winter cereal and vining pea crops. Ground-dwelling invertebrates were sampled across the study site using paired pitfall traps placed at 973 sampling points arranged in a grid pattern. The proportion of bare ground and that covered by weeds and the crop was measured each year around each sampling position. Two hundred emergence boxes were also established along alternate rows of sampling points in two of the fields to measure the spatial pattern of insect emergence from the soil. The spatial patterns of distribution and their association with biotic and abiotic factors, particularly vegetation cover and soil moisture, were determined using SADIE analytical techniques.

During the first two years of the project, mark-release-recapture experiments were conducted at the farm scale to determine to what extent hedgerows and crop rotations influenced the distribution and movement of *Pterostichus* species carabid beetles.

Key Results

The spatial scale and extent of the trapping grid used in this study made it possible for the first time to answer some key questions regarding the spatio-temporal dynamics of predatory invertebrates living on the soil surface and thereby to provide advice on how best to encourage the natural biocontrol provided by these generalist predators.

Early in the season (May and June) the predatory fauna was more diverse, being largely composed of those species that had overwintered in the margins as adults. In July, those species that had overwintered as larvae within the field itself (especially *Pterostichus* spp.) started to emerge as adults and these then dominated the species composition, while also being very numerous. The extent of spread through fields by margin-overwintering species varied from year to year and appeared to be influenced by aphid densities in the crop, although other factors may have been involved. The mid-field overwintering species, as expected, occurred across fields. For some species, patches of high density extended across several fields; while for others they were more restricted and were found only in certain fields or parts thereof. Thus it would appear that the spatial extent of a species' local population patch is species specific. **To ensure maximum biodiversity, broad-scale management treatments (eg. crop type and insecticide applications) across groups of contiguous fields should be avoided where possible.** Reinvasion from untreated fields is also likely to be faster if these are in close proximity to the treated ones.

Most species and predatory groups had a consistent spatial distribution pattern within each year. The total predatory effort, as indicated by numbers trapped, was stable within years but not between years, although there were exceptions. For example, the carabid beetle *P. melanarius* remained in the same location over the three years, and some other species and groups persisted in broadly the same place for two years. All of the species studied showed heterogeneous distribution patterns across the study area indicating that certain areas provided more attractive conditions. Consequently **the level of biocontrol within each field may be expected to vary between years**. Understanding why these changes occur is critical if we are to better manipulate generalist predators for biocontrol. When considering the potential for biological control it is the total number of predatory invertebrates that is important but this also varied spatially, with some fields having relatively even coverage across the whole field, while others revealed much more heterogeneous distribution patterns.

The distribution of invertebrates within farmland will be governed by historical and current management, along with abiotic and biotic factors that will be influenced to some extent by the management. In this study we examined whether the crop, weed cover or soil moisture influenced the predatory invertebrate distribution patterns. **Stronger associations were found between the distribution of broad-lead weeds and predatory invertebrates than total vegetation cover that included crop cover.** The optimal weed cover was between 10 and 14% when the total number of predators was considered, however, this could vary according to the species composition. Further studies in which weed cover was manipulated confirmed that the numbers of predatory invertebrates could be increased by reducing herbicide inputs. **The soil moisture levels in summer were less important to the distribution of active adults than those in the winter which strongly affected overwinter survival.**

The type of crop will influence many factors that are important to beneficial invertebrates and so particular crops will favour particular species according to their phenology, environmental requirements and diet. High numbers of predators were captured using pitfall traps in the pea fields in 2000 and 2001 and this crop may have favoured the survival of some species, especially the carabid beetle *P. madidus*, which was the numerically dominant species. The pitfall traps only provide a snapshot measurement of the invertebrate community whereas the emergence traps provided season-long activity. When they were used in pea and wheat crops, the emergence of Carabidae (including *Pterostichus* species) and Staphylinidae was higher from winter wheat compared to the spring-sown peas. The difference in the timing of the soil cultivations could have affected beetle survival. The species found here were autumn breeding species that have large larvae, and these were considered to be more susceptible to spring than autumn cultivations.

Our emergence trap data from 2002 show the accumulated population density of emerging beetles of all species to be at least 1 m^{-2}, while some species, e.g. *P. melanarius* and *P. madidus*, emerged at densities of nearly 30-40 m^{-2}. **Overall, carabids together with staphylinids emerged at population densities of 86 m^{-2}**

in one of the larger fields and almost double that density at a massive 157 m^{-2} in one of the smaller fields. These results highlight the important productivity of arable soils for these invertebrates. The **great biomass of these invertebrates will not only contribute to pest population suppression but also represents a major food resource for farmland birds and small mammals and, in some cases, each other.** There appeared to be a particular range of moisture conditions that was optimal for overwinter survival of several carabid and one staphylinid beetle species. There exists the possibility that certain soil types could best provide these optimal conditions, which could lead to management advice on the preservation of predatory invertebrates in such areas. **Strong spatial and numerical correlations were found between pitfall trap data and emergence trap data, justifying the use of pitfall traps and revealing that they were providing a measure of density.**

For cereal aphids, natural enemy impact early in the infestation period is considered important if an outbreak is to be prevented, and the evidence collected in this study indicates that the boundary overwintering species of predators are more likely to contribute to aphid control at this time. **We would therefore recommend that management practices to improve, increase and protect field boundaries/margins and allow the tussock forming grasses that provide the most suitable overwintering habitat for beetle survival should be encouraged.**

The extensive spatial scale at which this study was conducted, involving nearly 2000 traps in a grid covering nearly 70 ha, has allowed, for the first time, the spatial dynamics of carabid populations to be studied in detail at a scale approaching that of the whole farm. This is the spatial scale at which various agri-environment schemes are implemented, in which both crop and non-crop features are considered. It is also the relevant scale at which to study processes in spatially dynamic insect populations. Pitfall trap results suggested that the carabid beetle *P. madidus* is a more mobile species than its close relative *P. melanarius*. However, snapshot views of population distributions do not reveal whether aggregations appearing and disappearing in different fields are a result of mass movement of individuals between fields or of differences in the timing of emergence of populations in different fields.

Mark-release-recapture experiments enabled some questions concerning movement of individuals within populations to be addressed. Results confirmed that beetle species differed in their mobility, with that of *Pterostichus madidus* being twice that of *P. melanarius* despite their similar size. In the areas where *P. melanarius* were most abundant, emigration was least and vice versa. This suggests that **populations actively aggregate in high density patches where conditions are most favourable for them, either in terms of food availability, microclimate, or soil conditions for oviposition.** Field boundaries certainly function as barriers, retaining the majority of individuals within a field. However, they are not impenetrable and a certain amount of population exchange between fields does occur for these species.

In 2002, grain aphids were higher at 10 and 30 m from the set-aside strips compared to the crop edge, suggesting that set-aside strips were encouraging biological control. There was some evidence that predatory invertebrates were encouraged by the set-aside strips, possibly through a diversification of food resources and winter cover. However in 2003, the set-aside strips had the reverse effect with higher numbers of aphids occurring in transects adjacent to them. There were some changes in the vegetation within the strips between 2002 and 2003 that may have accounted for this. In 2002, the floral diversity was greater, and the vegetation was overall much shorter compared to 2003. In 2003 half of some strips had been resown with the orginal mixture, but because of the dry weather establishment and growth was poor. **The contrasting results for 2002 and 2003 indicate that there is potential for set-aside strips to increase levels of biological control within the adjacent crop, but the composition of plants needs to be carefully chosen if the habitat is to not act as a sink or to have no effect.**

In the pea fields the set-aside strips had no effect on the abundance of pea aphids. **The distribution of pea aphids was highly aggregated but also extremely ephemeral with patches appearing and disappearing between the four day sampling intervals.** Consequently, if crop scouting is to be accurate a large proportion of the field needs to be walked if the extent of an infestation is to be measured. Although overall pea aphid densities were high, **ground-active predators exerted a noticeable level of control with fewer pea aphids occurring where they were present.** Adequate pea aphid control was achieved through the use of a full rate of the selective aphicide `pirimicarb' instead of a full rate of a broad-spectrum pyrethroid, which should be less damaging to the beneficial invertebrates. Augmentation of non-crop habitats, through the establishment of beetle banks and wildflower strips would increase numbers of both ground- and crop-active predators and parasitoids within pea crops.

In 2000 and to a lesser extent in 2002, the type of field margin influenced the ground-active invertebrate community in the adjacent crop, with the presence of grasses encouraging beetle species that had used the margin as an overwintering habitat. Herbaceous forbs were associated with increased numbers of ladybirds, probably because the most abundant forb within the margins was stinging nettle, which supports large numbers of aphids that provide food for ladybird adults and larvae.

The results from this study have greatly improved our knowledge of invertebrate distribution and have provided insights into the spatial dynamic processes that occur across farmland. We have demonstrated that seasonal movement occurs from non-crop margin habitats but the extent of this can vary between fields and years. The reluctance of the boundary overwintering species of ground-dwelling predators to disperse across fields has implications for the extent and reliability of their contribution to pest control within fields, but there are ways in which their early dispersal could be encouraged and densities increased. Crops could be manipulated to provide more favourable environmental conditions for surface active species, and weed cover was identified as one key factor. Alternative prey can be increased through the application of organic manures, whilst field margin quality may be improved and the margin:field ratio increased. Annual seed mixtures for use in set-aside strips need to be examined as these could be rotated around the farm according to the cropping, so concentrating the biocontrol effort where it is most needed. There may also be potential benefits from mixing permanent and temporary habitats.

ASSESSMENT OF APHID PREDATION BY LINYPHIID SPIDERS AND CARABID BEETLES USING PCR TECHNIQUES

Methods

DNA was extracted from aphids, money spiders (Linyphiidae) and carabid beetles using commercially-available kits. Primers were designed to the aphid mitochondrial COII gene, and a primer pair was chosen that amplified a number of common UK species but did not amplifly DNA from predators, other insects or microbial contaminants found on predator surfaces. Cereal aphids (*Sitobion avenae, Metopolophium dirhodum* and *Rhopalosiphum padi*), as well as the peach-potato aphid *Myzus persicae*, were fed to spiders (*Lepthyphantes tenuis*), which were then sampled at various times after feeding (up to 8h) and subjected to PCR testing to determine if aphid DNA could be detected in the gut, and for how long after ingestion.

Spiders were also collected from cereal crops and a vining pea crop for PCR detection of aphid predation. Immediately after collection, linyphiid spiders were picked out of the sampling net using an entomological pooter or forceps and placed in Eppendorf tubes, and then frozen in crushed carbon dioxide ice. This procedure was done in the field to halt digestion of prey immediately after collection. The frozen spiders were then transported to the laboratory where they were transferred into a –80C freezer until analysis.

The carabid beetles *Pterostichus melanarius* and *Pterostichus madidus* were collected from wheat crops alongside margins with and without set-aside strips at the Cranborne study site in Dorset. Sampling was conducted once during the aphid population peak and beetles were frozen immediately after collection. Gut contents were extracted, weighed and refrozen and PCR analysis was done to determine the proportion of beetles that had consumed aphids.

Key Results

In the spider feeding trials, an aphid-specific band was still detected 8h after aphids had been consumed. Although the numbers of spiders caught at the field study sites declined with distance into the crop, spiders were shown to have fed on aphids with equal efficiency up to 100m into the crop, the maximum distance sampled. In 2001, around 25% of spiders were positive for aphid DNA, whilst in 2002, when aphid numbers were very low, 15% of spiders were positive. In the pea crop in 2003, 88% of spiders caught had eaten the pea aphid, *Acyrthosiphum pisum*. **These results provide evidence that linyphiid spiders were consuming a significant proportion of crop aphid pests, at least up to 100m away from botanically-**

diverse field margins. It is probable that the proportion of spiders feeding on aphid prey was influenced by aphid abundance, but even at low aphid densities spiders were functioning as important aphid predators in cereal crops. The much higher proportion of spiders detected feeding on aphids in the pea crop, compared with the cereal crop, was almost certainly due to the much greater aphid density in the former.

Out of a total of 233 carabid beetles (*Pterostichus* spp.) tested for the presence of aphid remains, 21% were found to have consumed aphids; 23% collected from fields with a set-aside strip and 18% from fields without a set-aside strip. The proportion of beetles that had consumed aphids was not significantly affected by distance from the margin, at least up to 100m, regardless of the presence of a set-aside strip.

HOVERFLY BEHAVIOUR STUDIES

Methods

The flower preferences of the hoverfly *Episyrphus balteatus* were tested in no choice and choice bioassays. A circle of twelve plants (all at the flowering stage) was arranged in flight cages (1m^3) such that each was equidistant from the centre of the cage and from its neighbours. A single newly emerged adult female hoverfly was released onto a platform in the centre of the cage. After a 5 minute settling period, the hoverfly was observed for a period of 30 minutes and the number of feeding visits to each plant and the length of each visit recorded. Experiments were replicated 20 times, using different hoverflies (to avoid problems of flower constancy) and different plants. The non-native plant *Phacelia tanacetifolia* was used as a standard in the experiments and a range of native UK flowering plants was screened.

The effects of flower choice on hoverfly oviposition rates were also investigated in cage bioassays. Flight cages were set out with a circle of six plants, each equidistant from its nearest neighbour. Four wheat plants that had been infested with a similar number of *Sitobion avenae* seven days previously were positioned in the centre of the circle to act as oviposition sites. Two, newly emerged, adult male and female hoverflies were released onto a platform at the centre of the cage, and the cage sealed and left undisturbed for 12 days, after which two pots of seedlings were removed and the number of hoverfly eggs counted. The other two pots were removed after 14 days and processed in the same way.

Cages were also used to investigate the foraging behaviour of female *E. balteatus* when searching for oviposition sites. Both no choice and choice bioassays were done using large and small aphid-infested wheat plants and large, uninfested plants. Plants were arranged in a triangle in the cage and individual female *E. balteateus* were released in the centre of the triangle and observed for a total of thirty-five minutes. No records of behaviour were made during the first five minutes, but during the remaining thirty minutes the length of time spent in various behaviours, and the number of eggs laid, were noted separately for each plant.

Key Results

The attractiveness of flowering plants to hoverflies was positively associated with the number of eggs that females subsequently developed and laid, supporting the hypothesis that female hoverflies select plant species that currently offer high quality food resources, which will result in increased egg load. These eggs gave rise to the aphidophagous stages of the hoverfly. **Therefore identification of preferred plant species and their inclusion in seed mixes developed for establishment of flower-rich field margins is important for the optimisation of conservation biological control**.

In no-choice tests, significant differences ($P<0.001$) were recorded between flower species in the number of feeding visits made during the 30 minute exposure period. **Three groups of plants were identified: the most preferred were species with umbelliferous or umbel-like flowers (yarrow, cow parlsey and hogweed) and white campion.** The second grouping consisted of three members of the daisy family with similar flower structures (cornflower, common knapweed and rough hawkbit), as well as field scabious and lady's bedstraw. The least preferred group included *Phacelia tanacetifolia*, ragged robin, red dead-nettle, cowslip and ox-eye daisy.

In all but one case, choice tests confirmed the preferences identified by no-choice tests. **A range of UK native plant species were shown to be equally or more attractive to hoverflies when compared to the non-native *Phacelia tanacetifolia*.** In particular, the umbellifer species listed above, yarrow and white campion were highly attractive to *E. balteatus* in the laboratory experiments, and subsequent observations of the rate at which these species are visited in the field have supported this finding. Field observations have also confirmed that hoverfly species other than *E. balteatus* are also attracted by these flower species**.** A second group of plants were also found to show high potential as components of flower-rich margins, including cornflower, field scabious, common knapweed, rough hawkbit and lady's bedstraw.

The range of species shown to be attractive to hoverflies in the current study have flowering times that collectively span the whole of the period in which aphidophagous hoverflies are both active in and around arable crops, and are developing their eggs. Provision of these species as part of the resource offered in managed field margins would therefore offer a plentiful supply of high quality pollen and nectar at the critical point in hoverfly life cycles. If such high quality resources are associated with increased egg load, then populations of the predatory larvae will be increased. This fact, coupled with behavioural responses to plant structure and signs of aphid presence that enable adult females to lay their eggs near to aphid colonies, may substantially increase the depression of aphid populations by hoverflies. **Thus the species of perennial wildflowers identified by this study should be considered as either valuable additions to seed mixes designed for establishment of flower-rich field margins or as species to be encouraged in other non-crop habitats,** as they offer advantages for increased farmland biodiversity, and also benefit a group of natural enemies that represent an important component of the beneficial fauna that contributes to conservation biocontrol.

When searching for oviposition sites, female hoverflies spent more time hovering in front of large infested cereal plants (at a growth stage present in fields during the period in which hoverflies are likely to be active) and large uninfested plants than in front of small infested plants (seedlings), but equal time hovering in front of large infested and large un-infested plants. After landing, they spent more time searching on large infested plants compared with both small infested and large un-infested plants, whilst significantly more eggs were laid on large than on small infested plants, and on both infested treatments compared with un-infested plants. This study has shown that *E. balteatus* females will react to plant structural cues and concentrate their initial searching behaviour (focussed hovering) on the larger plants in preference to the smaller plants, but will only progress through the rest of their oviposition behaviour if signs of aphid colonies are present. This reinforces the hypothesis that these **hoverflies have the potential to provide control of aphid populations as part of a natural predator complex.** Cereal crops are therefore a suitable subject for the management strategy investigated in this project. The searching efficiency for egg laying sites on other crops may also depend in part on the presence of appropriate visual cues, and therefore further work may be required before the management system developed in this project for cereals can be reliably transferred to new commodities.

KEY MESSAGES

- Field margins containing wild flower/grass mixtures can help to reduce aphid densities in adjacent cereal crops.
- Early activity by parasitic wasps (parasitoids), coinciding with aphid colonisation in spring, is a key component of natural biological control in cereals.
- Field margins and other non-crop habitats provide valuable reservoirs of aphid parasitoids.
- Aphid pheromones stimulate early spread of parasitoids into the crop and increase their impact on cereal aphid populations.
- Flower-rich field margins may increase the impact of aphid parasitoids on aphid populations in field brassicas.
- Umbellifer flowers, such as cow parsley and hogweed, as well as yarrow and white campion, provide the best food resources for adult hoverflies, whose larvae feed on aphids. These should be incorporated into field margin seed mixes or conserved in other non-crop habitats such as hedge bottoms and track verges, as appropriate.
- Hoverfly activity in fields with appropriate wild flower margins can result in substantial reductions in aphid numbers in cereal crops.
- Predatory hoverflies can significantly reduce aphid population development during early to mid summer, when the effect of parasitoids is declining.

- Both adult hoverflies and adult aphid parasitoids are highly mobile and can rapidly spread across large fields.
- The distribution of carabid beetles, which are valuable pest predators, varies through both space and time and is influenced by crop type and by crop and margin management.
- Field margins support ground-dwelling predatory invertebrates that subsequently distribute themselves through the crop. Large fields will be more slowly colonised than small fields, and the diversity of these predators will be lower in the centre of large fields.
- Large numbers of predatory invertebrates overwinter within the soil and autumn cultivations can reduce their numbers.
- Some species of generalist invertebrate predators, such as carabid beetles, have localised distribution patterns across and amongst fields and broad-scale insecticide applications should be avoided wherever possible if the chances of reinvasion are to be maximised.
- Predatory invertebrates are encouraged by weeds but 10-14% weed cover is optimal.
- Set-aside strips sown with game cover can encourage predatory invertebrates within the crop but sown mixtures need to be developed for this purpose.
- Ground-active invertebrate predators can contribute to pea aphid control.
- Money spiders are important predators of aphids, feeding on cereal and pea aphids for at least 100m into the crop even when aphid densities are low.
- Field margins provide valuable habitats for money spiders, which can rapidly spread into crops by ballooning on silk threads.
- Maintaining biodiversity on the farm aids natural aphid control, especially if a range of invertebrate predators and parasitoids are encouraged.
- Encouraging a diverse natural enemy community in agricultural ecosystems provides stability for natural biocontrol systems.
- A diverse range of field margins should be maintained on the farm as this adds to the diversity of invertebrate predators. There is not a single margin design that will suit all purposes.
- A dual margin consisting of a narrow strip of grassy uncut vegetation against the field boundary (around 1m), with a broader (at least 2m) flower-rich strip, cut in late summer, would probably benefit the greatest range of beneficial invertebrates.

TECHNICAL DETAIL

1. GENERAL INTRODUCTION

Agriculture is undergoing important changes as a result of CAP reform and continuing pressure to improve its environmental profile. Restrictions on pesticide use and the withdrawal of increasing numbers of compounds from the crop protection armoury mean it is essential to develop new, sustainable approaches to pest control. If the industry is to meet these challenges it is important that:

1. Agrochemical inputs are optimised and non-crop habitats are properly managed.
2. Natural pest control is maximised in integrated farming systems.
3. Productivity, competitiveness and product quality are maintained and preferably improved.
4. Biodiversity is encouraged to meet Rio summit commitments.

To achieve this, research is required to further promote the development of new, sustainable methods of crop protection and to improve our understanding of, and ability to manage, farmland ecosystems to ensure agriculture retains profitability whilst addressing environmental concerns.

This project was designed to build upon the following principles and recent developments, both in agricultural practices and pest control research, pertinent to the concept of ‘conservation biological control’. This approach is designed to maximise the impact of natural biological control agents operating within arable ecosystems as part of an integrated farm management strategy.

- *The conservation and manipulation of insect parasitoids and predators within the farmland ecosystem is the principal element of Integrated Pest Management (IPM), and new methods of enhancing beneficial insects are currently being developed.*

Biological control is the main component of IPM strategies and in arable crops this principally involves the exploitation of natural populations of parasitoids, predators and entomopathogens (diseases which infect and kill insects). Maintaining a diversity of habitats on farmland increases populations of beneficial insects but does not guarantee that these will arrive in the right place at the right time to have the maximum potential impact on pest populations in crops. However, manipulation techniques are being developed to concentrate natural enemies in crops and field margins at appropriate times of year (Powell, 1996; Powell et al., 1998). **It has been recognised for some time that field margins can play an important role in the development of novel manipulation techniques to enhance insect predators and parasitoids** (Powell, 1986).

At Rothamsted Research, the use of aphid sex pheromones to manipulate aphid parasitoids has recently been investigated in laboratory and small scale field experiments (Powell & Glinwood, 1998 - HGCA Project Report No. 155; Powell, 1998; Glinwood et al., 1998, 1999a; Powell & Pickett, 2003). Aphid sex pheromones attract a range of aphid parasitoids and it has been demonstrated that they can be used to increase parasitization rates in the field (Powell & Glinwood, 1998). Furthermore, hoverflies, many of which are important aphid predators, can be increased by planting patches of wild flowers in field margins (Cowgill, 1991; Cowgill et al, 1993; Hickman & Wratten, 1996; Holland & Thomas, 1996). Recent work at CSL has identified a number of key flower species as important sources of pollen and nectar for the adult flies, which need this food to mature their eggs. **It is essential to develop these approaches in a unified way and test them on a commercial field scale. The diversification of field margins through agri-environment schemes, primarily designed to increase farmland biodiversity, offers an ideal opportunity to do this. It is also important to determine how far into the crop the beneficial effects of field margin management and natural enemy manipulations extend.**

Field margins are also important habitats for other major predator groups, such as carabid beetles and spiders, and the diversification of margin habitats on farms (e.g. in arable stewardship schemes) will also affect these groups (Coombes & Sotherton, 1986; Holopainen, 1995). Previous HGCA research has indicated that these predator groups contribute to cereal pest control (Holland, 1997 - HGCA Project Report No. 148). Past research has indicated that **the combined action of a range of natural enemies is necessary for the successful natural control of aphid pests in arable field crops such as cereals** (Wratten & Powell, 1991; Sunderland et al., 1998). Therefore, any assessment of the impact of these new parasitoid and hoverfly manipulation strategies must consider effects of field margin management on other predatory groups. In addition, this project was designed to liaise closely with, and complement, an associated Sustainable Arable Link project at Rothamsted Research, which investigated novel strategies for aphid control using entomopathogenic fungi (Shah et al., 2004 – HGCA Project Report No. 336).

- *Recent attempts to reduce the impact of farming on the environment have involved the promotion of a range of field margin management options within agri-environment schemes, such as the Countryside Stewardship Scheme (CSS).*

Field margin management options that are being promoted in stewardship schemes and installed on demonstration farms, and which offer opportunities for incorporating beneficial insect manipulation, include wildlife strips, conservation headlands and wildflower and/or grass strips, as well as beetle banks across fields. The field margin is defined as the area between the field boundary (e.g. hedge, fence, ditch) and the crop proper, and sometimes may include crop plants, as in the case of conservation headlands. One of the main aims of these margin management options is to increase biodiversity in the countryside, since modern intensive farming is perceived as being one of the main causes of declining biodiversity. However, **there is considerable potential to manage such field margins to simultaneously increase pest control by natural control agents, particularly of aphids in crops such as cereals, in addition to enhancing biodiversity. These two aims are compatible and not mutually exclusive.** Field margin habitats around cereals and other arable crops are important refuge areas for insect predators and parasitoids, and provide essential resources for these beneficial insects and spiders at critical times of the year (Sotherton, 1984; Powell, 1986; Wratten & Powell, 1991). This has been demonstrated for management options such as beetle banks (Thomas et al., 1991), conservation headlands (Sotherton, 1991; De Snoo et al., 1995), wildflower strips (Baines et al., 1998; Thomas & Marshall, 1999), wildlife strips (Hawthorne, 1995) and grass strips (Kromp & Steinberger, 1992). Such margins, therefore, supply a reservoir of natural enemies, which move into adjacent crops to exploit aphid and other prey populations in spring and summer (Coombes & Sotherton, 1986; Riedel, 1992; Dennis & Fry, 1992; Holopainen, 1995). However, their impact on pest control needs to be adequately evaluated. **Botanical diversification of such margin habitats will lead to diversification of the margin fauna, including beneficial insects and spiders.**

- *The spatial distribution of insect predators and parasitoids over time within the field and its margins greatly affects their efficiency as biocontrol agents.*

Beneficial insects and spiders, in common with many other invertebrates, are not evenly distributed within farmland but show preferences for certain areas and especially non-crop habitats. This had been clearly demonstrated by collaborating participants in this project (Thomas et al., 1997, 1998; Holland et al, 1999). Insect interactions between field margin habitats and the crop and the overall density, diversity and distribution of both pests and beneficials are influenced not only by margin management (Dennis & Fry, 1992) but also by the crop husbandry practices employed in the field (e.g. cultivations, pesticide inputs, type of fertiliser). Local variations in factors such as soil type, soil pH, soil moisture, weed density, crop density and microclimate, as well as the type of field margin, can influence pest and predator distributions, (Speight & Lawton, 1976; Thiele, 1977; Hengeveld, 1979; Honek, 1988; Gruttke & Weigmann, 1990). Preliminary research by the Game Conservancy Trust and Long Ashton Research Station, using two-dimensional sampling grids, has revealed that beneficial invertebrates are frequently distributed in patches within fields and for some, such as carabid beetles, these are stable both annually and seasonally (Thomas et al., 1998). The type of field margin management affects the density and diversity of beneficial species that can be supported and consequently this influences their distribution, diversity and density within fields (Dennis & Fry, 1992; Cardwell et al., 1994; Kiss et al., 1997). **Measuring the extent of field margin influence on within-crop distributions and identifying which factors are the most important predictors of invertebrate distributions and diversity requires the collection and analysis of precise data.** New statistical techniques that allow us to map the distribution patterns of insects across a field and its margins and to analyse changes in those distribution patterns over time and in response to management practices have recently been developed at Rothamsted Research (Perry, 1998; Perry et al., 1999) The technique, technically called "Spatial Analysis by Distance IndicEs" and known as "SADIE" for short, has already been used successfully to investigate the distribution of insects in crops by Rothamsted Research and the Game Conservancy Trust (Winder et al., 1998, 1999). **In this project spatial distribution analyses were used to investigate the scale of the interactions between margin and crop and determine how crop,**

environmental and wildlife management can be more effectively integrated whilst maintaining profitability.

Serious environmental problems are now a recognised consequence of the intensification of agricultural production over the last 40 years. There is considerable evidence for the long-term decline of invertebrate abundance and diversity within arable ecosystems (Aebischer, 1991) and of the bird species dependent on them for food (Campbell et al., 1997). Non-crop habitats constitute one of the most important sources of biodiversity within farmland (Kretschmer et al., 1995) and their value to a wide variety of organisms has been demonstrated (Boatman, 1994), but their beneficial influence on adjacent crops has not been properly taken into account (Holland et al., 1998). In many arable areas, field margins are the only major non-crop habitat and act as the main source of beneficial species invading the crop in the spring and re-colonising after adverse agricultural operations such as pesticide treatments (Duffield & Aebischer, 1994; Holland et al., 1999). **This project aimed to develop management strategies that would allow farmers to fulfil their environmental commitments without jeopardising profitable crop production.**

1.1. OVERALL AIM

To use field margin management techniques to increase the abundance and diversity of beneficial insects and spiders and manipulate their distribution and dispersal on farmland for the control of aphid pests.

1.2. SPECIFIC OBJECTIVES

1. To provide farmers with advice on field margin management to optimise integrated pest management whilst maintaining biodiversity benefits and profitability.
2. To test and further develop a novel aphid control strategy involving the manipulation of parasitoids using aphid sex pheromones in field margins.
3. To develop and evaluate the use of specific native flowering plants in field margins to enhance the abundance and diversity of aphid-eating hoverflies in adjacent crops.
4. To measure the effects of margin and crop management on aphid and beneficial insect abundance, dispersal and spatial distribution in both the margin and adjacent crops.
5. To measure the spatial and temporal distribution of cereal aphids and the extent to which these are controlled by predatory and parasitic species.
6. To measure the impact of recently introduced field margin management options on the biodiversity of aphids and their natural enemies.

1.3. TARGET CROPS

The main target crop chosen for the study was winter cereals for a number of reasons:

- All scientific partners had considerable experience working in cereals

- The accumulated background knowledge of the ecology of cereal aphids and their natural enemies was far greater than for any other U.K. aphid pest
- There was strong evidence that cereal aphids were often prevented from reaching economic damage levels in summer by the action of natural enemies
- Cereal crops cover large areas of the countryside and are a dominant component of farmland ecosystems
- Sites were readily available near all the partner Institutes where established field margins bordered cereal crops.

For scientific reasons it was important to study the same crop for several years, but it was agreed that it would be useful to use the final field season to extend part of the study into field vegetable crops in order to gain some insight into the feasibility of extrapolating some of the findings to crops where aphid control presented a greater challenge. The aphid parasitoid and hoverfly manipulation field trials (Section 2) were extended to vining peas, organic broccoli and organic lettuce crops in 2003, whilst pea crops were also grown on some of the fields used in the intensive spatial distribution study (Section 3).

2. MANIPULATION OF APHID PARASITOID AND HOVERFLY ABUNDANCE AND DISTRIBUTION

2.1. INTRODUCTION

The concept of ‘conservation biological control’, involving enhancement of naturally-occurring populations of parasitoids and predators, is receiving increasing attention, especially for control of pests on field crops (Powell, 1986; Cortesero et al., 2000; Landis et al., 2000). This approach is based on the conservation of beneficial natural enemy populations within agro-ecosystems, by means of habitat manipulation, linked with the manipulation of insect behaviour to increase their impact on pest populations. The diversification of field margins within agri-environment schemes offers important opportunities for the manipulation of key aphid natural enemies. Strategies for the manipulation of aphid parasitoids and hoverflies, based on previous Defra-funded research carried out by scientific partners in the consortium, were developed and tested on commercial crop fields as a major component of the 3D Farming project. Parasitoid manipulation centred on the use of aphid sex pheromones to encourage overwintering reservoirs within field margins and then to stimulate the rapid colonisation of adjacent crops by parasitoids in spring. Hoverfly manipulation was based on the provision of essential nectar/pollen food sources for adult flies in field margins, in the form of selected native wild flowers.

2.1.1. Aphid Parasitoids

Ecological studies have shown that parasitoids are a key component of the natural enemy guild attacking cereal aphids but they need to be active in the crop at the time the aphids first colonise to be most effective (Wratten & Powell, 1991). Whilst searching for hosts to attack, aphid parasitoids make use of chemical information from both the host and the host plant, including semiochemicals generated by aphid-plant interactions (Powell et al., 1998). Recent identification of the semiochemicals involved in this host location behaviour provides exciting opportunities for manipulating parasitoid behaviour in order to enhance their impact on pests. One semiochemical that appears to be highly attractive to foraging female aphid parasitoids is a component of aphid sex pheromones.

Although pest aphids occur predominantly as all female, asexual populations, many pass through a sexual phase in the autumn that produces overwintering eggs, under appropriate climatic conditions. The sexual female attracts the winged male by releasing a sex pheromone, the main chemical components of which have been identified as (4*aS*,7*S*,7a*R*)-nepetalactone and (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (Dawson et al., 1987; Pickett et al., 1992). It was discovered that these compounds could be obtained from a species of catmint, *Nepeta cataria* L. (Dawson et al., 1989) and in early field trials with this plant-derived pheromone, female aphid parasitoids appeared to be strongly attracted (Hardie et al., 1991, 1994; Powell et al., 1993). Subsequent laboratory studies, involving electrophysiology (Wadhams et al., 1999) and behavioural bioassays (Powell et al., 1998; Glinwood et al., 1999a, 1999b), confirmed that females of a range of aphid parasitoid species showed strong responses to chemical components of aphid sex pheromones, especially to (4*aS*,7*S*,7a*R*)-nepetalactone. The potential of pheromone components for enhancing parasitization of aphid populations was then demonstrated in the field, using artificially-induced aphid infestations on potted trap plants (Powell et al., 1998; Glinwood et al., 1998). For example, in some of these trials, parasitization of the cereal aphid *Sitobion avenae* (F.), on potted wheat seedlings placed in field margins in the autumn, was more than ten times greater in the presence of the pheromone than on untreated control plants. Evidence demonstrating responses to aphid sex pheromones has now been accumulated, from both field and laboratory studies, for a range of economically important parasitoid species (Table 2.1).

Table 2.1. Parasitoids of economically-important aphids for which behavioural and/or electrophysiological responses to aphid sex pheromones have been recorded.

Parasitoid	**Pest Aphid Hosts**	**Evidence of Response**
Aphidius rhopalosiphi	Cereal aphids	Field Experiments Laboratory Bioassays Electrophysiology
Aphidius ervi	Pea Aphid Cereal Aphids Glasshouse Aphids	Field Experiments Laboratory Bioassays Electrophysiology
Aphidius eadyi	Pea Aphid	Field Experiments Laboratory Bioassays
Aphidius matricariae	Glasshouse Aphids	Electrophysiology
Diaeretiella rapae	Brassica Aphids	Field Experiments Laboratory Bioassays Electrophysiology
Praon volucre	Wide range of hosts	Field Experiments Laboratory Bioassays Electrophysiology
Ephedrus plagiator	Wide range of hosts	Laboratory Bioassays

A strategy for using these pheromones to manipulate aphid parasitoid populations was devised, based on the hypothesis that early season parasitoid activity within the crop leads to effective biological control of cereal aphids by restricting initial aphid population growth rates. Because aphid populations increase exponentially, if they escape this early mortality they can increase rapidly enough to exceed economic damage thresholds before other natural enemies, such as hoverflies and ladybirds, can have an impact. The strategy involves the use of the pheromone compound, nepetalactone, to stimulate early parasitoid activity in the crop, firstly by placing pheromone lures in field margins in autumn to encourage overwintering populations of parasitoids in this sheltered habitat, and then using the pheromone in the crop in spring to stimulate rapid parasitoid colonisation to coincide with aphid immigration. Although small plot field trials at Rothamsted Research had been encouraging, it was essential to test the effects of the pheromone on parasitoid abundance and spatial distribution at larger spatial scales, in real commercial crops, and to measure any consequent effects on aphid densities. The project would also provide the opportunity to test the validity of the hypothesis that early parasitoid activity plays an important role in the natural control of aphid pests in field crops.

2.1.2. Hoverflies

Hoverflies (Diptera: Syrphidae) are also important aphid predators in both arable and horticultural crops (Wratten et al., 1995; Hickman & Wratten, 1996), and have the potential to compliment parasitoids in an IPM strategy for cereals. Many species lay their eggs near aphid colonies and it is their larvae that are aphidophagous (Hickman & Wratten, 1996; Sadeghi & Gilbert, 2000c; Scholz & Poehling, 2000; Sutherland et al., 2001; Yang et al., 2002). Within arable land in Great Britain, *Episyrphus balteatus* and *Metasyrphus corollae* are the most commonly recorded hoverflies (Dean, 1982). Decline in cereal aphid numbers has been shown to be associated with the presence of hoverfly larvae, in both commercial fields (Chambers et al., 1986) and experimental trials. For example, Tenhumberg & Poehling (1991) found that an 80-90% reduction in cereal aphid populations, mainly *Sitobion avenae,* occurred 6 days after second instar *E. balteatus* were released into field cages, even where the ratio of prey to predator was as high as 245:1. Analysis of hoverfly life history indicates that egg laying and hatching of the predatory larvae of the most common aphidophagous species occurs during June and July. They therefore offer temporal complimentarity with the early season activity of parasitoids, which suppress aphid population development soon after colonisation of cereals in spring but often become less effective later in the summer.

Adult hoverflies, arriving either from locally overwintering populations or migrating from other locales, appear in cereal fields from late May onwards. Before seeking egg laying sites, they feed on flowers from which they need nectar for energy and the protein from pollen for sexual maturation and egg development (Hickman & Wratten, 1996). The amount and quality of the pollen and nectar available to hoverflies can have a direct consequence on the viable egg load of adult females (Scholz & Poehling, 2000). Gut analysis indicates that hoverflies fall into two categories; some species are highly specific to a small range of flowers, whereas others are polyphagous (Haslett, 1989). In the latter category, those flower species that currently offer the best resources are visited most frequently. As nectar flow in these species reduces and pollen availability changes, making other flowers more attractive, hoverfly populations adjust their feeding habits (Cowgill et al., 1993). Thus, egg laying and therefore predatory capacity of local hoverfly populations is enhanced by the availability of a range of plants offering high quality pollen and nectar flows, and which flower in sequence throughout late spring and summer.

Beneficial insectary planting is a form of conservation biological control that involves introducing flowering plants into agricultural and horticultural systems to increase the nectar and pollen resources required by some natural enemies of insect pests. Surveys of naturally occurring weed and wild plant compositions in agroecosystems have associated florally abundant, non-crop habitats with significantly higher numbers of pollen and nectar feeding natural enemies in and around fields (Cowgill 1989; Cowgill et al., 1993) and orchards (Leius 1967). Several studies have demonstrated the potential of establishing flowering plants in or around fields to attract natural enemies and enhance biological control of crop pests in adjacent fields (Harwood et al., 1994; Hickman & Wratten, 1996).

Many adult hoverflies, as well as parasitoids, exhibit a high degree of selectivity to flowers from which they feed (Leius, 1960; MacLeod, 1992), and this varies inter-specifically. However, little work has been reported on specific host plant preferences for feeding of each major hoverfly species. A few flowering plants have been experimentally evaluated as insectary plants including phacelia (*Phacelia tanacetifolia*), coriander (*Coriandrum sativa*) and buckwheat (*Fagopyrum esculentum*) (Colly & Luna, 2000). Yellow and white flowers are also often included in lists of attractive species because these colours have been shown to elicit feeding in hoverflies (Cowgill, 1989). Umbelliferous flowers, coriander and fennel have short corollae, facilitating nectar availability (Gilbert, 1981), also making them good insectary plants. Cowgill (1990) produced a host plant feeding preference index for *E. balteatus*. It was found that in field boundaries during June, creeping thistle (*Cirsium arvense*) and sowthistle (*Sonchus oleraceus*) were the most preferred, followed by red dead-nettle (*Lamium purpureum*) and white campion (*Silene latifolia*). White dead-nettle (*L. album*) and bladder campion (*S. vulgaris*) were avoided. **Further work is required to ascertain the value of selected flowering plant species (particularly common UK native species that are readily available in current wildflower seed mixes) to aphidophagous hoverflies.** This will facilitate the design of a field margin seed mixture that will contain a suitable balance of species in order to promote the maximum benefit from a suite of natural predators throughout the growing season.

The positioning of insectary planting in relation to crops has been widely debated, but further research is needed before firm conclusions can be drawn. However, field margins have been shown to support a greater diversity and density of hoverflies than within-crop wildflower patches, despite being reported to have a lower flower head density. For example, the hoverfly *E. balteatus* has been shown to demonstrate a very positive habitat association with a field margin and was rarely reported from in-field wildflower patches (Sutherland et al., 2001). This may be due to (non-floral) resources that field margins offer, namely additional aphids, shelter from predation and suitable flight paths for dispersing adults (Colley & Luna, 2000). Therefore, areas with diverse field boundaries may offer greater potential for biological control of aphids by hoverflies compared to landscapes with smaller proportions of field boundary diversification (Krause & Poehling, 1995). In addition, economic considerations may also favour the use of field boundaries for insectary planting. Chaney et al. (1999) found that yields of field margins were 38% lower than those in the centre of the field, thus promotion of environmental schemes focusing on field margins may have lower impact on farm profit margins, whilst significantly benefiting local biodiversity.

Several authors have suggested that hoverflies may accumulate in florally rich field margins during their flower feeding phase, and subsequently fail to disperse into the crop effectively during their egg laying period. For example, MacLeod (1999) showed that *E. balteatus* disperse more slowly from flower-rich margins than from grass margins. However, ultimately effective dispersion into the field in search of egg laying sites has been shown to occur. **Once again further whole crop studies are required to confirm this.**

The impact of hoverflies on cereal aphids not only depends on the number of adults and dispersion into the crop (MacLeod, 1999), but also on searching efficiency of egg laying females (McDowall, 2002). Following a period of flower feeding, females seek out colonies of prey, deposit their eggs nearby and the resultant larvae feed on the aphids until pupation. A crucial aspect of oviposition is host plant choice, especially as the newly hatched offspring are unable to move a great distance to search for the appropriate prey. Such offspring must generally feed on the host plant and aphids previously selected by the mother. Factors involved in the selection of oviposition sites by *E. balteatus* are poorly understood but include; chemical stimuli, the presence of honeydew, aphid colony size and host plant characteristics (Vanhaelen et al., 2001). **Further work is required to investigate if plant cues will result in selection of cereal plants by hoverflies for egg laying during the critical growth stages.**

Hence, habitat manipulation by the addition of flowering boundary strips can lead to higher numbers of hoverflies, higher oviposition rates, and fewer numbers of aphids on crops (Hickman & Wratten, 1994).

This project will

1. investigate if plant cues leading to egg laying by hoverflies will result in substantial egg laying on cereal crops during the critical GS 59-83.
2. ascertain the value of certain flowering plant species (particularly common native species that are readily available in current wildflower seed mixes) to aphidophagous hoverflies.
3. quantify the effects of flowering margins on both the number and in-field distribution/dispersal of aphidophagous hoverflies and their effects on cereal aphid populations in full scale commercial crops.

2.2. MATERIALS & METHODS

2.2.1. Field Sites

In 2000, 2001 and 2002, field trials were done on cereal crops at four sites:

1. Radcot Bridge Farm, near Faringdon, Oxfordshire, farmed by Mr. Andrew Hichens. This site was chosen because it was already the site of a number of Agroecology trials managed by Marek Nowakowski of United AgriProducts (now of the Farmed Environment Company). These trials had involved the establishment of flower-rich field margins which were suitable for use in the 3D Farming study. The experimental work at this site was managed by Rothamsted Research (RRes).
2. Colworth Farm, Unilever Research Colworth, Sharnbrook, Bedfordshire, farmed by Mr. Alan Green and then by Mr. Innes McEwen. Unilever Research is a commercial partner in the 3D Farming project and their trials farm at Colworth provided an ideal site for the project, which complemented existing work by Unilever on Sustainable Agriculture. The farm had already installed extensive field margins and a new flower-rich margin was sown for the 3D Farming study, using a seed mix provided by Marek Nowakowski. The experimental work at this site was managed by Rothamsted Research.
3. Manor Farm, Eddlethorpe, Malton, North Yorkshire, farmed by Mr. Chris Rigley. Manor Farm is the site of a major project demonstrating that practical wildlife conservation and profitable farming can be effectively integrated, set up by Marek Nowakowski, and originally run by UAP but now run by the Farmed Environment Company (FEC). This site again provided established flower-rich margins and was readily accessible to one of the 3D Farming scientific partners. The experimental work at this site was managed by the Central Science Laboratory (CSL).
4. West Fenton Farm, North Berwick, East Lothian, farmed by Mr. Garth Morrison who had already established a number of flower-rich field margins on the farm. The experimental work at this site was managed by the Scottish Agricultural College (SAC), Edinburgh.

In 2003, a further cereal trial was done at the Colworth site, whilst trials on various field horticultural crops were done at four new sites. The Horticultural Development Council (HDC) and the Processor's and Growers Research Organisation (PGRO) were instrumental in locating these sites.

1. Wallington Farm, Morden Grange, near Royston, Cambridgeshire, farmed by Mr. Alan Hannah. A reduced trial, testing the effects of the aphid sex pheromone, was done on a vining pea crop and the experimental work was managed by Rothamsted Research.
2. Dimmocks Cote Farm, Strettam, near Ely, Cambridgeshire, farmed by Mr. David Norman for GSShropshire. A trial was done on an organic lettuce crop and the experimental work was managed by Rothamsted Research.
3. Muirton Farm, Drem, North Berwick, East Lothian, farmed by Mr. Charles Russell. A trial was done on a vining pea crop and the experimental work was managed by the Scottish Agricultural College, Edinburgh.

4. Holmes Farm, Epworth, Doncaster, Yorkshire, farmed by Mr. Peter Cornish for Loveden Estates Limited. A trial was done on an organic broccoli crop and the experimental work was managed by the Central Science Laboratory.

2.2.2. Field Treatments

For all the cereal trials, three fields were selected each year at each site:

1. A field with a tussocky grass margin, along which the pheromone lures were deployed in autumn, followed by pheromone deployment in the adjacent crop in spring.
2. A field with a flower-rich field margin to encourage hoverflies.
3. A field with neither pheromones nor a flower-rich margin to act as a control.

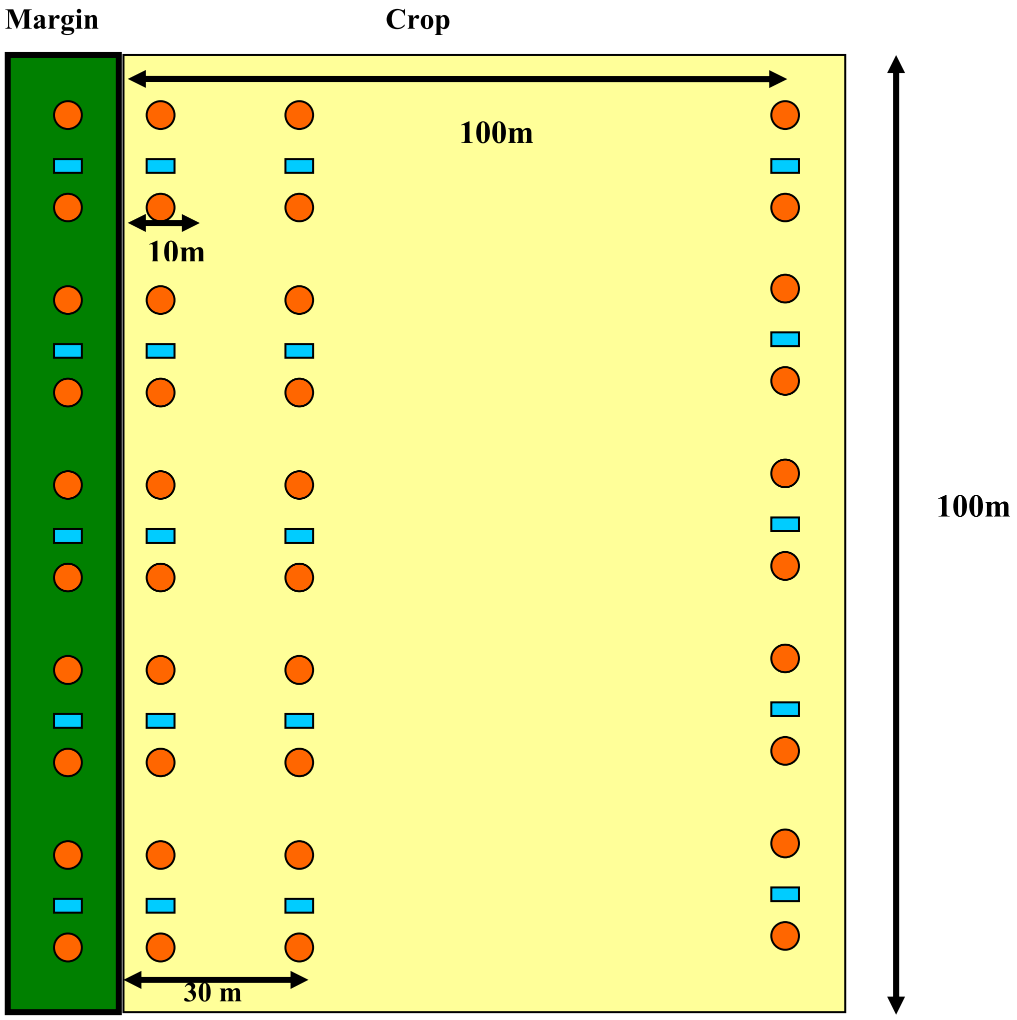
At the four cereal trial sites, where trials were done over the first three years of the project, the vegetation within the treatment margins of the three study fields was surveyed. Due to individual farm cropping plans, it was not always possible to use the same fields for all treatments over the three years.

In 2003, it proved impossible to find sites with three separate fields containing the same horticultural crop, planted around the same time, and including one with a flower-rich field margin. Therefore, the field treatments had to be modified to accommodate the available conditions. At the organic lettuce site in Cambridgeshire, a single large field was used, which was bordered by a flower-rich embankment. The pheromones were deployed at one end of the field and the opposite end was used as an untreated control area. A similar design was employed at the Royston pea site, where a flower-rich margin along the edge of a single large field was used, with pheromones deployed at one end. At the pea field site at Drem in East Lothian a single very large field was used, which was large enough to allow three different sides to be used, one of which had a flower-rich margin bordering a burn. At the organic broccoli site in Yorkshire a flower rich border along a hedgerow and roadside was used for the flower margin treatment, whilst opposite sides of a second field were used for the pheromone and control treatments.

2.2.3. Insect Sampling

In each study field, four 100m sampling transects were established, one in the margin and three in the crop, parallel to the margin, at 10m, 30m and 100m away from the margin. Thus all insect sampling was done in a 100m length of margin and an adjacent 100m x 100m area of crop (Fig. 2.1). The only exception was the organic lettuce site in 2003, where the small size of the cropped area necessitated a reduction in the length of the sample transects to 50m and the omission of the 100m crop transect. Insects were assessed weekly over an 8-10 week period covering the main summer aphid infestation period. Sampling protocols were prepared and circulated to all scientific partners at the start of the project.

Figure 2.1. Insect sampling transects in fields at the parasitoid and hoverfly manipulation study sites. Circles = pitfall trap positions. Rectangles = water trap positions.



Cereal aphids were counted *in situ* on 25, randomly selected tillers along each of the three sampling transects in the crop. Pea aphids were assessed by counting on 25 plants per transect (Drem site) or, when numbers were too high, by beating plants over a plastic tray along ten 1m row lengths along each of the three transects (Royston site). In the case of lettuce aphids, ten whole plants were removed from the field along each of the two sampling transects (10m & 30m) and examined for aphids in the laboratory. Aphids on the broccoli crop were counted *in situ* on one large leaf of each of 25 plants along each sampling transect. In all cases, aphids were identified to species and counted, and the presence of parasitized aphids (mummies), fungus-killed aphids (cadavers) and aphid predators was recorded.

Suction samplers (Vortis/D-vac) were used to sample adult parasitoids by sweeping along 20m row lengths of the crop. Five samples were taken along each of the sampling transects in both the margin and the crop areas. Each sample was placed into a polythene bag and taken back to the laboratory for sorting. All parasitic Hymenoptera were removed, placed in an alcohol preservative and sent to RRes for extraction and identification of adult aphid parasitoids. Suction samplers cannot be used efficiently when the vegetation is wet and so in weeks when the weather was unsuitable, these samples had to be omitted.

Adult hoverflies were sampled using water traps placed at crop canopy height. These consisted of plastic bowls (24cm diameter x 9cm deep), painted yellow and white, and part filled with water containing a mild detergent and a preservative (water sterilisation tablets). In preliminary trials conducted by CSL, traps painted with alternating yellow and white quarters proved to be the most efficient colour for attracting hoverflies. The traps were emptied weekly by straining the contents through a muslin cloth and taking the catch to the laboratory for sorting. Adult hoverfly samples were sent to CSL for identification. Five traps were positioned along each sampling transect in both the margin and crop areas (Fig. 2.1).

Carabid beetles were sampled using ten conventional pitfall traps, evenly spaced along each sampling transect in both the margin and crop areas (Fig. 2.1). Each trap consisted of a plastic beaker embedded in the soil with the aid of a plastic sleeve and part filled with water containing ethylene glycol as a preservative. The traps were changed weekly and taken to the laboratory for sorting and carabid identification.

Table 2.2. Numbers of insect samples taken per week at each parasitoid and hoverfly manipulation trial site, and the total numbers of samples taken at all these sites over the four years of the study.

Year	***Site***	***Crop***	***Aphids***	***Parasitoids (Suction Samples)***	***Hoverflies (Water Traps)***	***Carabids (Pitfall Traps)***
2000, 2001 & 2002	Colworth	Cereal	225 tillers	60	60	120
	Radcot	Cereal	225 tillers	60	60	120
	Manor Farm	Cereal	225 tillers	60	60	120
	W. Fenton	Cereal	225 tillers	60	60	120
2003	Colworth	Cereal	225 tillers	60	60	120
	Strettam	Lettuce	40 plants	30	30	60
	York	Cabbage		60	60	120
	Drem	Peas	225 plants	60	60	120
	Royston	Peas	60x1m rows	40	-	-
Total Samples[1]				**5150**	**6210**	**12420**

[1] All sites, all years and all weeks

A total of 23,780 insect samples were collected and processed during this part of the project, in addition to the *in situ* aphid counts (Table 2.2).

2.2.4. Pheromone Deployment

The aphid sex pheromone component, (4*aS*,7*S*,7a*R*)-nepetalactone, was supplied by AgriSense BSC *via* an associated project (CSA 4473) in the ‘Competitive Industrial Materials from Non-Food Crops’ LINK Programme, entitled “Nepeta spp. as a non-food, crop-derived feedstock for the production of semiochemicals for aphid pest control”. The pheromone was formulated into strips of PVC polymer. A standard lure length of 4.0cm of this polymer strip, designed to release at least 200 micrograms of nepetalactone for approximately six weeks, was used throughout the study.

Lures were attached to thin canes, using twist ties, so that the pheromone was released on a level with the top of the vegetation in either the margin or the crop. The pheromone was deployed at two times during the year; in the margin in autumn and in the crop in spring, except at the organic lettuce site where it was only deployed in the crop, soon after planting in summer. The autumn deployment, consisting of 10 lures evenly spaced along the 100m margin sampling transect, was made in 2000, 2001 and 2002. In addition, in spring 2001, 10 lures were evenly spaced along each of the three 100m sampling transects in the crop (at 10m, 30m & 100m from the margin), whilst in 2002, 49 lures were placed in a 7x7 grid covering the 100m x 100m crop sampling area, or the 35m x 50m sampling area in the case of the 2003 lettuce trial. The timing of deployment of the pheromone in the crop was determined by the timing of aphid immigration in the spring/summer, based on RRes Insect Survey suction trap data. Sixteen of these traps are positioned across the U.K. and continuously monitor aphid aerial movements.

2.2.5. Data Handling and Analysis

Data sets were sent to RRes for final collation and analysis. Data were collated onto standard spreadsheets and analysed using an ANOVAR programme prepared for the project by statisticians at RRes. The ANOVAR programme was a modified version of that used to analyse the large datasets generated by the Farmscale Evaluation Study of herbicide-tolerant GM crops, which also used some of the same insect sampling methods.

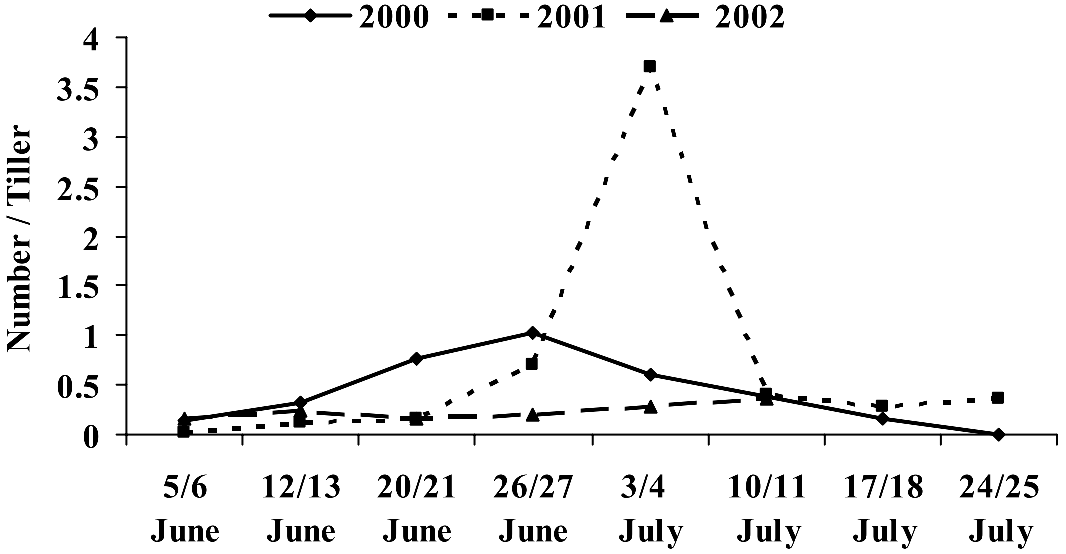
2.3. RESULTS

2.3.1. Cereal Aphid Population Trends

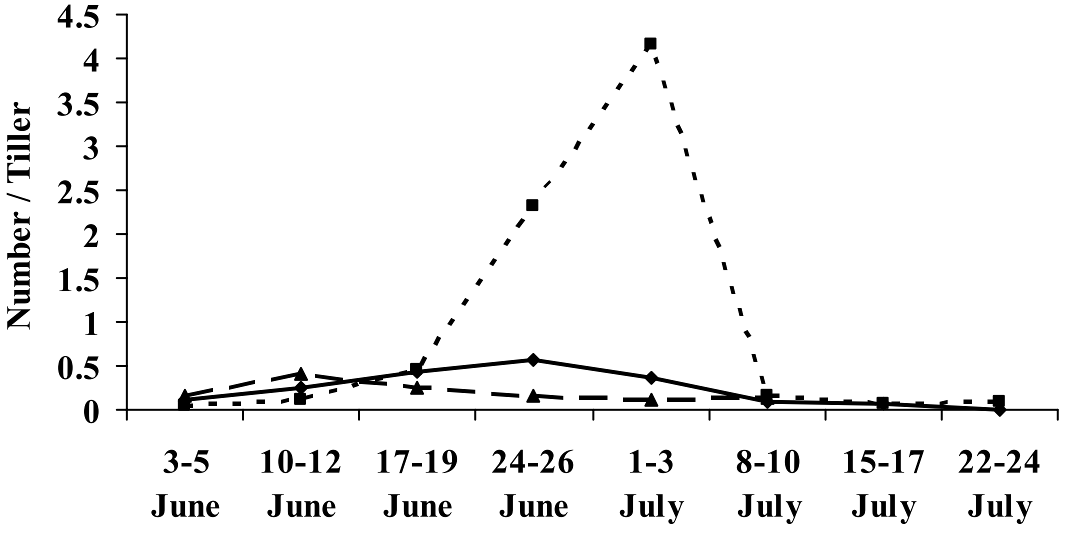
At the two southern English sites, in Bedfordshire and Oxfordshire, the pattern of cereal aphid population development within the crop varied dramatically from year to year (Fig. 2.2). In 2000 and 2002, aphid populations remained very low throughout the season and never exhibited the exponential growth curves typical of aphid outbreaks.

Figure 2.2. Cereal aphid density (mean number / tiller) at the two southern English study sites; (a) Colworth, (Beds) and (b) Radcot, (Oxon) in 2000 (solid line), 2001(dotted line) and 2002 (dashed line).

(a) Colworth



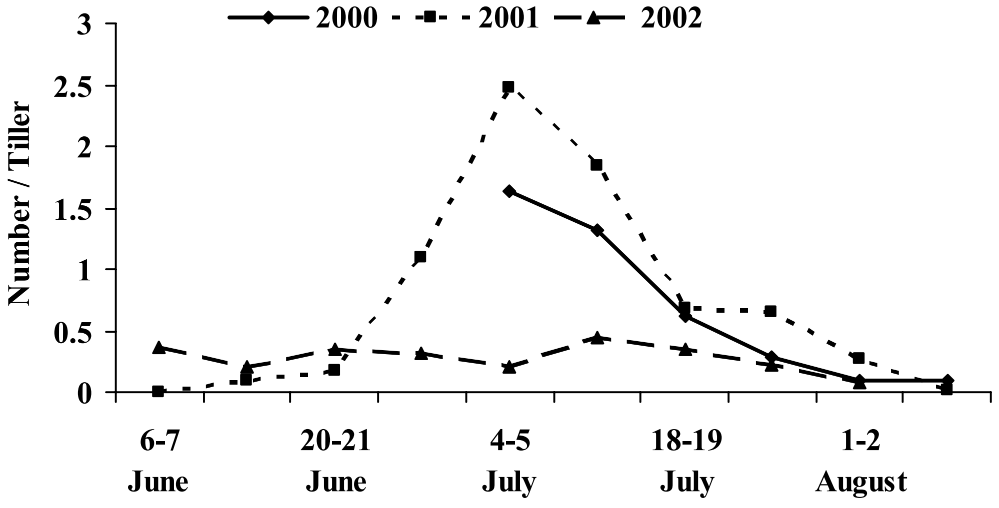
(b) Radcot



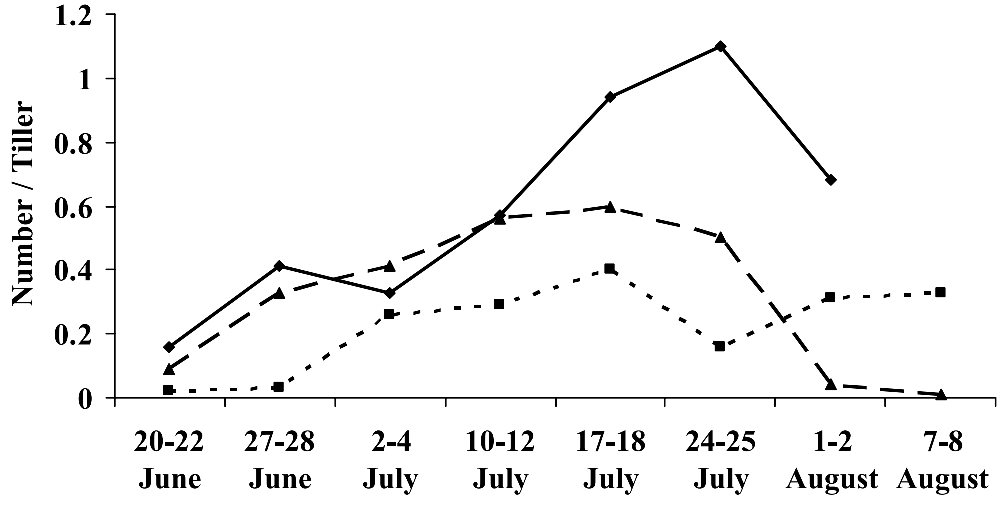
This suggests that natural control was working well in these two years. However, in 2001, aphid numbers remained low until mid-June when they began to increase exponentially, reaching a peak in early July, after which numbers crashed dramatically. The period of exponential growth suggests a lack of natural control factors operating at this time (see Discussion section 2.4.1.1.). At the northern English site near York, the 2001 population peaked at the same time as those at the more southerly sites, but reached a lower level and suffered a less dramatic decline (Fig. 2.3a). However, at the southern Scottish site, populations remained low in all three years, including 2001 (Fig. 2.3b).

Figure 2.3. Cereal aphid density (mean number / tiller) at (a) the northern English site, Manor Farm (Yorks), and (b) the southern Scottish site, West Fenton Farm, (Lothian), in 2000 (solid line), 2001(dotted line) and 2002 (dashed line).

(a) Manor Farm



(b) West Fenton



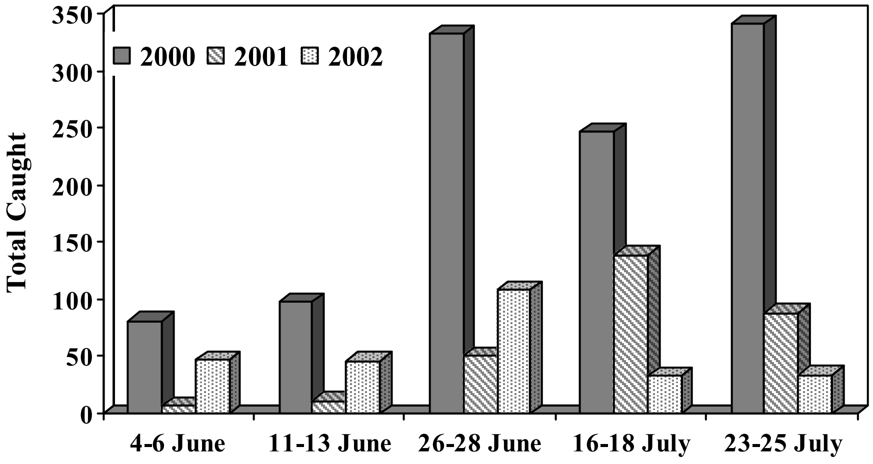
2.3.2. Cereal Aphid Parasitoids

2.3.2.1. *Parasitoid population dynamics*

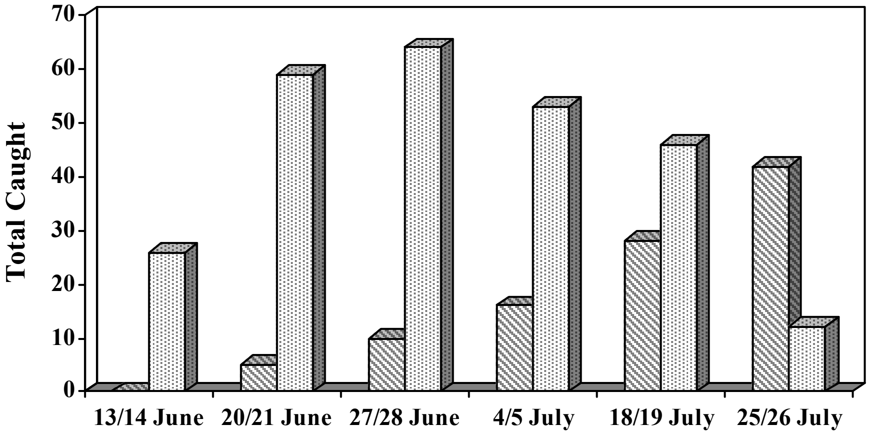
The aphid sex pheromone was first deployed in the autumn of 2000, so the first full treatment season was summer 2001. However, the 2000 field season was used to evaluate the sampling protocol and collect baseline data on parasitoid population dynamics in cereal crops at the two southern English sites. The data show that parasitoids were active in the crop early in the season in 2000, coinciding with the early stages of aphid colonisation (Fig. 2.4a). In contrast, the cold, wet weather in the spring/early summer of 2001 prevented early parasitoid activity and parasitoid populations did not get established in the crop until later in the season, well

Figure 2.4. Total numbers of adult aphid parasitoids caught in Vortis suction samples at (a) the two southern English sites, Colworth (Beds) and Radcot (Oxon), in 2000 (solid bars), 2001 (hatched bars) and 2002 (stippled bars) and (b) the northern English site, Manor Farm (Yorks) in 2001 and 2002 (suction samples were not taken at Manor Farm in 2000).

(a) Colworth & Radcot



(b) Manor Farm



after the initial aphid colonisation. In 2002, parasitoid activity was again evident at the time of aphid colonisation early in the season (Fig. 2.4a). Data from the Yorkshire site again indicate very little parasitoid activity in the wet spring of 2001 but much greater activity at the time of aphid colonisation in 2002 (Fig. 2.4b). Prolonged wet weather through the summer of 2001 in southern Scotland prevented suction sampling in most weeks but a sample was taken in the first week of July which caught only six adult aphid parasitoids in the three treatment fields combined, compared with 342 in the same week in 2002.

The critical factor in efficient biological control is not the absolute numbers of natural enemies present but the pest:natural enemy ratio. The relative (not absolute) aphid:parasitoid ratio can be compared for the 3 years at the same site by comparing the numbers of aphids counted at the start, peak and collapse of the aphid population with the numbers of adult parasitoids caught in the suction samples at the same times. This is exemplified by the data for the Colworth site, which shows that there were far more aphids per parasitoid, particularly at the start and peak of the aphid infestation, in 2001, when the aphid population showed an exponential growth phase, than in the other two years (Table 2.3). These are not the actual ratios of aphids to parasitoids present in the crop but are a relative measure based on sample data, which allows comparison between the three years.

Table 2.3. Relative cereal aphid:adult parasitoid ratios at the start, peak and during the collapse of the aphid infestation at Colworth (Beds) in 2000, 2001 and 2002.

	Start	**Peak**	**Collapse**
2000	1.2	1.1	1.9
2001	7.0	19.4	4.3
2002	1.1	3.3	2.9

2.3.2.2. *Parasitoid species abundance*

Five species of aphid parasitoids that are known to attack cereal aphids were caught in the suction samples; *Aphidius rhopalosiphi, Aphidius ervi, Aphidius picipes, Praon volucre* and *Ephedrus plagiator*. All five species were caught at all four cereal sites used in the study (Table 2.4). The most abundant species overall was the cereal aphid specialist *A. rhopalosiphi*, which dominated catches, except at West Fenton in 2001, where *P. volucre* was more abundant, and at Manor Farm in 2002, where *A. picipes* was equally abundant.

The relative abundance of the different species changed with time in a consistent way, the cereal aphid specialist *A. rhopalosiphi* strongly dominating at the beginning of the season (Figs. 2.5 & 2.6). In early June, over 80% of suction sampler catches consisted of this species (Fig. 2.5). The other two *Aphidius* species were also usually present in significant numbers during June but the two species with the greatest aphid host ranges, *P. volucre* and *E. plagiator*, did not build up until aphid populations were already declining (Fig. 2.6).

Table 2.4. Relative abundance of the five main parasitoids of cereal aphids caught in suction samples taken within the crop in all treatment fields at each of the four cereal study sites.

Year		**Colworth**	**Radcot**	**Manor Farm**	**West Fenton**
2000	Number of sample weeks	5	5	0	5
	Aphidius rhopalosiphi	588	233	-	706
	Aphidius ervi	66	52	-	107
	Aphidius picipes	10	11	-	56
	Praon volucre	61	66	-	26
	Ephedrus plagiator	9	1	-	2
	All Species	734	363	-	897
	% Aphidius rhopalosiphi	**80%**	**64%**	**-**	**79%**
2001	Number of Sample Weeks	8	8	8	3
	Aphidius rhopalosiphi	81	196	213	158
	Aphidius ervi	31	35	49	149
	Aphidius picipes	23	18	27	14
	Praon volucre	16	58	24	219
	Ephedrus plagiator	11	2	9	1
	All Species	162	312	322	541
	% Aphidius rhopalosiphi	**50%**	**63%**	**66%**	**29%**
2002	Number of Sample Weeks	7	6	7	6
	Aphidius rhopalosiphi	151	48	90	1243
	Aphidius ervi	34	28	76	312
	Aphidius picipes	21	26	93	279
	Praon volucre	9	15	47	295
	Ephedrus plagiator	14	4	7	37
	All Species	229	121	313	2166
	% Aphidius rhopalosiphi	**66%**	**40%**	**29%**	**57%**
All Years	**% Aphidius rhopalosiphi**	**73%**	**60%**	**48%**	**58%**

Figure 2.5. Relative abundance of *Aphidius rhopalosiphi* in cereal crops compared to all other aphid parasitoid species, expressed as percentage of *A. rhopalosiphi* in suction sample catches over time. C=Colworth, R=Radcot, MF=Manor Farm, WF=West Fenton

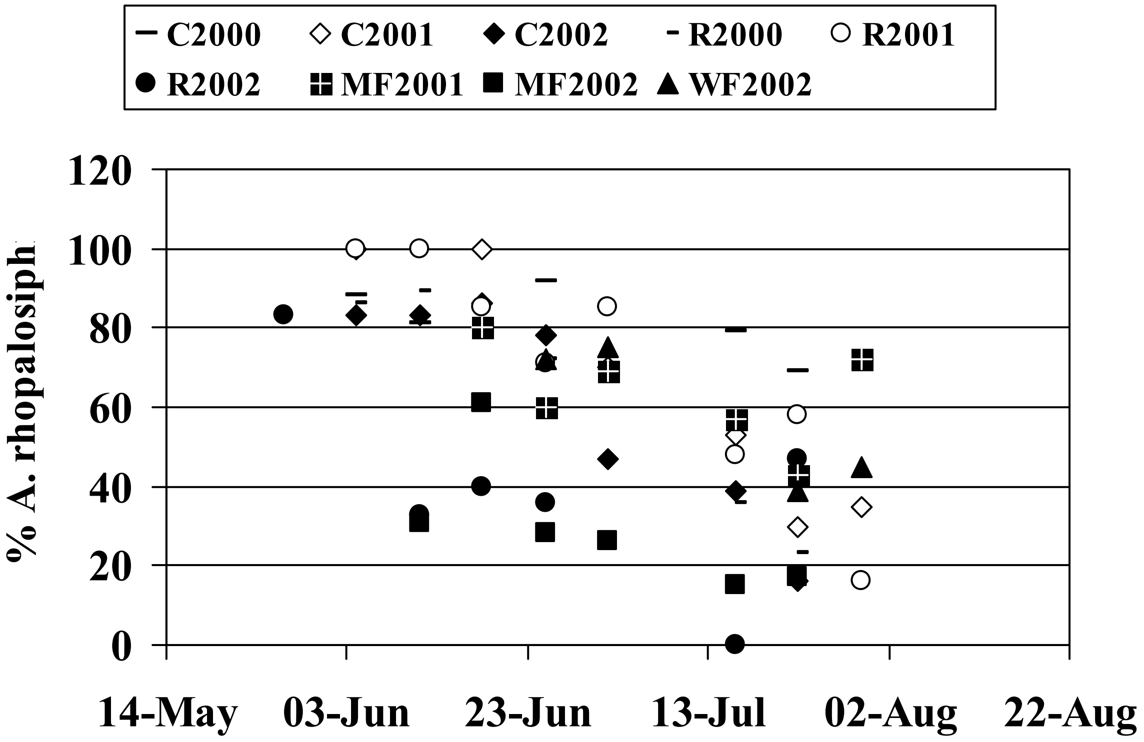
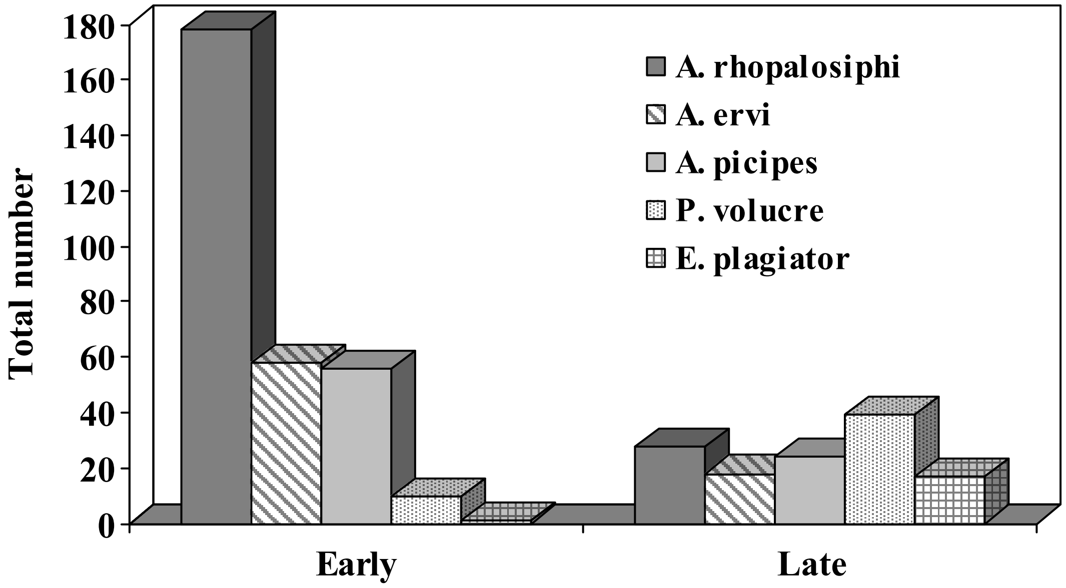


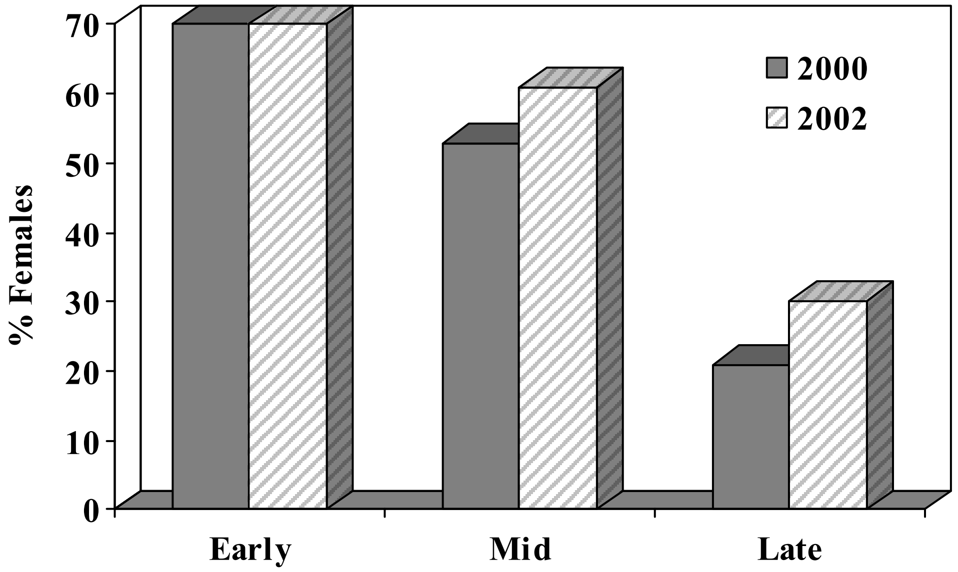
Figure 2.6. Relative abundance of parasitoid species in suction samples taken in 2002 from cereal crops in the early and late stages of cereal aphid infestation. Data for all sites combined.



2.3.2.3. *Parasitoid sex ratios*

The sex ratio of adult aphid parasitoids caught in the cereal crops changed during the course of the season. During the period of aphid colonisation and early infestation there was a strong female bias with around 70% of the parasitoid population consisting of females (Figure 2.7). During the main aphid infestation period, the sexes were caught in approximately equal numbers, with only a slight female bias (50-60%), whilst during the aphid population crash the sex ratio became strongly male biased with only 20-30% females.

Figure 2.7. Sex ratio of aphid parasitoids, expressed as % females in suction samples taken in early, mid & late periods of aphid infestation in cereal crops at Colworth (Beds) & Radcot (Oxon) in 2000 (solid bars) and at all sites in 2002 (hatched bars).



2.3.2.4. *Effect of aphid sex pheromone*

The aphid sex pheromone lures were deployed for the first time in tussocky grass field margins at the four sites after harvest 2000 and in the crop in spring 2001. Unfortunately the cool, wet conditions in spring 2001 prevented parasitoid activity at the critical time, making adequate assessment of the effects of the pheromone impossible. However, conditions were good in the 2002 season, with plenty of parasitoid activity, allowing any effects of the pheromone on parasitoid numbers and spatial distribution to be measured. Figure 2.8 shows the numbers of parasitoids caught along the three sampling transects within the crop at all sites during the first two sampling weeks, which represents the critical aphid colonisation period when parasitoid activity is important for preventing rapid aphid population growth (see section 2.3.1). The overall numbers of parasitoids caught in control fields and pheromone-treated fields were similar but their spatial distributions differed. In the control fields, numbers were greatest nearest to the field margin and declined with increasing distance into the crop, but the distribution pattern was different where the pheromone was present, with greater numbers caught further into the crop (Fig. 2.8). However, the combined data are strongly dominated by the data for the Scottish site (West Fenton) where much greater numbers were caught than at the other sites. When the data for the four individual sites are considered, the effects of the pheromone on early parasitoid distribution was evident at both West Fenton and Manor Farm (Fig. 2.9c,d), but not at the two southern English sites (Fig. 2.9a.b), although meaningful interpretation of the data from the Radcot site is not possible because of the very low numbers of adult parasitoids present in the samples (Fig. 2.9b).

Figure 2.8. Effect of the aphid sex pheromone compound, nepetalactone, on the numbers of adult aphid parasitoids caught in cereal crops at 10m, 30m and 100m away from the field margin during the first two weeks after cereal aphid colonisation in 2002. Data for all sites combined. (Control field – solid bars; Pheromone-treated field – hatched bars).

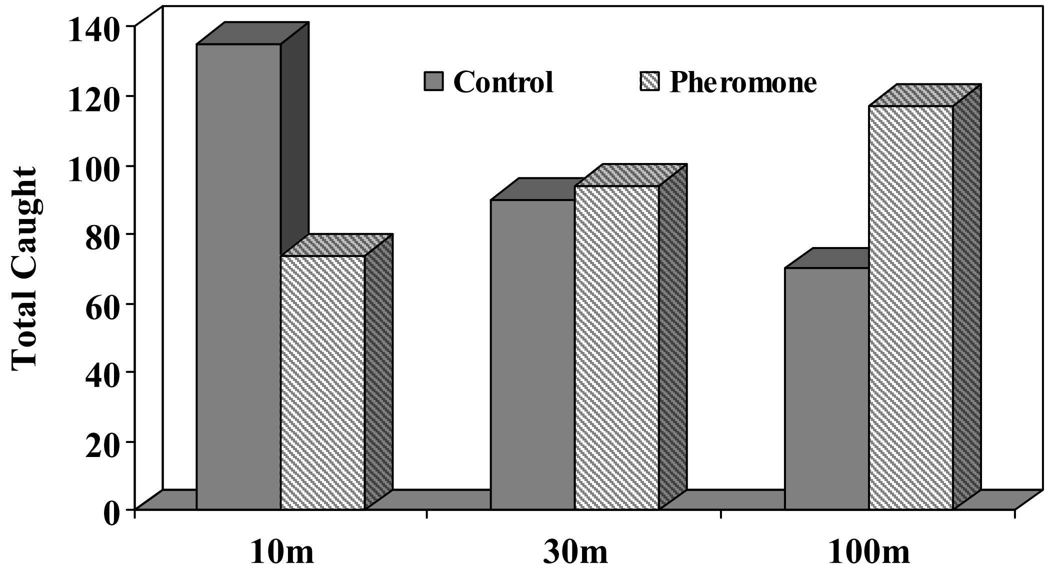
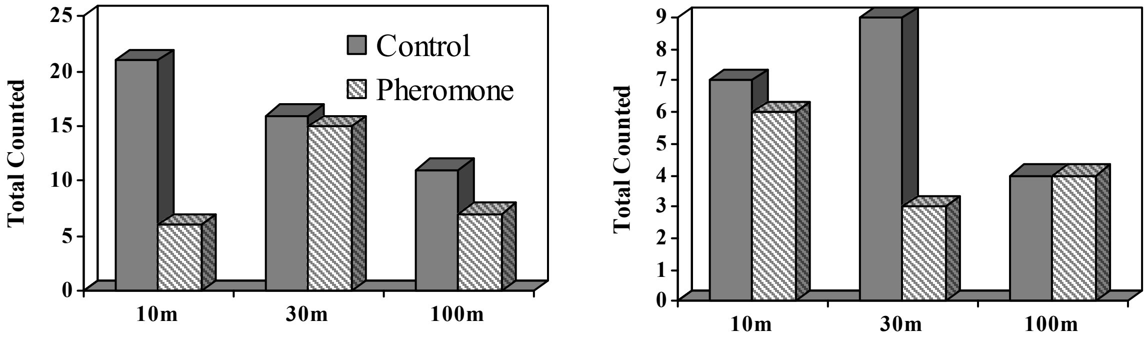
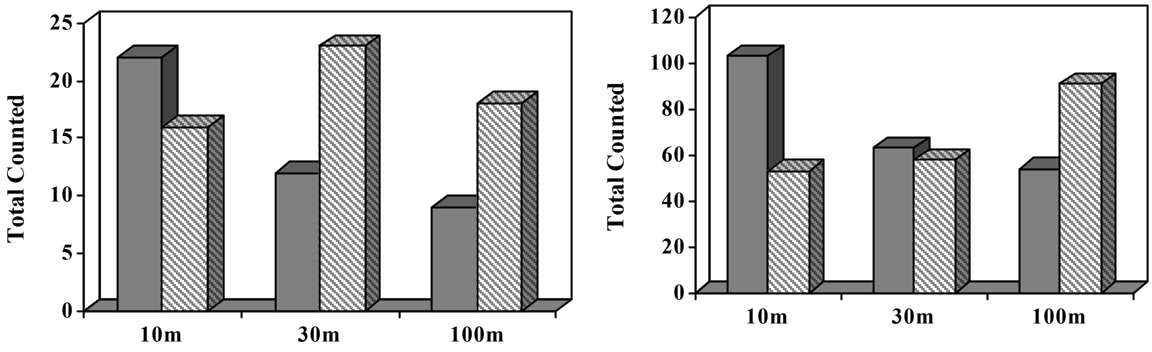


Figure 2.9. Effect of the aphid sex pheromone compound, nepetalactone, on the numbers of adult aphid parasitoids caught in cereal crops at 10m, 30m and 100m away from the field margin during the first two weeks after cereal aphid colonisation in 2002 at the four study sites. (Control fields – solid bars; Pheromone-treated fields – hatched bars).



(a) Colworth

(b) Radcot

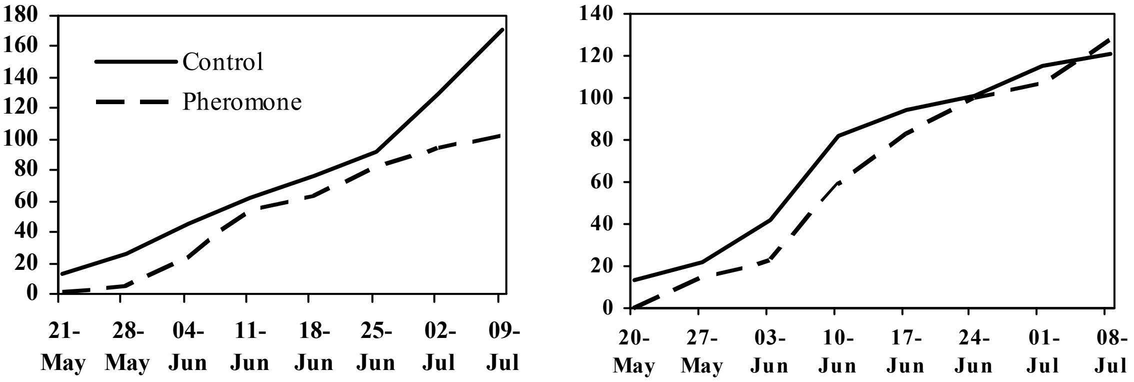


(c) Manor Farm

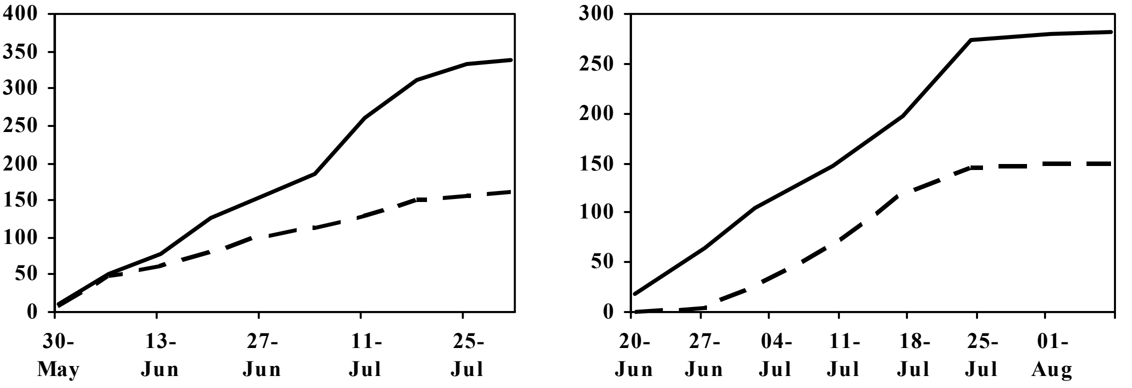
(d) West Fenton

At the two sites where the pheromone appeared to induce rapid movement of adult parasitoids into the crop during the early aphid colonisation period (West Fenton & Manor Farm), the cumulative numbers of aphids recorded in the crop through the season were significantly lower ($p<0.01$) in the pheromone-treated fields than in the control fields (Fig. 2.10c,d). At both sites, the total aphid count over the season was twice as great in the control fields as in the pheromone-treated fields, even though the aphid population remained low throughout the season. In contrast, at the Radcot site, where there were very few parasitoids and aphids and no obvious effect of the pheromone on early parasitoid distribution, there was no difference in the cumulative aphid numbers between the control and pheromone-treated fields (Fig. 2.10b). At the Colworth site, cumulative aphid numbers over the season where slightly lower in the pheromone-treated field compared with the control field, but the difference was not statistically significant (Fig. 2.10a) and there was no strong evidence of early effects on parasitoid distributions. When aphid populations during the first three weeks after colonisation are considered, there were consistently more aphids present in control fields than in pheromone-treated fields (p<0.01) across all sites (Fig. 2.11).

Figure 2.10. Effect of the aphid sex pheromone compound, nepetalactone, on the cumulative numbers of cereal aphids counted on 75 tillers per week in 2002 in pheromone-treated (dashed line) and control (solid line) fields at the four study sites. p<0.01 for Manor Farm and West Fenton



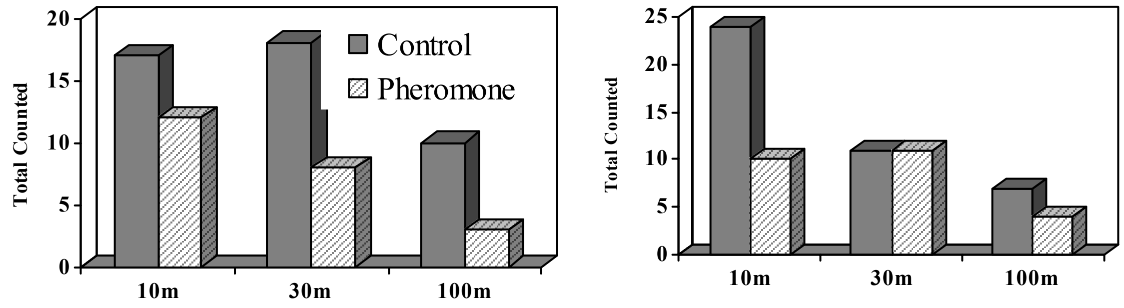
(a) Colworth **(b) Radcot**



(c) Manor Farm **(d) West Fenton**

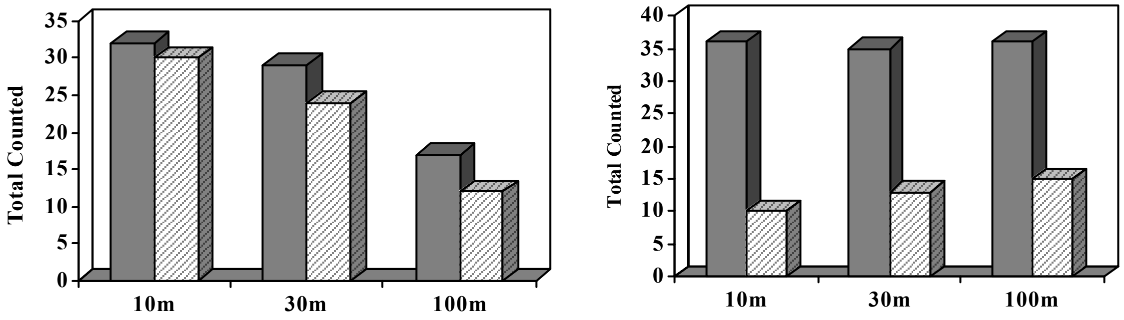
The greatest effect of the pheromone in 2002 appeared to occur at the Scottish site (West Fenton) where populations of both aphids and parasitoids were greater than at the other three sites. However, if the numbers of adult parasitoids caught in the suction net samples at West Fenton are viewed in isolation, it is obvious that more parasitoids were caught in the control field than in the pheromone-treated field, implying that the pheromone had a negative impact on parasitoid numbers (Fig. 2.12).

Figure 2.11. Effect of the aphid sex pheromone compound, nepetalactone, on the number of cereal aphids counted on 75 tillers during the first three weeks after aphid colonisation of control (solid bars) and pheromone-treated (hatched bars) fields in 2002 at the four study sites.



(a) Colworth

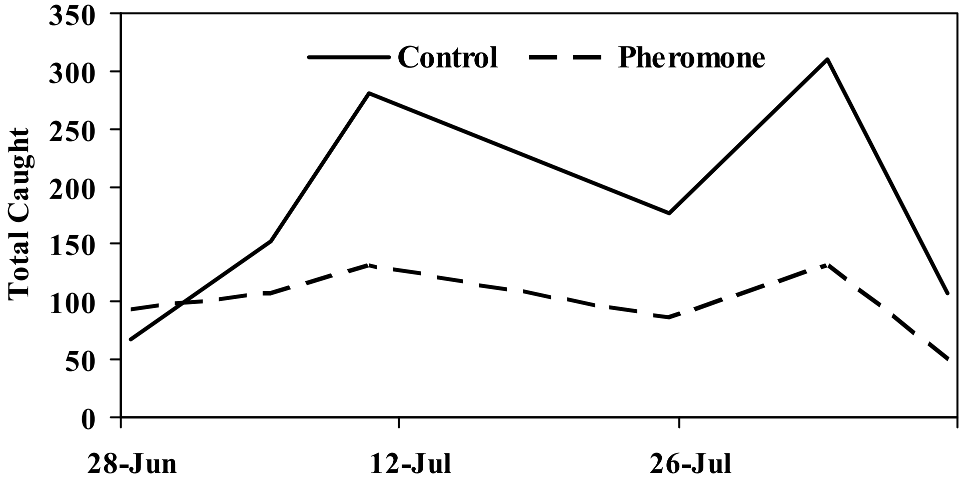
(b) Radcot



(c) Manor Farm

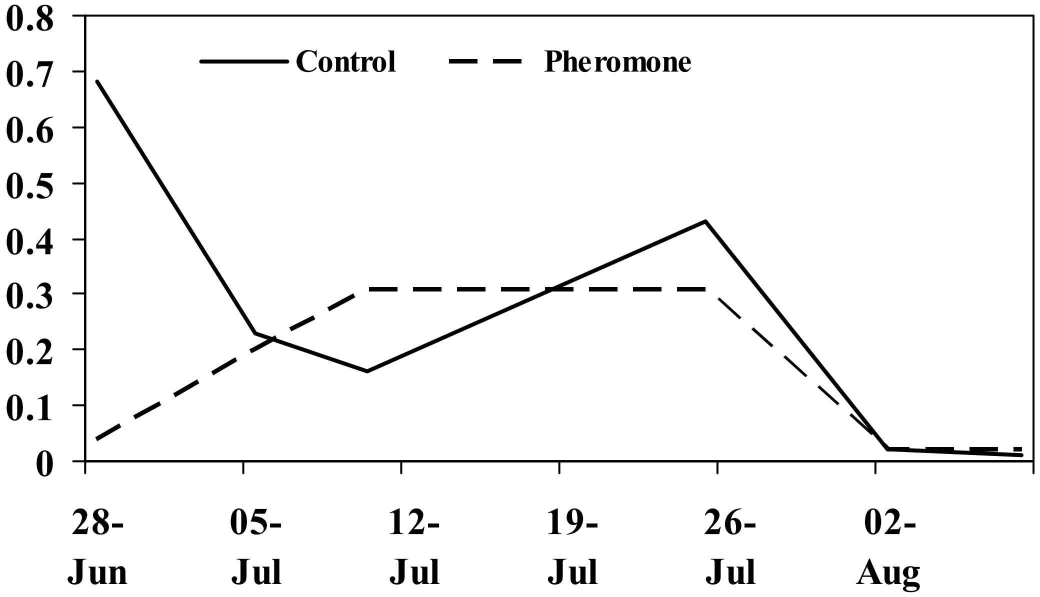
(d) West Fenton

Figure 2.12. Numbers of adult parasitoids caught in suction net samples at West Fenton in 2002 in the control (solid line) and pheromone-treated (dashed line) fields.



However, if the ratio of aphids recorded in the tiller counts to adult parasitoids caught in the suction net samples is considered, it is apparent that the ratios are very similar through most of the season, except at the beginning of the aphid infestation when there was a much more favourable ratio in the pheromone-treated field (Fig. 2.13).

Figure 2.13. Ratio of aphids recorded in tiller counts to adult parasitoids caught in suction net samples at West Fenton in 2002 in the control (solid line) and pheromone-treated (dashed line) fields.

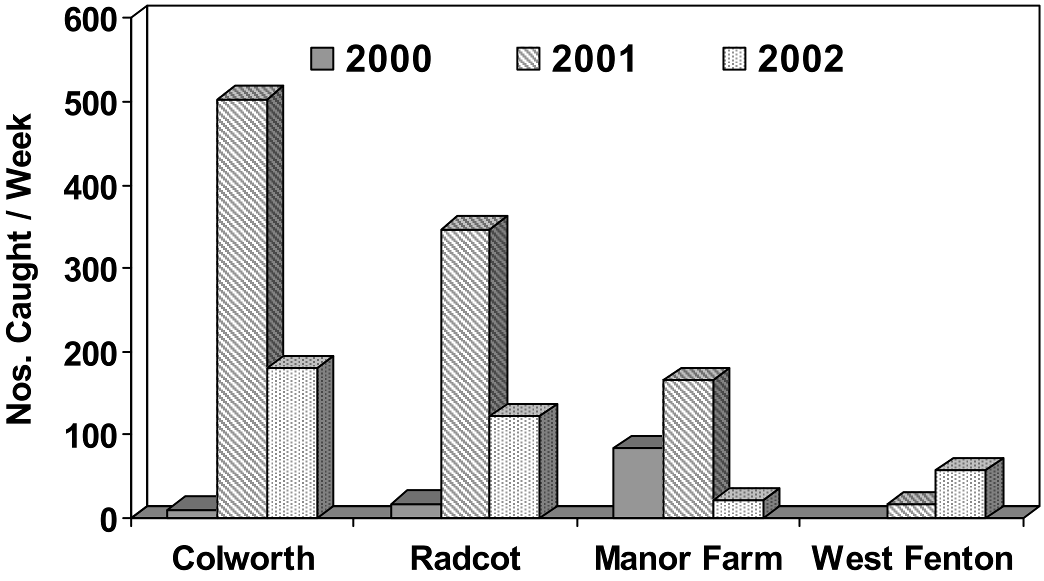


2.3.3. Hoverflies in Cereals

2.3.3.1. *Hoverfly population dynamics*

Hoverfly populations varied considerably between years, with low numbers of adults of aphidophagous species caught in the water traps in 2000 compared with very large numbers at all sites except West Fenton in 2001 (Fig. 2.14). Catches also varied between sites each year. In 2000, when traps were operated at the three English sites only, more were caught at the Yorkshire site (Manor Farm) than at the two more southerly sites (Colworth and Radcot). In contrast, in 2001 catches were very large at the two southern English sites but much smaller at the Scottish site (West Fenton), whilst in 2002, fewest were caught at Manor Farm.

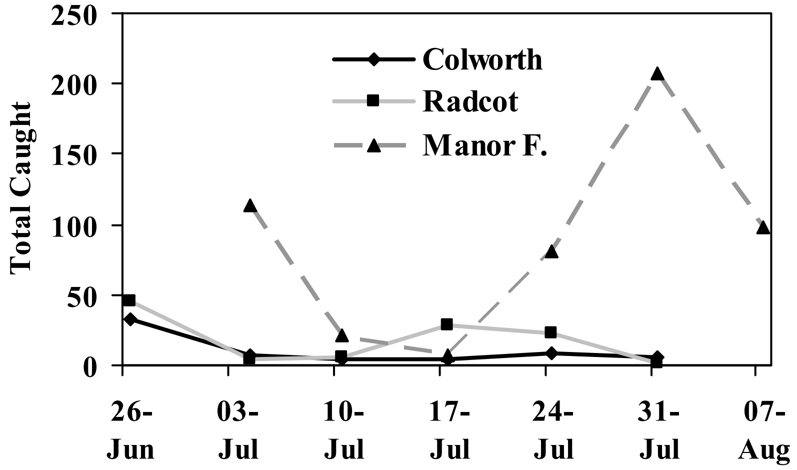
Figure 2.14. Mean number of adult aphidophagous hoverflies caught per trapping week in water traps placed within the cereal crop in control fields at the four sampling sites in 2000 (solid bars), 2001 (hatched bars) and 2002 (stippled bars) (water traps were not available at West Fenton in 2000).



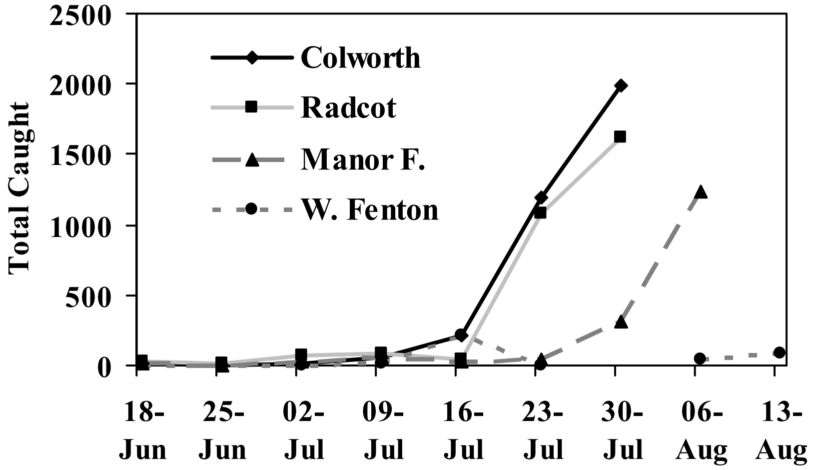
In 2001, the water trap catches of adult hoverflies began to increase dramatically in mid July at the sites in Bedfordshire (Colworth) and Oxfordshire (Radcot) and about a week later at the Yorkshire site (Manor Farm), but at the Scottish site (West Fenton) this dramatic increase in the catches did not occur (Fig. 2.15b). This increase in is almost certainly caused by the emergence of a new generation of adults arising from larvae that had bred on the summer aphid populations.

Figure 2.15. Numbers of adult aphidophagous hoverflies caught in weekly water trap samples within the cereal crop in control fields at the four sites. (a) 2000, (b) 2001, (c) 2002.

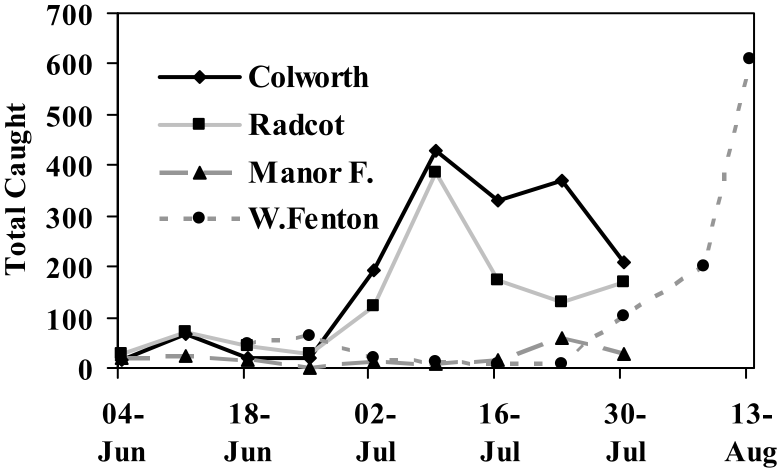
(a) 2000



(b) 2001



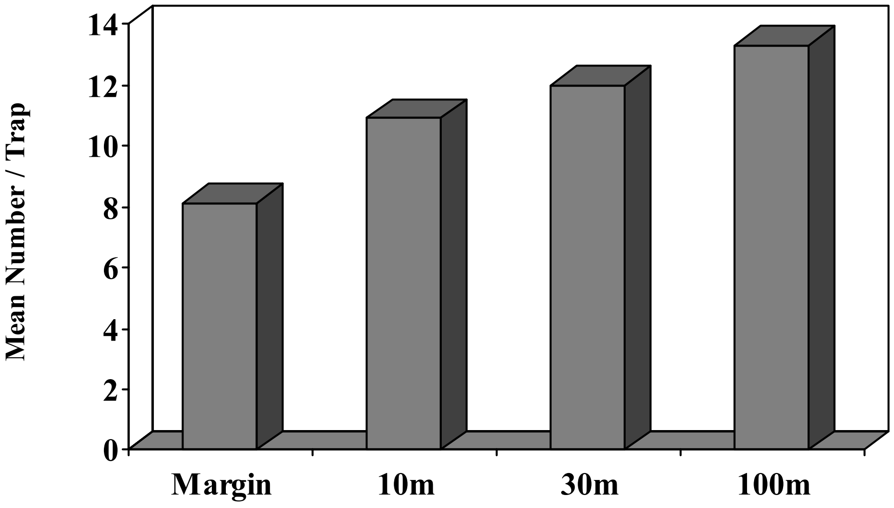
(c) 2002



A similar, obvious increase in numbers of adult aphidophagous hoverflies caught in the crop occurred at three of the four sites in 2002 (Fig. 2.15c). At the two southern English sites the increase began at the end of June, about three weeks earlier than in 2001, whilst at the Scottish site it began at the end of July, but at Manor farm in Yorkshire catches remained low until the beginning of August when sampling was terminated. In contrast, during the project establishment year of 2000, when water traps were run at the three English sites only, an obvious rise in numbers of adults caught within the crop only occurred at Manor Farm, in mid July (Fig. 2.15a)

Analysis of Variance of the 2001 water trap data revealed a highly significant ($p<0.001$) within field spatial affect on the distribution of adult hoverflies. The numbers caught increased with increasing distance from the field margin (Fig.2.16). There was also a highly significant ($p<0.001$) interaction between distance into the crop and field treatment due to this effect being most evident in the fields with a flower-rich margin. A highly significant ($p<0.001$) interaction between distance into the field and site reflected the absence of an obvious effect at the Scottish site, where numbers remained low throughout the season.

Figure 2.16. Abundance of adult aphidophagous hoverflies caught in water traps in field margins and at increasing distances into adjacent cereal crops in 2001. Data are for all sites and fields combined.



2.3.3.2. ***Hoverfly species abundance***

Twenty-five species of aphidophagous hoverflies were caught in water traps positioned in the 3 transects within the cereal crop in the control fields across the four study sites. The two most abundant species overall were *Episyrphus balteatus* and *Metasyrphus corollae* (Table 2.5). In 2001, when hoverflies were unusually abundant, *E. balteatus* dominated the catches, constituting more than 70% of all aphidophagous hoverflies caught at the three English sites (Table 2.5; Fig. 2.17). It is obvious from the data that this migratory species made a significant contribution to the increased hoverfly abundance in that year, as the combined numbers of the remaining aphidophagous species were similar in 2001 and 2002 at the three English sites and greater in 2002 than 2001 at West Fenton (Fig. 2.18). *Episyrphus balteatus* also constituted 59% of the aphidophagous hoverfly catch in the control field at Manor Farm in 2000 (Table 2.5). However, in 2002 *M. corollae* was the most abundant species caught in the crop, with *E. balteatus* constituting less than 20% of the catches at Radcot and West Fenton (Table 2.5)

Table 2.5. Aphidophagous hoverfly species that represent >20% of individuals caught in water traps within the cereal crop in control fields at the four study sites. C=Colworth; R=Radcot; MF=Manor Farm; WF=West Fenton

	2000			**2001**				**2002**			
Site	**C**	**R**	**MF**	**C**	**R**	**MF**	**WF**	**C**	**R**	**MF**	**WF**
No. Sample Weeks	6	6	6	7	7	10	8	9	9	10	9
Total No. caught	60	70	510	3187	2082	1119	92	1591	1142	198	1064
% Episyrphus balteatus	37	31	59	72	73	86	34	25		31	
% Metasyrphus corollae	28		29	26				33	54	32	68
% Platycheirus peltatus		27									
% Platycheirus manicatus										21	
% Melanostoma scalare							34				

Figure 2.17. Percentage of the marmalade hoverfly *Episyrphus balteatus* in water trap catches of adult aphidophagous hoverflies within the cereal crop in control fields at the four sampling sites in 2000 (solid bars), 2001 (hatched bars) and 2002 (stippled bars) (water traps were not available at West Fenton in 2000)

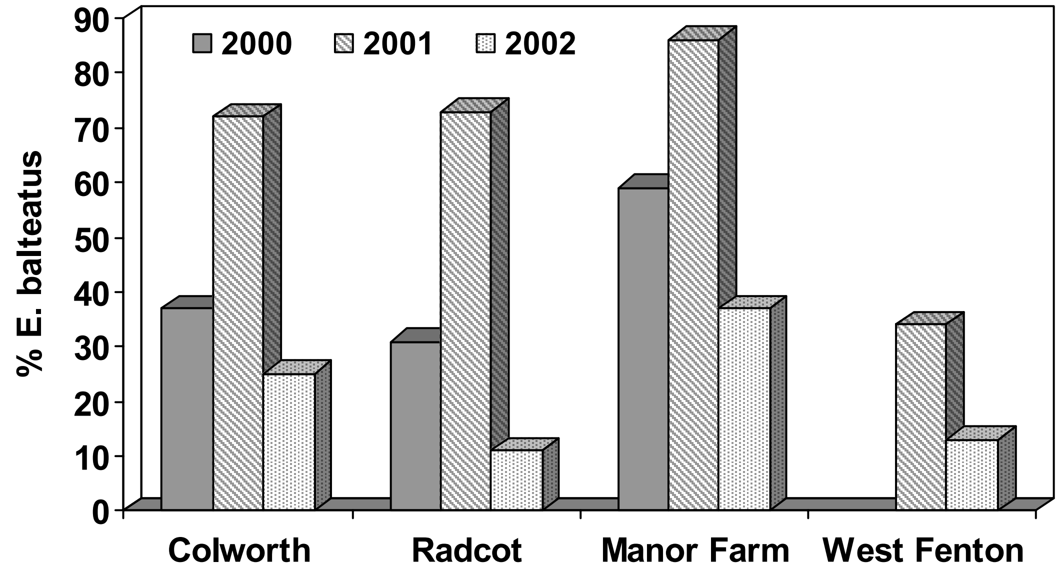
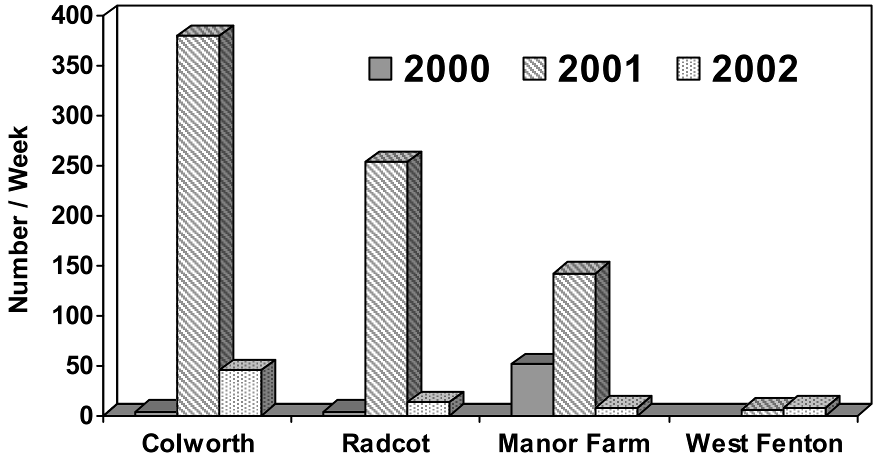
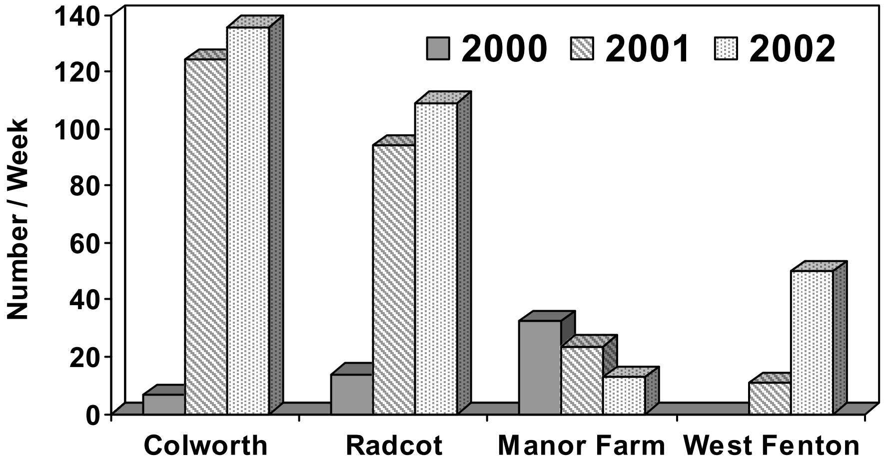


Figure 2.18. Mean number of adult (a) *Episyrphus balteatus* and (b) other aphidophagous hoverflies caught per trapping week in water traps placed within the cereal crop in control fields at the four sampling sites in 2000 (solid bars), 2001 (hatched bars) and 2002 (stippled bars) (water traps were not available at West Fenton in 2000).

(a) *Episyrphus balteatus*



(b) Other aphidophagous hoverflies



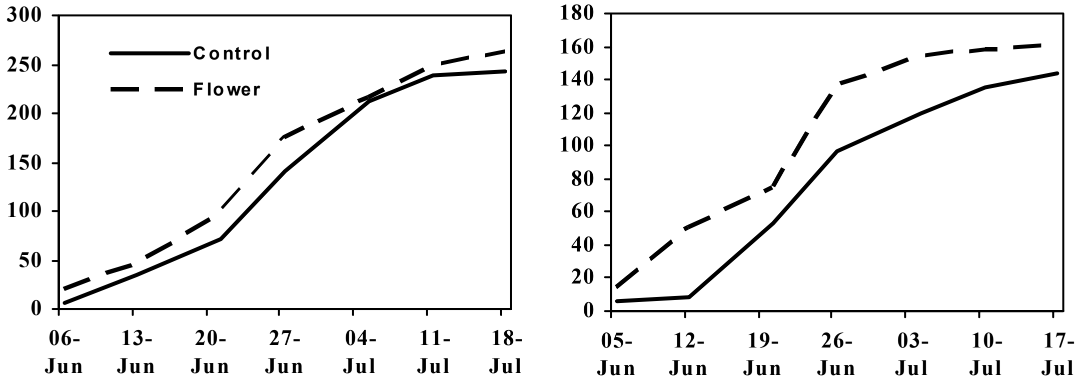
2.3.3.3. *Effect of flower margins*

One of the potential benefits of flower-rich field margins is the provision of nectar and pollen food resources for beneficial insects, including aphidophagous hoverflies. Such food resources should increase the fitness and reproductive capacity of adult female hoverflies, resulting in more effective control of aphids by hoverfly larvae on adjacent crops. In 2000, there were significantly fewer aphids in the field with a flower margin than in the control field at Manor Farm ($p<0.05$) but not at the other three sites (Fig. 2.19). Hoverfly activity was low in 2000 with comparatively few aphidophagous species being caught within the cereal crop, although they were much more abundant at Manor Farm (Yorkshire) than at the two southern sites of

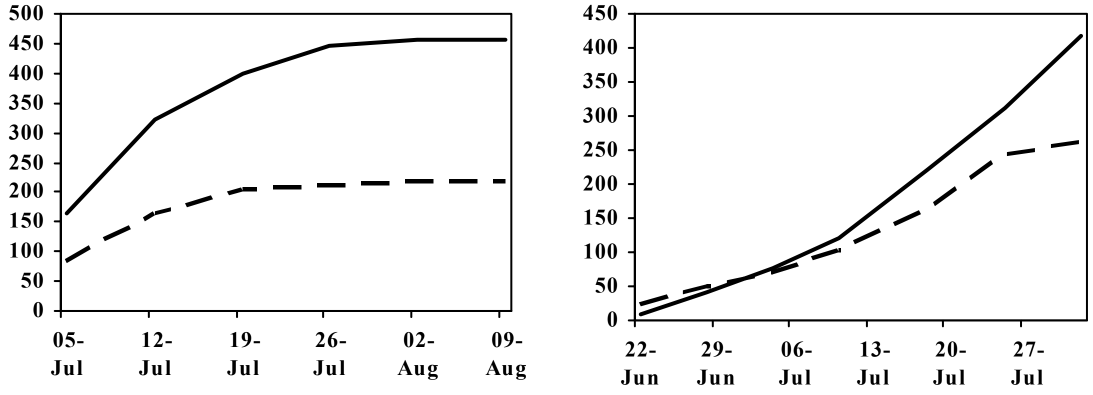
Colworth (Bedfordshire) and Radcot (Oxfordshire) (Table 2.5). Also, Manor Farm was the only site where there was a noticeable increase in the numbers of adult hoverflies caught in the crop transects later in the summer, suggesting active breeding had occurred within the crop (Fig.2.15a).

Figure 2.19. Effect of a flower-rich field margin on the cumulative numbers of cereal aphids counted on 75 tillers per week in 2000 at the four study sites (Control field – solid line; field with Flower Margin – dashed line). p<0.05 for Manor Farm

a) Colworth **(b) Radcot**

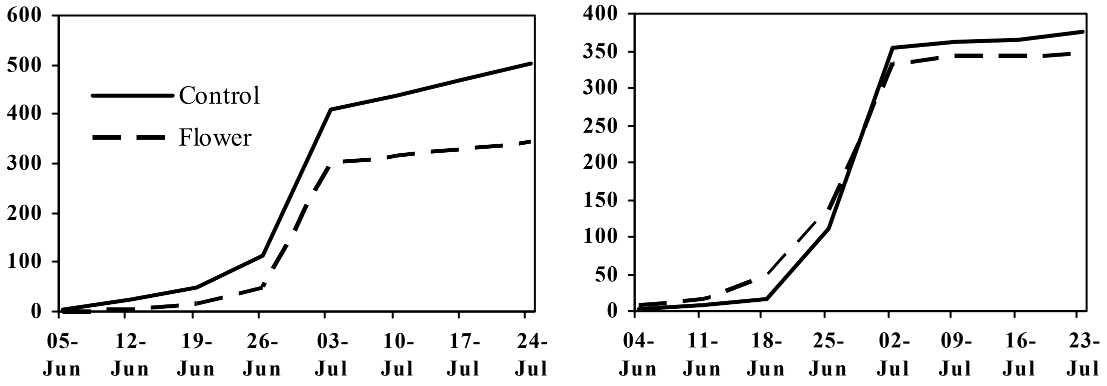


(c) Manor Farm **(d) West Fenton**

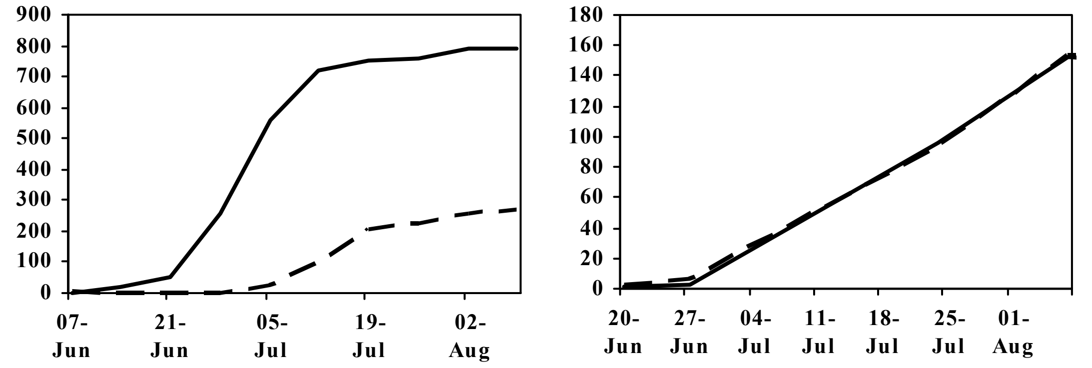


In 2001, when aphidophagous hoverflies were unusually abundant at the three English sites, there were fewer aphids in the field with the flower-rich margin than in the control field at Manor Farm (p<0.01) and, to a lesser extent, at Colworth (Fig. 2.20). However, there was no apparent effect at either Radcot or West Fenton. At West Fenton, far fewer aphidophagous hoverflies were caught in the crop compared with the three English sites (Table 2.5) and there was no increase in catches associated with significant breeding in the crop at the Scottish site (Fig. 2.15b), which could explain the lack of effects on aphid numbers.

Figure 2.20. Effect of a flower rich field margin on the cumulative numbers of cereal aphids counted on 75 tillers per week in 2001 at the four study sites (Control field – solid line; field with Flower Margin – dashed line). p<0.01 for Manor Farm

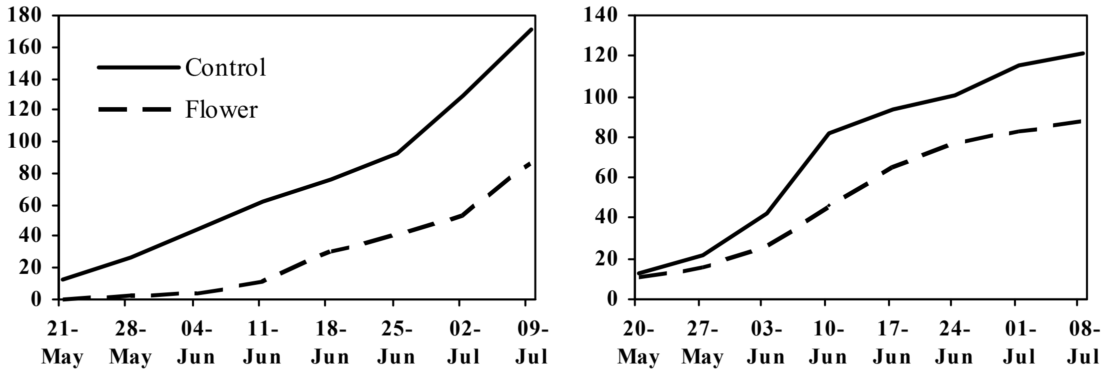


(a) Colworth **(b) Radcot**

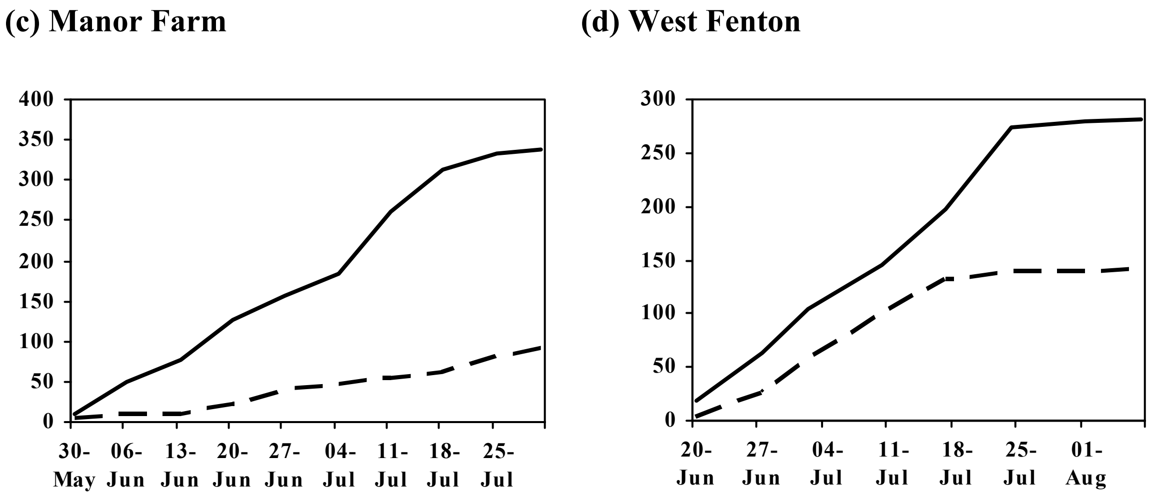


(c) Manor Farm **(d) West Fenton**

Figure 2.21. Effect of a flower-rich field margin on the cumulative numbers of cereal aphids counted on 75 tillers per week in 2002 at the four study sites (Control field – solid line; Field with Flower Margin – dashed line). p<0.001 for the combined site data.



(a) Colworth **(b) Radcot**

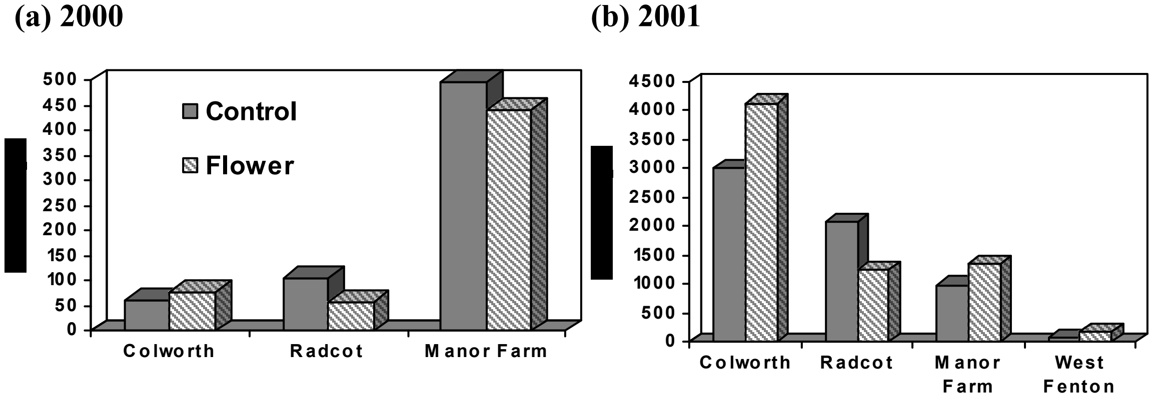


(c) Manor Farm

(d) West Fenton

In 2002, analysis of variance revealed a highly significant effect of treatment on aphid numbers (p<0.001). There were significantly fewer aphids recorded in the field with a flower-rich margin than in the control field at all four study sites (Fig. 2.21), even though at Manor Farm catches of aphidophagous hoverflies were small (Table 2.5) and there was no evidence of significant breeding within the crop as there was no increase in numbers of adults caught in late summer (Fig. 2.15c).

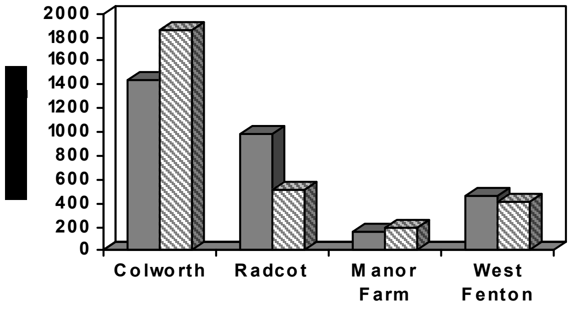
Figure 2.22. Numbers of adult aphidophagous hoverflies caught in water traps placed in the cereal crop in control fields (solid bars) and fields with a flower-rich margin (hatched bars).



(a) 2000

(b) 2001

(c) 2002

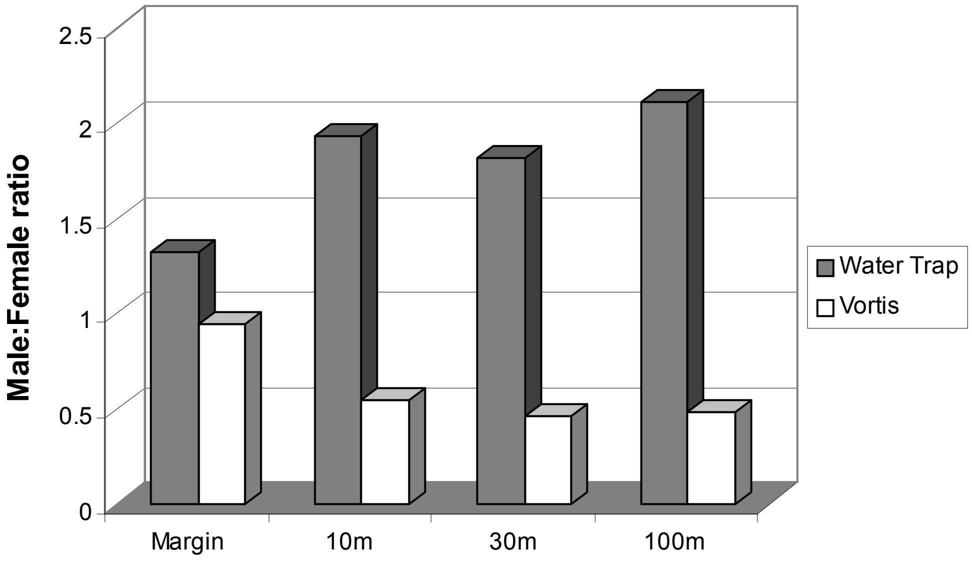


Although aphid populations were significantly reduced by the presence of a flower-rich field margin in seven site-years out of twelve, and on no occasion were there significantly fewer aphids in control fields than in those with flower margins, the numbers of adult aphidophagous hoverflies caught in traps within the crop did not differ greatly between the two fields in any site year, including at Manor Farm where the biggest effects on aphid populations occurred (Fig. 2.22).

2.3.3.4. ***Sampling methods and hoverfly sex ratio***

For aphidophagous hoverflies to be useful as a biological control agent, it is essential that the females travel into the crop to lay their eggs near aphid colonies. Therefore, the observation from the preliminary work in 2000, that many more males than females were being captured in the water traps in the crop required further investigation. In 2001, when there were very high numbers of *E. balteatus* in the crop (Fig. 2.15b) the opportunity arose to compare the sex ratio of the hoverflies in the water traps in the crop with that from the Vortis suction samples. Figure 2.23 shows that in the field margin the sex ratio of aphidophagous hoverflies trapped from both sampling methods was around 1:1. However, in the samples from within the crop a big difference is apparent, with the water traps showing a bias towards males of 1.8:1 to 2:1 and the suction samplers showing a bias towards females with ratios of around 0.5:1.

Figure 2.23. Sex ratio of adult aphidophagous hoverflies caught in water traps and in the Vortis suction samples in field margins and at increasing distances into adjacent cereal crops in 2001. Data are for Colworth and Radcot, all dates and fields combined.



Laboratory experiments were conducted to test the hypothesis that searching gravid female hoverflies were not as strongly attracted to flowers (and therefore coloured water traps) as equivalent aged males, potentially explaining the different sex ratios produced by the two trap types. Equal numbers (20) of two day old adults were starved for four hours and released individually into a laboratory flight cage containing the standard coloured water trap used in the field experiments. Each hoverfly was observed continuously for 30 minutes and the number of visits to the trap was recorded. A visit was defined as landing on the trap. The experiment was repeated using 12 day old adult hoverflies. Data were subjected to analysis of variance.

No significant difference was recorded between the number of visits to the water trap by two day old females (which were searching for flowers as pollen and nectar sources) and males (Table 2.6). However, twelve day old females (searching for egg laying sites) made significantly (P<0.05) fewer visits to the coloured traps than equivalent aged males.

Table 2.6. Mean (± Standard Error) number of visits by two and twelve day old male and female *E. balteatus* to standard yellow water traps during a half-hour exposure in laboratory flight cages.

Treatment	N	Mean	SE
2 Day/Male	20	8.9	2.1
2 Day/Female	20	9.2	2.0
12 Day/Male	20	7.1	2.2
12 Day/Female	20	0.9	0.3

2.3.4. Carabid Beetles in Cereals

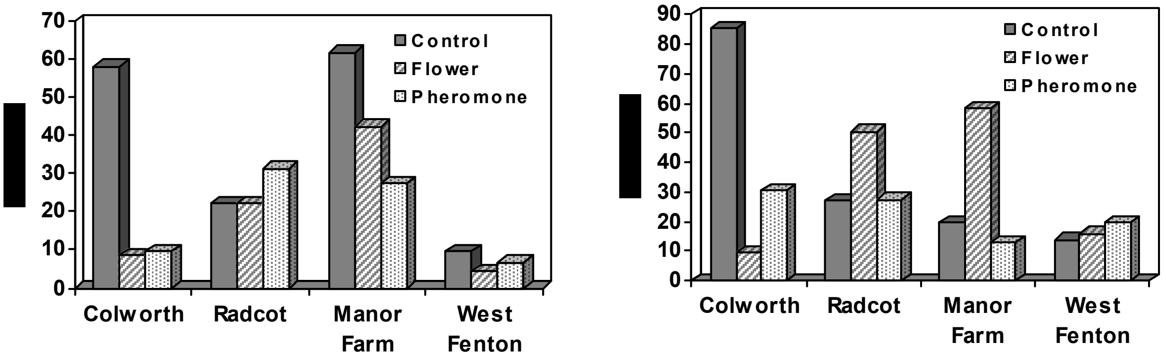
Although the target groups for manipulation in this part of the project were aphid parasitoids and hoverflies, carabid beetles are an important component of the natural enemy community affecting aphid populations and are known to be influenced by field margins. Therefore, it was important to monitor carabids in case they were also affected by the treatments aimed at the two target groups. This was essential in order to adequately interpret any recorded effects of treatments on aphid populations.

2.3.4.1. *Carabid abundance*

At all sites, overall carabid abundance in pitfall trap samples varied dramatically amongst the different fields sampled, independent of treatments (Figs. 2.24 & 2.25). It must be remembered that the relative abundance of species caught in pitfall traps does not indicate the actual abundance of species present in the field. This is because a much greater proportion of large active species are caught compared with smaller, often very abundant, species that have much smaller areas of activity. The data for the traps situated in the crop itself (Fig. 2.24) actually reflect the abundance of a few *Pterostichus* species, which tend to dominate pitfall catches in arable fields, due to their high levels of activity. For example, at the two southern English sites, 13,015 and 12,487 carabid beetles, respectively, were caught in pitfall traps in the three study fields during 2000. These consisted of 32 species at Colworth, of which three *Pterostichus* species formed 74% of the catch, and 35 species at Radcot, of which three *Pterostichus* species formed 86% of the catch. These large *Pterostichus* species, which breed within the field, were not significantly affected by the field margins, forming the same percentage of the catch in the margin traps as in the crop itself.

However, the relative abundance of carabids caught in pitfalls in the three fields at any one site was often different in the crop area and in the margin (compare Figs. 2.24 & 2.25). This indicates that species other than the dominant *Pterostichus* species were differentially affected by the treatments. Using the data for the Radcot site in 2001 as an example, it can be seen that the pattern of catches through the season within the cereal crop itself was very similar for the total carabid populations of the three fields and for the populations of the large *Pterostichus* species; catches were consistently higher in the field with the flower margin than in the other two (Fig. 2.26). However, the catches show a different pattern if the *Pterostichus* species are omitted, with catches now being highest in the pheromone treated field in the early part of the season (Fig. 2.27). These catches are now dominated by *Harpalus rufipes* and the pattern of catches for this species alone is very similar to that of the total catch excluding *Pterostichus* (Fig. 2.27).

Figure 2.24. Mean number of carabid beetles caught per pitfall trap in cereal crops at the four study sites over the summer aphid season in 2000, 2001 and 2002 (Control Fields – solid bars; Fields with Flower-rich Margin – hatched bars; Pheromone-treated Fields – stippled bars). Note: The pheromone treatment had not yet been applied in summer 2000.



(a) 2000 **(b) 2001**

(c) 2002

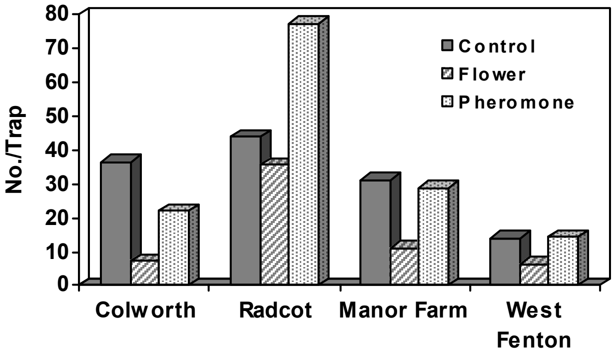
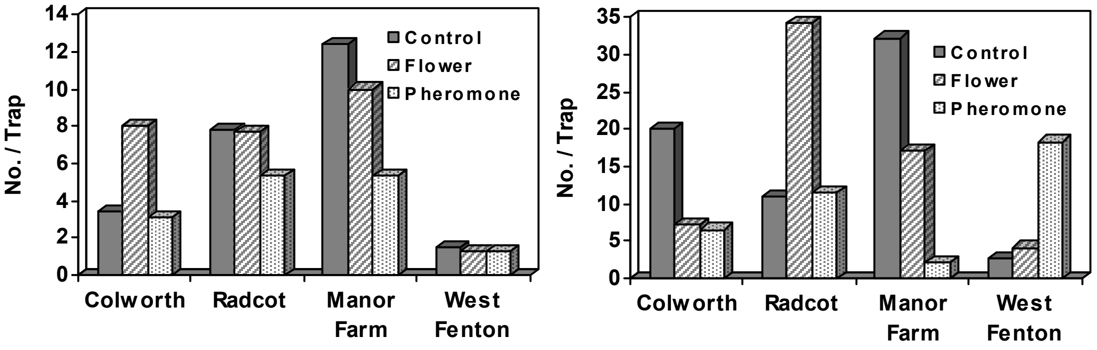


Figure 2.25. Mean number of carabid beetles caught per pitfall trap in cereal crop margins at the four study sites over the summer aphid season in 2000, 2001 and 2002 (Control Fields – solid bars; Fields with Flower-rich Margin – hatched bars; Pheromone-treated Fields – stippled bars). Note: The pheromone treatment had not yet been applied in summer 2000.

(a) 2000 **(b) 2001**



(c) 2002

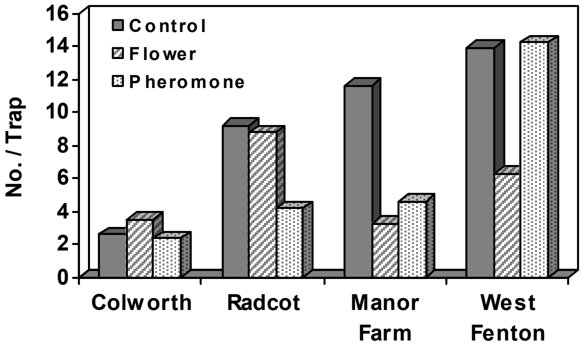
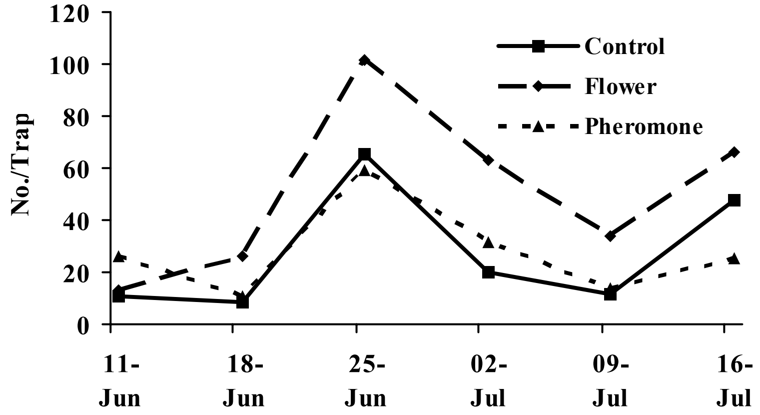


Figure 2.26. Mean number of carabid beetles caught per pitfall trap in cereal crops in the three study fields at Radcot in 2001. (a) Total carabids; (b) *Pterostichus* species only. Control Fields – solid line; Fields with Flower-rich Margin – dashed line; Pheromone-treated Fields – dotted line.

(a) Total



(b) *Pterostichus* spp.

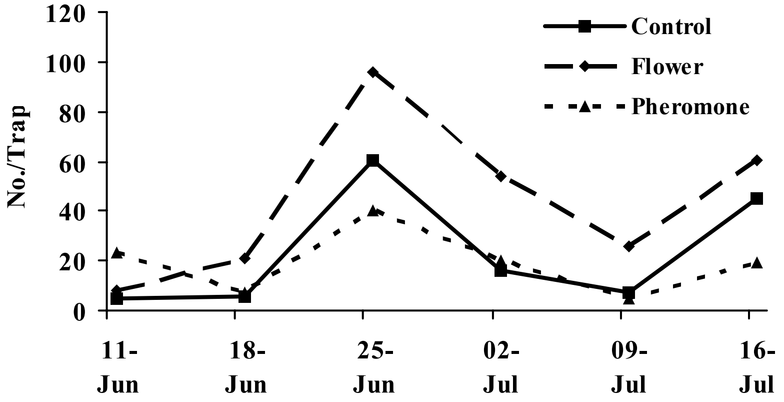
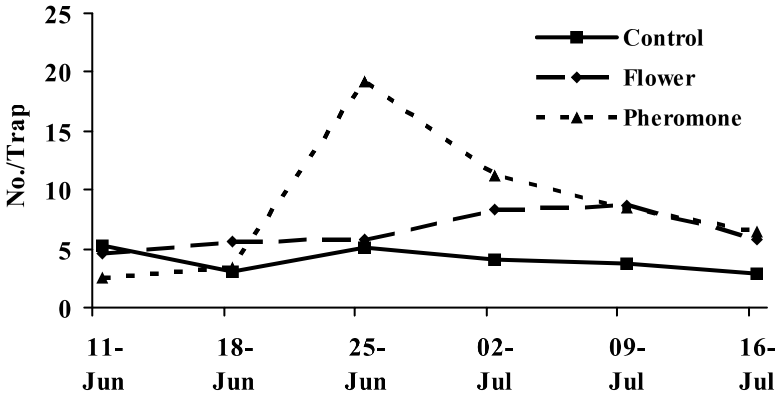
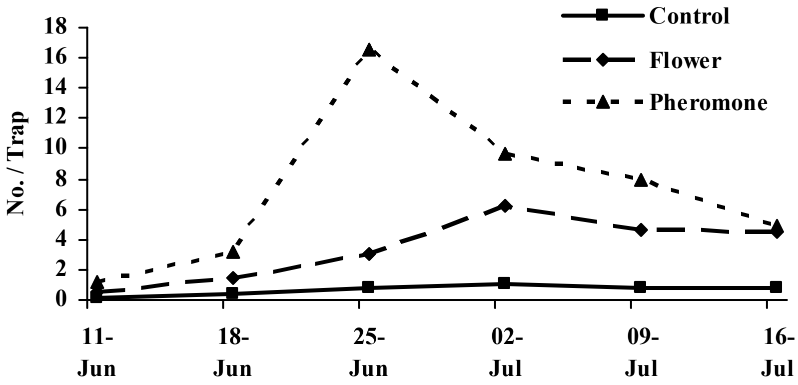


Figure 2.27. Mean number of carabid beetles caught per pitfall trap in cereal crops in the three study fields at Radcot in 2001. (a) Total carabids excluding *Pterostichus* species; (b) *Harpalus rufipes* only. Control Fields – solid line; Fields with Flower-rich Margin – dashed line; Pheromone-treated Fields – dotted line.

(a) Total excluding *Pterostichus* spp.



(b) *Harpalus rufipes*



2.3.4.2. *Effect of aphid sex pheromone on* Harpalus rufipes

When catches of the carabid beetle *Harpalus rufipes* are considered for all the site/years in which the aphid sex pheromone, nepetalactone, was deployed in the crop, many more were caught in the pheromone-treated field than in the control field in five out of the nine occasions (Fig. 2.28). On three of the other four occasions, very low numbers of this species were caught in all fields making any treatment effects impossible to detect. Electrophysiological experiments indicated that *H. rufipes* could physiologically detect the pheromone, and so in 2003 the *H. rufipes* data from the only cereal site (Colworth) used that year were examined in more detail. The beetles caught were sexed and the proportion of males in the catches compared for the three fields. Catches from the pheromone-treated field consistently contained a higher proportion of males than catches from the other two fields (Fig. 2.29) and this difference was statistically significant ($p<0.001$).

Figure 2.28. Numbers of the carabid beetle *Harpalus rufipes* caught in pitfall traps in cereal crops in pheromone-treated (hatched bars) and control fields (solid bars) in all site/years when the pheromone was deployed. Data standardised as number caught per trap per week. (Col=Colworth; Rad=Radcot; M.F.=Manor Farm; W.F.=West Fenton)

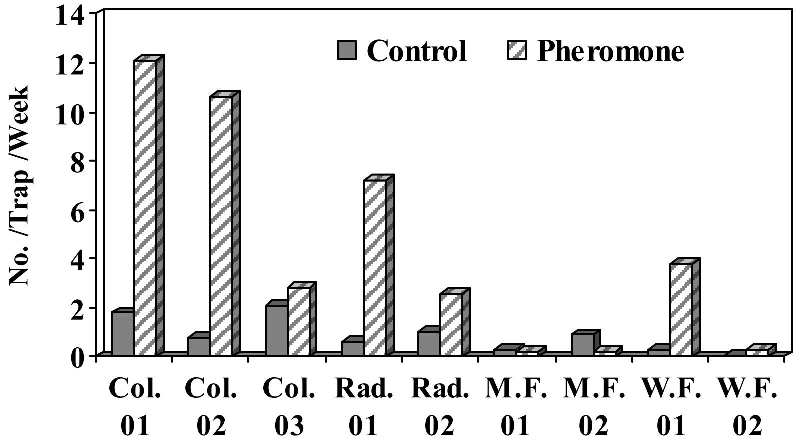
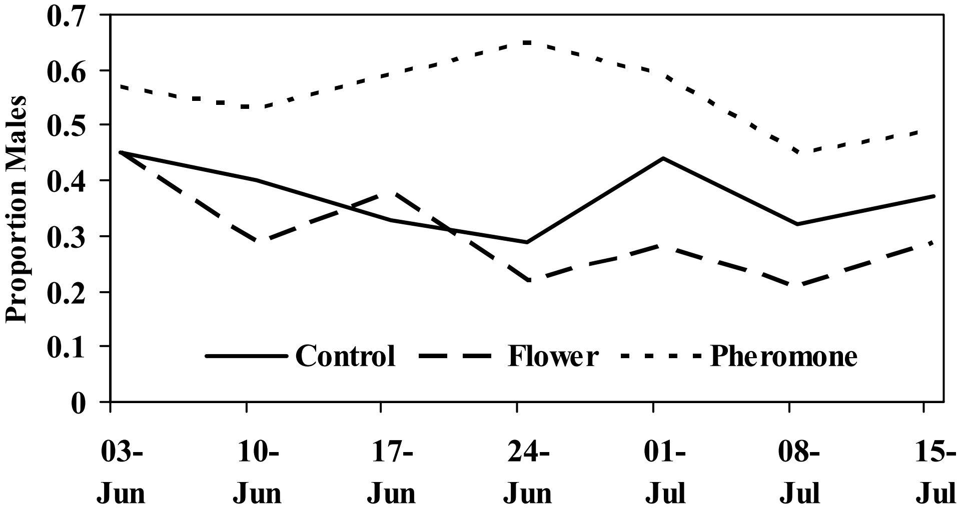


Figure 2.29. Proportion of males in pitfall trap catches of the carabid beetle *Harpalus rufipes* in cereal crops in the pheromone-treated field (dotted line), the field with a flower margin (dashed line) and the control field (solid line) at the Colworth site in 2003.



2.3.5. Non-Cereal Sites

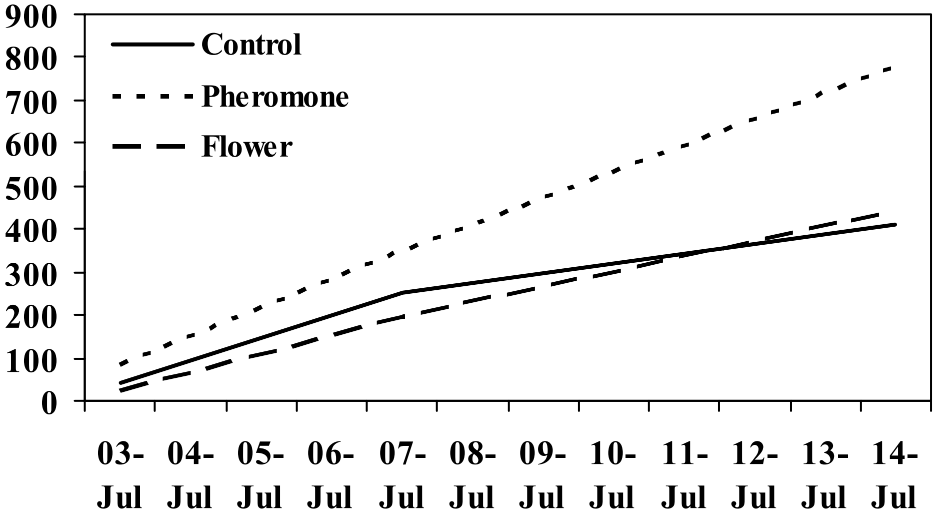
In the final year, 2003, pilot trials were done at four sites to explore the possibilities and identify the difficulties of adapting the hoverfly and parasitoid manipulation approaches, initially developed for cereal aphid control, for use in horticultural field crops. These trials involved vining pea crops at two sites, in East Lothian and Cambridgeshire, an organic broccoli crop in Yorkshire and an organic lettuce crop in Cambridgeshire.

2.3.5.1. *Vining peas*

At the East Lothian site (Drem) separate fields were not available for the three treatments, which therefore were established along three different sides of a single very large field, one side of which was bordered by a flower-rich margin alongside a stream.

Pea aphid numbers increased rapidly from late June until mid-July when the farmer applied an aphicide (Aphox) on 18th July, after which no aphids were recorded in the weekly plant counts. The presence of the flower-rich margin appeared to have little effect on aphids in the adjacent crop area (Fig. 2.30). However, more aphids were recorded in the area where the pheromones were deployed than in the control area (Fig. 2.30).

Figure 2.30. Cumulative numbers of pea aphids counted on 75 plants in three sample areas within a single large pea field at Drem, East Lothian in 2003. One sample area bordered a flower-rich field margin (dashed line), one area was treated with aphid sex pheromone lures (dotted line) and the third acted as a control area (solid line). An aphicide was applied by the farmer on 18th July.



Aphid parasitoids at Drem were dominated by *Aphidius ervi*, which comprised more than 90% of the individuals caught. The pea aphid is regarded as the main host of this parasitoid, although it attacks a range of other species, including cereal aphids. There were no significant differences amongst the three treatment areas in the numbers of adult aphid parasitoids caught in suction samples during the aphid infestation period (Fig. 2.31).

Figure 2.31. Numbers of adult aphid parasitoids in suction net samples taken from three treatment areas in a pea crop at Drem, East Lothian in 2003. (Control Area – solid bars; Area with Flower-rich Margin – hatched bars; Pheromone-treated Area – stippled bars).

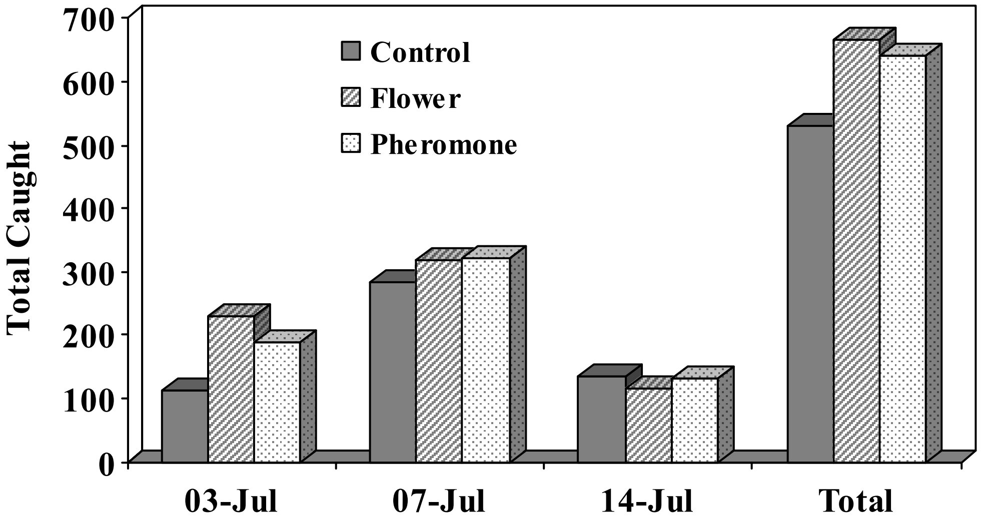
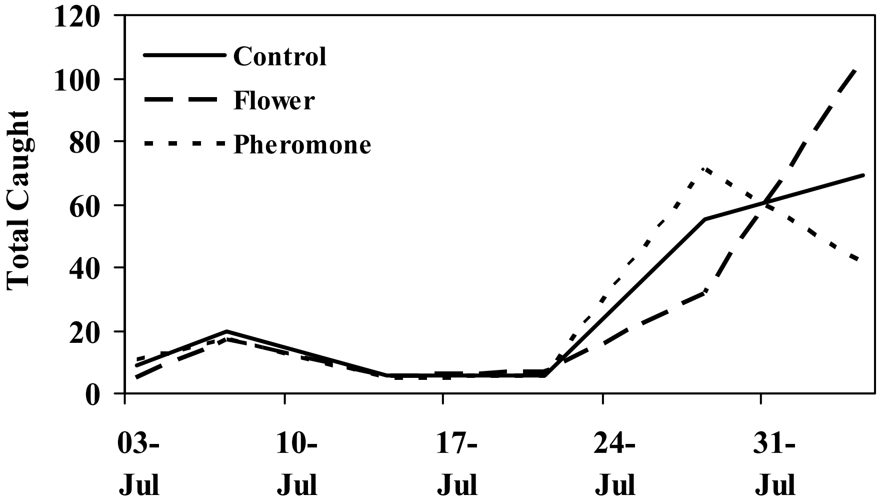
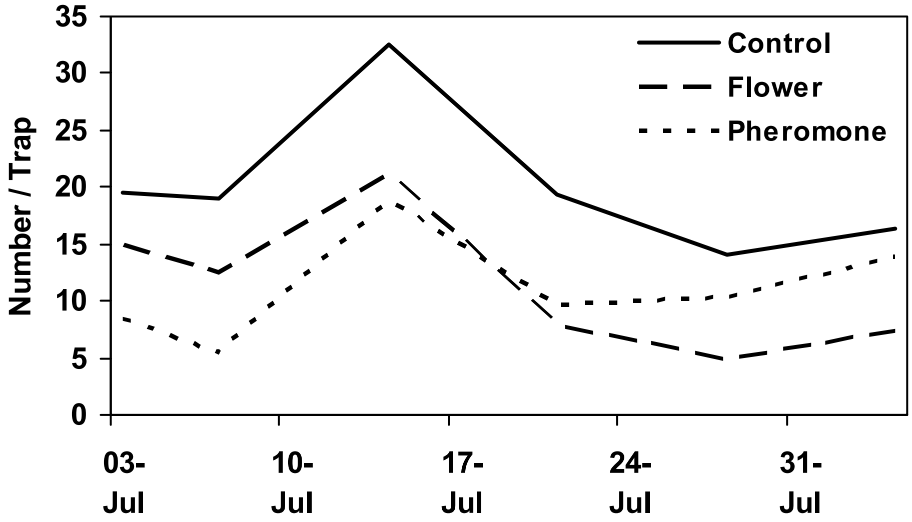


Figure 2.32. Adult aphidophagous hoverflies caught in water traps placed in three treatment areas in a pea crop at Drem, East Lothian in 2003. (Control Area – solid line; Area with Flower-rich Margin – dashed line; Pheromone-treated Area – dotted line).



There were no significant differences between the total numbers of adult aphidophagous hoverflies caught in water traps placed in the crop in the three treatment areas: control-165, adjacent to flower margin-173, pheromone-treated-151. The majority were caught in late July/early August and probably represent second generation adults that had developed as larvae feeding on the aphids in the crop (Fig. 2.32). The aphids were killed by a pirimicarb (Aphox) application on 18th July, so most of these hoverflies must have reached the pupal stage by that time. Hoverfly pupae can be a problem contaminant in pea crops because their size and shape hinder automatic sorting of contaminants in harvested peas.

Figure 2.33. Carabid beetles caught in pitfall traps placed in three treatment areas in a pea crop at Drem, East Lothian in 2003. (Control Area – solid line; Area with Flower-rich Margin – dashed line; Pheromone-treated Area – dotted line).



Pitfall traps placed in the pea crop caught more carabid beetles in the control area than in the area adjacent to the flower-rich margin and the pheromone-treated area, especially in the period before the crop was treated with an aphicide (18th July) when twice as many were caught in the control area compared to the pheromone-treated area (Fig. 2.33). The catch was very much dominated by *Pterostichus melanarius*, which formed 94% of the beetles caught before the aphicide application.

At the Cambridgeshire site (Royston) an additional, reduced, pea trial was carried out in a single large field. Two sample areas were set up alongside a flower-rich margin, one of which was treated with aphid sex pheromone lures and the other acted as a control. Pea aphids increased more rapidly in the area treated with pheromones, particularly in the first two weeks of June when there were significantly ($p<0.05$) more aphids in the pheromone-treated area (Fig. 2.34).

Figure 2.34. Cumulative numbers of pea aphids counted in beating tray samples taken in two sample areas within a single large pea field at Royston, Cambridgeshire in 2003. Both sample areas bordered a flower-rich field margin. One area was treated with aphid sex pheromone lures (dotted line) and the other acted as a control area (solid line).

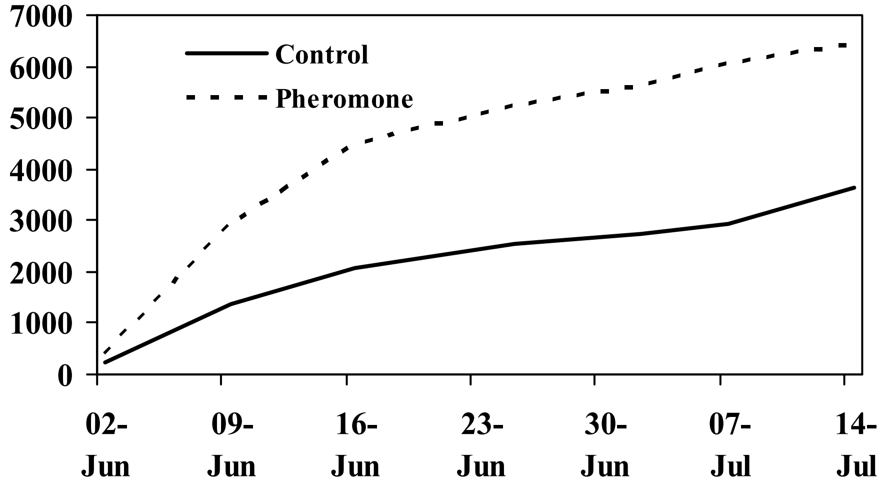
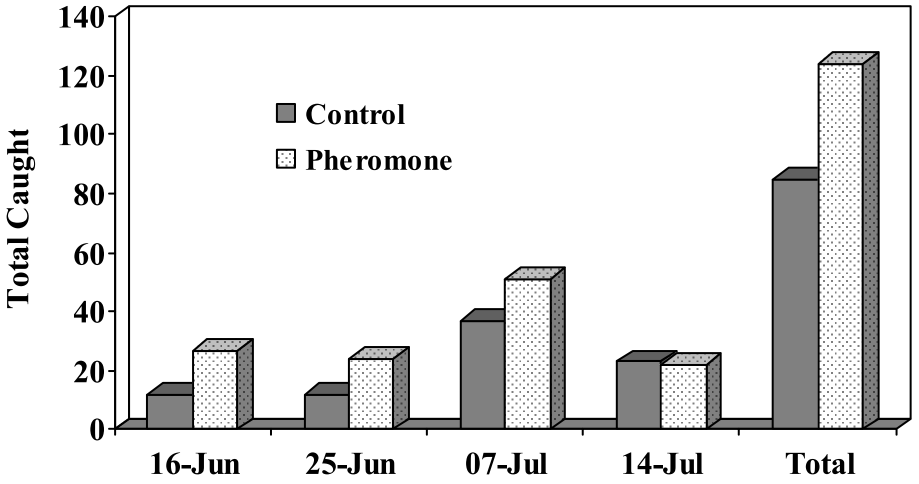
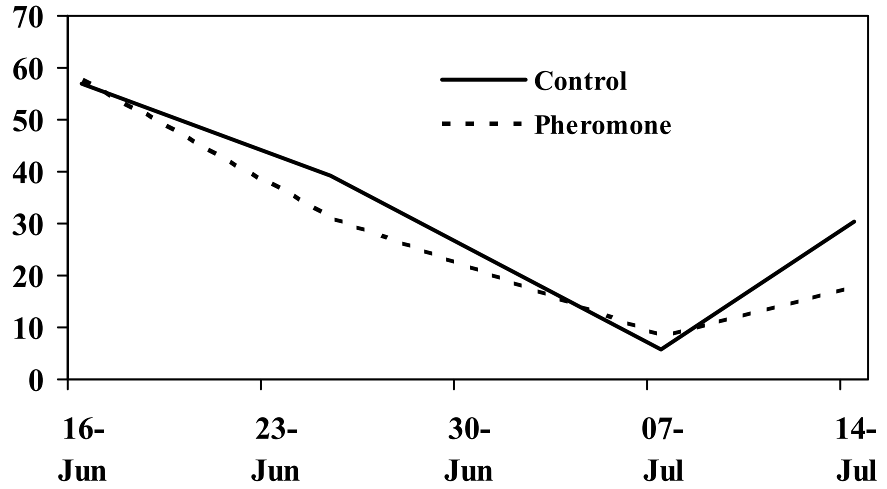


Figure 2.35. Numbers of adult aphid parasitoids in suction net samples taken from two treatment areas in a pea crop at Royston, Cambridgeshire in 2003. (Control Area – solid bars; Pheromone-treated Area – stippled bars). Both areas were bordered on one side by a flower-rich margin.



More adult aphid parasitoids were caught in the pheromone-treated area than in the control area, especially at the beginning of the sampling period (Fig. 2.35), but the ratios of aphids:parasitoids in the respective samples were almost identical in the pheromone-treated and control areas, indicating that the slightly greater numbers caught in the pheromone-treated area was a response to the greater aphid numbers rather than a response to the pheromone (Fig. 2.36). At this site, the dominant parasitoid species was *Aphidius eadyi*, a species that has only ever been recorded from pea aphids and so appears to be a specialist on this host. This species formed 80% of the total catch at Royston.

Figure 2.36. Ratio of aphids recorded in plant counts to adult parasitoids caught in suction net samples in control (solid line) and pheromone-treated (dashed line) areas of a pea crop at Rotston, Cambridgeshire in 2003. Both areas were bordered on one side by a flower-rich margin.



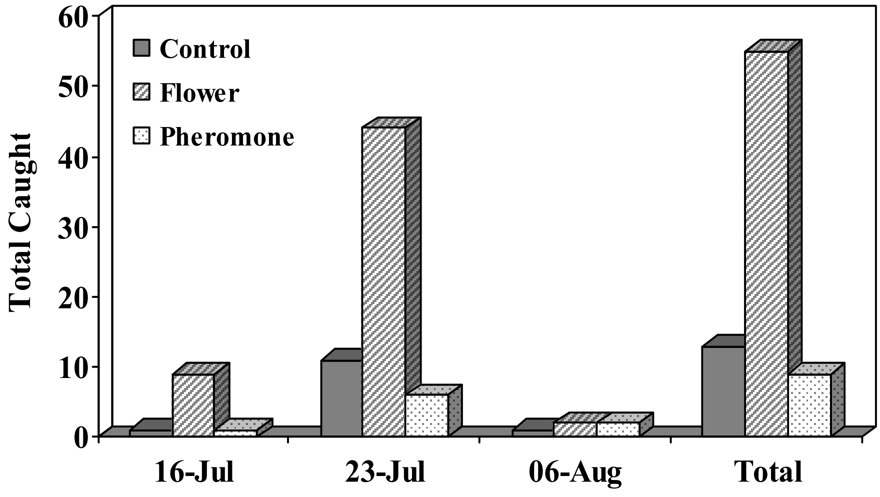
In this additional, reduced trial, hoverflies and carabid beetles were not monitored due to lack of resources, which were concentrated on the main trial sites.

2.3.5.2. ***Organic broccoli***

At the organic broccoli site in Yorkshire (Epworth) two fields were used for the trial, one of which had a flower-rich border alongside a hedgerow and road. The pheromone lures were deployed at one end of a second field and the opposite end of this field was used as the control area.

Aphids were counted on one large leaf from each of 25 plants along each of the three sampling transects (10m, 30m, 100m from margin) weekly. At the first count on 16th July, there were almost twice as many aphids in the control treatment (4.0 per sample leaf) than alongside the flower-rich margin (2.3 per leaf), with intermediate numbers in the pheromone treatment (3.1 per leaf). Both the peach-potato aphid, *Myzus persicae*, and the cabbage aphid, *Brevicoryne brassicae*, were present but the latter species formed only 10% of the aphids sampled. After the first sample, the crop was treated with soap every 7-10 days, which greatly reduced the aphids in all three sample areas.

Figure 2.37. Adult aphid parasitoids caught in vortis suction samples in organic broccoli crops at Epworth, Yorkshire in 2003. The pheromone-treated area (stippled bars) and control areas (solid bars) were at opposite sides of the same field but the flower-rich margin treatment (hatched bars) was in a separate field.



The adult aphid parasitoid catches were dominated by *Diaeretiella rapae* (86% of the total catch), a species that specialises in attacking aphids on brassicaceous plants. Significantly more ($p<0.01$) parasitoids were caught in the sample area next to the flower-rich margin than in the control and pheromone-treated sample areas, especially early in the sampling period (Fig. 2.37). However, the flower margin treatment was in a different field from the control and pheromone treatments. There was no significant difference in the numbers of adult aphid parasitoids caught in the control and pheromone-treated areas (Fig. 2.37). The abundance of parasitoids in the broccoli next to the flower-rich margin is also reflected in the numbers of parasitized aphids (mummies) present on the plants in this area before the soap solutions were applied (Fig. 2.38).

Figure 2.38. Numbers of parasitized aphids (mummies) counted on leaves from 75 broccoli plants (one leaf per plant) during aphid assessments at Epworth, Yorkshire in 2003. The pheromone-treated area (stippled bars) and control areas (solid bars) were at opposite sides of the same field but the flower-rich margin treatment (hatched bars) was in a separate field.

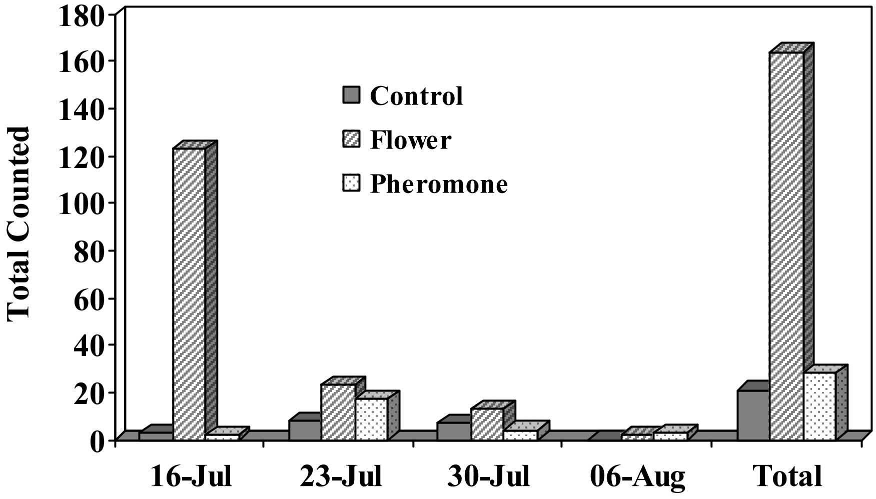
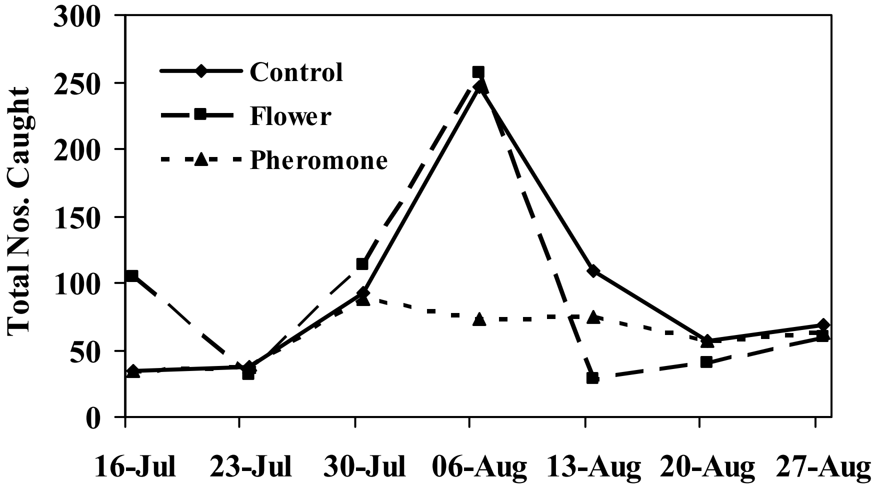


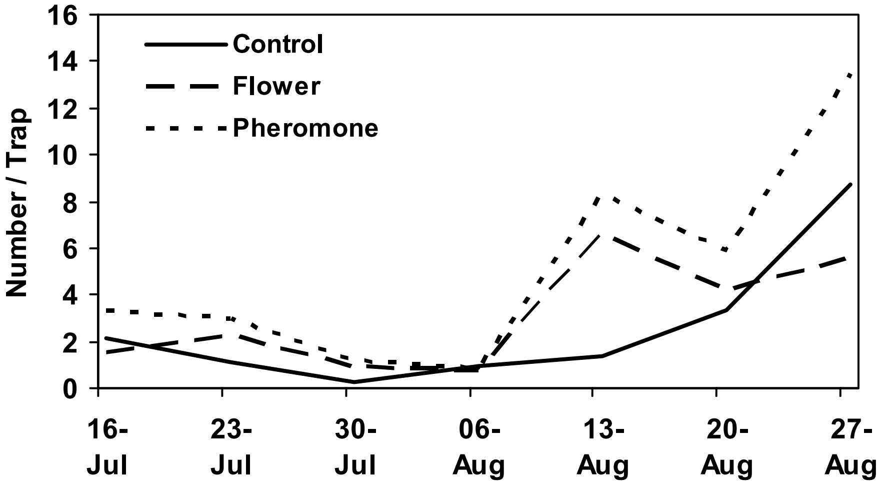
Figure 2.39. Numbers of adult aphidophagous hoverflies caught in weekly water trap samples within organic broccoli crops at Epworth, Yorkshire in 2003. The pheromone-treated area (dotted line) and control areas (solid line) were at opposite sides of the same field but the flower-rich margin treatment (dashed line) was in a separate field.



Catches of adult aphidophagous hoverflies were very similar in the three sample areas except on the first sampling date (16th July) when more were caught in the area adjacent to the flower-rich margin than in the other two sample areas and on 6th August when far fewer were caught in the pheromone-treated area than in the other two sample areas (Fig. 2.39).

There were no significant effects of treatments on the numbers of carabid beetles caught in pitfall traps within the broccoli crops. Carabid catches were low in all three treatment areas from the start of sampling in mid-July until mid-August (Fig. 2.40).

Figure 2.40. Carabid beetles caught in pitfall traps placed in three treatment areas in organic broccoli crops at Epworth, Yorkshire in 2003. The pheromone-treated area (dotted line) and control areas (solid line) were at opposite sides of the same field but the flower-rich margin treatment (dashed line) was in a separate field.



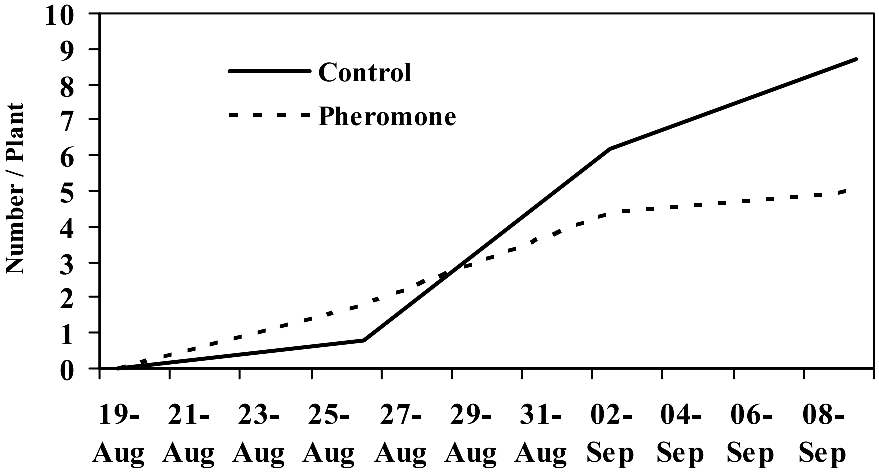
2.3.5.3. *Organic lettuce*

Due to significant differences in planting dates amongst fields sown with organic lettuce crops at the Ely site, the trial had to be conducted on a single field. The design was the same as that used at the Royston pea site, i.e. two sample areas were set up alongside a flower-rich margin, one of which was treated with aphid sex pheromone lures and the other acted as a control. Also, the small size of the planted area only allowed two sampling transects within the crop, at 10m and 30m from the field margin.

Very few aphids were recorded on the lettuces, probably due to the very hot dry conditions prevailing over the crop growth period in August and early September 2003 (Fig. 2.41). Out of a total of 200 plants sampled over the five week sampling period, aphids were found on only twenty-six. *Nasonovia ribisnigri* was the only species recorded. There was no significant difference between aphid numbers in the two treatment areas.

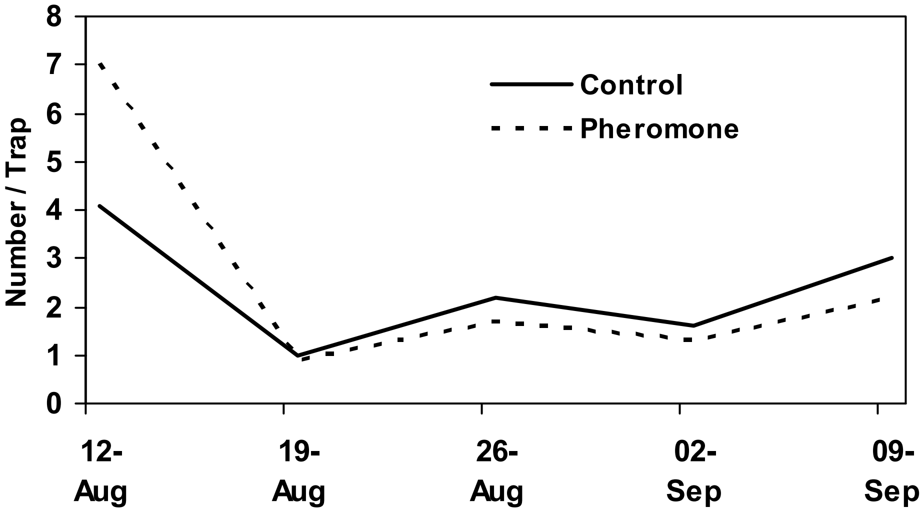
Only fifteen adult aphid parasitoids were caught in Vortis suction net samples taken within the lettuce crop during the sampling period, most of which were probably associated with aphids on plants within the adjacent field margin.

Figure 2.41. Cumulative numbers of aphids counted on 20 whole lettuce plants in control (solid line) and pheromone-treated (dotted line) areas of an organic lettuce crop at Ely, Cambridgeshire in 2003. Both areas were bordered on one side by a flower-rich margin.



Similar numbers of adult aphidophagous hoverflies were caught in water traps placed in the pheromone-treated (95) and control (83) plots. Very few carabid beetles were caught in pitfall traps within the lettuce crop and here was also no significant difference in the numbers caught in the control and pheromone-treated plots (Fig. 2.42).

Figure 2.42. Carabid beetles caught in pitfall traps placed within an organic lettuce crop at Ely, Cambridgeshire in 2003. Control plot – Solid line; Pheromone-treated plot – Dotted line.



2.4. DISCUSSION

2.4.1. Cereals

2.4.1.1. *Cereal aphid and parasitoid populations*

One of the main factors that contribute to the pest status of many aphids is their capacity for rapid population growth. Aphid populations can develop remarkably quickly because they exist for most of the time as all female, asexual populations, with every individual adult capable of producing several daughters per day. When an aphid is born it already has its own developing embryos inside it. Thus, aphid populations increase exponentially, which simply means that the rate of increase is continually accelerating. Therefore, for any biological control to be effective, it must impact on the aphid population very early in its growth curve before the rate of increase becomes so fast that it outstrips the control agents. Nevertheless, cereal aphid populations often fail to increase to economic damage levels due to the impact of natural control factors, principally a range of biological control agents (predators, parasitoids and pathogens) and weather factors such as heavy rain.

Studies of the ecology of aphid natural enemies in arable crops, funded by DEFRA (formerly MAFF), led to the conclusion that natural control of cereal aphids depends upon the activities of a range of predators, parasitoids and pathogens and that parasitoids (parasitic wasps) were a key component of this natural enemy community (Wratten & Powell, 1991). Detailed studies of aphid and parasitoid population dynamics led to the hypothesis that parasitoids needed to be present in the crop to coincide with initial aphid colonisation to have a significant impact. This initial parasitoid activity appeared to retard early aphid population growth and prevent exponential development, thereby allowing other natural enemies in the system subsequently to retain aphid numbers below damage thresholds (Wratten & Powell, 1991; Powell *et al*., 1998; Powell, 2000). The 3D Farming LINK project provided an ideal opportunity to test this hypothesis and to evaluate the potential of using aphid pheromones to induce early parasitoid activity in the crop.

Data from the first three years of the project, when the focus was on cereal crops, provided interesting contrasts in cereal aphid population development curves, particularly at the two southern English sites of Colworth in Bedfordshire and Radcot in Oxfordshire. **In 2000 and 2002, aphid populations remained at low levels throughout the summer and showed no signs of exponential growth. In contrast, in 2001 typical exponential growth began in mid-June followed by a population crash in early July. In 2000 and 2002, there was a significant parasitoid presence in the crop during the early stages of aphid colonisation, whereas in 2001 parasitoids were virtually absent at this time, providing strong evidence in support of the hypothesis that early parasitoid activity can hinder aphid population development sufficiently to prevent exponential growth.** The important factor preventing early parasitoid activity in 2001 was prolonged cold, wet, weather conditions in spring and early summer. This prevented the parasitoids from flying and foraging for aphid hosts and because this first generation, which had emerged from overwintering diapause, was unable to reproduce effectively, parasitoid populations remained depressed throughout the season.

Two factors prevented a damaging aphid outbreak in 2001; firstly the cold, wet, weather conditions at the beginning of the season caused significant aphid mortality and hindered early population growth and, secondly, there was a large immigration of hoverflies, principally the migratory marmalade hoverfly *Episyrphus balteatus*, during the summer (see Section 2.3.3.). So, although the aphid population began to increase exponentially as soon as the weather improved in June, this population 'take-off' had been delayed and the hoverflies arrived in time and in sufficient numbers to curtail the outbreak. **This emphasises the importance of maintaining a diverse natural enemy community in agricultural ecosystems, as this provides stability for natural biocontrol systems in the face of environmental variability, particularly variability in climatic conditions.**

The relationship between early parasitoid activity levels and subsequent aphid population development patterns was also apparent in the 2001 and 2002 data from the Manor Farm site in Yorkshire. However, at the Scottish site of West Fenton in E. Lothian, there was no evidence of exponential growth in the aphid population in 2001, which remained low throughout the season, despite the absence of early parasitoid activity. This was probably due to the persistence of wet weather conditions throughout the entire summer season at this site.

2.4.1.2. *Parasitoid diversity*

Five species of parasitoid known to attack cereal aphids were recorded in all study fields at all sites in the suction net samples taken within the cereal crops. The dominant species at all sites in all three years, except at West Fenton in 2001 and Manor Farm in 2002, was *Aphidius rhopalosiphi*, which is a cereal aphid specialist (i.e. only attacks aphids occurring on graminaceous plants). This agrees with earlier studies of parasitoid species abundance in cereal crops both in the U.K. and elsewhere in northern Europe (Dean *et al*., 1981; Wratten & Powell, 1991). ***Aphidius rhopalosiphi* was always the most abundant species early in the season at the 3D Farming study sites and so can be regarded as the most important species for cereal aphid control.** The other two *Aphidius* species, *A. ervi* and *A. picipes*, were also often present in smaller, but significant, numbers during the critical early period of the season, whereas the two most polyphagous (attacking a wide variety of aphids) species, *Praon volucre* and *Ephedrus plagiator*, tended to appear in the crop later in the season when they contributed to the aphid population crash.

Aphidius rhopalosiphi overwinters in its immature stages, including the mummy stage, in a range of graminaceous aphids in both crop and semi-natural habitats and can be active very early in the season, even emerging from diapause during mild periods in winter and early spring (Powell, 1983; Vorley, 1986). **Therefore, habitats that include a high proportion of grasses, such as pasture and grass-rich field**

margins are valuable reservoirs of cereal aphid parasitoids. Although these habitats obviously also support populations of cereal aphids, these are often non-pest species such as *Metapolopium festucae* and *Sitobion fragariae* and the benefits of these habitats as parasitoid reservoirs outweigh any negative effects as pest sources.

2.4.1.3. *Parasitoid sex ratios*

Analysis of the sex ratio of adult aphid parasitoids caught in suction net samples through the summer season revealed that the ratio changes dramatically during the course of the season. Early in the season, during the critical period of aphid colonisation, the sex ratio of cereal aphid parasitoids caught within the crop was consistently female biased. Parasitic wasps have a distinctive haplo-diploid reproductive system, which means that females develop from fertilised eggs and males develop from unfertilised eggs (and therefore have half the chromosomes of females). This means that females can reproduce without mating, but all their offspring will be male. In a batch of parasitoids of even age, males tend to emerge before females and the males normally remain at the emergence site waiting to intercept and mate with the emerging females. Emerging females, however, disperse in pursuit of hosts, into which they can lay their eggs, regardless of whether or not the eggs have been fertilised. **The female-biased sex ratio in cereal crops early in the season suggests that a significant proportion of the population of parasitoids foraging within the crop have immigrated from surrounding semi-natural habitats, which have acted as overwintering sites for diapausing parasitoids.**

In contrast, samples collected within the crop during the aphid population crash at the end of the season tended to be male-biased. This suggests that the mobile females have emigrated from the crop because of the rapid decline in host availability as aphid populations crash, leaving the more sedentary males behind. During mid-season, when aphid hosts are still available within the crop, sex ratios tended to be more stable at approximately 50:50. **Emigrating females will seek hosts in field margins and other semi-natural habitats within the farming ecosystem. The cereal aphid specialist *A. rhopalosiphi* attacks aphids on grasses in these habitats and in pasture (Vorley, 1986), but a significant proportion of the population enters a summer diapause at the mummy stage, possibly triggered by the declining nutritional quality of aphid hosts on the ripening cereal plants. Diapausing mummies within the crop will be largely destroyed at harvest, emphasising the importance of non-crop habitats for maintaining viable populations of this key species.**

2.4.1.4. *Effect of aphid sex pheromone*

The aphid sex pheromone was not deployed in the crop in the first summer field season of 2000 when the sampling protocols were being verified, the first pheromones being placed in field margins in autumn 2000. No effects of the pheromone were evident in 2001 due to the virtual absence of parasitoid activity during the critical aphid colonisation period in early summer, as a result of the cool, wet, weather conditions prevailing at that time. However, conditions in 2002 were much more conducive to both aphid and parasitoid activity, allowing good data on the effects of the pheromone to be obtained. The aim of using the pheromone was to increase the impact of parasitoids on the aphid population in the first couple of weeks of the infestation in order to prevent early exponential population growth. There was good early parasitoid activity in 2002 in both treated and untreated fields and aphid populations remained small at all sites, with no signs of exponential growth, indicating that natural biological control worked well that year. Nevertheless, at the Yorkshire and Scottish sites, where aphid numbers were greater than at the two southern English sites, twice as many aphids were counted in the crop over the season in the control fields than in the pheromone-treated fields. Interestingly, at these sites, **the pheromone did not appear to cause a significant increase in the number of parasitoids caught in the samples but, importantly, it did significantly affect their spatial distribution within the crop at the start of the season; more were caught further out into the crop where the pheromone was present, whereas there was a distinct edge effect in the control fields. This indicates that the pheromone stimulated rapid spread of parasitoids through the crop at the critical time when aphids were beginning to invade.** It is encouraging that even at low aphid densities it was possible to detect an effect of the pheromone on both parasitoid distribution and aphid numbers, at least at two of the four sites.

There was no evidence that deploying the pheromone in field margins in autumn significantly increased parasitoid activity the following spring. As mentioned above, adult parasitoids were not caught in significantly greater numbers within the crop in the pheromone-treated fields compared with the control fields at the beginning of sampling in spring. Also numbers caught in the margins themselves were not significantly greater where the pheromone had been deployed the previous autumn. It is likely, therefore, that adult parasitoids dispersing from harvested fields in late summer and autumn colonise suitable field margins effectively as these are the first non-crop habitats they are likely to encounter. **The use of aphid sex pheromones directly in the crop at the time of aphid colonisation, therefore, appears to be the most effective strategy.**

When assessing the effects of the pheromone treatment, interpretation of the sample data must be done with care; the numbers of adult parasitoids caught in suction net samples cannot be considered alone but must be assessed together with data on aphid densities. This is exemplified by the data for West Fenton. Increased parasitoid efficiency at the start of the aphid infestation, due to more rapid dispersal throughout the crop, resulted in lower aphid numbers throughout the rest of the summer in the pheromone-treated field. However, because there were more aphids in the control field, this led to increased parasitoid populations later in the season, although the aphid:parasitoid ratio remained similar to that in the pheromone field at this time. Thus, at the beginning of the season similar numbers of parasitoids were present but the aphid:parasitoid ratio was much lower in the pheromone field as a result of the better spatial distribution of parasitoids, whereas later in the season the aphid:parasitoid ratio was similar in the two fields but parasitoid numbers were greater in the control field due to the presence of a larger aphid population.

These results strongly suggest that female parasitoids immigrating into the crop in spring, when aphid densities were still very low, responded strongly to the aphid sex pheromone and so moved further into the crop more rapidly. However, when aphid numbers increased and the parasitoids were already established in the crop, female parasitoids responded more to host densities, probably utilising aphid-induced plant volatiles during foraging.

2.4.1.5. *Hoverfly populations*

Some hoverflies are entirely plant-feeders, but the larvae of many species eat aphids (Hickman & Wratten, 1996) and these are important members of the natural enemy complex that helps to control aphid populations on crops. The adult hoverflies feed on nectar and pollen and females require these food sources in order to develop their eggs, which are then laid amongst aphid colonies in the case of aphidophagous species. Access to good food sources will also increase the fitness of the adult flies allowing them to live longer, fly further and lay more eggs (Scholz & Poehling, 2000). Field margins can supply these food sources in the form of wild flowers and so the presence of flower-rich margins should enhance the impact of hoverflies on aphid populations in nearby crops, by increasing hoverfly abundance and/or increasing their reproductive fitness.

During the course of this study, hoverfly abundance, as measured by water trap catches of adult flies, varied considerably both amongst sites and amongst years. Very large numbers were caught at the three English sites during 2001 and this was partly due to an abundance of the marmalade hoverfly, *Episyrphus balteatus*. This species is known to be migratory (Stubbs & Falk, 2002) and the population in 2001 may have been boosted by migratory individuals from continental Europe. There are two possible reasons why far fewer hoverflies were caught at West Fenton in southern Scotland than at the three English sites in 2001. Firstly, the influence of immigrating *E.balteatus* was probably much less than it was further south; this species formed only 34% of the total aphidophagous hoverflies caught at West Fenton compared with over 70% at the other sites. Secondly, the weather in southern Scotland remained very cool and wet throughout most of the summer season in 2001 and this will have significantly hindered hoverfly activity.

The sudden increase in catches of adult aphidophagous hoverflies in mid summer that occurred in cereal crops in seven of the eleven site-years for which hoverfly data were available, was almost certainly due to the emergence of the second generation, which had developed as larvae feeding on the summer aphid population in and around the crop. This second generation was very large in 2001, dominating the seasons water trap catches. **There was a highly significant trend of increasing numbers caught with distance into the crop, suggesting that these highly mobile insects disperse and distribute their eggs throughout the crop. In addition, many of these hoverflies probably developed within the crop itself and therefore had**

fed predominantly on cereal aphids. Sutherland *et al*., (2001) questioned the suitability of *E. balteatus* as a candidate for biological control via augmentation as their study found that this hoverfly seemed to be concentrated in the field margins and was found less frequently in the field. However, they were sampling in within-field wildflower patches rather than in the crop itself and the observation may have been due to the effect of (non-floral) resources that field margins offer, namely additional aphids (when compared to in-field wildflower patches), shelter from predation and flight corridors for flower-seeking adults (Colley & Luna, 2000). Aphid populations on the crop itself will offer a richer resource for adults seeking egg-laying sites than within-crop wildflower patches. This work has shown that not only is *E. balteatus* found up to at least 100 metres into the crop, but also that this species and other aphidophagous hoverflies are trapped in greater numbers in the crop than in the margin. There is evidence of a geographical influence on the timing of this second generation emergence, with the main emergence occurring 1-2 weeks later in Yorkshire than in Bedfordshire and Oxfordshire in 2001 and about a month later in southern Scotland than at the two southern English sites in 2002.

2.4.1.6. *Hoverfly species abundance*

By far the most common hoverflies trapped at all sites were the two species normally associated with arable land, *E. balteatus* and *M. corollae* (Dean, 1982). Larvae from all the aphidophagous species named in Table 2.5 and a further five species trapped during this study have been found feeding on aphids in cereal fields (Chambers *et al*., 1986). Although the majority of work in this study has focussed on the behaviour of the most common species (*E. balteatus*) it is recognised that other aphidophagous species are potentially important natural predators and that a range of flower types should be included in the field margin seed mixture to ensure that there is a suitable selection of flower types for hoverflies with different mouthpart morphologies and flower preferences. As *E. balteatus* is a migratory species, arriving into cereal crops in June and July, natural predation from hoverflies in May and early June must rely on other species. The provision of early flowering plants in the margin to enhance the potential of other species such as *M. corollae,* whose larvae have been found in fields of winter wheat in late May (Chambers *et al*., 1986), will improve the temporal spread of the natural control of aphids by hoverflies.

2.4.17. *Effect of flower margins*

There was strong evidence that the presence of a flower-rich margin along at least one side of the field can have a significant impact on aphid numbers in cereal crops. There were significantly fewer aphids present on the crop in fields with such margins than in control fields for seven out of twelve site-years and for no site-years were there significantly fewer aphids in the control field. The amount of food resource available to the adult hoverflies could account for the apparent similarity in numbers in the two fields. Hickman *et al.,* (2001) highlighted the possibility that the difference in food resource in flower rich sites and control sites would lead to a higher proportion of the hoverfly population being trapped in the control field as the trap represents a food signal to hoverflies and would attract hungry individuals. Other studies have also found similar trap catches of adult hoverflies in flower rich and control sites, even though observed numbers and oviposition rates have been much greater in the flowering sites (Hickman & Wratten, 1996; MacLeod, 1994).

It is possible therefore that the hoverfly populations in the flower rich sites were larger than the control fields and this combined with the increased reproductive efficiency due to better adult nutrition, provided by nectar and pollen resources in the margin led to an increase in the number of predaceous hoverfly larvae developing in the crop and an associated reduction in the aphid populations. However, adult aphidophagous hoverflies were not caught in significantly greater numbers in fields with flower-rich margins than in control fields. In addition, adult hoverflies are fast fliers and extremely mobile (as indicated by the ability of *E. balteatus* to migrate into the U.K. from continental Europe) so the emerging second generation is likely to have rapidly dispersed across the whole farm, making the detection of local differences in emergence densities difficult with the trapping method used. Finally, it must be remembered that the field margins are likely to have had a beneficial effect on the abundance and fitness of other aphid natural enemies, contributing to the apparent impact on aphid populations.

This evidence of an impact of flower margins on cereal aphid populations was apparent at all four study sites in 2002 but at only two of the sites, Manor Farm and Colworth, in 2001. At West Fenton the main aphid control factor in 2001 was the weather, with the persistent cool, wet conditions keeping aphid numbers very low in all fields throughout the season (low aphid populations would not attract extensive egg-laying by hoverflies). At Radcot, the failure to detect an effect of the field margin was due to a site problem beyond our control. Due to the farm cropping regime, a suitable cereal control field was not available that year and we compromised by using the opposite end of the flower margin field from that where the flower-rich margin was situated. This was a large field, allowing a gap of more than 200 metres between the control and flower margin treatment sample areas. However, the high mobility of the hoverflies almost certainly allowed them to have an impact across the whole field. The first year of the project, 2000, was an establishment year and the flower-rich margin at the Colworth site was newly sown in that year and so was not expected to have any effect until at least 2001. Also, very little hoverfly activity was recorded at either Colworth or Radcot in 2000.

2.4.1.8. ***Hoverfly sex ratio***

The bias in the sex ratio detected by the two sampling methods (yellow water traps and a within canopy suction sampler) can be accounted for by the hypothesis that the yellow water trap represents a food signal to the hoverflies (Hickman *et al.,* 2001) and that the majority of females that fly out into the crop from margins are responding to oviposition signals rather than food signals. This hypothesis was reinforced by laboratory trials, which showed that gravid females showed very little response to the traps (food signals) even though they had previously been starved. The two-day old, non-gravid, females however were as strongly attracted to the traps as the males. It is suggested, therefore, that all the males in the crop and only the newly emerged females were responding to food signals, resulting in a greater number of males being captured in the water traps and that the more mature gravid females were more likely to be responding to oviposition signals and searching amongst the canopy for aphid colonies. Their position in the crop canopy would therefore be accessible by the suction sampler as it was swept through the crop leading to a much greater proportion of females being captured via this method.

2.4.1.9. *Carabid beetles*

It was important to monitor carabid beetles at the sites where hoverfly and parasitoid manipulation was being trialled in order to detect any effects of the treatments on this important group of insect predators. Any effects of flower-rich margins or the deployment of aphid sex pheromones on carabid activity within the crop needed to be taken into account when interpreting data on aphid numbers. More detailed studies of the effects of margin and crop management on the spatial distribution of carabid beetles and other ground-dwelling predators were done at a further study site on the Hampshire/Dorset border and are reported in Section 3.

Carabid monitoring was done using pitfall traps and it is important to remember that pitfall trap catches do not directly reflect the actual abundance of the different species but are a function of both abundance and activity. Highly mobile beetles that move around over large areas are much more likely to be caught than beetles that restrict their activity to a small spatial area. This has been demonstrated in field trials that compared restricted area trapping with conventional pitfall trapping (Sunderland *et al.*, 1987a). In conventional traps, large mobile *Pterostichus* species dominated, whereas traps placed in small areas, restricted by physical barriers, caught predominantly small *Bembidion* species. Therefore it is not possible to compare the abundance of different species using pitfall trap data, as some very abundant species can be caught in much smaller numbers than other less abundant but very mobile species. However, it is possible to compare catches of the same species or group of species from traps placed in the same habitat type to detect the effects of crop management treatments.

It is obvious from the data that total carabid catches can vary dramatically amongst different fields at the same site. This variability bore no relation to the field treatments or to recorded treatment effects on aphid populations. For example, in 2002 when the presence of a flower-rich margin significantly reduced aphid numbers at all four sites (Fig. 2.21), fewer carabids were caught in the fields with flower margins than in control fields (Fig. 2.23), indicating that the reduction in aphids was not primarily due to carabid predation. Similarly, at the Manor Farm and West Fenton sites in 2002, where the pheromone treatment appeared to reduce aphid numbers compared with the control (Fig. 2.10), there was no difference between the two fields in total carabid catches.

2.4.1.10. *Effect of aphid pheromone on* Harpalus rufipes

Pitfall trap catches of the carabid beetle *Harpalus rufipes* appeared to be increased by the aphid pheromone, nepetalactone, in some site/years. The reasons for this are unknown. This was first noticed when the data for 2001 were being processed and so some supplementary laboratory studies were initiated in 2002 to test whether or not this was a real effect. Electrophysiological studies using an electroantennogram detected a physiological response to the pheromone. Catches of this species from the only cereal site used in 2003 (Colworth) were sorted according to sex and the proportion of males in the catch compared between the three fields to detect any sex difference in the response. **Analysis revealed a significantly greater proportion of males in the pheromone-treated field than in the other two fields, suggesting that males were responding more than females.** The nature of any behavioural response by male *H. rufipes* to the pheromone remains unclear but laboratory bioassays are being conducted to try to confirm that a behavioural response to aphid sex pheromone exists in this species and to elucidate the nature of such a response. Increased pitfall catches in the presence of the pheromone could result from an accumulation of beetles in the treated area due to an attraction/arrestment response or alternatively could result from increased beetle activity due to an irritant/repellent effect. However, until a behavioural response has been definitely confirmed, the field results, even though they are statistically significant, should be treated with caution, as there still remains a possibility that the results are simply due to chance.

Regardless of whether there is a real effect of the pheromone on this carabid or not, the increased abundance/activity of *H. rufipes* in some pheromone-treated fields did not appear to affect cereal aphid numbers. There was no significant effect of pheromone treatment on aphid numbers in 2001 even though much greater numbers of *H. rufipes* were caught in the pheromone-treated fields than in the control fields at three of the four sites (Fig. 2.27). Conversely, significantly fewer aphids were recorded in pheromone-treated fields than in control fields at Manor Farm and West Fenton in 2002 (Fig. 2.10) but there was no significant difference in the numbers of *H. rufipes* caught (Fig. 2.27).

2.4.2. Non-Cereal Crops

The aim of the trials in the final year of the project was to extend the work into several high value, non-cereal, field crops to evaluate the potential of the natural enemy manipulation approach, based on flower-rich field margins and aphid sex pheromones, for development in these crops. These trials were designed to highlight problems specifically associated with field vegetable crops and identify areas that would need to be addressed in further work in order to adapt the approach developed for cereal aphid control. Three crops were chosen for study after consultation with project partners at PGRO and HDC: vining peas, organic broccoli and organic lettuce. Field vegetable crops present a far greater challenge for biological control of aphids than do cereals, principally because of the very low tolerance levels for aphid contamination and crop damage. Also, it was not possible to conduct the trials on three separate fields at each site, as had been done in the cereal trials, and so compromises had to be made in trial design. Because of this and the lack of spatial and temporal replication, interpretation of the data from these trials was much more problematical. Nevertheless, the trials provided valuable information for steering the direction of future work.

2.4.2.1. *Vining peas*

Data from the pea trials at both the main site at Drem in East Lothian and the supplementary site at Royston in Cambridgeshire do not reveal any obvious effects of the aphid sex pheromone, nepetalactone, on pea aphid populations. In fact, at both sites aphid numbers were greater where the aphid sex pheromone was deployed than in the control areas. There is no obvious reason why the presence of the pheromone should cause an increase in aphid numbers and it is probable that the differences between the single treated and untreated plots at the two sites was simply due to chance. There was also no evidence that the pheromone significantly affected aphid parasitoid numbers or spatial distribution at either site. Although more adult parasitoids were caught in the pheromone-treated area at Royston than in the control area, the aphid:parasitoid ratio was the same in both areas indicating that the increased parasitoid catches simply reflected increased aphid presence and the pheromone had no discernible effect.

Two main compounds, nepetalactone and nepetalactol, occur in the natural aphid sex pheromones that have been identified so far. The sex pheromone of cereal aphids contains only nepetalactone whereas that of pea aphids consists of a 50:50 mixture of the two compounds. **The pheromone lures used in this project only released nepetalactone and it is possible that lures releasing both compounds, and therefore more closely matching the natural pea aphid pheromone, would be more effective in pea crops.** Evidence that supports this is provided by preliminary data emerging from a collaborative experiment being conducted in Japan in 2004. Combined nepetalactone and nepetalactol lures placed in lucerne crops appear to be having significant effects on aphid parasitoids leading to reductions in populations of legume aphids (Yoshitaka Nakashima, personal communication).

Hoverflies were not monitored at the supplementary Royston site, due to lack of resources, but **there was no evidence that the presence of a flower-rich margin had any significant effects either on pea aphid numbers or on adult aphidophagous hoverfly abundance in the crop at the main pea site in Scotland**.

2.4.2.2. ***Organic broccoli***

The most striking result from the broccoli trial was the large numbers of aphid parasitoids in the crop alongside the flower-rich margin. Before the grower treated the crop with soap solution, the density of aphids on the crop near the flower margin was almost half that in the control plot and it is possible that the high parasitoid activity, as indicated by both the adult catches and the mummies present on the plants, would have prevented significant aphid damage if the soap treatment had not been applied. Because the flower margin treatment was in a separate field from that used for the control and pheromone treatments and it was not possible to replicate at the spatial scale used, it is not possible to be sure that the flower margin itself positively affected parasitoid numbers. However, this merits further investigation.

Very few adult aphid parasitoids were present in the field containing the pheromone-treated and control plots, with only eighteen parasitoids caught in Vortis suction net samples in the two plots combined during the whole sampling period. Therefore, **it was not possible to assess the potential of the pheromone for manipulating the main brassica aphid parasitoid *Diaeretiella rapae*.** However, it is known from laboratory studies that this species responds to aphid sex pheromone components, particularly nepetalactone, and traps baited with the pheromone have caught large numbers of *D. rapae* in small scale field trials in the UK and Poland (Gabrys *et al*., 1997; Glinwood, 1998; Powell, 2000). **In view of the positive effects of the pheromone in the cereal trials, further trials in brassica crops are recommended.**

Although there were significantly more adult aphidophagous hoverflies caught in water traps within the crop adjacent to the flower-rich margin than in the control plot during the first week of sampling, catches later in the season were not significantly different between the two treatments. There is therefore no evidence that the hoverflies bred more in the crop near the flower margin. This was probably due to the great reduction in aphid prey caused by the soap applications made by the grower soon after sampling began.

2.4.2.3. ***Organic lettuce***

The organic lettuce trials were very disappointing due mainly to the unusually hot and dry weather conditions prevailing during the trial period in August-early September 2003. As a consequence, very few aphids colonised the crop, with only 13% of plants sampled over a five week period being infested. The lack of aphids inevitably resulted in a lack of aphid parasitoids and only fifteen adult parasitoids were caught in suction net samples taken within the crop. **Therefore, as in the broccoli trial, it was not possible to assess the effects of the aphid sex pheromone treatment.** However, discussions with the growers revealed that they have released commercially-reared aphid parasitoids into organic lettuce crops in the past in an attempt to biologically control lettuce aphids. Random releases of parasitoids into open field crops are unlikely to be effective due to the probability that the parasitoids would rapidly disperse from the crop if aphid populations were not high enough. However, waiting until aphid densities were sufficient to retain released parasitoids in the crop before releasing would defeat the object. It is possible that the aphid sex pheromone could be used to retain released parasitoids in the crop for longer and the potential of this approach is currently being investigated in Defra-commissioned research at Rothamsted. Organic lettuce would be an ideal crop in which to test this approach if the initial strategic work demonstrates its feasibility.

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