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The results and conclusions in this report are based on a series of experiments conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

## AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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## GROWER SUMMARY

There is considerable potential to manage field margins to increase pest control by natural control agents, in addition to enhancing 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 lay their eggs in the crop near aphid colonies. Hoverflies played an important role in maintaining control of pest aphids, their numbers and therefore effect being greatest after the impact of parasitoids (an early season control agent) began to wain in mid-summer. Thus, the effects of parasitoids and hoverflies were complimentary 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 but the significance of this remains unknown and requires further investigation. 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.

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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 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<sup>-2</sup>.

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*,7*aR*)-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-leaf 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<sup>-2</sup>, while some species, e.g. *P. melanarius* and *P. madidus*, emerged at densities of nearly 30-40 m<sup>-2</sup>. **Overall, carabids together with staphylinids emerged at population densities of 86 m<sup>-2</sup>**

in one of the larger fields and almost double that density at a massive 157 m<sup>-2</sup> 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 that 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 vagile 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 original 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 affect.**

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 amplify 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 detectable 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, *Acyrtosiphum pisum*. **These results provide evidence that linyphiid spiders are 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 is influenced by aphid abundance, but even at low aphid densities spiders are 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, is 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<sup>3</sup>) 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 eclosed, 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. balteatus* 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, was 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 give rise to the aphidophagous stages of the hoverfly, and 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 parsley 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.

## **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*,7*aR*)-nepetalactone and (1*R*,4*aS*,7*S*,7*aR*)-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*,7*aR*)-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.

<b>Parasitoid</b>	<b>Pest Aphid Hosts</b>	<b>Evidence of Response</b>
<i>Aphidius rhopalosiphi</i>	Cereal aphids	Field Experiments Laboratory Bioassays Electrophysiology
<i>Aphidius ervi</i>	Pea Aphid Cereal Aphids Glasshouse Aphids	Field Experiments Laboratory Bioassays Electrophysiology
<i>Aphidius eadyi</i>	Pea Aphid	Field Experiments Laboratory Bioassays
<i>Aphidius matricariae</i>	Glasshouse Aphids	Electrophysiology
<i>Diaeretiella rapae</i>	Brassica Aphids	Field Experiments Laboratory Bioassays Electrophysiology
<i>Praon volucre</i>	Wide range of hosts	Field Experiments Laboratory Bioassays Electrophysiology
<i>Ephedrus plagiator</i>	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 complementarity 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, Stretton, 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.

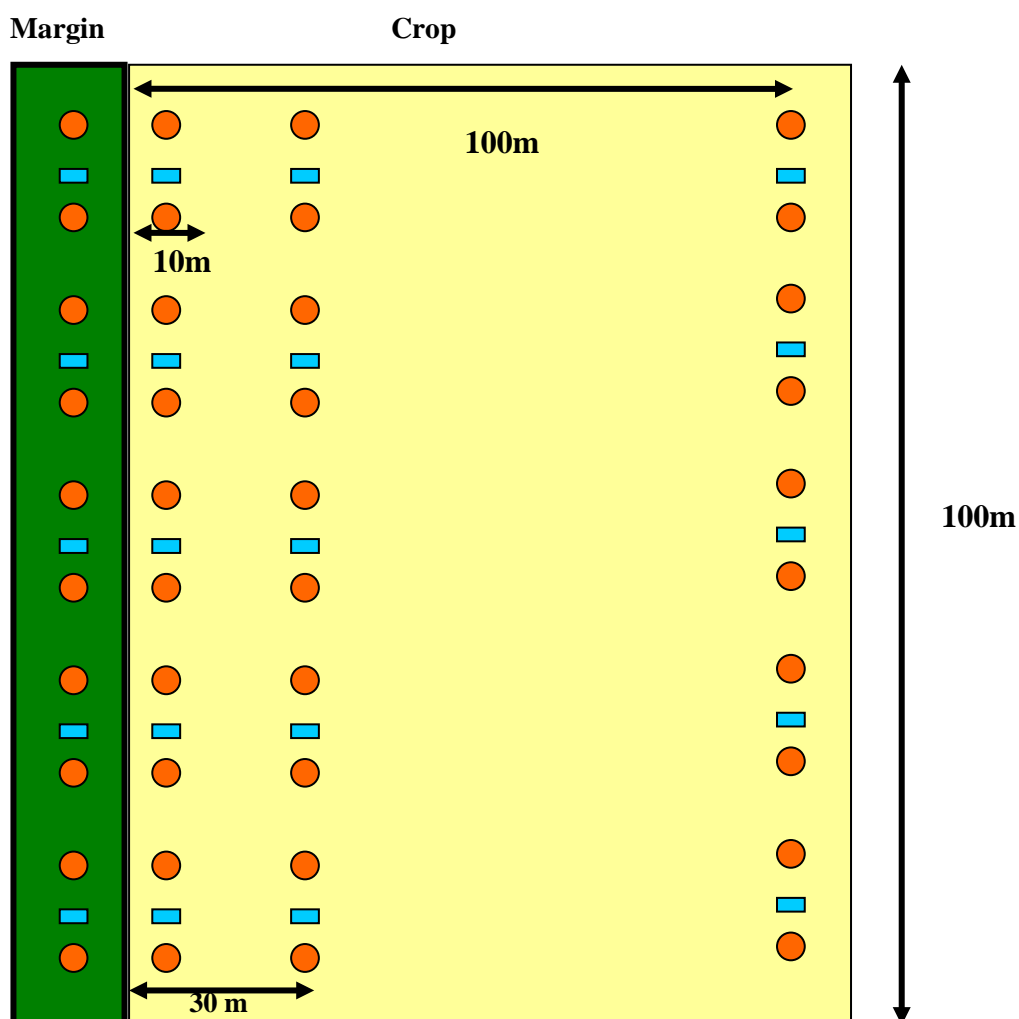
At the four cereal trial sites, where trials were done over the first three years of the project, the vegetation within the treatment margins of the three study fields was surveyed. Due to individual farm cropping plans, it was not always possible to use the same fields for all treatments over the three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

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

<sup>1</sup>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*,7*aR*)-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.

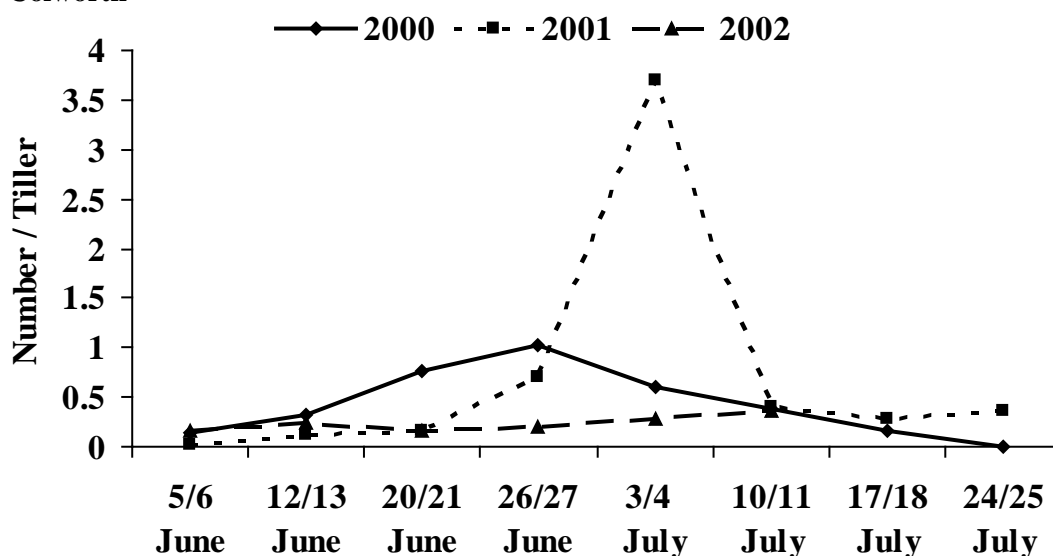
## 2.3. RESULTS

### 2.3.1. Cereal Aphid Population Trends

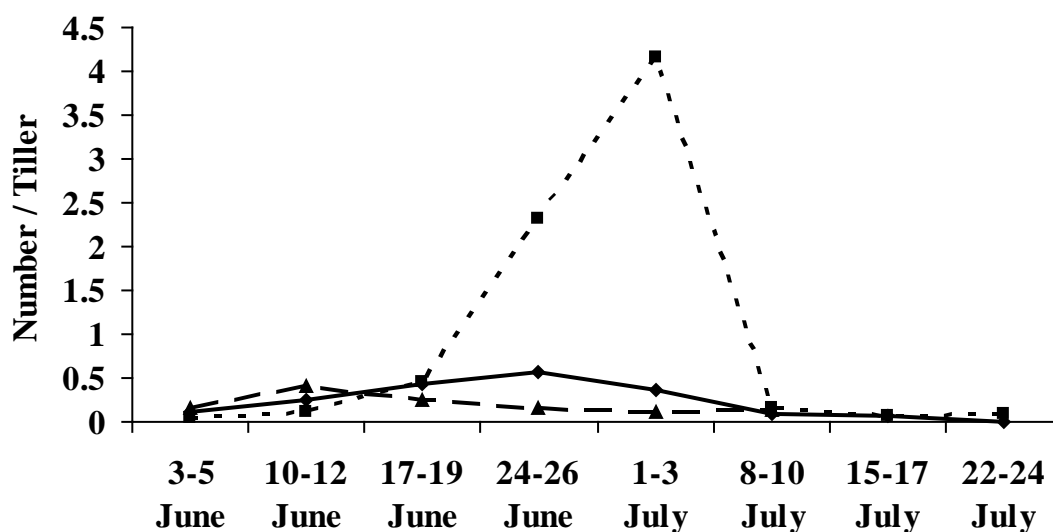
At the two southern English sites, in Bedfordshire and Oxfordshire, the pattern of cereal aphid population development within the crop varied dramatically from year to year (Fig. 2.2). In 2000 and 2002, aphid populations remained very low throughout the season and never exhibited the exponential growth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



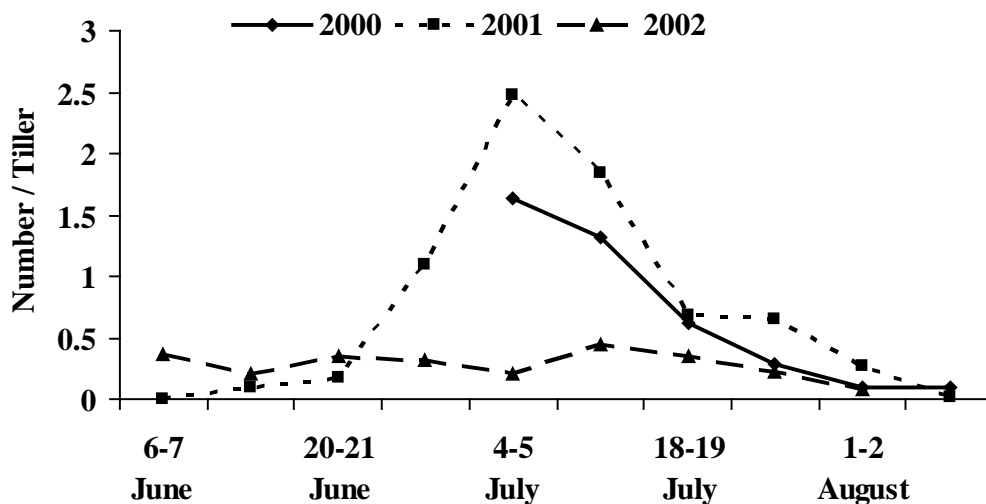
#### (b) Radcot



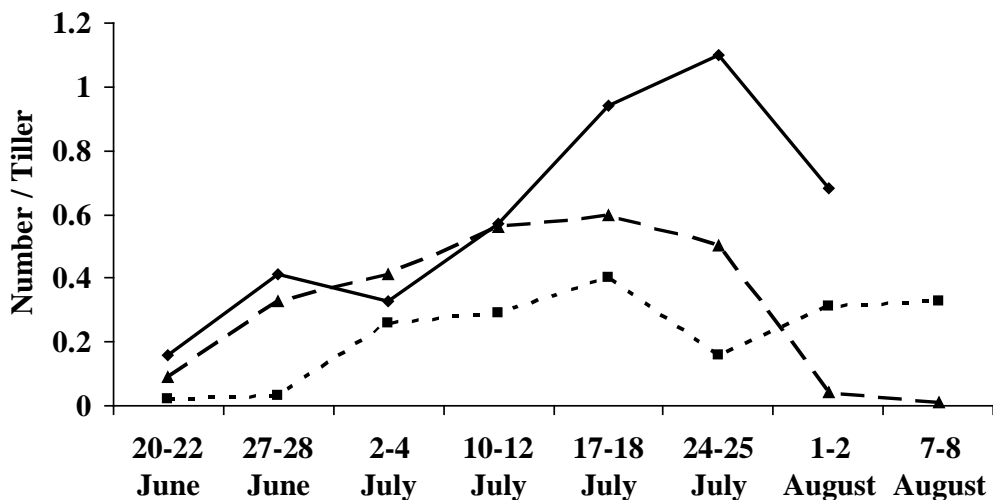
This suggests that natural control was working well in these two years. However, in 2001, aphid numbers remained low until mid-June when they began to increase exponentially, reaching a peak in early July, after which numbers crashed dramatically. The period of exponential growth suggests a lack of natural 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**



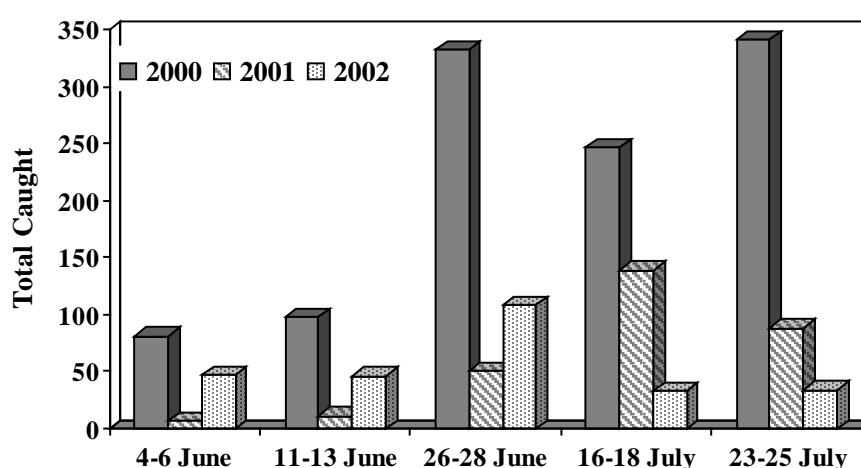
## 2.3.2. Cereal Aphid Parasitoids

### 2.3.2.1. Parasitoid population dynamics

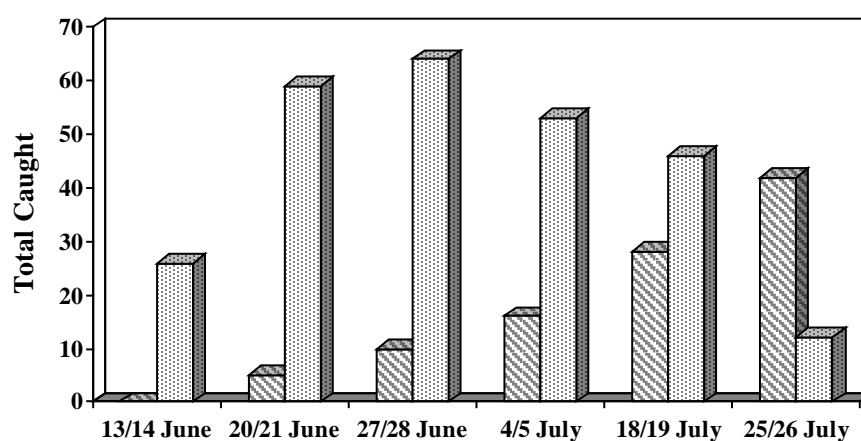
The aphid sex pheromone was first deployed in the autumn of 2000, so the first full treatment season was summer 2001. However, the 2000 field season was used to evaluate the sampling protocol and collect baseline data on parasitoid population dynamics in cereal crops at the two southern English sites. 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
<b>2000</b>	1.2	1.1	1.9
<b>2001</b>	7.0	19.4	4.3
<b>2002</b>	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.

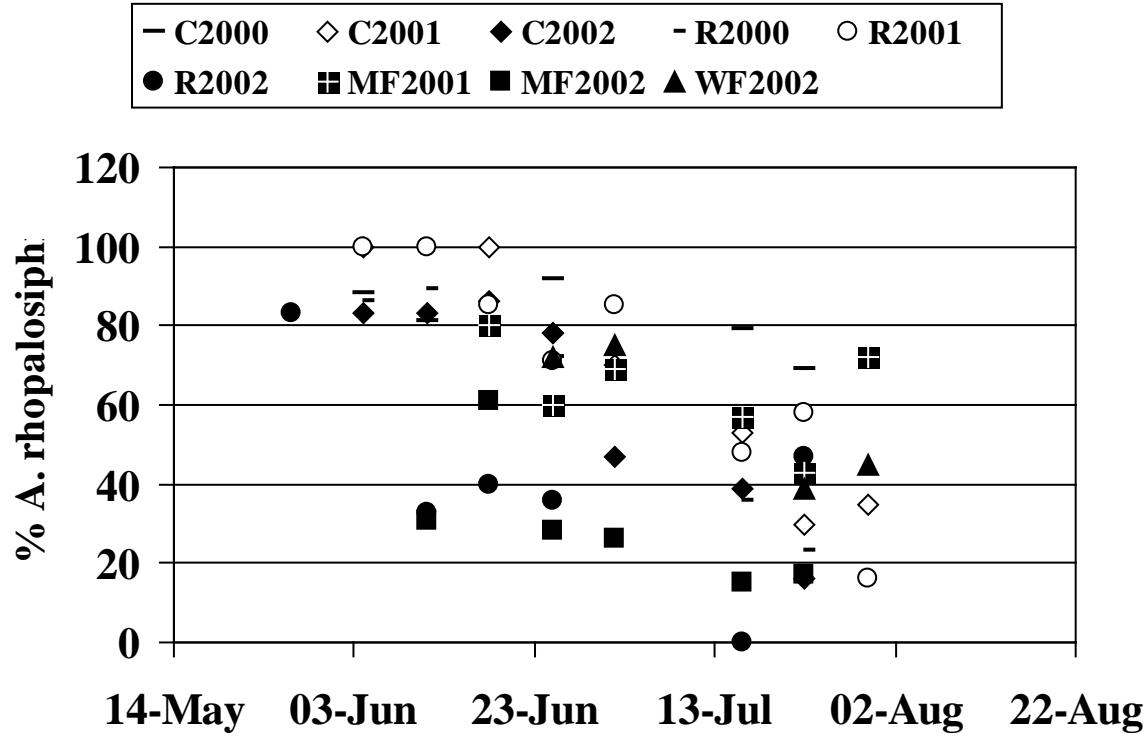
The relative abundance of the different species changed with time in a consistent way, the cereal aphid specialist *A. rhopalosiphi* strongly dominating at the beginning of the season (Figs. 2.5 & 2.6). In early June, over 80% of suction sampler catches consisted of this species (Fig. 2.5). The other 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).

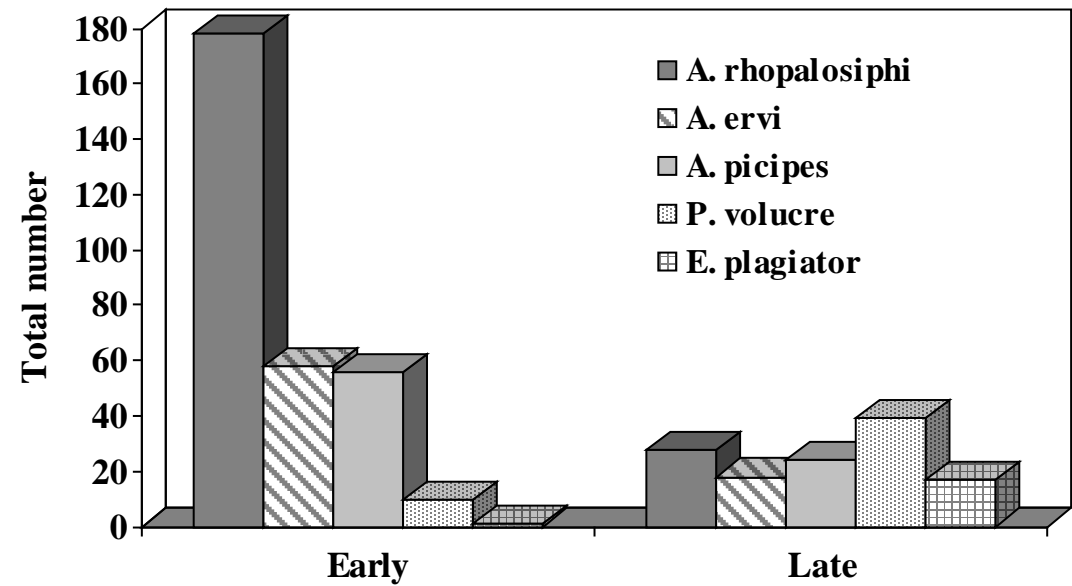
**Table 2.4.** Relative abundance of the five main parasitoids of cereal aphids caught in suction samples taken within the crop in all treatment fields at each of the four cereal study sites.

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

**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



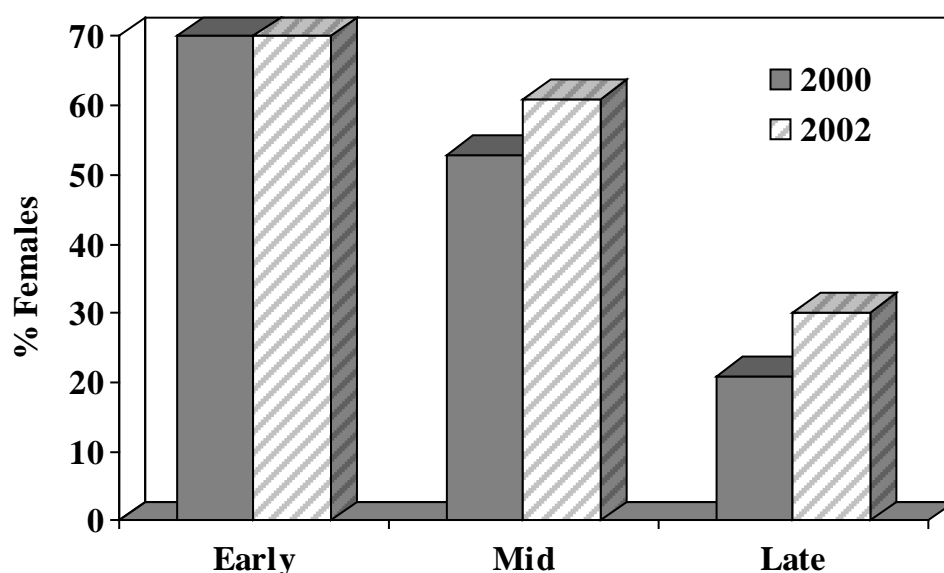
**Figure 2.6.** Relative abundance of parasitoid species in suction samples taken in 2002 from cereal crops in the early and late stages of cereal aphid infestation. Data for all sites combined.



### 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).

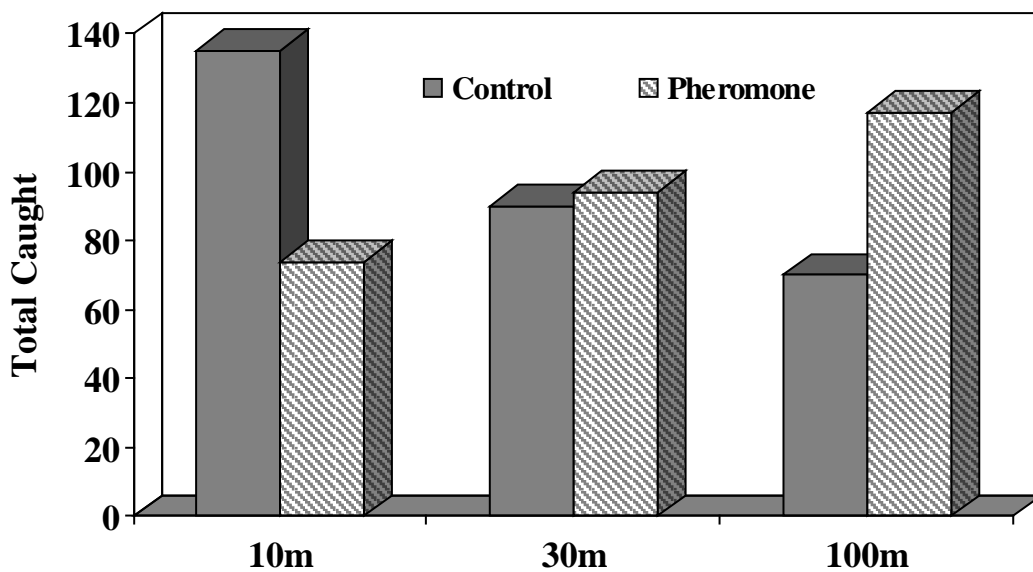


### 2.3.2.4. Effect of aphid sex pheromone

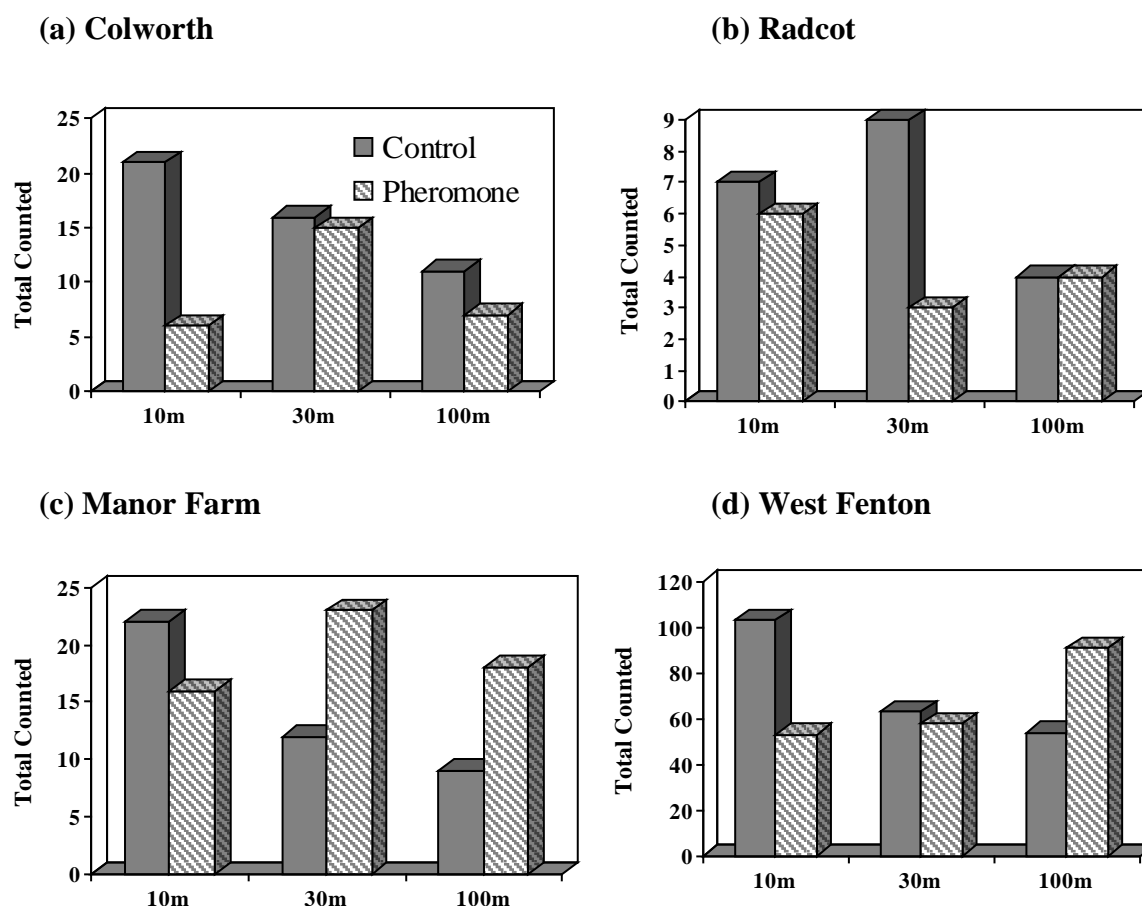
The aphid sex pheromone lures were deployed for the first time in tussocky grass field margins at the four sites after harvest 2000 and in the crop in spring 2001. Unfortunately the cool, wet conditions in spring 2001 prevented parasitoid activity at the critical time, making adequate assessment of the 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).

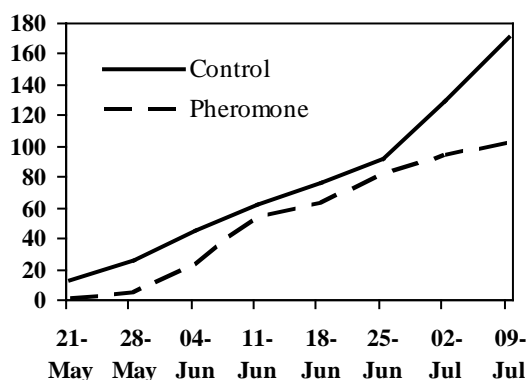


At the two sites where the pheromone appeared to induce rapid movement of adult parasitoids into the crop during the early aphid colonisation period (West Fenton & Manor Farm), the cumulative numbers of aphids recorded in the crop through the season were significantly lower ( $p < 0.01$ ) in the pheromone-treated 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 were 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

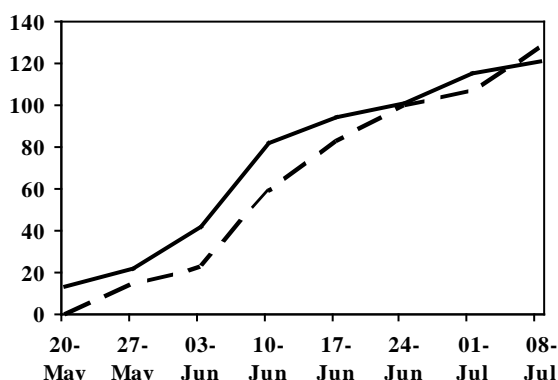
weeks after colonisation are considered, there were consistently more aphids present in control fields than in pheromone-treated fields ( $p < 0.01$ ) across all sites (Fig. 2.11).

**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

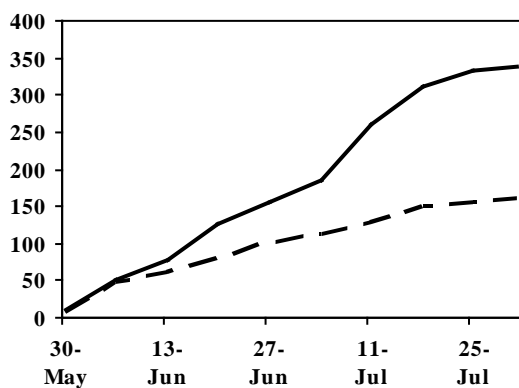
**(a) Colworth**



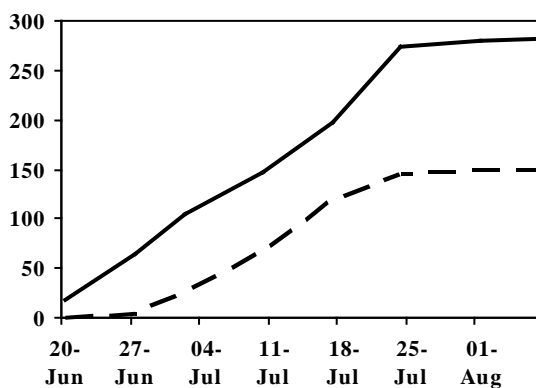
**(b) Radcot**



**(c) Manor Farm**



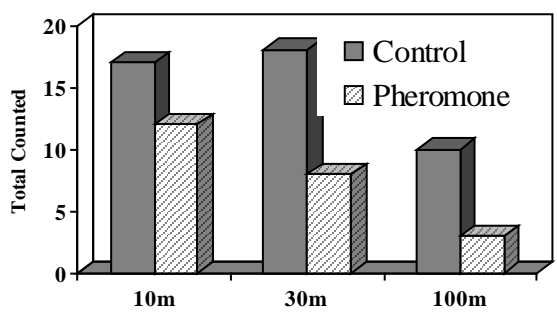
**(d) West Fenton**



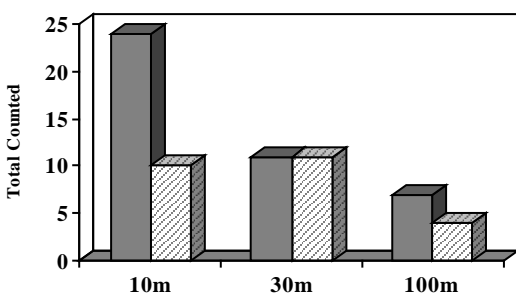
The greatest effect of the pheromone in 2002 appeared to occur at the Scottish site (West Fenton) where populations of both aphids and parasitoids were greater than at the other three sites. However, if the numbers of adult parasitoids caught in the suction net samples at West Fenton are viewed in isolation, 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.

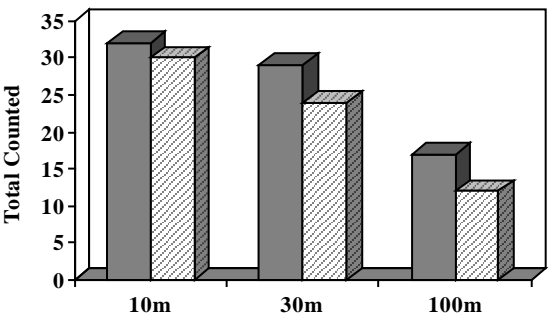
**(a) Colworth**



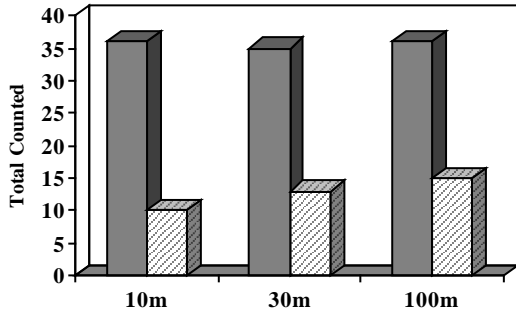
**(b) Radcot**



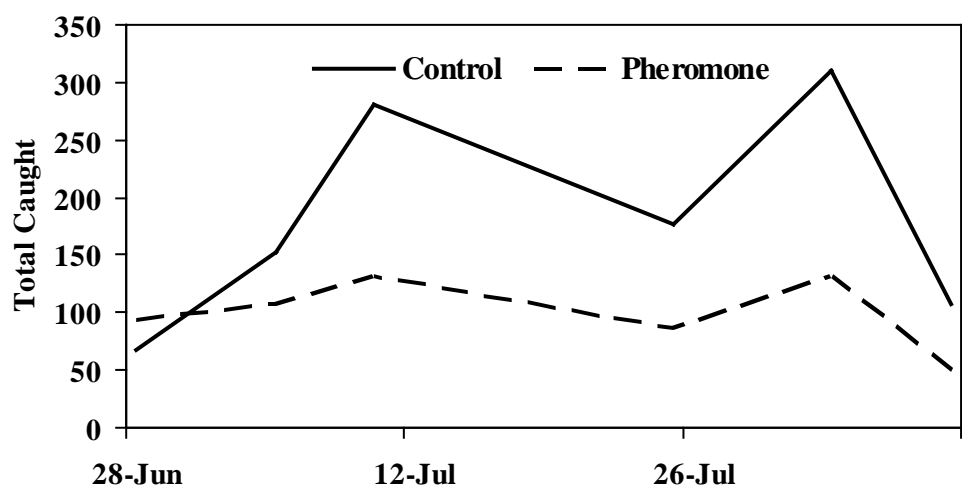
**(c) Manor Farm**



**(d) West Fenton**



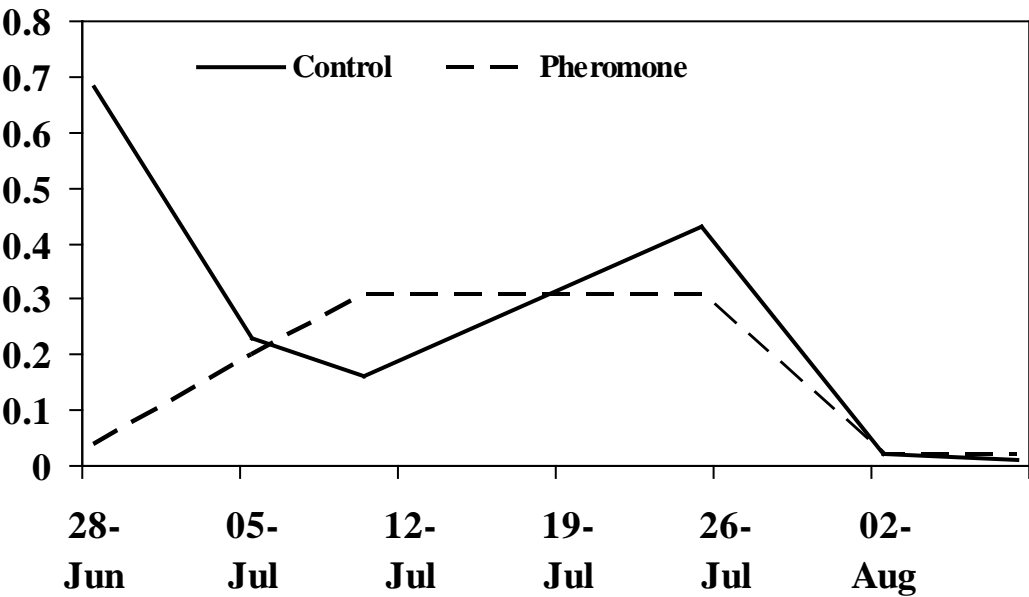
**Figure 2.12.** Numbers of adult parasitoids caught in suction net samples at West Fenton in 2002 in the control (solid line) and pheromone-treated (dashed line) fields.





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.

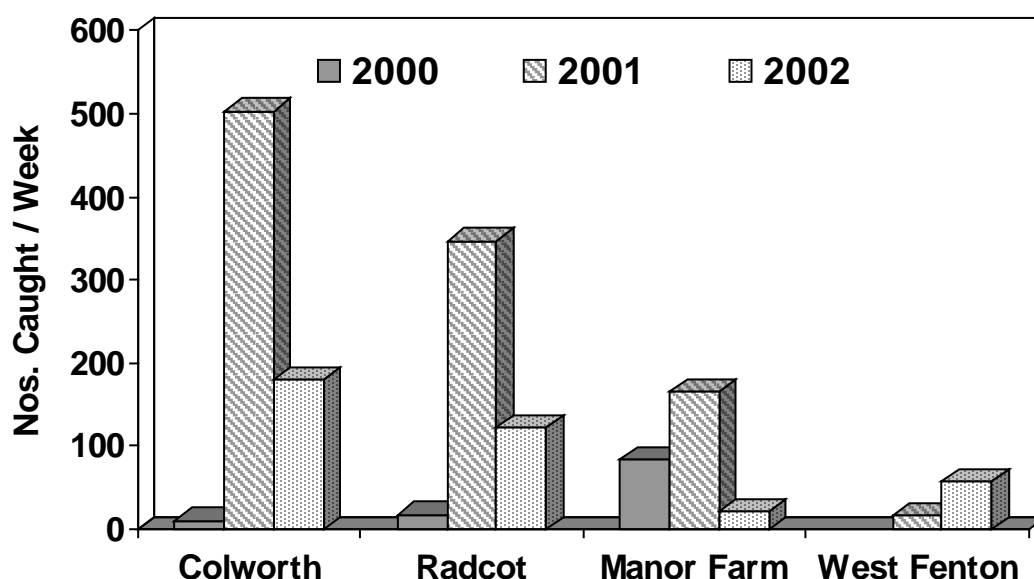


### 2.3.3. Hoverflies in Cereals

#### 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.

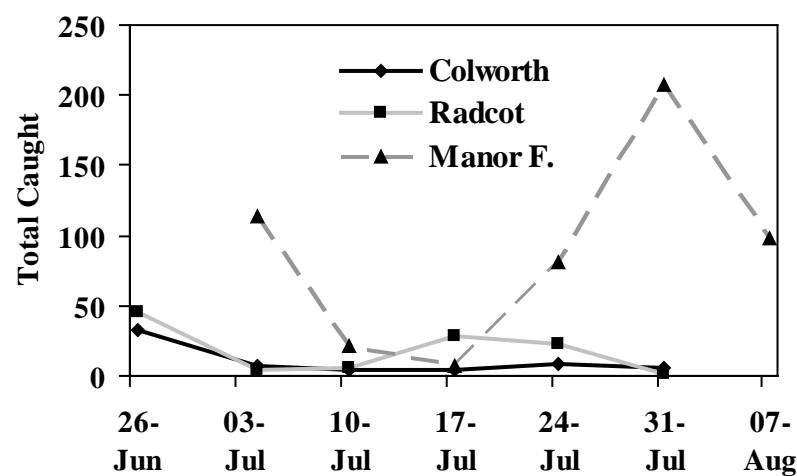
**Figure 2.14.** Mean number of adult aphidophagous hoverflies caught per trapping week in water traps placed within the cereal crop in control fields at the four sampling sites in 2000 (solid bars), 2001 (hatched bars) and 2002 (stippled bars) (water traps were not available at West Fenton in 2000).



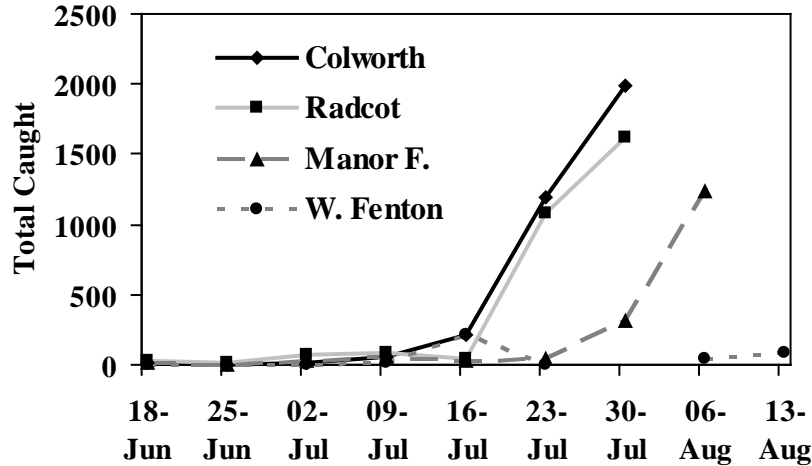
In 2001, the water trap catches of adult hoverflies began to increase dramatically in mid July at the sites in Bedfordshire (Colworth) and Oxfordshire (Radcot) and about a week later at the Yorkshire site (Manor Farm), but at the Scottish site (West Fenton) this dramatic increase in the catches did not 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.

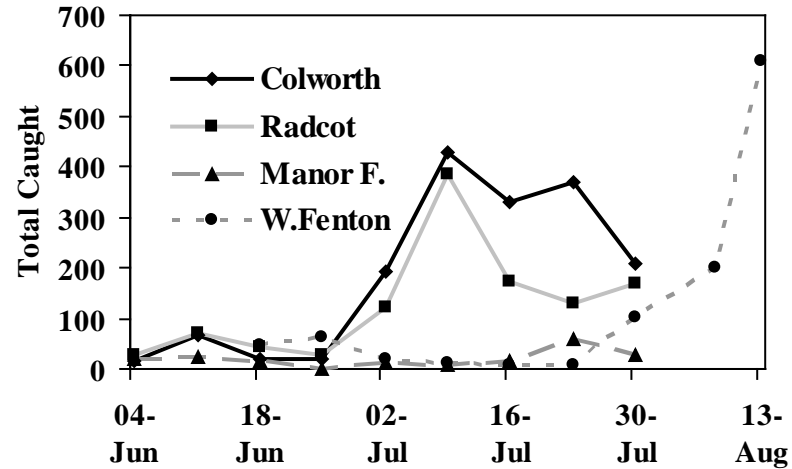
**(a) 2000**



**(b) 2001**



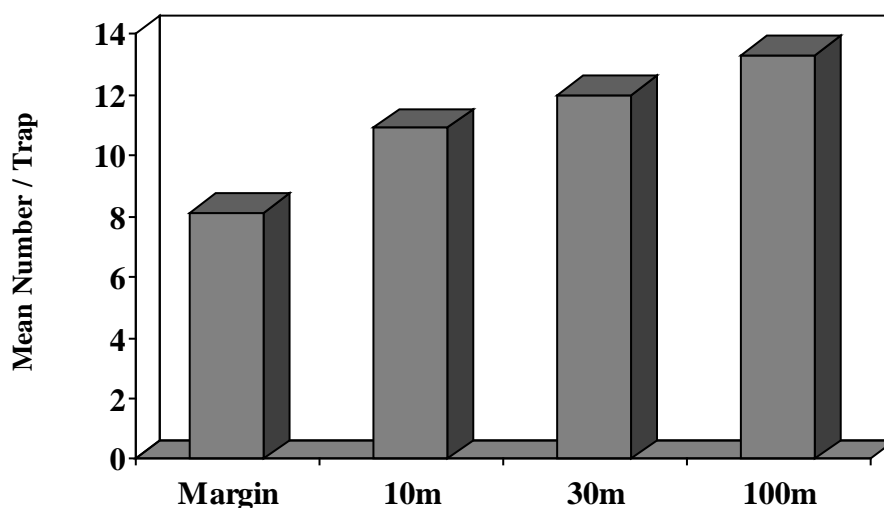
**(c) 2002**



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

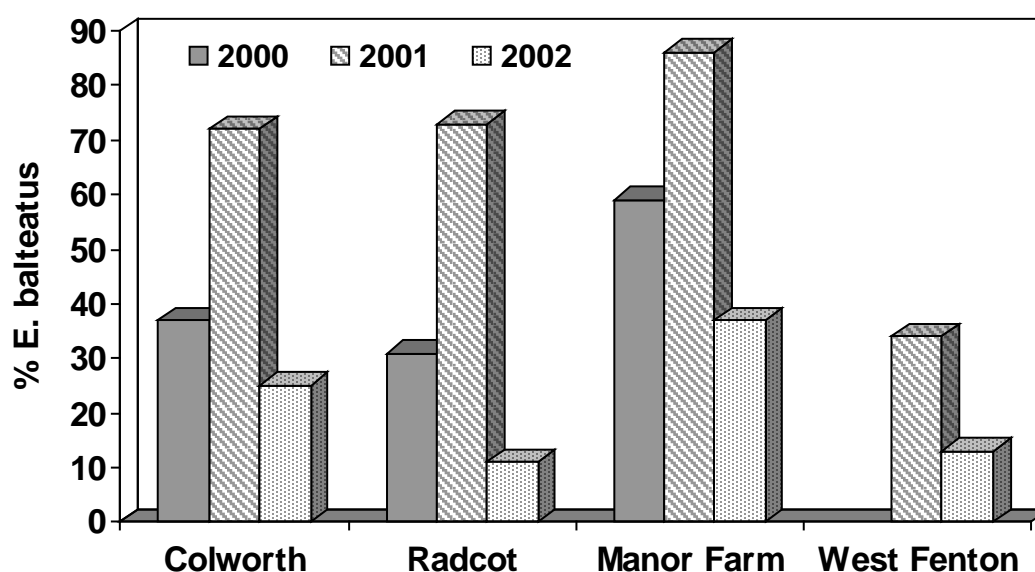
Twenty-five species of aphidophagous hoverflies were caught in water traps positioned in the 3 transects within the cereal crop in the control fields across the four study sites. The two most abundant species overall were *Episyrphus balteatus* and *Metasyrphus corollae* (Table 2.5). In 2001, when hoverflies 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

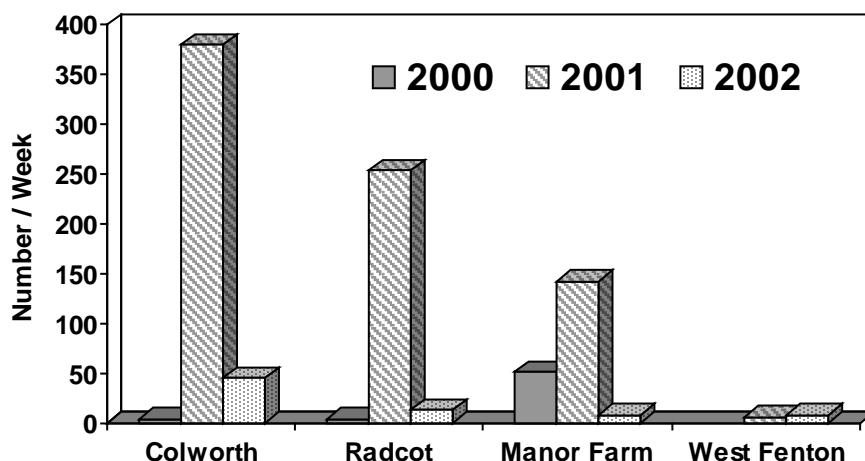
	2000			2001				2002			
Site	C	R	MF	C	R	MF	WF	C	R	MF	WF
No. Sample Weeks	6	6	6	7	7	10	8	9	9	10	9
Total No. caught	60	70	510	3187	2082	1119	92	1591	1142	198	1064
% <i>Episyrphus balteatus</i>	37	31	59	72	73	86	34	25		31	
% <i>Metasyrphus corollae</i>	28		29	26				33	54	32	68
% <i>Platycheirus peltatus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

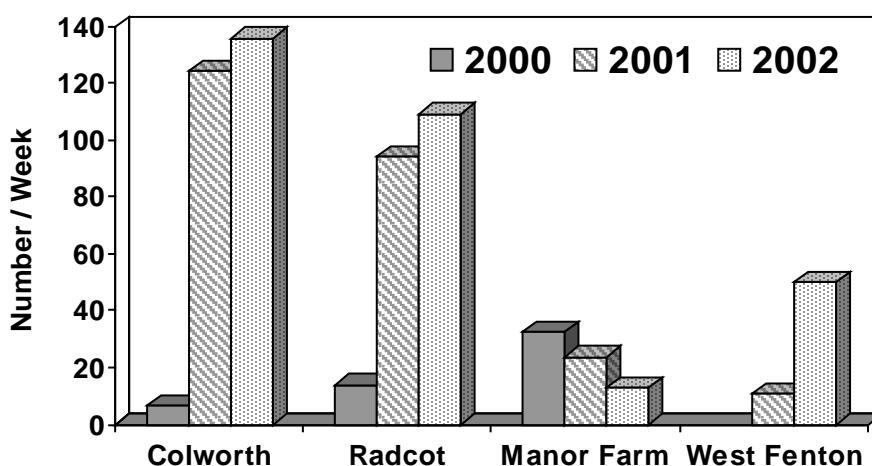


**Figure 2.18.** Mean number of adult (a) *Episyrphus balteatus* and (b) other aphidophagous hoverflies caught per trapping week in water traps placed within the cereal crop in control fields at the four sampling sites in 2000 (solid bars), 2001 (hatched bars) and 2002 (stippled bars) (water traps were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

(a) *Episyrphus balteatus*



(b) Other aphidophagous hoverflies



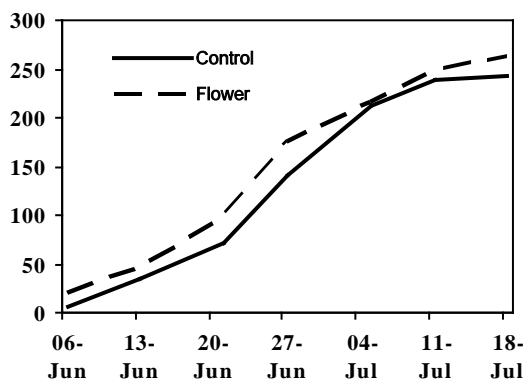
#### 2.3.3.3. Effect of flower margins

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

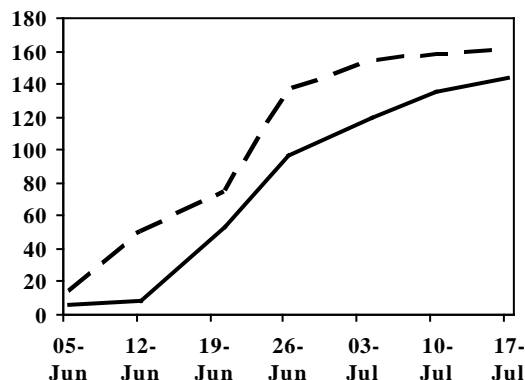
Colworth (Bedfordshire) and Radcot (Oxfordshire) (Table 2.5). Also, Manor Farm was the only site where there was a noticeable increase in the numbers of adult hoverflies caught in the crop transects later in the summer, suggesting active breeding had occurred within the crop (Fig.2.15a).

**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

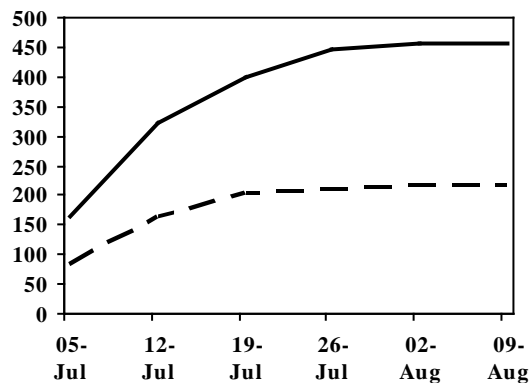
**a) Colworth**



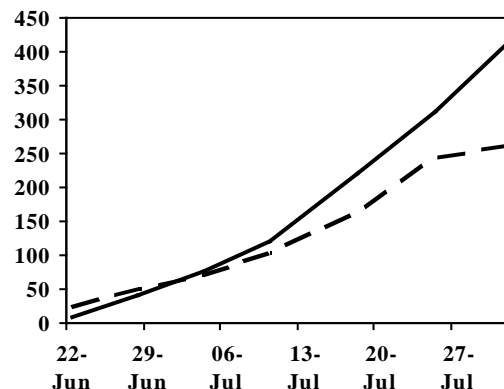
**(b) Radcot**



**(c) Manor Farm**



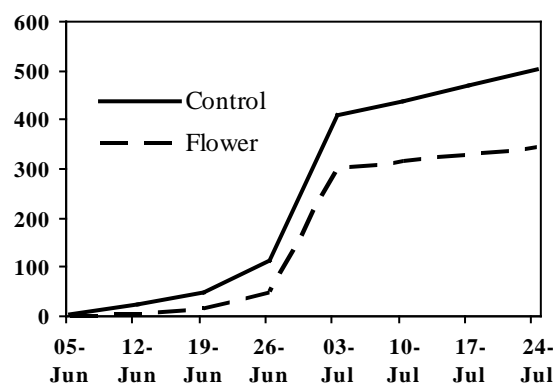
**(d) West Fenton**



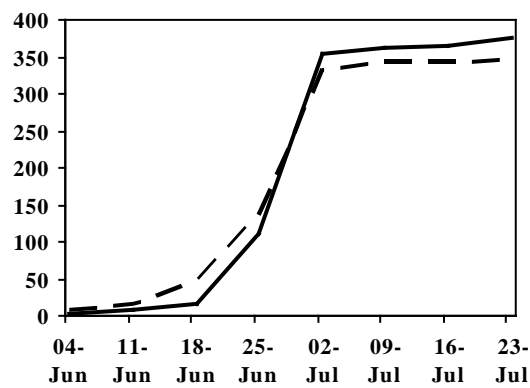
In 2001, when aphidophagous hoverflies were unusually abundant at the three English sites, there were fewer aphids in the field with the flower-rich margin than in the control field at Manor Farm ( $p < 0.01$ ) and, to a lesser extent, at Colworth (Fig. 2.20). However, there was no apparent effect at 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

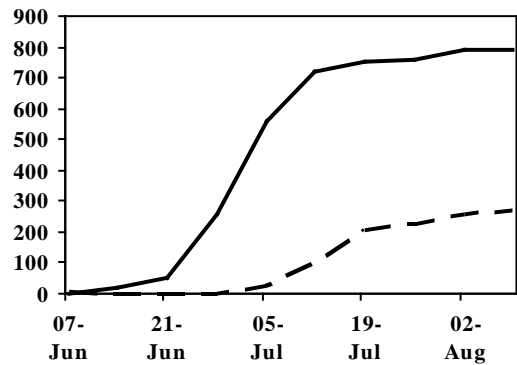
**(a) Colworth**



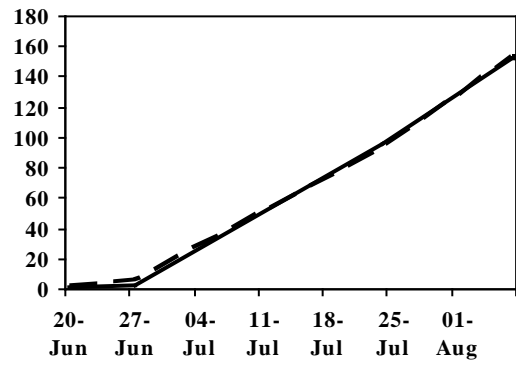
**(b) Radcot**



**(c) Manor Farm**

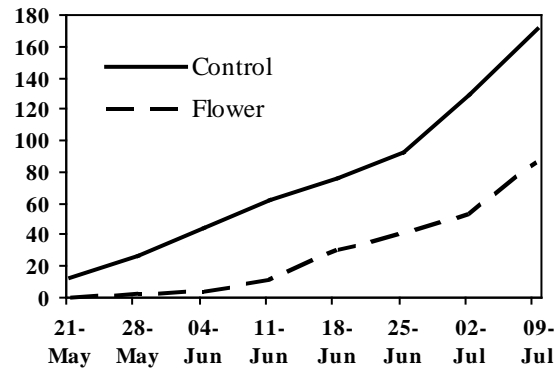


**(d) West Fenton**

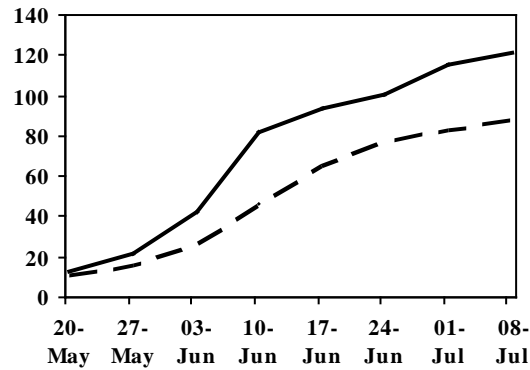


**Figure 2.21.** Effect of a flower-rich field margin on the cumulative numbers of cereal aphids counted on 75 tillers per week in 2002 at the four study sites (Control field – solid line; Field with Flower Margin – dashed line).  $p < 0.001$  for the combined site data.

**(a) Colworth**

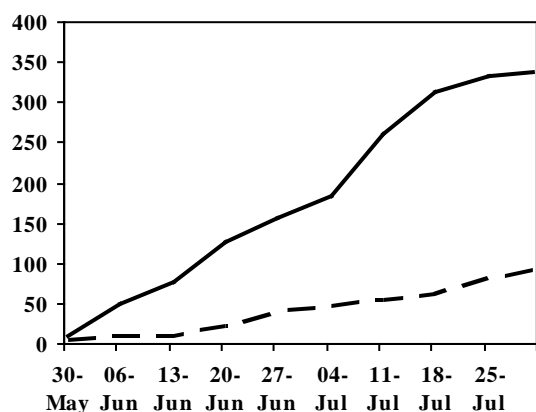


**(b) Radcot**

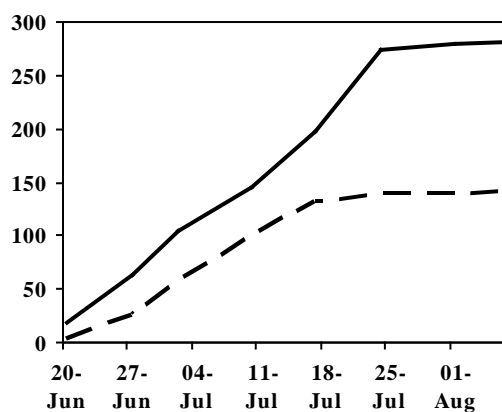




(c) Manor Farm



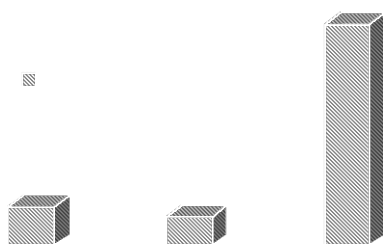
(d) West Fenton



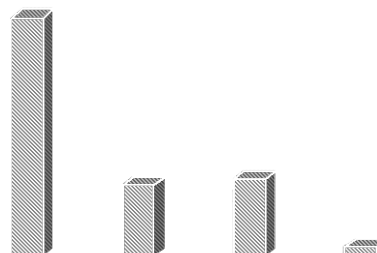
In 2002, analysis of variance revealed a highly significant effect of treatment on aphid numbers ( $p < 0.001$ ). There were significantly fewer aphids recorded in the field with a flower-rich margin than in the control field at all four study sites (Fig. 2.21), even though at Manor Farm catches of aphidophagous 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).

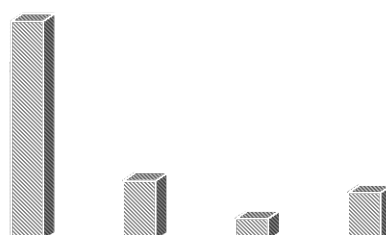
(a) 2000



(b) 2001



(c) 2002

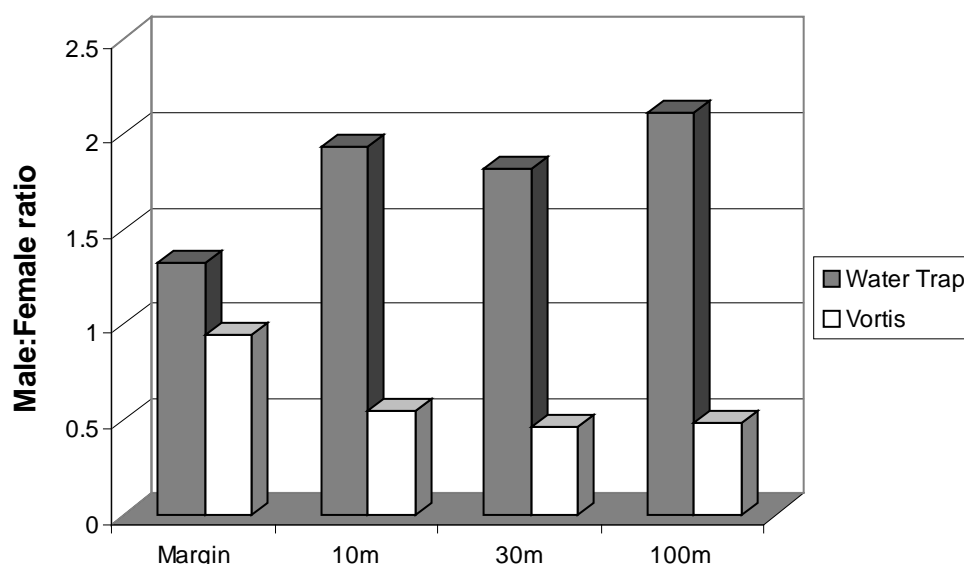


Although aphid populations were significantly reduced by the presence of a flower-rich field margin in seven site-years out of twelve, and on no occasion were there significantly fewer aphids in control fields than in those with flower margins, the numbers of adult aphidophagous hoverflies caught in 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 ( $\pm$  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

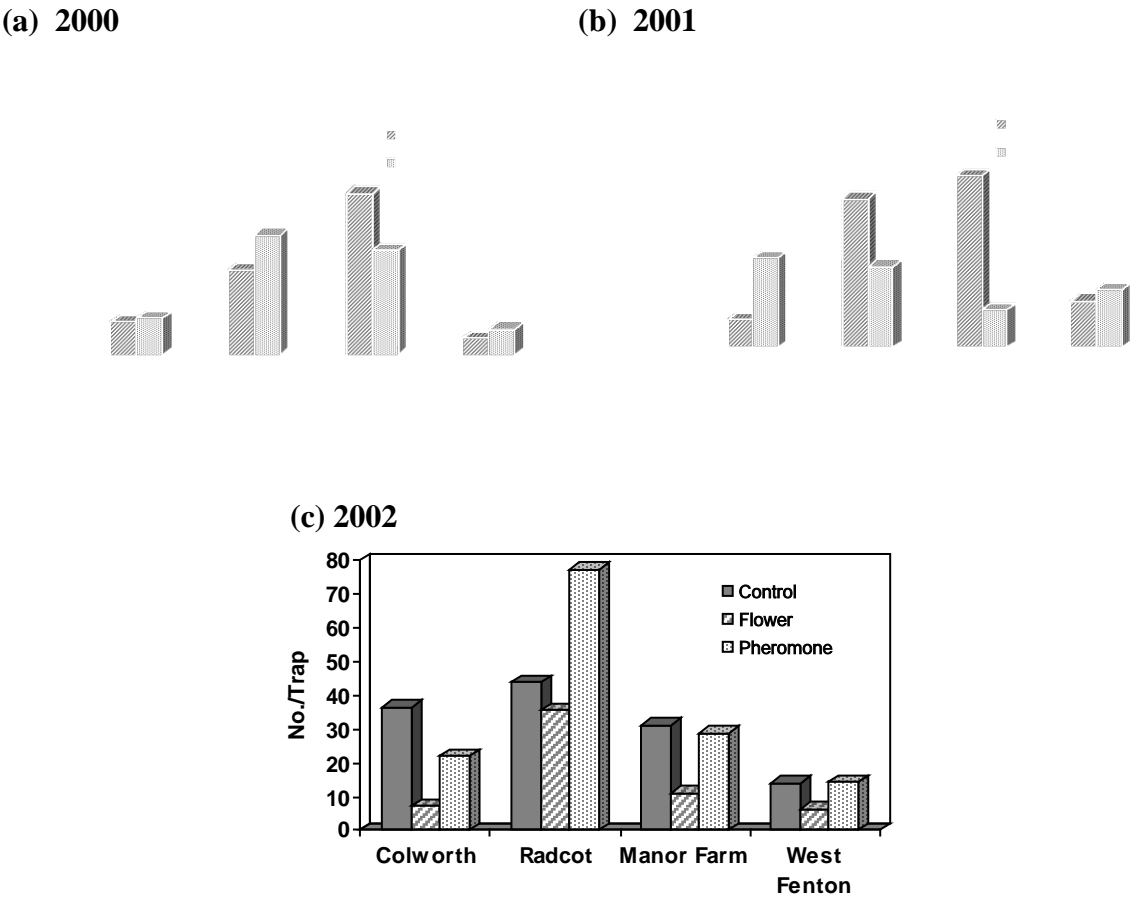
Although the target groups for manipulation in this part of the project were aphid parasitoids and hoverflies, carabid beetles are an important component of the natural enemy community affecting aphid populations and are known to be influenced by field margins. Therefore, it was important to monitor 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.

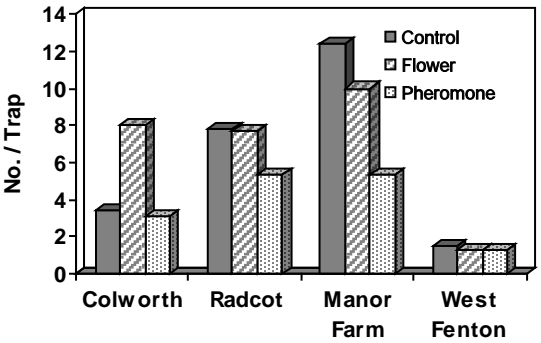
However, the relative abundance of carabids caught in pitfalls in the three fields at any one site was often different in the crop area and in the margin (compare Figs. 2.24 & 2.25). This indicates that species other than the dominant *Pterostichus* species were differentially affected by the treatments. 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.

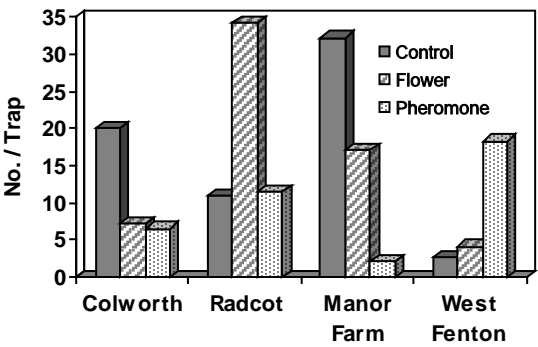


**Figure 2.25.** Mean number of carabid beetles caught per pitfall trap in cereal crop margins at the four study sites over the summer aphid season in 2000, 2001 and 2002 (Control Fields – solid bars; Fields with Flower-rich Margin – hatched bars; Pheromone-treated Fields – stippled bars). Note: The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

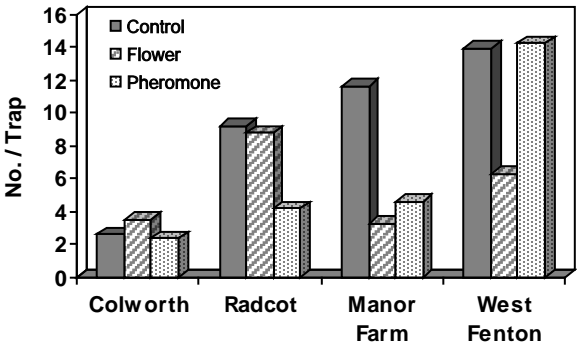
**(a) 2000**



**(b) 2001**

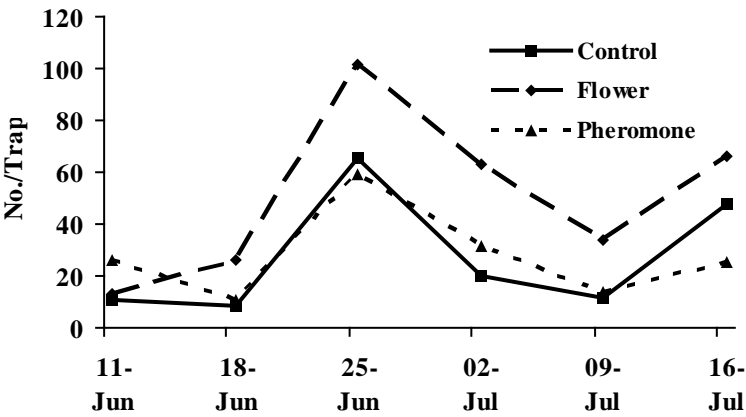


**(c) 2002**

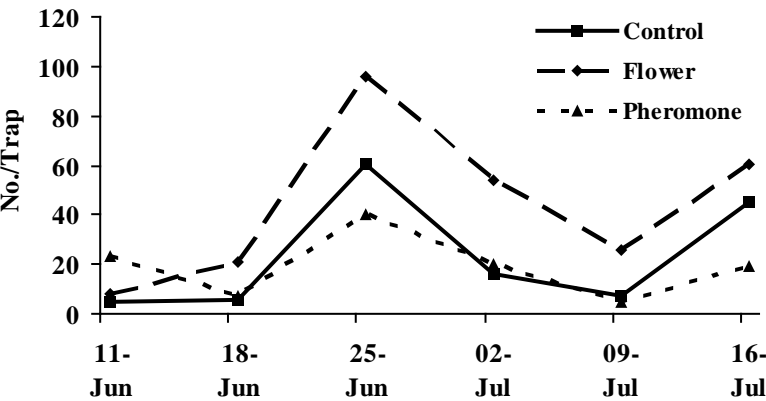


**Figure 2.26.** Mean number of carabid beetles caught per pitfall trap in cereal crops in the three study fields at Radcot in 2001. (a) Total carabids; (b) *Pterostichus* species only. Control Fields – solid line; Fields with Flower-rich Margin – dashed line; Pheromone-treated Fields – dotted line.

**(a) Total**

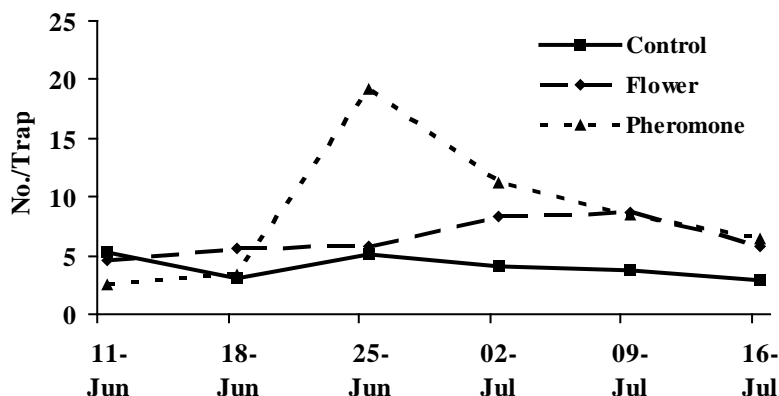


**(b) *Pterostichus* spp.**

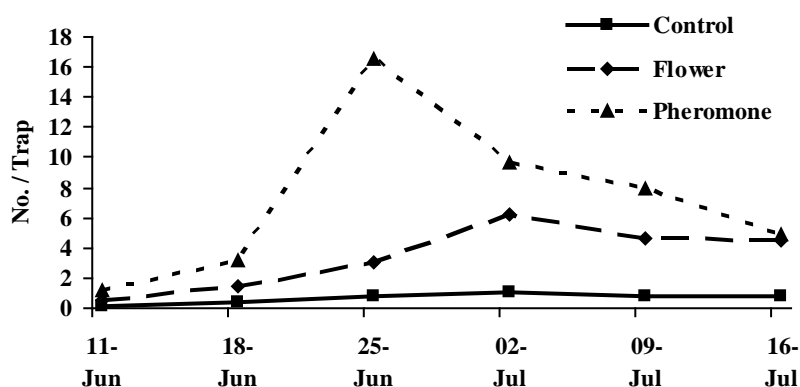


**Figure 2.27.** Mean number of carabid beetles caught per pitfall trap in cereal crops in the three study fields at Radcot in 2001. (a) Total carabids excluding *Pterostichus* species; (b) *Harpalus rufipes* only. Control Fields – solid line; Fields with Flower-rich Margin – dashed line; Pheromone-treated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

**(a) Total excluding *Pterostichus* spp.**



**(b) *Harpalus rufipes***

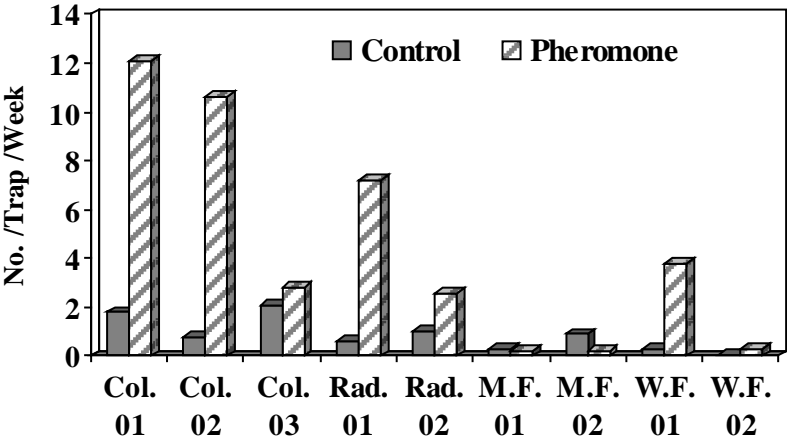


**2.3.4.2. Effect of aphid sex pheromone on *Harpalus rufipes***

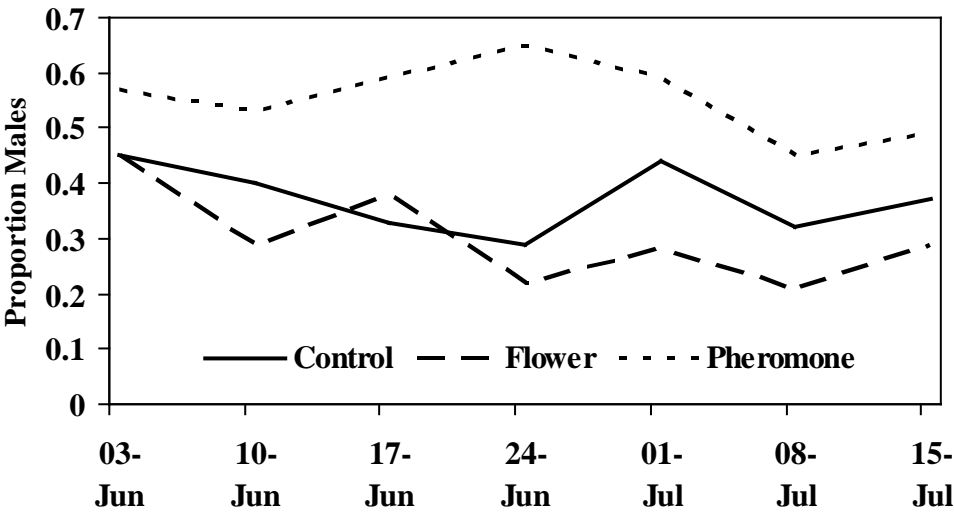
When catches of the carabid beetle *Harpalus rufipes* are considered for all the site/years in which the aphid sex pheromone, nepetalactone, was deployed in the crop, many more were caught in the pheromone-treated field than in the control field in five out of the nine occasions (Fig. 2.28). On three 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

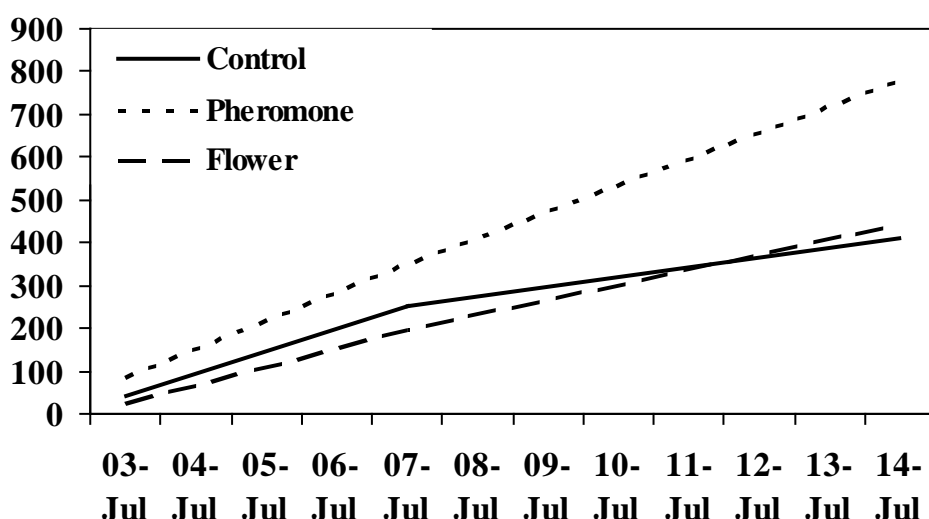
In the final year, 2003, pilot trials were done at four sites to explore the possibilities and identify the difficulties of adapting the hoverfly and parasitoid manipulation approaches, initially developed for cereal aphid control, for use in horticultural field crops. These trials involved vining pea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

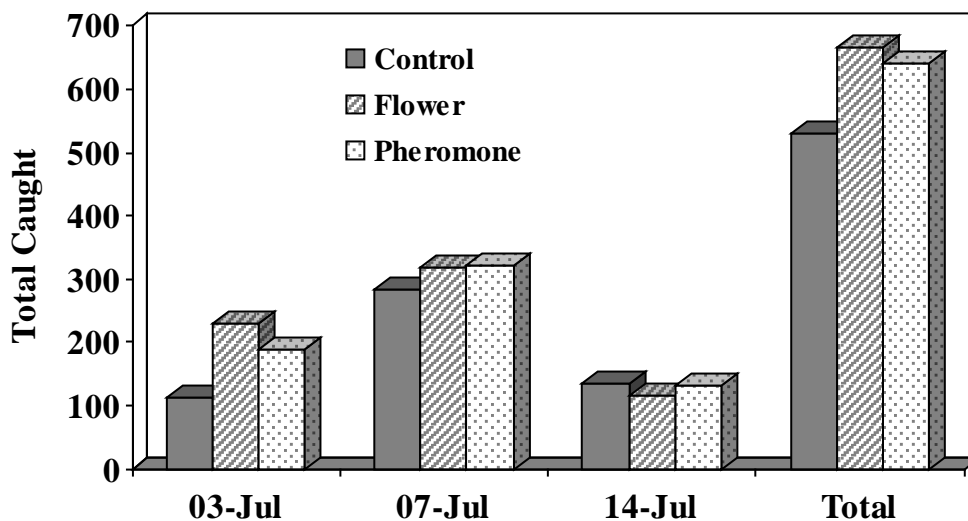
Pea aphid numbers increased rapidly from late June until mid-July when the farmer applied an aphicide (Aphox) on 18<sup>th</sup> July, after which no aphids were recorded in the weekly plant counts. The presence of the flower-rich margin appeared to have little effect on aphids in the adjacent crop area (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 18<sup>th</sup> July.

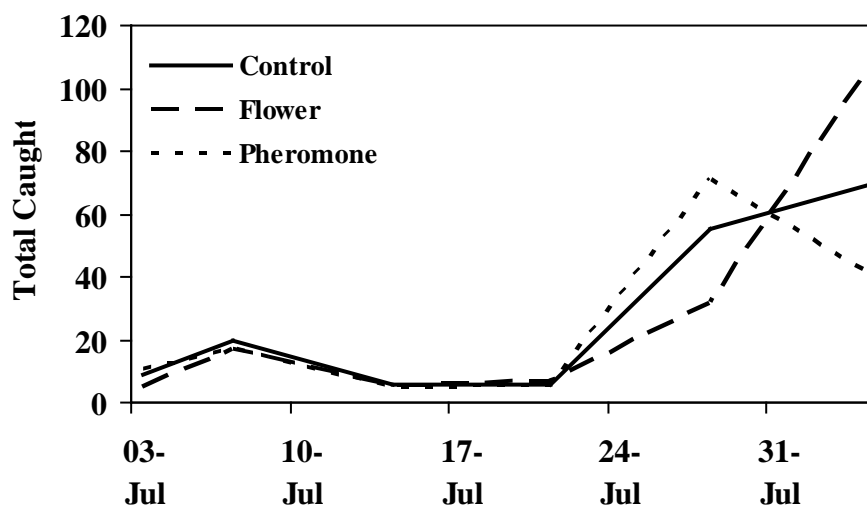


Aphid parasitoids at Drem were dominated by *Aphidius ervi*, which comprised more than 90% of the individuals caught. The pea aphid is regarded as the main host of this parasitoid, although it attacks a range of other species, including cereal aphids. There were no significant differences amongst the 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).

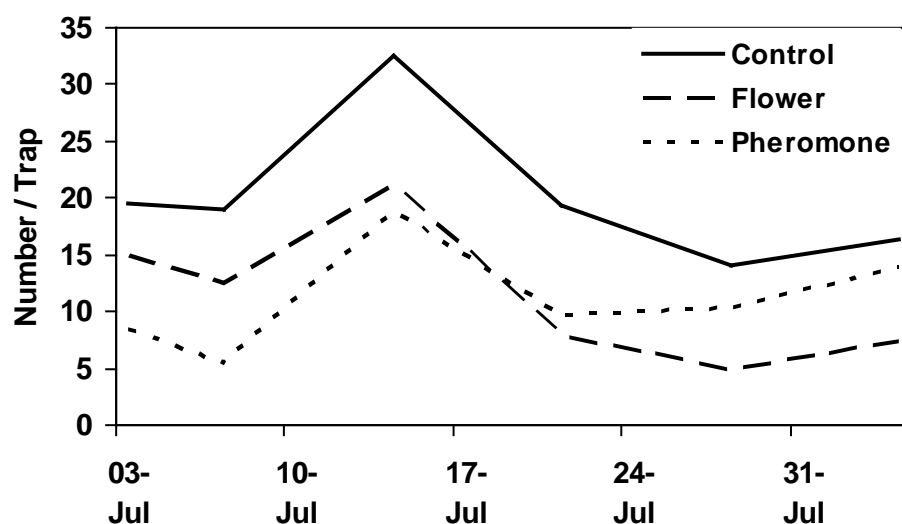


**Figure 2.32.** Adult aphidophagous hoverflies caught in water traps placed in three treatment areas in a pea crop at Drem, East Lothian in 2003. (Control Area – solid line; Area with Flower-rich Margin – dashed line; Pheromone-treated Area – dotted line).



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 18<sup>th</sup> 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.

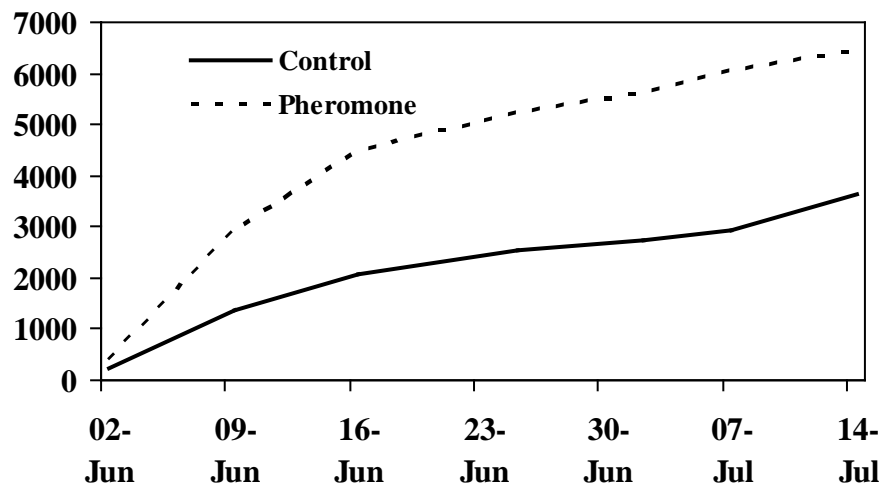
**Figure 2.33.** Carabid beetles caught in pitfall traps placed in three treatment areas in a pea crop at Drem, East Lothian in 2003. (Control Area – solid line; Area with Flower-rich Margin – dashed line; Pheromone-treated Area – dotted line).



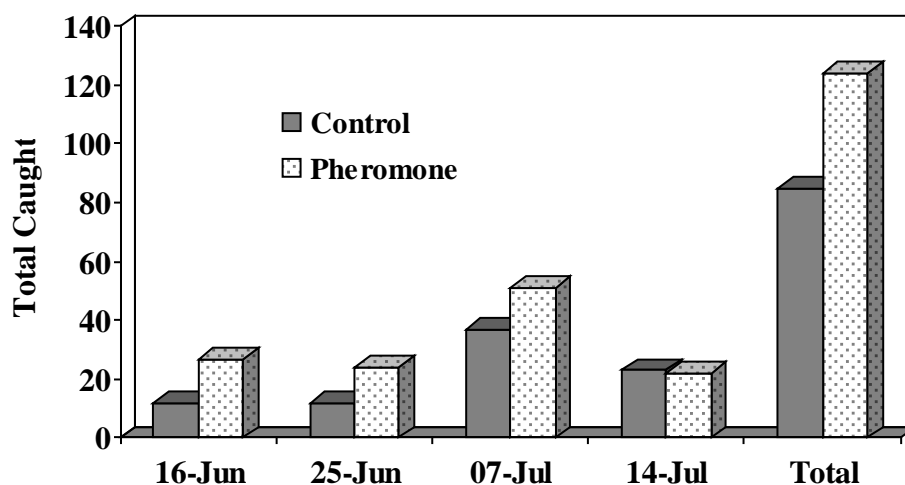
Pitfall traps placed in the pea crop caught more carabid beetles in the control area than in the area adjacent to the flower-rich margin and the pheromone-treated area, especially in the period before the crop was treated with an aphicide (18<sup>th</sup> July) when twice as many were caught in the control 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).



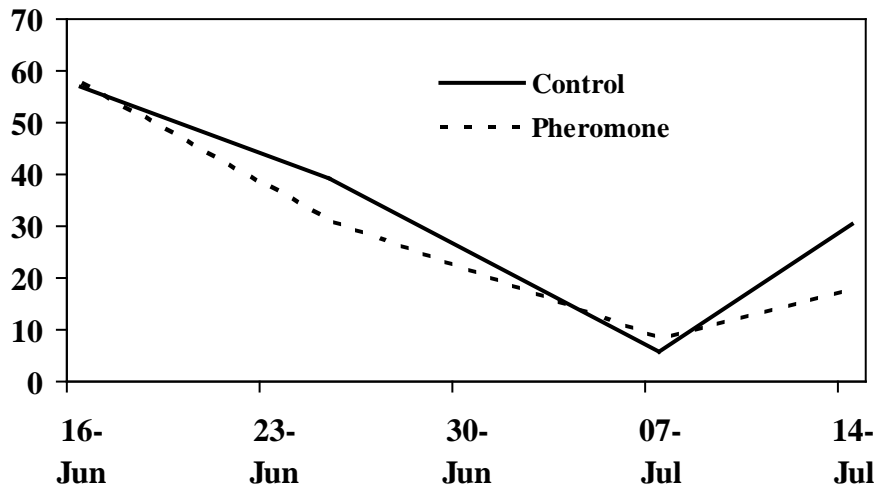
**Figure 2.35.** Numbers of adult aphid parasitoids in suction net samples taken from two treatment areas in a pea crop at Royston, Cambridgeshire in 2003. (Control Area – solid bars; Pheromone-treated Area – stippled bars). Both areas were bordered on one side by a flower-rich margin.



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.



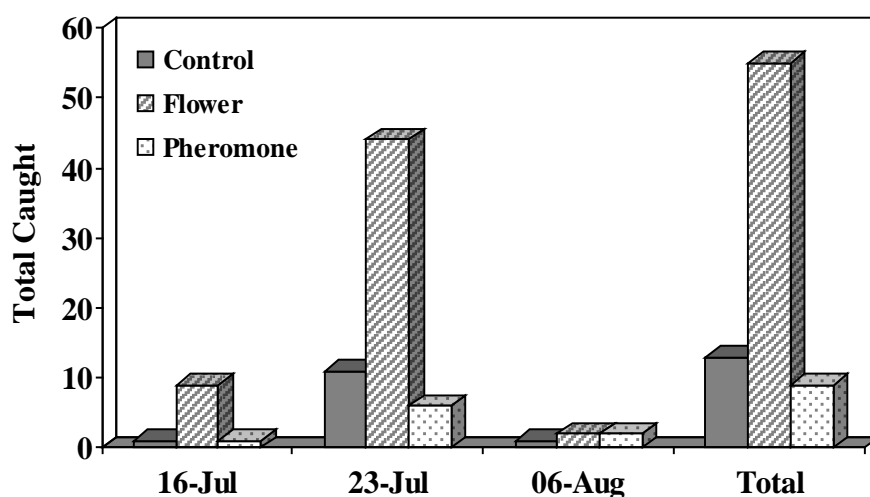
In this additional, reduced trial, hoverflies and carabid beetles were not monitored due to lack of resources, which were concentrated on the main trial sites.

### 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 16<sup>th</sup> 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.

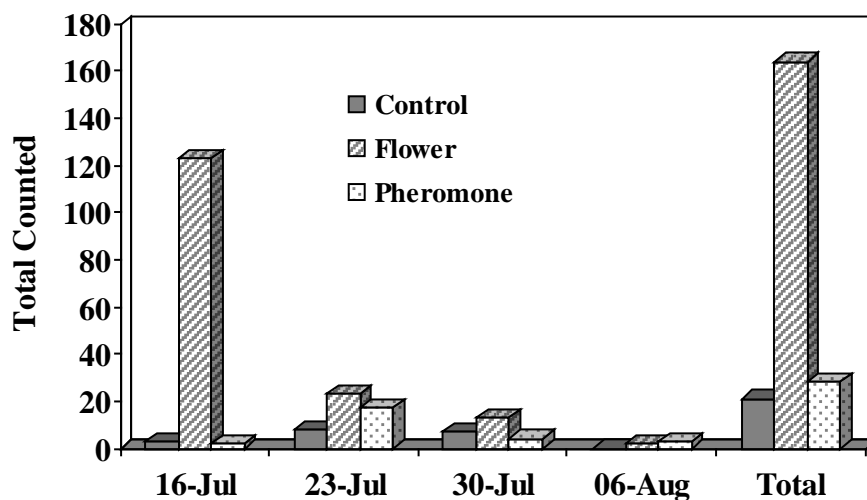
**Figure 2.37.** Adult aphid parasitoids caught in vortis suction samples in organic broccoli crops at Epworth, Yorkshire in 2003. The pheromone-treated area (stippled bars) and control areas (solid bars) were at opposite sides of the same field but the flower-rich margin treatment (hatched bars) was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



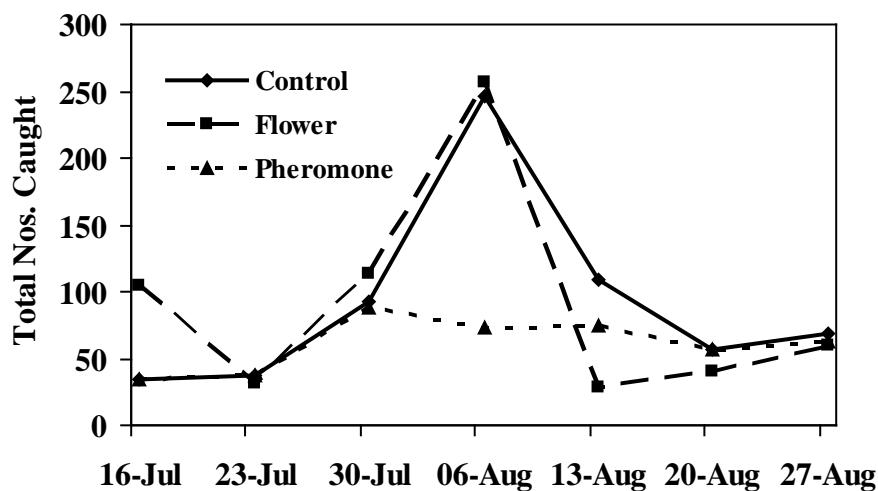
The adult aphid parasitoid catches were dominated by *Diaeretiella rapae* (86% of the total catch), a species that specialises in attacking aphids on brassicaceous plants. Significantly more ( $p < 0.01$ ) parasitoids were caught in the sample area next to the flower-rich margin than in the control and 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.

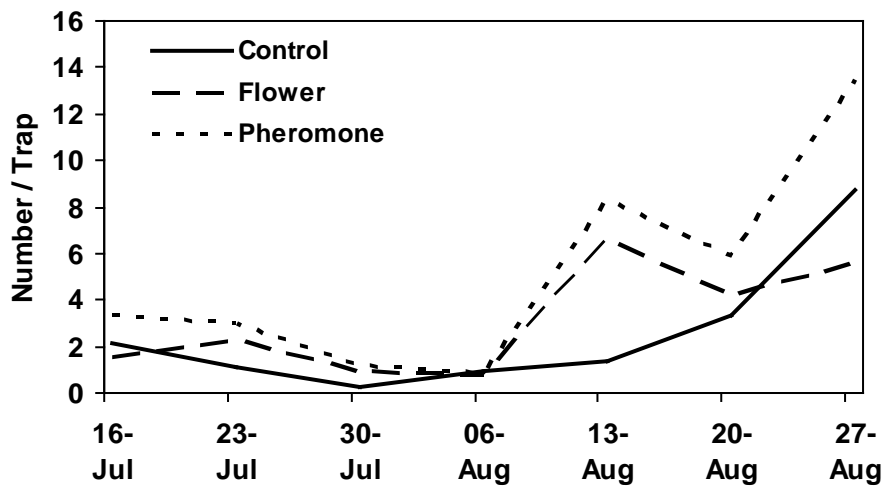




Catches of adult aphidophagous hoverflies were very similar in the three sample areas except on the first sampling date (16<sup>th</sup> July) when more were caught in the area adjacent to the flower-rich margin than in the other two sample areas and on 6<sup>th</sup> August when far fewer were caught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



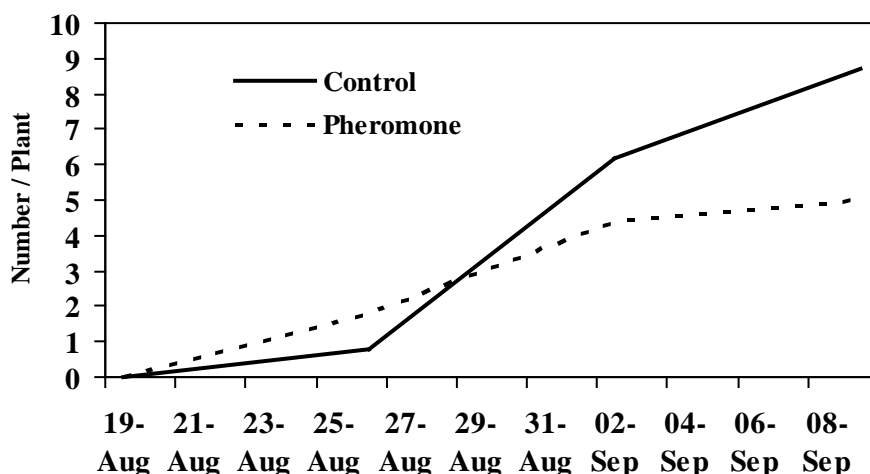
### 2.3.5.3. Organic lettuce

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.

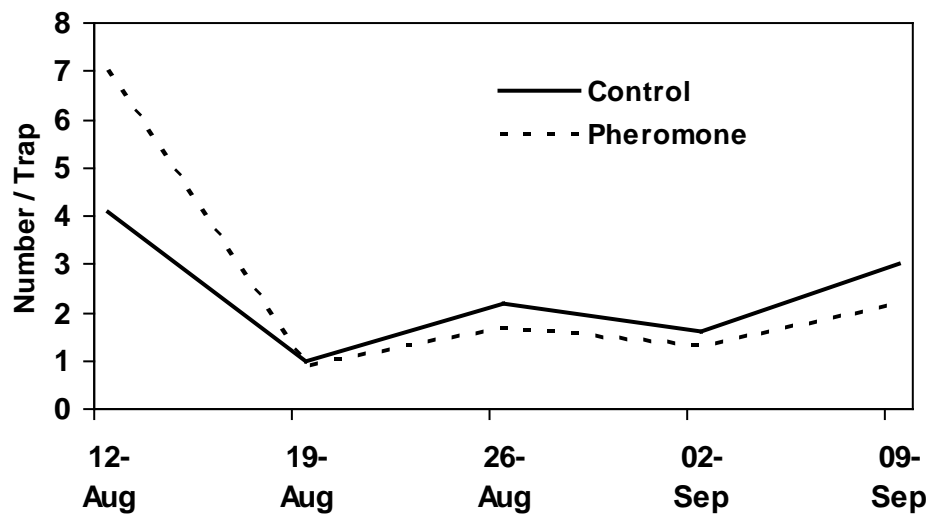
Only fifteen adult aphid parasitoids were caught in Vortis suction net samples taken within the lettuce crop during the sampling period, most of which were probably associated with aphids on plants within the adjacent field margin.

**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 *Metopolophium 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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### **3. INVESTIGATIONS OF APHID AND BENEFICIAL INSECT ABUNDANCE, DISPERSAL AND SPATIAL DISTRIBUTION ACROSS FIELDS**

#### **3.1. INTRODUCTION**

It is now well recognised that insects exist as discrete patches of local populations that together constitute a metapopulation and it has been demonstrated that this phenomenon occurs in agricultural landscapes for some predatory groups (reviewed for Carabidae by Thomas et al., 2002) and pests (Winder et al., 1999). The size of these local populations within a metapopulation may differ depending on the species and can extend over several hectares (Holland et al., 1999). However, most studies have been conducted within parts or across whole fields (Holland et al., 1999; Thomas et al., 2002) and the extent to which these metapopulations extend across boundary features (e.g. field boundaries, tracks, watercourses and roads) is not understood. For some species, such non-crop areas form stable habitats that are seasonally essential for aestivation or overwintering (Sotherton, 1984). Recolonisation of the ephemeral cropped areas then occurs when conditions there become more suitable (Wissinger, 1997). Habitats at field margins can also act as refuges from agricultural operations that may impose certain mortality risks to individuals within the field. That part of a population taking refuge within non-crop habitats may then act as an important source population for the rapid re-colonisation of fields following catastrophic disturbances (Holland et al., 2000). However, for species that inhabit the cultivated areas alone, features such as field boundaries and roads may act as barriers to movement and dispersal between fields (Mader et al., 1990; Mauremootoo et al., 1995; Thomas et al., 1998), and consequently may influence epigeal arthropod distribution patterns or impose metapopulation structure on their populations by fragmenting them into local populations. Few studies have investigated how non-crop habitats and their distribution in fragmented farmland affect the spatial dynamics of beneficial insects. Moreover, the extent to which non-crop habitats at field boundaries restrict insect movement between fields has rarely been studied extensively. There is also little definitive evidence to explain why insects are frequently heterogeneously distributed within fields, even though investigating how arthropods are distributed in agricultural landscapes can provide important and useful insights into how best to encourage them for pest control (Thomas et al., 2001). Within fields, abiotic and biotic factors may influence spatial dynamics and, consequently, levels of natural biological control. The most influential abiotic factors are likely to be those related to crop management practices (eg. pesticide inputs, crop rotations and tillage). Biotic factors include suitability and location of overwintering habitats, soil type and moisture, cover by vegetation within fields and food availability. The issues relating to invertebrate spatial dynamics were reviewed by Thomas et al. (2002) for Carabidae but the principles discussed are applicable to a wider range of invertebrates.

Funding to establish additional non-crop habitats within farmland is now available through various agri-environment schemes. Some of these habitats will be colonised by beneficial invertebrates and this may increase levels of biocontrol in nearby fields. However, little information is available on where these should



be located to maximise their benefits for biocontrol and biodiversity. Studies of insect spatial pattern in relation to non-crop habitats will provide guidance on the location of non-crop habitats.

Insecticides are known to cause mortality of many non-target species but some are able to re-colonise after spraying from unsprayed refuges such as field margins and adjacent unsprayed fields (Duffield et al.; Holland et al., 2000). The extent and rapidity of this process depends upon the mobility of individual species, the extent and location of unsprayed refuges and to what extent there are barriers to their movement. A better understanding of insect distributions and movement will aid the insecticide risk assessment process by allowing the potential for reinvasion to be estimated. Species or groups of invertebrates at greatest risk may be identified, and mitigation measures developed to reduce the impact on the population.

### **3.1.1. Generalist Beneficial Invertebrates**

The studies of spatial distribution patterns focussed on generalist predatory invertebrates, as other groups such as parasitoids and the specialist aphid predators, hoverflies and ladybirds, are highly mobile, active fliers and so their spatial distribution on farmland needs to be studied at greater spatial scales. The manipulation of parasitoids and hoverflies with respect to field margins was investigated in other parts of the project (section 2). Generalist predators are numerically dominant within agroecosystems and occupy a wide range of niches and so are important members of the natural enemy community attacking pests in arable field crops. Manipulative experiments demonstrated that generalist predators alone can keep cereal aphid populations below damage thresholds (Edwards et al., 1979; Carter et al., 1980; Chambers et al., 1982; Chiverton, 1986; Holland & Thomas, 1997). Moreover, because they are present when aphids first appear they have the potential to slow down or prevent the development of aphid outbreaks (Edwards et al., 1979; Chiverton 1986), especially when aphid numbers increase slowly (Carter et al., 1980; Chambers et al., 1982). They also inhabit the field and the boundaries throughout the year and are consequently influenced by a wide range of agricultural practices, the response varying according to the species ecology and phenology.

Studies of generalist predator spatial dynamics have focussed on quantification of spatial pattern within fields or parts thereof (Ericson, 1978; Hengeveld, 1979; Holland et al., 1999; Thomas et al., 2001), their capacity to penetrate field boundaries (Thomas et al., 1998; 2001), relationship to pests (Warner et al., 2000; Winder et al., 2001), the impact of changes in cropping (Brown, 2000) and response to insecticide applications (Thomas & Jepson, 1997; Holland et al., 2000). The spatial dynamics of generalist predators within the same field over more than one year have only been investigated once (Brown, 2000). Moreover, the majority of these studies were on carabid beetles (Carabidae), although other insects such as rove beetles (Staphylinidae) are known to be important for pest control (Dennis & Wratten, 1991). All studies of generalist predator distribution patterns have used pitfall traps, which measure a combination of activity/density. To what extent these relate to actual density has always been debatable.

### **3.1.2. Pests**

The spatio-temporal dynamics of several pest species has been investigated, including that of cereal aphids (Schotzko & Knudsen, 1992; Winder et al., 1999), slugs (Bohan et al., 2000), pea leaf weevil (Schotzko & Quisberry, 1999), brassica pod midge (Warner et al., 2000) and cabbage stem weevil, pollen beetle and cabbage seed weevil (Ferguson et al., 2003). Most data exists for cereal aphids, and in the UK their distribution has been examined in detail using a grid based sampling approach within eight cereal fields (Winder et al., 1999 & unpublished data). These have shown that pest species exhibit heterogeneous distribution patterns within fields, but the location of areas of high and low density may vary through time. Redistribution may occur following initial colonisation in response to a range of factors, but predation, environmental conditions and host suitability have all been shown to have an effect (Winder et al., 2001; Ferguson et al., 2003). Knowledge of a pest's spatial dynamics can assist when developing integrated management strategies. Crop scouting can be improved to ensure that spatial pattern is taken into account (Alexander et al., submitted), thereby gaining the most accurate measurement of infestation levels for the minimum cost. Chemical intervention can be targeted at those areas of highest risk, for example for those species invading from the field edges. Finally, the relative importance of abiotic and biotic factors may be determined if appropriate additional sampling is conducted and this can lead to the development of alternative cultural methods of control.

In this project the large-scale, spatio-temporal dynamics and movement of beneficial epigeal insects was investigated along with measurements of some of the most likely influential biotic factors over three years (Section 3.2-3.4). The landscape was also manipulated to increase the proportion of non-crop habitat through the use of set-aside strips. Experimental studies were then conducted to confirm the influence of some biotic factors (Section 3.5-3.7).

### 3.2. INVESTIGATION OF THE LARGE-SCALE, SPATIO-TEMPORAL DYNAMICS OF PREDATORY EPIGEAL INVERTEBRATES IN ARABLE FARMLAND.

This study formed one of the main components of the overall project and aimed to investigate a number of issues in relation to the population Density, Distribution and Dispersal (hence 3D Farming) of predatory invertebrates in field crops. The primary aims were to investigate:

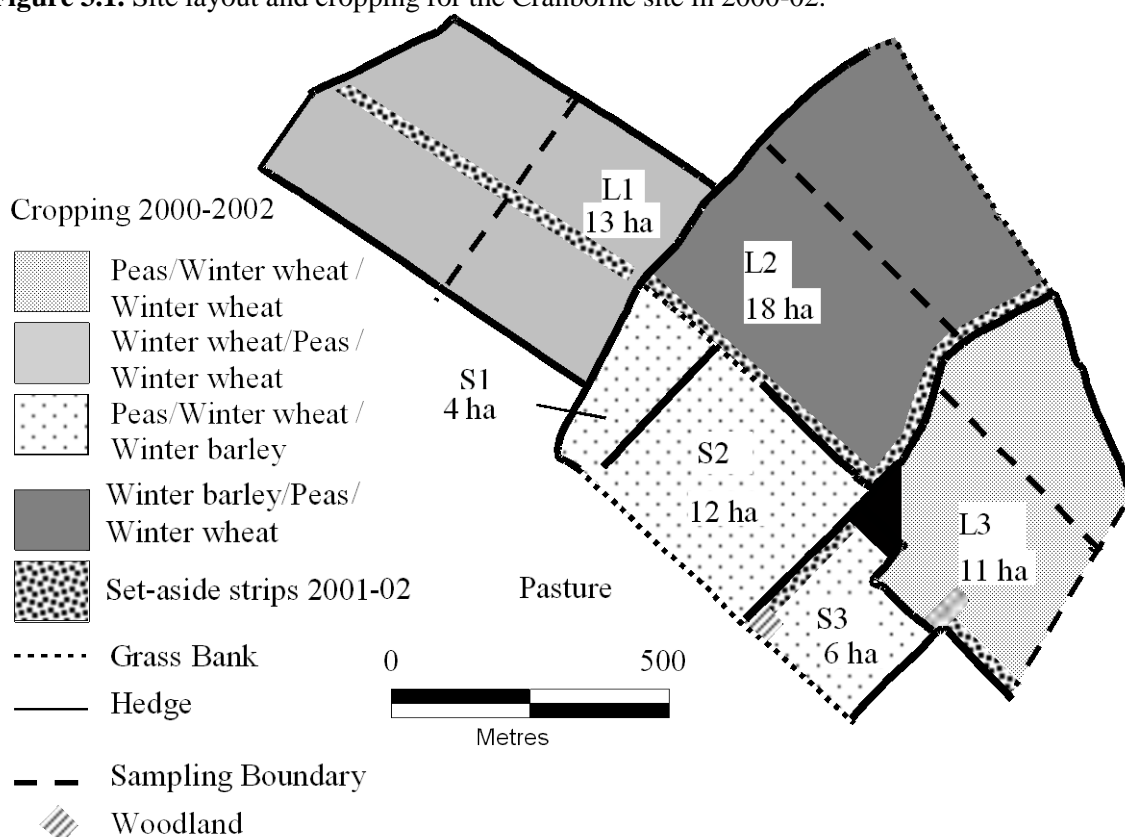
- The spatial pattern of predatory invertebrate distributions across a contiguous block of fields
- The within and between year stability of predatory invertebrate aggregations
- The scale of these aggregations for different species and groups of invertebrates
- The association with biotic and abiotic factors in the field
- The relative contribution of different field boundary types to invertebrate diversity within the field
- The extent of the influence of field boundaries on invertebrate abundance and diversity within the cropped area.

Beneficial invertebrates were sampled using pitfall traps in conjunction with measurements of plant cover and soil moisture. To investigate within-year spatial changes sampling was conducted four times in 2000 while between-year changes were determined by sampling twice in the following two years. An additional post-harvest sample was taken in some fields in 2001.

#### 3.2.1 Materials and Methods

##### 3.2.1.1. Field site

**Figure 3.1.** Site layout and cropping for the Cranborne site in 2000-02.



The study area, covering 66 ha in Dorset, comprised six arable fields separated by mature hedgerows or grassy banks (Fig. 3.1). The majority of hedgerows comprised a hedge with an herbaceous/grass bank. In many places the dominant species were barren brome (*Anisantha sterilis*) and stinging nettle (*Urtica dioica*). A beetle bank was established across one of the larger fields and formed a boundary to the sampling area. The crop rotation was of winter wheat, spring-sown forage peas and winter barley. The three smaller fields were all at the same stage of the crop rotation while that of the three large fields differed. After the first year of sampling a number of 24 m wide set-aside strips, sown with a mixture of kale, millet, *Phacelia tanacetifolia*, quinoa, triticale, sunflowers and yellow sweet clover, were established around the edge of four of the fields. The insecticide 'pirimicarb' (0.27 kg ha<sup>-1</sup>) was applied on 17/6/2000 to fields S1-S3 & L3 and to L2 on 24/6/2001.

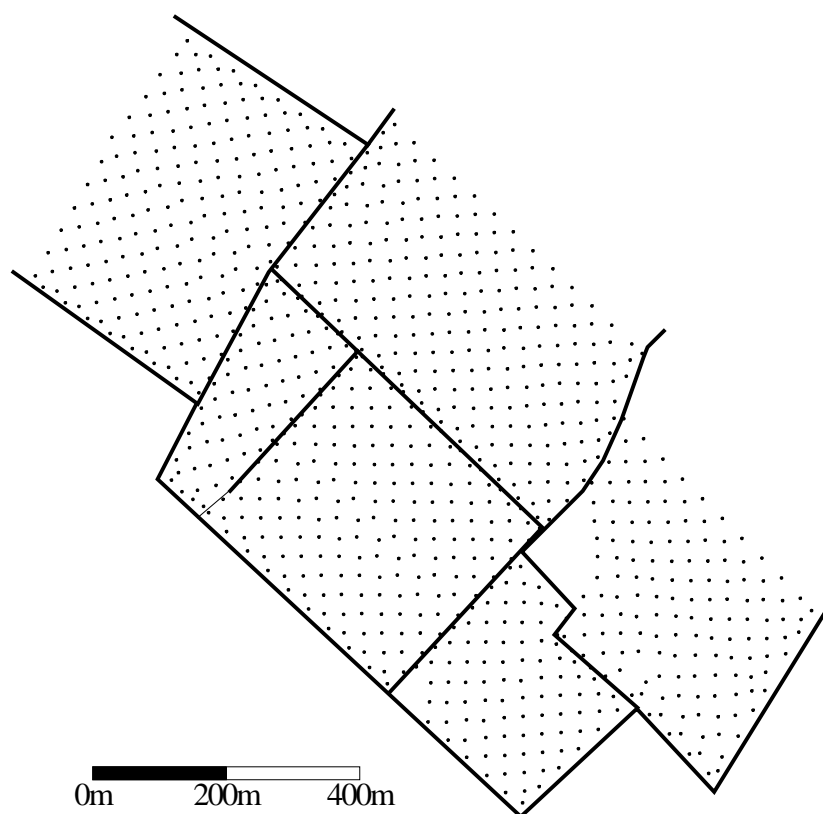
### 3.2.1.2. Insect sampling

Epigeal invertebrates were sampled simultaneously across the study site using paired pitfall traps arranged in a grid pattern with 973 sampling points (Fig. 3.2). The pitfall traps were arranged in an offset grid pattern with 40 x 20 m spacing within each field. The grid extended across the whole of the three smaller fields, but only half the area of each large field was covered (Fig. 3.2). At each sampling point there were two pitfall traps (6 cm diam.), partly filled with water and detergent, and these were opened for one week on four occasions in 2000 and twice in 2001 and 2002 (Table 3.1). Each sample location was surveyed and located using the national grid reference using a differential Global Positioning System (Geoexplorer 3, Trimble, California, USA). After collection all arthropods were removed and stored in 70% alcohol. The majority of the catch comprised carabid beetles (Coleoptera; Carabidae), rove beetles (Coleoptera; Staphylinidae) and spiders (Arachnida; Araneae). The Carabidae and Staphylinidae were identified to species; other genera or families were also identified in each sample and are listed in Table 3.2 along with their overwintering location. Numbers of 19 species of Carabidae, 10 staphylinid species and 7 families were listed. Many money spiders (Araneae; Linyphiidae) were also caught but pitfall trapping provides an unreliable estimate of their abundance and they were not identified (Topping & Sunderland, 1992).

**Table 3.1.** Sampling dates for insect distribution studies.

Pitfall trapping		Weed cover	
Cereals	Peas	Cereals	Peas
2-9/5/2000	16-23/5/2000	4/5/2000	18/5/2000
6-13/6/2000	6-13/6/2000	6/6/2000	7/6/2000
28/6-5/7/2000	28/6-5/7/2000		
12-19/7/2000	12-19/7/2000	14/7/2000	18/7/2000
4-11/6/2001	4-11/6/2001	7-12/6/2001	7-8/6/2001
9-16/7/2001	9-16/7/2001	11-13/7/2001	10-11/7/2001
5-12/9/2001	5-12/9/2001		
10-17/6/2002	10-17/6/2002	13/6/2002	13/6/2002
8-15/7/2002	8-15/7/2002		

**Figure 3.2.** Sampling positions within each field.



#### **3.2.1.3. *Ground cover***

The proportion of ground covered by vegetation and soil moisture are both factors that are considered to influence the distribution of epigeal invertebrates and therefore measurements of these were made across the study area. The proportion of bare ground and that covered by broad-leaf and grass weeds and the crop was measured each year around each sampling position. Estimates were taken at five points within a 5m area of the pitfall sampling position on three occasions in 2000, twice in 2001 and once in 2002 (Table 3.1).

#### **3.2.1.4. *Soil moisture***

Soil moisture was estimated by measuring electrical conductivity using a soil probe (Theta Probe). Measurements were taken in 2000 (26/6-13/7). Three measurements were taken within 2m of each sampling position in four of the fields. However, owing to equipment failure in the other two fields (L1 and L2) measurements were taken at each sampling position along alternate rows only.

In autumn 2001 the fields were surveyed for electrical conductivity by Magnascan (Turftrax), from which soil moisture was estimated. Soil type was also measured by combining information from Magnascan with soil sampling across the study area. Soil moisture for each sampling position was estimated by entering the data into a GIS system and calculating the mean soil moisture around each sampling position using voronoi correction.

**Table 3.2.** Invertebrates recorded in spatial studies 2000-02 and their overwinter location where known.

<b>Taxa</b>	<b>Overwinter location</b>
<b>Carabidae</b>	
<i>Agonum dorsale</i>	Boundary
<i>Agonum muelleri</i>	Boundary
<i>Amara</i> spp.	Boundary & Field (varies between species)
<i>Asaphidion flavipes</i>	Boundary
<i>Bembidion lampros</i>	Boundary
<i>Bembidion obtusum</i>	Boundary & Field
<i>Calathus fuscipes</i>	Field
<i>Calathus melanocephalus</i>	Field
<i>Carabus</i> spp.	Boundary
<i>Demetrias</i> spp.	Boundary
<i>Harpalus affinis</i>	Field
<i>Harpalus rufipes</i>	Field
<i>Loricera pilicornis</i>	Field
<i>Nebria brevicollis</i>	Boundary
<i>Notiophilus biguttatus</i>	Field
<i>Pterostichus cupreus</i>	Field (larvae) & Boundary (as adults)
<i>Pterostichus madidus</i>	Field (larvae) & Boundary (as adults)
<i>Pterostichus melanarius</i>	Field (larvae) & Boundary (as adults)
<i>Trechus quadristriatus</i>	Field
Carabid larvae	Field & Boundary
Total Carabidae	
Number of carabid species	
Boundary overwintering Carabidae	
<b>Staphylinidae</b>	
<i>Paederus</i> spp.	Boundary
<i>Philonthus cognatus</i>	Field
<i>Philonthus</i> spp.	
<i>Stenus</i> spp.	Boundary
<i>Tachinus</i> spp.	Boundary
<i>Tachyporus chrysomelinus</i>	Boundary
<i>Tachyporus hypnorum</i>	Boundary
<i>Tachyporus nitidulus</i>	Boundary
<i>Tachyporus obtusum</i>	Boundary
<i>Xantholinus</i> spp.	Boundary
Staphylinid larvae	Field & Boundary
Total Staphylinidae	
Number of Staphylinid species	
Elateridae	Field
Elaterid larvae	Field
Curculionidae	Boundary
Coccinellidae	Boundary
<i>Gastrophysa polygoni</i> (Chrysomelidae)	Boundary
Other Chrysomelidae	Boundary
Other Coleopteran larvae	
Lycosidae	Boundary & Field
Total predatory invertebrates	
Number of carabid & staphylinid species	
Boundary overwintering invertebrates	Boundary

Both the above techniques identify relative changes in soil moisture but cannot be used to determine actual soil moisture levels. In addition, the soil moisture levels determined in the autumn may not reflect those that occur in the preceding or following summer because soil moisture can be transitory, the soil type having a strong influence.

### 3.2.1.5. Data analysis

To determine whether the distribution of insects or the environmental parameters were spatially aggregated into patches of higher than average numbers or gaps of lower than average numbers their distribution was analysed using SADIE analysis (Spatial Analysis by Distance IndicEs) (Perry et al. 1999), termed “red/blue” analysis. This calculates the degree of clustering in the form of (i) 'patches' of large counts, using the overall index  $\bar{v}_i$  and its associated probability  $P_i$ , or (ii) 'gaps' of small counts, using the overall index  $\bar{v}_j$  and its associated probability  $P_j$  (Perry et al., 1999). For a particular set, if all of these indices have values around unity, conformation of the data to the null hypothesis of spatial randomness is indicated; a value of at least one index well above unity indicates spatial non-randomness of some form. Distribution data are presented as two-dimensional contour maps from counts, drawn using the package Surfer for Windows Version 6.04 (Golden Software Inc, Colorado, USA). Analyses were conducted for data from across the whole study area and in some cases for each field.

To test whether two data sets were spatially correlated, the correlation coefficient,  $X$ , between the clustering indices of each set was calculated according to the method described by Perry & Dixon (2002). Hence, if the indices of set one are denoted  $z_{i1}$ , with mean  $q_1$  and those of set two  $z_{i2}$ , with mean  $q_2$ , then a measure of local spatial association for position  $i$  is given by:

$$\chi_i = n(z_{i1} - q_1)(z_{i2} - q_2) / [\sum_i (z_{i1} - q_1)^2 \sum_i (z_{i2} - q_2)^2]^{1/2}$$

The overall spatial association is the mean of these local values,  $X = \sum_i \chi_i / n$ . The significance of  $X$  was tested against values  $X_{\text{rand}}$  from a randomisation test that included a Dutilleul (1993) adjustment procedure to provide a probability value  $P_D$ .

There may also be differences in the invertebrate community composition between crops and between years. To test for crop differences, multivariate analyses were conducted on the pitfall data using routines in PRIMER (PRIMER 5.2.9, PRIMER-E Ltd). These analyses are relatively robust as they are non-parametric and make very little assumption about the nature of the data (Clarke & Warwick, 2001). A two way crossed ANOSIM was used to test for significant differences (inferred from similarities) between the invertebrate community composition of the samples according to crop, removing any year differences. Data was averaged across fields so that 'field' was the basic unit of analysis; June and July data were analysed separately. ANOSIM is based on a similarity matrix, which in this case was calculated using fourth root transformed data and the Bray-Curtis similarity co-efficient. The R statistic (global or pair-wise) that is generated reflects

the observed differences between the groups (in this case crops), contrasted with differences among replicates within them. The test is based on rank similarities between samples in the similarity matrix. R falls between 0 and 1 giving an absolute measure of how separated the groups are; 0 indicates that they are virtually indistinguishable and 1 indicates that all similarities within groups are less than any similarity between groups (i.e. groups are very different). The R statistic itself is a useful comparative measure of the degree of separation of sites, and its value is at least as important as its statistical significance, if not more so (Clarke & Warwick, 2001). As with standard univariate tests, it is possible for R to be significantly different from zero yet inconsequentially small if there are many replicates at each site. (Clarke & Warwick, 2001). Where a significant difference was found, SIMPER was used to characterise that difference. This identified which species discriminated the invertebrate communities between crops, based on rank abundance. The limitation is that the routine compares two groups at a time and this can complicate interpretation of the analysis.

### **3.2.2 Results**

Spatial distribution data were collected for 29 species from seven invertebrate families over three years. In total 501,963 individual invertebrates were identified over the three years. Some invertebrate groupings were also compiled and these included: boundary overwintering Carabidae, total boundary overwintering invertebrates, number of carabid species, number of staphylinid species, number of carabid and staphylinid species, total number of predatory invertebrates and total species richness. Data from across the six fields for all of these species/families/groupings was analysed to determine the extent to which spatial patterning existed and the extent of spatial association between and within years. Association with the key environmental parameters of soil moisture and vegetation cover was also examined for many of these invertebrate groups. In addition, data for individual fields was analysed for some groups. Owing to the enormity of the data output, only a selection of the data can be presented here. These were selected as being either typical for an invertebrate family or being highly relevant to the overall aims of the project.

#### **3.2.2.1. Abundance and distribution patterns in 2000**

The abundance of carabid beetles increased from May through to July but the species richness declined (Table 3.3). In contrast, numbers and species richness of staphylinid beetles peaked in June. Thus in June the pitfall catch comprised Carabidae (44%) and Staphylinidae (42%), but in July the fauna comprised primarily Carabidae (94%). Two species, *Pterostichus madidus* and *P. melanarius*, which are large generalist predatory species, formed 91% of the pitfall trap catch at this time. The fauna was more diverse earlier in the summer with a more even species composition. The species that overwinter as adults in the field boundaries were most abundant early in the year (May and June). In contrast, those that overwinter as larvae within fields and emerge as adults were most abundant in July. The latter group were most abundant overall and, as a consequence, the total number of invertebrates was highest in July.

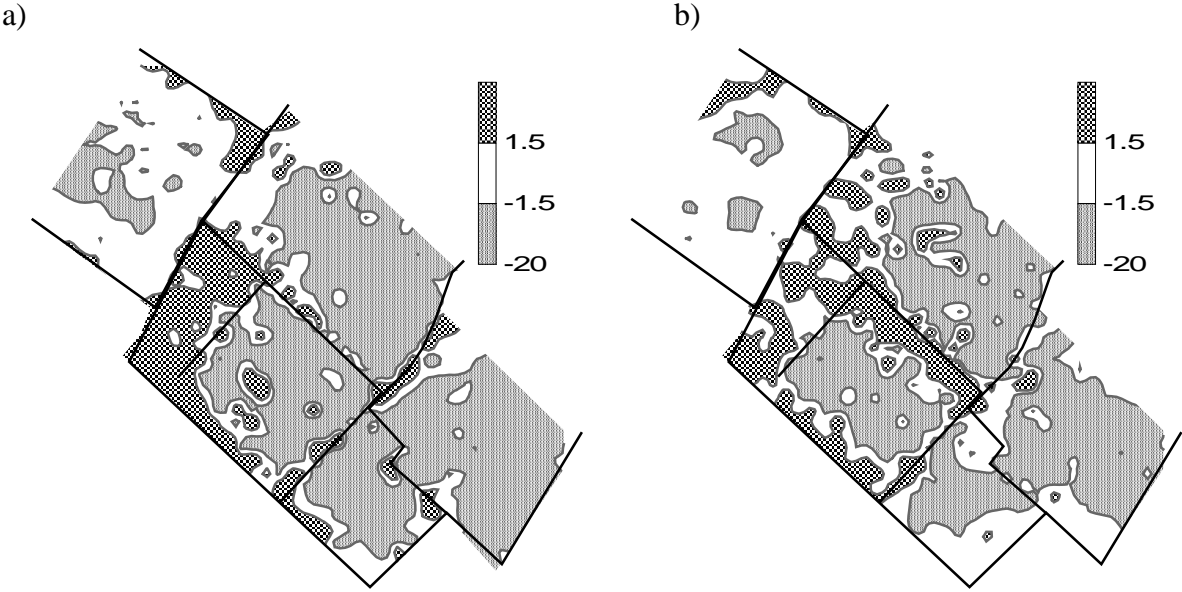


**Table 3.3.** Mean and 1 standard error (SE) for each predatory arthropod group across the six fields in 2000.

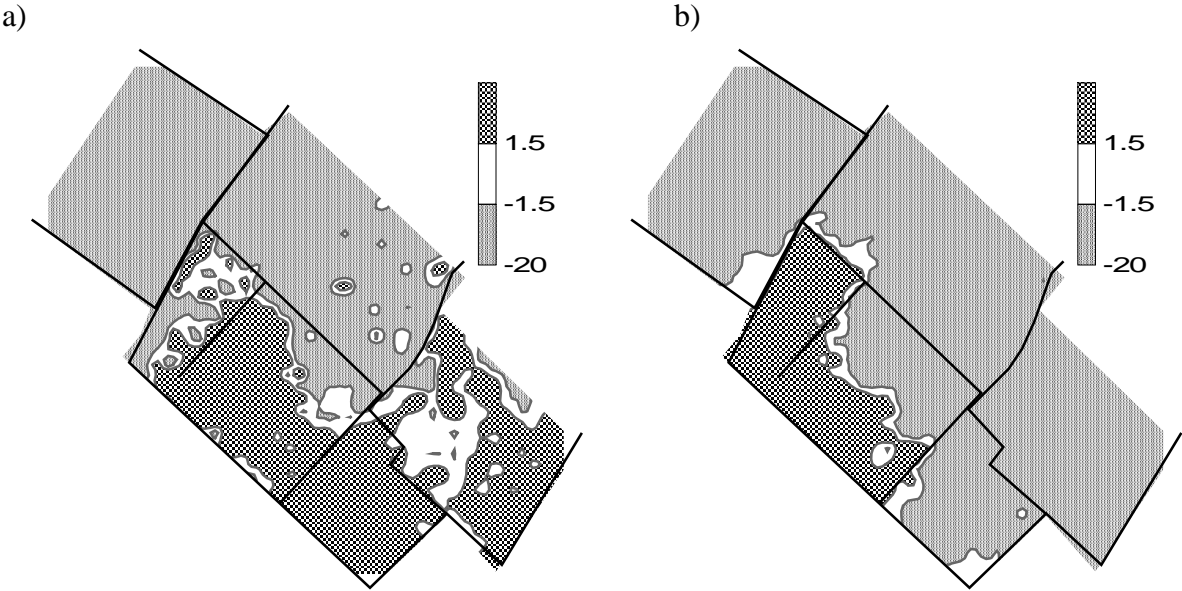
	May		June		early July		mid-July	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<i>Agonum dorsale</i>	0.73	0.04	1.41	0.07	0.30	0.02	0.26	0.02
<i>Bembidion lampros</i>	5.94	0.27	2.14	0.12	0.19	0.02	0.26	0.03
<i>Bembidion obtusum</i>	0.87	0.05	0.48	0.04	0.03	0.01	0.03	0.01
<i>Nebria brevicollis</i>	2.79	0.17	2.00	0.10	0.06	0.01	0.08	0.01
<i>Poecilus cupreus</i>	0.70	0.08	1.50	0.18	0.21	0.03	0.07	0.02
<i>Pterostichus madidus</i>	1.17	0.05	4.35	0.36	113.70	4.25	89.99	2.87
<i>Pterostichus melanarius</i>	0.14	0.01	12.46	1.20	10.96	0.92	9.91	0.83
Total Carabidae	17.30	0.46	26.95	1.49	127.53	4.44	103.02	3.16
No. carabid species	5.35	0.06	5.44	0.07	3.21	0.05	3.48	0.06
Boundary overwintering Carabidae	10.37	0.36	6.54	0.22	0.62	0.04	0.70	0.04
<i>Philonthus cognatus</i>	10.22	0.54	21.20	0.54	8.59	0.28	3.27	0.09
Total Staphylinidae	16.87	0.50	25.55	0.53	9.20	0.28	3.68	0.10
No. Staphylinid species	1.79	0.03	2.35	0.03	1.31	0.03	1.20	0.02
Lycosidae	1.40	0.08	3.33	0.22	0.54	0.04	0.48	0.04
No. carabid & staphylinid species	7.14	0.07	7.14	0.07	4.52	0.06	4.68	0.06
Total predatory invertebrates	36.09	0.66	60.72	1.71	139.62	4.49	109.08	3.16
Boundary overwintering	16.88	0.44	10.19	0.27	1.09	0.06	1.00	0.05

The red/blue SADIE analysis was used to test whether the distribution of invertebrate species or groupings was clustered into patches or whether gaps existed where few were present. There was evidence of significant clustering across the study area into patches, with gaps between these, for all the species and groups analysed, with a few exceptions on each date in 2000 (Table 3.4), as was also the case in 2001 (Table 3.5) and 2002 (Table 3.6). The strength of clustering varied between species and groups. Most species were also heterogeneously distributed within fields (data not presented here). In 2000, the boundary overwintering Carabidae and total invertebrates showed strong clustering on all four sampling occasions, but there were some differences in their distribution patterns. The boundary overwintering Carabidae remained largely associated with the field boundaries throughout the summer and only spread across the whole field in field S1 and to some extent in field S2 (Fig. 3.3). Patches of the group that included all boundary overwintering species were similarly distributed.

**Figure 3.3.** Spatial clustering for boundary overwintering Carabidae in a) June 2000 and b) early July 2000. The maps indicate clusters of relatively high counts ( $v_i > 1.5$ ) and small counts ( $v_j < -1.5$ ).



**Figure 3.4.** Spatial clustering in early July 2000 for a) *P. madidus* and b) *P. melanarius*. The maps indicate clusters of relatively high counts ( $v_i > 1.5$ ) and small counts ( $v_j < -1.5$ ).



**Table 3.4.** Degree of clustering into 'patches' using overall index  $\bar{v}_i$  and associated probability  $P_i$ , or of 'gaps' using overall index  $\bar{v}_j$  and associated probability  $P_j$  for each predatory arthropod group across the six fields in 2000. (\*\*\*=P<0.001, \*\*= P<0.01, \*= P<0.05).

	May				June				early July				mid-July			
	$\bar{v}_j$	$P_i$	$\bar{v}_i$	$P_j$	$\bar{v}_j$	$P_i$	$\bar{v}_i$	$P_j$	$\bar{v}_j$	$P_i$	$\bar{v}_i$	$P_j$	$\bar{v}_j$	$P_i$	$\bar{v}_i$	$P_j$
<i>Agonum dorsale</i>	-4.1	***	3.8	***	-4.9	***	5.6	***	-2.4	***	2.6	***	-2.9	***	3.0	***
<i>Bembidion lampros</i>	-3.0	***	3.2	***	-2.4	***	2.4	***	-2.0	***	2.0	***	-1.7	**	1.7	**
<i>Bembidion obtusum</i>	-1.4	*	1.3		-2.3	***	2.2	***	-1.1		1.2		-5.6	***	5.6	***
<i>Nebria brevicollis</i>	-3.4	***	3.9	***	-2.3	***	1.4	***	-1.4	*	1.4	*	-1.2		1.3	
<i>Poecilus cupreus</i>	-3.2	***	2.9	***	-3.3	***	3.3	***	-3.3	***	3.3	***	-2.3	***	2.4	***
<i>Pterostichus madidus</i>	-2.8	***	2.8	***	-3.9	***	3.8	***	-5.2	***	5.0	***	-6.3	***	6.4	***
<i>Pterostichus melanarius</i>	-2.6	***	2.5	***	-4.2	***	4.7	***	-4.3	***	4.6	***	-4.4	***	4.9	***
Total Carabidae	-2.3	***	2.2	***	-3.8	***	3.3	***	-4.8	***	4.6	***	-5.7	***	5.8	***
No. carabid species	-2.6	***	2.6	***	-4.9	***	5.1	***	-4.5	***	5.3	***	-5.0	***	5.3	***
Boundary overwintering Carabidae	-2.2	***	2.1	***	-3.3	***	3.6	***	-2.9	***	2.8	***	-2.6	***	2.3	***
<i>Philonthus cognatus</i>	-8.6	***	9.2	***	-7.4	***	8.0	***	-2.6	***	2.5	***	-2.5	***	2.7	***
Total Staphylinidae	-6.8	***	7.5	***	-6.8	***	7.5	***	-2.5	***	2.5	***	-3.1	***	3.4	***
No. Staphylinid species	-1.6	**	1.6	**	-1.6	**	1.6	**	-1.8	**	2.2	***	-4.3	***	4.6	***
Lycosidae	-1.6	***	1.6	**	-1.5	*	1.5	*	-1.6	*	1.5	*	-2.3	***	2.2	***
No. carabid & staphylinid species	-3.5	***	3.5	***	-3.5	***	3.5	***	-5.0	***	5.5	***	-5.9	***	6.2	***
Total predatory invertebrates	-5.9	***	6.3	***	-3.8	***	3.1	***	-4.7	***	4.5	***	-3.8	***	3.1	***
Boundary overwintering invertebrates	-3.1	***	3.2	***	-2.8	***	2.6	***	-2.5	***	2.9	***	-3.2	***	2.8	***

**Table 3.5.** Degree of clustering into 'patches' using overall index  $\bar{v}_i$  and associated probability  $P_i$ , or of 'gaps' using overall index  $\bar{v}_j$  and associated probability  $P_j$  for each predatory arthropod group across the six fields in 2001. (\*\*\*=P<0.001, \*\*= P<0.01, \*= P<0.05).

	June				July			
	$\bar{v}_j$	$P_i$	$\bar{v}_i$	$P_j$	$\bar{v}_j$	$P_i$	$\bar{v}_i$	$P_j$
<i>Agonum dorsale</i>	-3.8	***	3.5	***	-1.8	***	1.8	**
<i>Bembidion lampros</i>	-2.1	***	2.1	***	-1.8	***	1.8	**
<i>Bembidion obtusum</i>	-3.4	***	3.6	***	-0.8		0.9	
<i>Nebria brevicollis</i>	-5.4	***	5.1	***	-1.9	**	1.9	**
<i>Poecillus cupreus</i>	-3.0	***	3.1	***	-1.6	*	1.6	*
<i>Pterostichus madidus</i>	-6.5	***	5.3	***	-3.7	***	3.5	***
<i>Pterostichus melanarius</i>	-3.5	***	3.3	***	-4.4	***	4.7	***
Total Carabidae	-4.1	***	4.3	***	-3.0	***	2.8	***
No. carabid species	-1.9	***	2.1	***	-3.5	***	3.5	***
Boundary overwintering Carabidae	-5.3	***	5.1	***	-2.5	***	2.6	***
<i>Philonthus cognatus</i>	-3.3	***	3.0	***	-3.5	***	3.0	***
Total Staphylinidae	-5.6	***	5.5	***	-2.4	***	2.2	***
No. Staphylinid species	-4.6	***	5.1	***	-2.4	***	2.5	***
Lycosidae	-1.9	***	2.0	***	-1.4	*	1.4	*
No. carabid & staphylinid species	-4.3	***	4.5	***	-2.4	***	2.7	***
Total predatory invertebrates	-5.3	***	5.2	***	-2.6	***	2.4	***
Boundary overwintering invertebrates	-5.8	***	5.4	***	-1.8	**	1.9	**

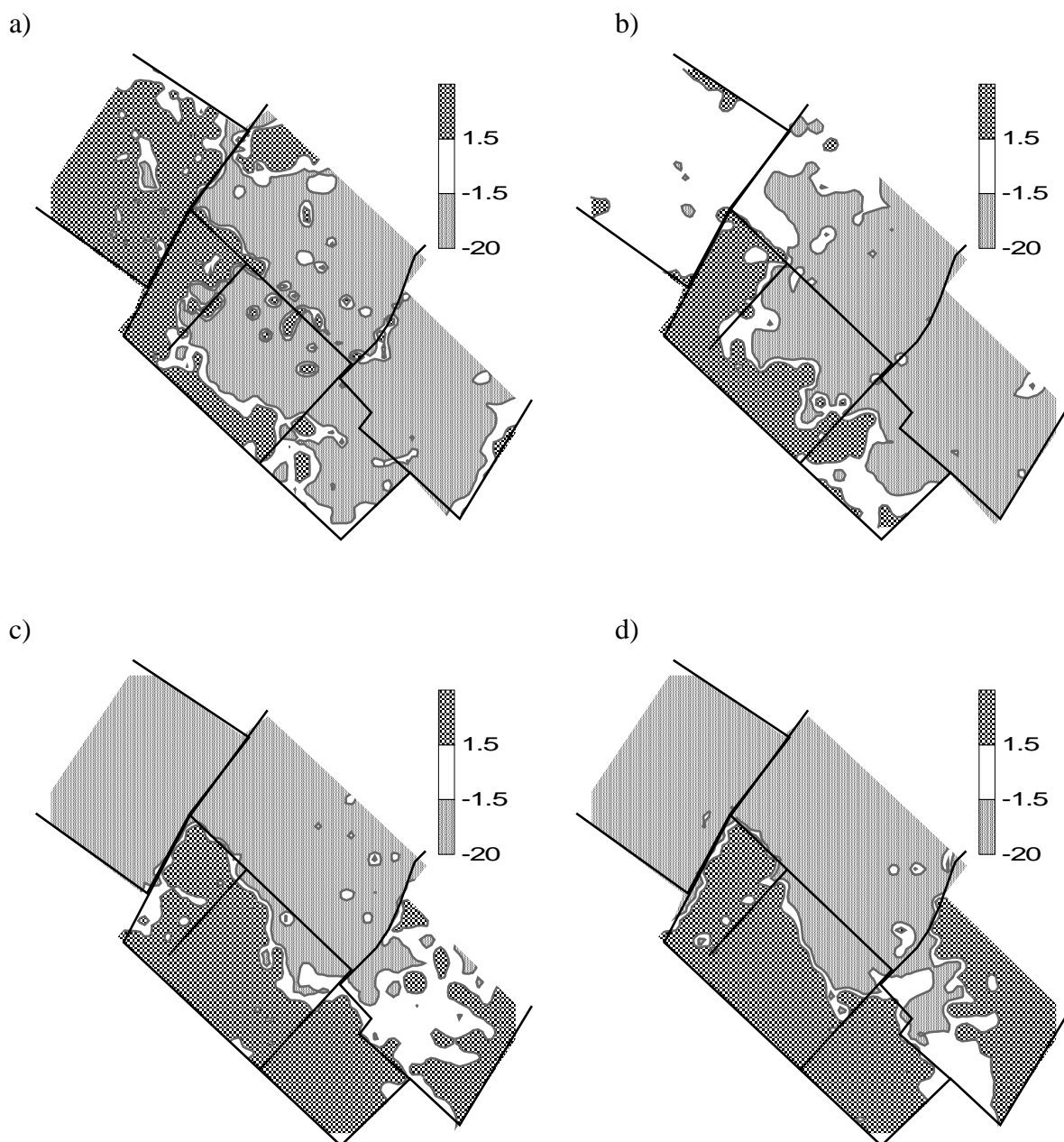
**Table 3.6.** Degree of clustering into 'patches' using overall index  $\bar{v}_i$  and associated probability  $P_i$ , or of 'gaps' using overall index  $\bar{v}_j$  and associated probability  $P_j$  for each predatory arthropod group across the six fields in 2002. (\*\*\*=P<0.001, \*\*= P<0.01, \*= P<0.05).

	June				July			
	$\bar{v}_j$	$P_i$	$\bar{v}_i$	$P_j$	$\bar{v}_j$	$P_i$	$\bar{v}_i$	$P_j$
<i>Agonum dorsale</i>	-3.4	***	3.1	***	-3.0	***	3.4	***
<i>Bembidion lampros</i>	-3.0	***	3.1	***	-1.6	**	1.7	**
<i>Bembidion obtusum</i>	-3.0	***	3.2	***	-1.5	*	1.5	*
<i>Nebria brevicollis</i>	-2.3	***	2.2	***	Insufficient data			
<i>Poecillus cupreus</i>	-1.2		1.2		-1.6	*	1.6	*
<i>Pterostichus madidus</i>	-6.3	***	5.8	***	-4.4	***	3.9	***
<i>Pterostichus melanarius</i>	-4.1	***	4.4	***	-3.6	***	3.6	***
Total Carabidae	-5.6	***	4.6	***	-4.0	***	3.5	***
No. carabid species	-3.9	***	4.1	***	-6.4	***	6.0	***
Boundary overwintering Carabidae	-3.6	***	3.5	***	-3.7	***	3.7	***
<i>Philonthus cognatus</i>	-3.3	***	3.0	***	-2.7	***	2.7	***
Total Staphylinidae	-3.5	***	3.5	***	-2.5	***	2.5	***
No. Staphylinid species	-4.1	***	3.7	***	-2.3	***	2.3	***
Lycosidae	Insufficient data				-1.5	*	1.5	*
No. carabid & staphylinid species	-4.8	***	5.0	***	-5.6	***	6.1	***
Total predatory invertebrates	-5.1	***	3.9	***	-3.9	***	3.5	***
Boundary overwintering invertebrates	-3.4	***	3.5	***	-3.3	***	3.8	***

Those beetle species that overwintered as larvae within fields and emerged as adults, for example the carabids *P. madidus*, *P. melanarius* and the staphylinid *Philonthus cognatus*, showed the strongest evidence of clustering into patches and gaps when they were most abundant (Tables 3.4, 3.5 & 3.6). The distribution patterns of the two *Pterostichus* species were quite different; *P. madidus* occurred throughout the study area whereas *P. melanarius* was confined to two fields (Fig. 3.4). The distribution and movement of these two species are more fully described in Holland *et al.* (2002; 2004).

The number of predatory invertebrates peaked in early July then started to decline. Numbers were highest in fields L1 (winter wheat), S2 (peas) and S3 (peas) during May and June but in July were highest in the pea fields S2, S3 and L3 (Fig. 3.5).

**Figure 3.5.** Spatial clustering for total predators in 2000. a) May, b) June, c) early July and d) late July. The maps indicate clusters of relatively high counts ( $v_i > 1.5$ ) and small counts ( $v_j < -1.5$ ).



#### 3.2.2.2. Abundance and distribution patterns in 2001 and 2002

In 2001, fewer predatory arthropods were caught compared to the previous year (Tables 3.3 & 3.7). In June, the pitfall catch comprised Carabidae (49%) and Staphylinidae (40%), by July the Carabidae (74%) were the most numerous compared to Staphylinidae (21%). *P. melanarius* and *P. madidus* were not as abundant as in the previous year but still formed 60% of the catch.

**Table 3.7.** Mean and 1 standard error (SE) for each predatory arthropod group across the six fields in 2001.

	June		July	
	Mean	SE	Mean	SE
<i>Agonum dorsale</i>	0.73	0.05	0.74	0.05
<i>Bembidion lampros</i>	0.62	0.04	0.77	0.08
<i>Bembidion obtusum</i>	0.09	0.01	0.01	0.00
<i>Nebria brevicollis</i>	4.89	0.25	0.03	0.01
<i>Poecillus cupreus</i>	0.76	0.09	0.40	0.07
<i>Pterostichus madidus</i>	0.84	0.04	18.90	0.73
<i>Pterostichus melanarius</i>	0.45	0.06	1.75	0.14
Total Carabidae	9.80	0.32	25.14	0.72
No. carabid species	3.35	0.06	4.99	0.08
Boundary overwintering Carabidae	6.38	0.26	1.72	0.10
<i>Philonthus cognatus</i>	8.44	0.52	5.86	0.23
Total Staphylinidae	11.38	0.56	9.74	0.33
No. Staphylinid species	4.37	0.12	3.71	0.06
Lycosidae	2.49	0.16	0.41	0.05
No. carabid & staphylinid species	7.72	0.14	8.71	0.11
Total predatory invertebrates	24.73	0.79	34.19	0.82
Boundary overwintering	7.72	0.29	2.64	0.13

The number of predators caught in 2002 was higher than in 2001 but lower than in 2000 (Table 3.8 compared with 3.3 & 3.7). In June, the predators comprised Carabidae (68%) and Staphylinidae (29%), but by July the Carabidae were again the most prolific group, comprising 94% of the catch. Again the two *Pterostichus* species comprised a large proportion of the catch (51% in June, 82% in July).

The location of some species and groups changed between 2000 and 2001. In 2001 and 2002, the patches of boundary overwintering Carabidae and of total invertebrates were found around and within the larger fields (Figs. 3.6 & 3.7) and were less evident in the smaller fields. In 2002, boundary overwintering Carabidae were found throughout most of fields L1 and L2 (Fig. 3.7). The total number of boundary overwintering invertebrates showed considerable changes in the location of patches in June from the smaller fields in 2000, to L3 in 2001 and to L1 and S1 in 2002. For July, the change was predominantly from the smaller fields in 2000 to L1 and L2 in 2001 and 2002 (Figs. 3.5, 3.6 & 3.7).

In 2001, patches of *P. madidus* were found in the three largest fields (two growing peas and one winter wheat) and in 2002 in L2 and L3 (Fig. 3.8). The patches of *P. melanarius* persisted in the same fields over the three years. *P. madidus* formed a large proportion of the total number of predators and consequently their distribution showed the same pattern, as described more fully in Holland *et al.* (2003).

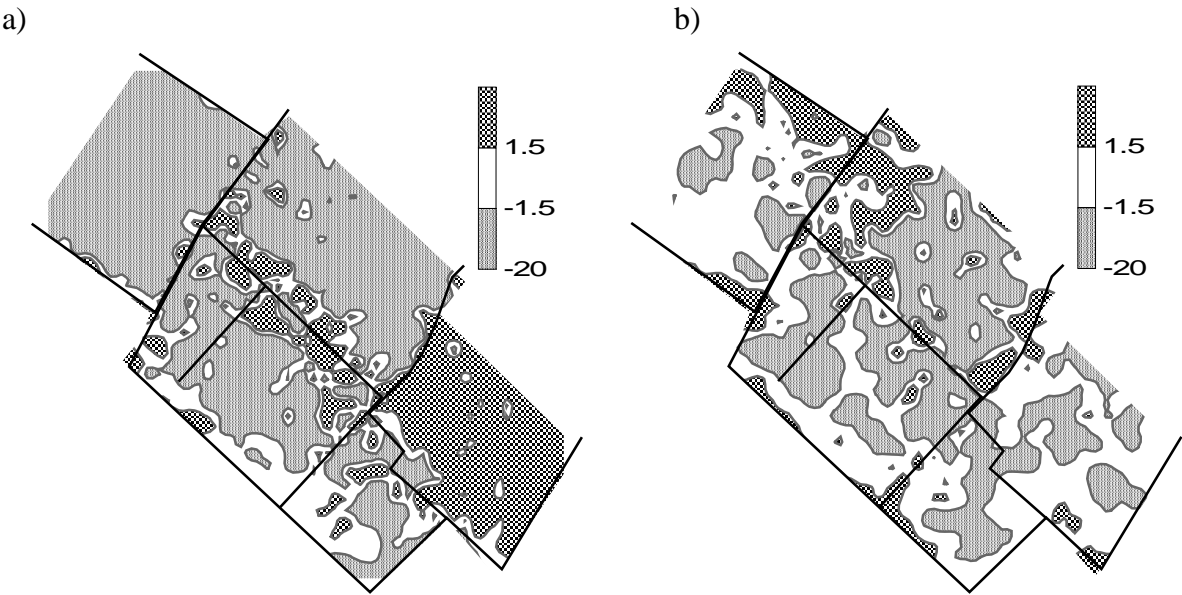
**Table 3.8.** Mean and 1 standard error (SE) for each predatory arthropod group across the six fields in 2002.

	June		July	
	Mean	SE	Mean	SE
<i>Agonum dorsale</i>	4.61	0.19	1.05	0.08
<i>Bembidion lampros</i>	0.39	0.05	0.24	0.02
<i>Bembidion obtusum</i>	0.11	0.01	0.02	0.01
<i>Nebria brevicollis</i>	0.69	0.03	0.28	0.02
<i>Poecillus cupreus</i>	0.74	0.10	0.33	0.07
<i>Pterostichus madidus</i>	26.29	1.58	46.11	1.95
<i>Pterostichus melanarius</i>	2.13	0.22	1.15	0.12
Total Carabidae	38.42	1.54	54.56	1.99
No. carabid species	4.80	0.07	4.68	0.07
Boundary overwintering Carabidae	5.81	0.20	1.77	0.09
<i>Philonthus cognatus</i>	13.67	0.37	1.91	0.11
Total Staphylinidae	17.57	0.44	3.65	0.14
No. Staphylinid species	2.76	0.05	1.48	0.04
Lycosidae	1.61	0.15	0.31	0.04
No. carabid & staphylinid species	7.56	0.09	6.15	0.09
Total predatory invertebrates	55.48	1.55	57.38	2.01
Boundary overwintering	6.91	0.21	2.06	0.10

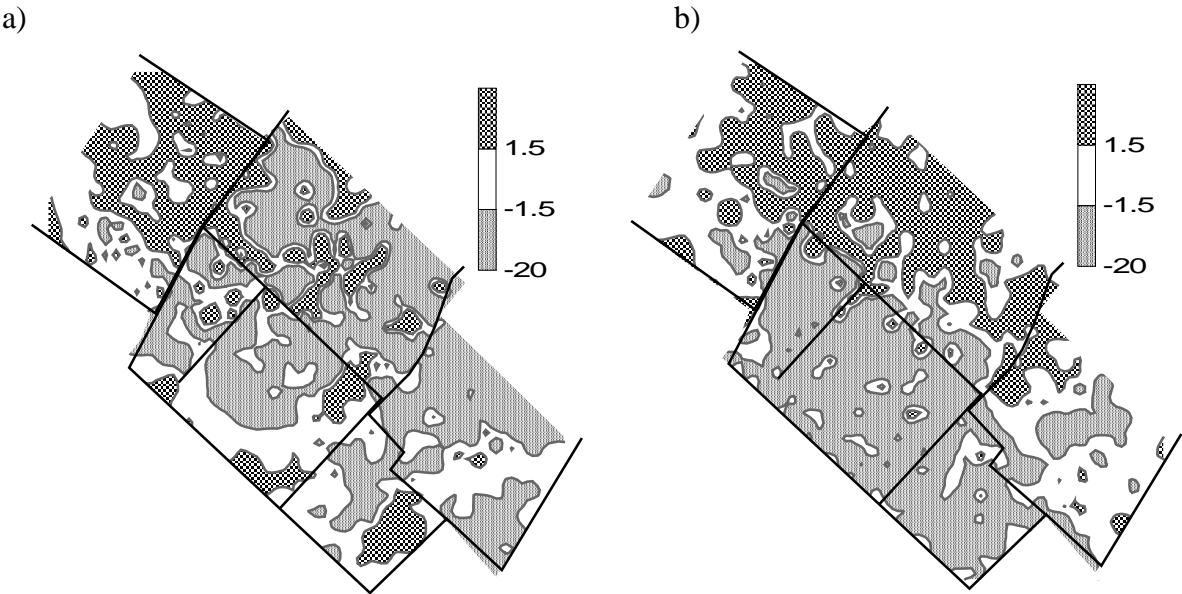
In June 2001, most predators were captured in L3 (winter wheat) and in the smaller fields S1 and S2, whereas in July, the largest patches of high predator numbers were present in L2 and L3 (Fig. 3.9). In June 2002, the area containing the highest number of predators was in L3 (winter wheat), as found in the previous year and this extended across most of the sampled area (Fig. 3.10). Patches were also present in S1, S2 and L2. In July large patches of high numbers were found in L2 and L3.



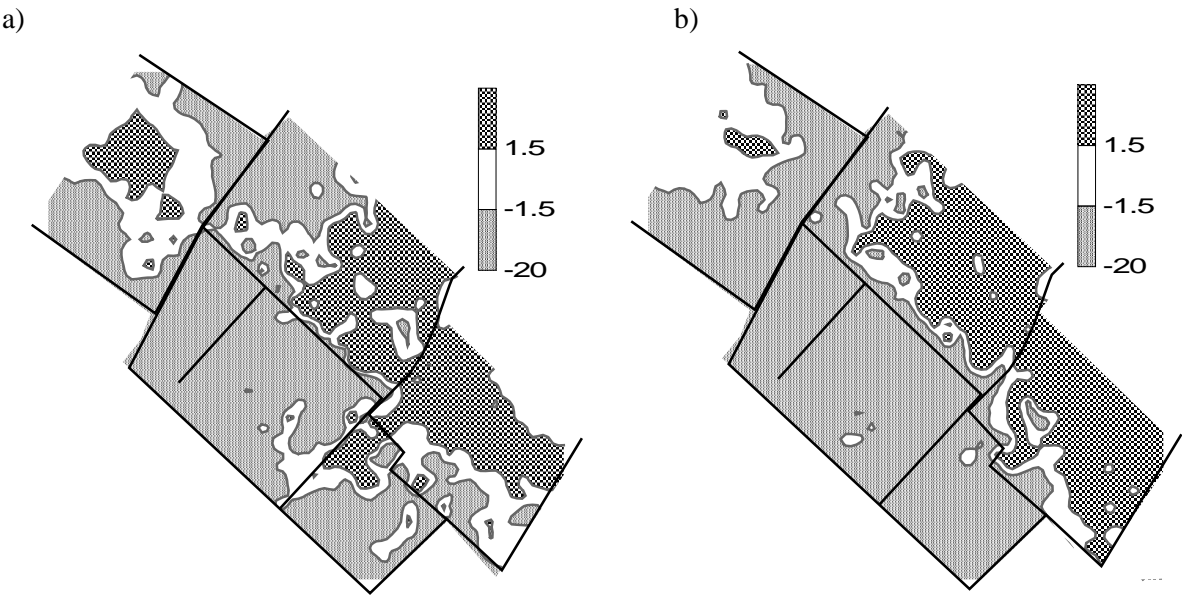
**Figure 3.6.** Spatial clustering for boundary overwintering Carabidae in a) June 2001 and b) July 2001. The maps indicate clusters of relatively high counts ( $v_i > 1.5$ ) and small counts ( $v_j < -1.5$ ).



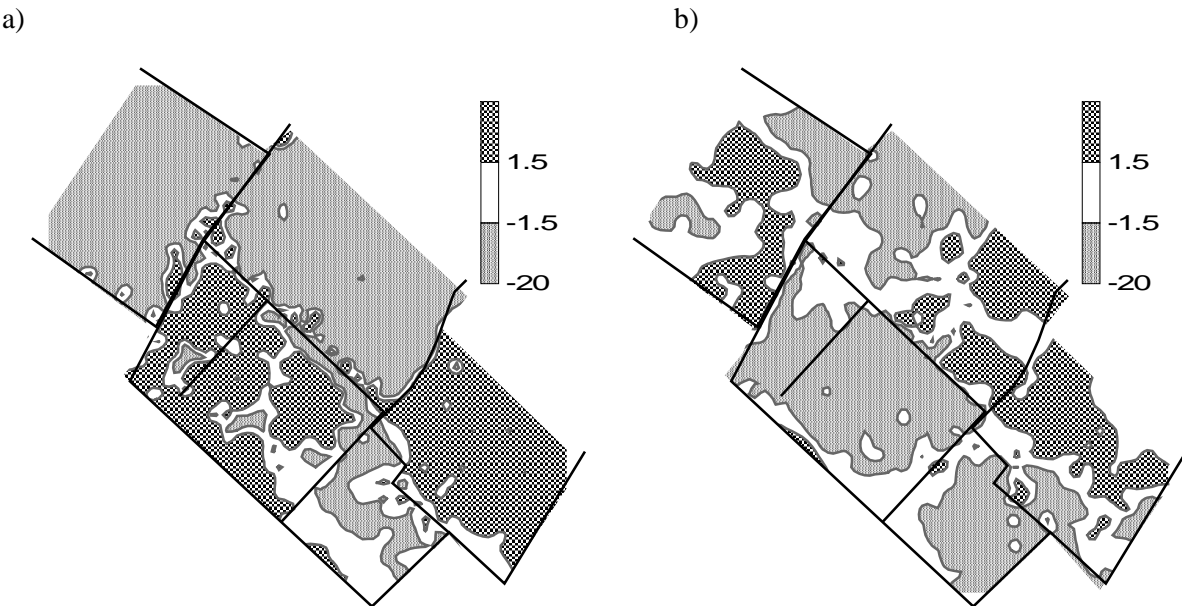
**Figure 3.7.** Spatial clustering for boundary overwintering Carabidae in a) June 2002 and b) July 2002. The maps indicate clusters of relatively high counts ( $v_i > 1.5$ ) and small counts ( $v_j < -1.5$ ).



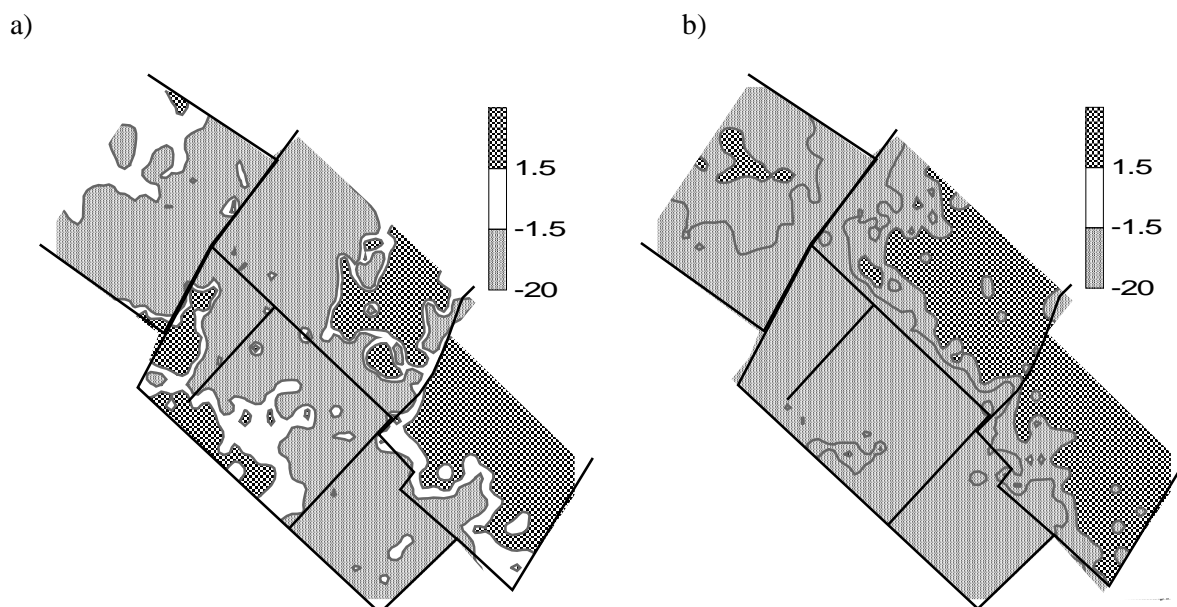
**Figure 3.8.** Spatial clustering for *P. madidus* in a) July 2001 and b) July 2002. The maps indicate clusters of relatively high counts ( $v_i > 1.5$ ) and small counts ( $v_j < -1.5$ ).



**Figure 3.9.** Spatial clustering for total predators in a) June 2001 and b) July 2001. The maps indicate clusters of relatively high counts ( $v_i > 1.5$ ) and small counts ( $v_j < -1.5$ ).



**Figure 3.10.** Spatial clustering for total predators in a) June 2002 and b) July 2002. The maps indicate clusters of relatively high counts ( $v_i > 1.5$ ) and small counts ( $v_j < -1.5$ ).



#### 3.2.2.3. The stability of spatial pattern within years

Within year stability was tested by comparing the spatial distribution between the four sampling occasions in 2000, and two occasions in 2001 and 2002, using the SADIE association test. The location of patches and gaps remained consistent between sampling occasions for most of the species analysed (Table 3.9). The distribution of *P. madidus* changed between May and June, because in May adults that had overwintered were captured, whereas the new generation of adults were captured from June onwards. The absence of spatial stability for *P. cognatus* in June to July coincided with a decline in abundance.

All the various groups, with the exception of the staphylinid species, showed considerable spatial stability in 2000 (Table 3.10). The boundary overwintering Carabidae and total Staphylinidae differed in their location between June and mid July in 2001 and 2002.

#### 3.2.2.4. The stability of spatial pattern between years

The degree of stability varied between the species and invertebrate groups but also between June and July (Tables 3.11 & 3.12). For example, the location of *P. melanarius* was consistent between years in June but not in July for 2000 and 2001. The change in the location of *P. madidus* may have been linked to cropping. In 2000, *P. madidus* was most abundant in S1-3 and L3 (Fig. 3.4) and in 2001 in L1 and L2 (Fig. 3.8), which were all in peas. However, they were also high in the winter wheat grown in L3 in 2001 (Fig. 3.8). In 2002 only cereal crops were available. Some species e.g. *Agonum dorsale*, a boundary overwintering species, showed no consist trend, with association, disassociation and no significant difference all occurring.

**Table 3.9.** Association indices comparing distribution of the insect species within years.

(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

		2000		2001		2002	
		X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
<i>Agonum dorsale</i>							
May-June		0.45	***				
June-early July		0.47	***	0.24	***	-0.01	NS
Early-mid July		0.48	***				
May-mid July		0.45	***				
<i>B. lampros</i>							
May-June		0.32	***				
June-early July		0.38	***	0.38	***	0.25	***
Early-mid July		0.37	***				
May-mid July		0.10	**				
<i>Philonthus cognatus</i>							
May-June		0.56	***				
June-early July		-0.02	NS	-0.10	NS	0.02	NS
Early-mid July		0.28	***				
May-mid July		0.14	**				
<i>Poecillus cupreus</i>							
May-June		0.62	***				
June-early July		0.61	***	0.48	***	0.07	NS
Early-mid July		0.47	***				
May-mid July		0.48	***				
<i>P. madidus</i>							
May-June		-0.28	***				
June-early July		0.68	***	0.25	***	0.72	***
Early-mid July		0.83	***				
May-mid July		-0.37	***				
<i>P. melanarius</i>							
May-June		0.57	***				
June-early July		0.88	***	0.70	***	0.80	***
Early-mid July		0.91	***				
May-mid July		0.52	***				

There was little consistency in the results comparing distribution between years for the various groups, with the exception of the number of carabid species in July (Table 3.12). This group showed a positive association between each year indicating that patches and gaps with the highest and lowest number of species respectively, remained in the same location between years.

**Table 3.10.** Association indices comparing distribution of the insect groups within years.

(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

		2000		2001		2002	
		X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
<b>Boundary Carabidae</b>							
May-June		0.36	***				
June-early July		0.40	***	-0.03	NS	0.07	*
Early-mid July		0.29	***				
May-mid July		0.16	***				
<b>Total Carabidae</b>							
May-June		0.45	***				
June-early July		0.35	***	0.11	*	0.63	***
Early-mid July		0.83	***				
May-mid July		0.34	***				
<b>Carabid spp.</b>							
May-June		0.34	***				
June-early July		0.48	***	0.2	***	0.22	***
Early-mid July		0.46	***				
May-mid July		0.25	***				
<b>Total Staphylinidae</b>							
May-June		0.55	***				
June-early July		0.03	0.36	-0.06	NS	-0.27	***
Early-mid July		0.27	***				
May-mid July		0.17	***				
<b>Staphylinid spp.</b>							
May-June		-0.04	0.79				
June-early July		0.02	0.34	0.22	***	0.10	*
Early-mid July		0.19	***				
May-mid July		0.24	***				
<b>Carabid &amp; Staphylinid spp.</b>							
May-June		0.99	***				
June-early July		0.34	***	0.18	***	0.19	***
Early-mid July		0.47	***				
May-mid July		0.33	***				
<b>Boundary species</b>							
May-June		0.43	***				
June-early July		0.13	0.005	-0.16	NS	0.1	**
Early-mid July		0.29	***				
May-mid July		0.21	***				
<b>Total predators</b>							
May-June		0.36	***				
June-early July		0.40	***	0.03	NS	0.38	***
Early-mid July		0.29	***				
May-mid July		0.16	***				

**Table 3.11.** Association indices comparing distribution of insect species between years for sampling conducted in June and July each year.

(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

	2000-01		2001-02		2000-02	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
<i>Agonum dorsale</i>						
June	-0.01	NS	-0.09	NS	0.27	***
July	0.003	NS	-0.11	**	-0.13	**
<i>B. lampros</i>						
June	0.09	NS	0.33	***	0.22	***
July	0.09	NS	0.22	***	0.18	**
<i>Philonthus cognatus</i>						
June	-0.44	>0.999	0.28	**	0.2	**
July	0.09	NS	0.13	**	0.08	NS
<i>Poecillus cupreus</i>						
June	0.28	***	0.03	NS	0.22	***
July	-0.18	**	0.15	*	0.03	NS
<i>P. madidus</i>						
June	0.43	***	0.38	***	0.17	***
July	<0.001	NS	0.65	***	-0.06	NS
<i>P. melanarius</i>						
June	0.75	***	0.77	***	0.88	***
July	-0.19	**	-0.13	NS	0.78	***

### 3.2.2.5. Association between invertebrate distribution and weed cover

Total vegetation and weed cover increased from May through to July in 2000, with patches developing along the field margins and within some fields (Fig. 3.11 a-c). From 2000 to 2002 weed cover increased within field L3 but decreased in the other fields (Fig. 3.11 d-f). The set-aside strips (Fig. 3.1) developed very high weed cover. The SADIE analysis confirmed that the total vegetation and weed cover were aggregated into patches (Table 3.13).

**Table 3.12.** Association indices comparing distribution of insect groups between years for sampling conducted in June and July each year.

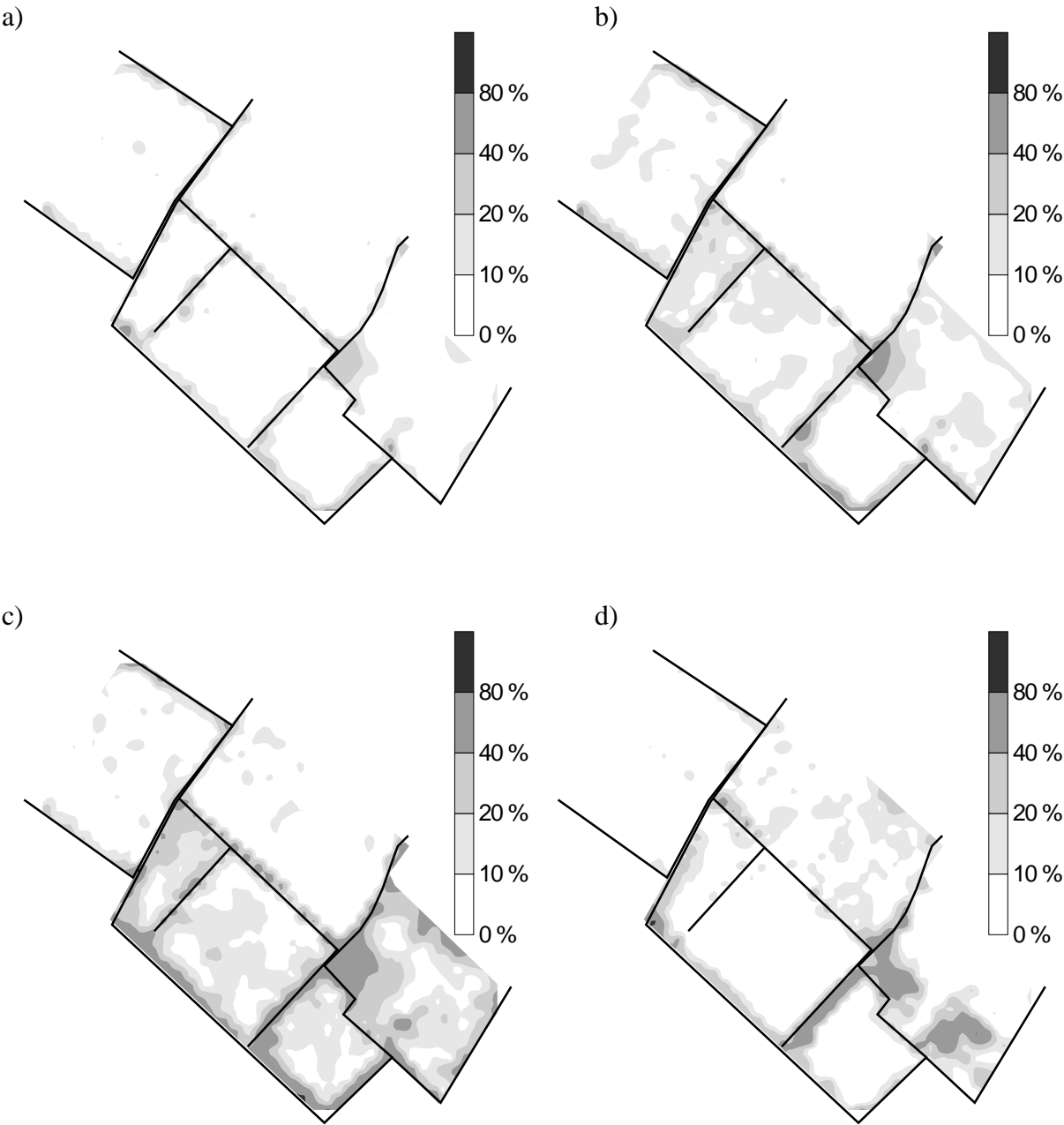
(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

	2000-01		2001-02		2000-02	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
<b>Boundary Carabidae</b>						
June	-0.13	**	-0.16	***	0.13	***
July	0.12	**	0.15	***	-0.01	NS
<b>Total Carabidae</b>						
June	0.06	NS	0.19	***	-0.38	***
July	-0.05	0.7	0.57	***	-0.17	NS
<b>No. Carabid spp</b>						
June	0.08	*	0.09	NS	0.26	***
July	0.22	***	0.16	***	0.23	***
<b>Total Staphylinidae</b>						
June	-0.39	***	0.21	*	0.31	***
July	-0.07	NS	0.16	**	-0.06	NS
<b>No. Staphylinid spp.</b>						
June	-0.11	**	0.03	NS	0.12	**
July	0.09	NS	-0.14	NS	0.16	***
<b>No. Carabid &amp; Staphylinid spp.</b>						
June	-0.05	NS	0.03	NS	0.33	***
July	0.29	***	0.10	**	0.21	***
<b>Boundary species</b>						
June	0.16	***	-0.13	***	0.12	**
July	0.13	***	0.06	NS	-0.08	*
<b>Total predators</b>						
June	-0.13	NS	0.31	***	-0.28	**
July	-0.22	**	0.46	***	-0.32	**

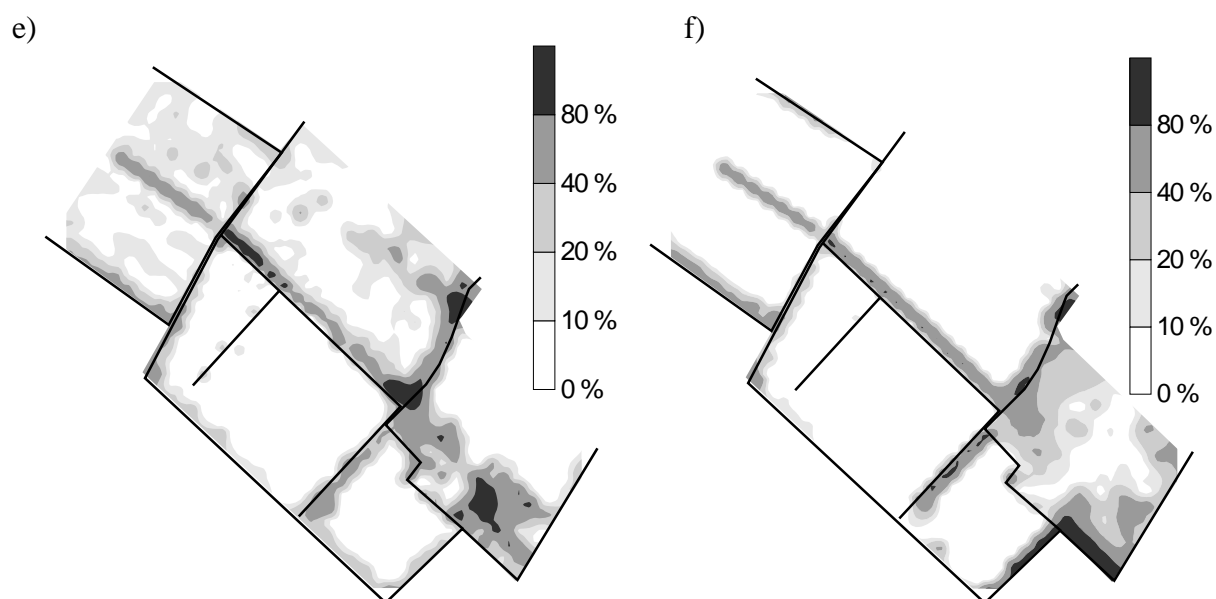
**Table 3.13.** Degree of clustering into 'patches' using overall index  $\bar{v}_i$  and associated probability  $P_i$ , or of 'gaps' using overall index  $\bar{v}_j$  and associated probability  $P_j$  for total vegetation and weed cover across the six fields in each year. (\*\*\*= $P < 0.001$ , \*\*= $P < 0.01$ , \*= $P < 0.05$ ).

<b>Total vegetation cover</b>						
	May-00	Jun-00	Jul-00	Jun-01	Jul-01	Jun-02
$\bar{v}_j$	-4.51	-2.84	-8.11	-2.04	-1.93	-6.66
$P_j$	***	***	***	***	***	***
$\bar{v}_i$	4.33	2.74	8.54	1.94	1.92	6.98
$P_i$	***	***	***	***	***	***
<b>Total weed cover</b>						
$\bar{v}_j$	-1.29	-1.81	-3.46	-3.35	-1.98	-3.29
$P_j$	*	**	***	***	***	***
$\bar{v}_i$	1.35	2.01	3.71	3.10	1.94	4.10
$P_i$	*	***	***	***	***	***

**Figure 3.11.** Percentage weed cover in a) May 2000, b) June 2000, c) July 2000, d) June 2001 e) July 2001 and f) June 2002.







The insect species tested varied in their level of association with total vegetation and weed cover (Tables 3.14 & 3.15). Some species, e.g. *P. madidus*, were consistently associated with weed cover, whereas others showed disassociation or no consistent relationship. Overall, twice as many positive significant associations compared to disassociations were found for the species and total vegetation cover. However, all but one of the significant associations were positive when weed cover was compared, indicating that this was influencing the distribution pattern more than total vegetation cover.

**Table 3.14.** Association indices comparing distribution of insect species with that of total vegetation cover for sampling conducted in each year.

(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

	May-00		June-00		early July-00		mid July-00	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
<i>A. dorsale</i>	-0.28	***	0.13	*	-0.02	NS	-0.13	*
<i>B. lampros</i>	-0.19	***	0.08	NS	0.01	NS	0.15	**
<i>P. cupreus</i>	-0.09	NS	0.16	*	0.50	***	0.42	***
<i>P. madidus</i>	0.44	***	0.35	***	0.72	***	0.73	***
<i>P. melanarius</i>	0.09	NS	0.06	NS	0.16	*	0.14	NS
<i>P. cognatus</i>	0.36	***	-0.30	***	0.45	***	0.05	NS
	June-01		July-01		June-02		July-02	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
<i>A. dorsale</i>	-0.14	NS	-0.05	NS	-0.11	*	0.01	NS
<i>B. lampros</i>	0.21	**	0.26	***	-0.12	*	-0.03	NS
<i>P. cupreus</i>	0.05	NS	0.21	**	0.22	***	0.04	NS
<i>P. madidus</i>	-0.01	NS	0.20	**	0.40	***	0.31	***
<i>P. melanarius</i>	-0.15	NS	-0.17	**	-0.40	***	-0.45	***
<i>P. cognatus</i>	-0.24	**	0.42	***	-0.14	**	0.02	NS

**Table 3.15.** Association indices comparing distribution of insect species with that of total weed cover for sampling conducted in each year.

(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

	May-00		June-00		early July-00		mid July-00	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
<i>A. dorsale</i>	0.22	***	0.28	***	0.09	0.03	0.13	*
<i>B. lampros</i>	0.20	***	0.30	***	0.21	***	0.15	**
<i>P. cupreus</i>	0.14	**	0.32	***	0.39	***	0.42	***
<i>P. madidus</i>	-0.08	NS	0.23	***	0.40	***	0.73	***
<i>P. melanarius</i>	0.16	***	0.22	***	0.18	*	0.14	NS
<i>P. cognatus</i>	-0.01	NS	-0.05	0.80	0.31	**	0.05	NS
	June-01		July-01		June-02		July-02	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
<i>A. dorsale</i>	0.18	**	0.03	NS	-0.08	NS	0.12	NS
<i>B. lampros</i>	0.00	NS	0.18	***	0.05	NS	0.03	NS
<i>P. cupreus</i>	-0.16	***	0.07	NS	0.30	***	0.08	NS
<i>P. madidus</i>	0.21	***	0.20	**	0.23	***	0.24	***
<i>P. melanarius</i>	-0.01	NS	-0.18	**	-0.36	***	-0.40	***
<i>P. cognatus</i>	0.17	**	0.31	***	-0.13	NS	0.15	**

**Table 3.16.** Association indices comparing distribution of insect groups with that of total vegetation cover for sampling conducted in each year.

(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

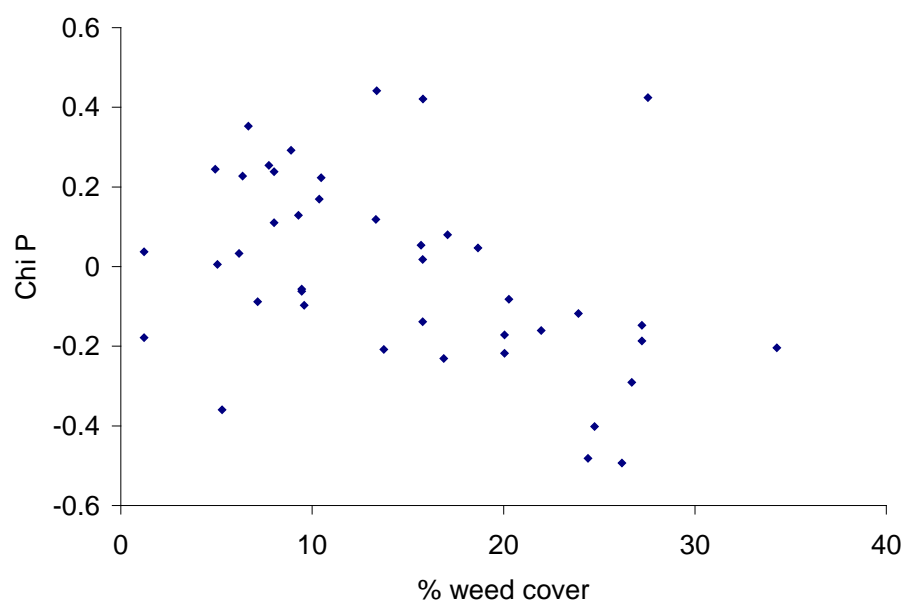
	May-00		June-00		early July-00		mid July-00	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
Total Carabidae	-0.28	***	0.18	*	0.72	***	0.73	***
Carabid spp.	-0.07	NS	0.00	NS	-0.18	***	-0.18	***
Boundary Carabidae	-0.32	***	0.00	NS	-0.12	**	-0.07	NS
Total Staphylinidae	0.20	***	-0.31	***	0.43	***	0.04	NS
Staphylinid spp.	0.34	***	-0.03	NS	0.08	NS	-0.19	***
Carabid & Staphylinid spp.	0.08	NS	0.06	NS	-0.14	**	-0.20	***
Boundary species	-0.38	***	0.30	***	-0.06	NS	-0.02	NS
Total predators	0.01	NS	-0.03	NS	0.73	***	0.72	***
	June-01		July-01		June-02		July-02	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
Total Carabidae	-0.02	NS	0.26	***	0.42	***	0.28	***
Carabid spp.	0.03	NS	0.22	***	-0.28	***	-0.35	***
Boundary Carabidae	0.04	NS	0.13	**	-0.14	**	-0.07	NS
Total Staphylinidae	-0.21	*	0.35	***	-0.14	**	0.01	NS
Staphylinid spp.	-0.09	NS	0.14	**	-0.24	***	-0.16	***
Carabid & Staphylinid spp.	-0.10	NS	0.25	***	-0.32	***	-0.34	***
Boundary species	-0.01	NS	0.13	**	-0.10	**	-0.02	NS
Total predators	-0.17	NS	0.37	***	0.37	***	0.26	***

**Table 3.17.** Association indices comparing distribution of insect groups with that of total weed cover for sampling conducted in each year.

(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

	May-00		June-00		early July-00		mid July-00	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
Total Carabidae	0.33	***	0.33	***	0.43	***	0.73	***
Carabid spp.	0.24	***	0.27	***	-0.03	NS	-0.18	***
Boundary Carabidae	0.34	***	0.27	***	0.05	NS	-0.07	NS
Total Staphylinidae	-0.07	NS	-0.03	NS	0.32	***	0.04	NS
Staphylinid spp.	0.00	NS	0.08	NS	0.07	NS	-0.19	***
Carabid & Staphylinid spp.	0.19	***	0.16	**	-0.01	NS	-0.20	***
Boundary species	0.17	**	0.30	***	0.09	NS	-0.02	NS
Total predators	0.15	**	0.23	***	0.42	***	0.15	**
	June-01		July-01		June-02		July-02	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
Total Carabidae	0.38	***	0.25	**	0.26	**	0.23	**
Carabid spp.	0.08	*	0.20	**	-0.18	***	-0.21	***
Boundary Carabidae	0.28	***	0.11	**	-0.07	NS	0.02	NS
Total Staphylinidae	0.38	***	0.27	***	-0.11	NS	0.16	**
Staphylinid spp.	0.20	***	0.15	**	-0.16	***	-0.04	NS
Carabid & Staphylinid spp.	0.21	***	0.21	**	-0.17	***	-0.15	***
Boundary species	0.29	***	0.12	*	-0.05	NS	0.04	NS
Total predators	0.23	***	0.35	***	0.24	***	0.22	**

**Figure 3.12.** Relationship between weed cover and strength of predator to weed cover correlation.



For the different insect groups, the relationship with total vegetation cover was often inconsistent between years and consequently no firm conclusions could be made based upon this level of analysis (Table 3.16). Cover increased during the summer and thus the environmental conditions may have changed making areas more or less suitable for each species. A field by field analytical approach may be more suitable because cover varied quite considerably between fields and such an analysis is underway. In contrast, stronger correlations were found with weed cover, notably for total Carabidae and total predators (Table 3.17). To examine this further the percentage weed cover was plotted in relation to the correlation value between weed cover and predators (Fig. 3.10). This indicated that there may be an optimum level of weed cover (10-14%), beyond which the number of predators declined.

### 3.2.2.6 Association between invertebrate distribution and soil moisture.

In the summer of 2000, the soil moisture was highest in L1, with wetter patches of relatively small sizes occurring within the other fields (Fig. 3.11). Dry patches occurred where the chalk was exposed on the higher ground. Few significant correlations were found for the individual species but there was a trend towards disassociation, suggesting that there was a negative relationship between soil moisture and beetle abundance (Table 3.18). Only significant disassociation was found for the invertebrate groups indicating further that there was a negative relationship between the majority of the epigeal invertebrates and soil moisture in the summer (Table 3.19).

To test whether the wetter areas persisted in the same location from year to year and from winter to summer, the data from the summer of 2000 and the autumn of 2001 were compared for each field and for the whole study area using SADIE. There was no significant association (Table 3.20), except in fields S1 and S2. S1 had a relatively high soil moisture content across the whole field on both occasions. S2 was characterised by an area of low soil moisture across half of the field, where the soil had a high chalk content.

**Table 3.18.** Association indices comparing distribution of insect species with that of soil moisture in 2000.

(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

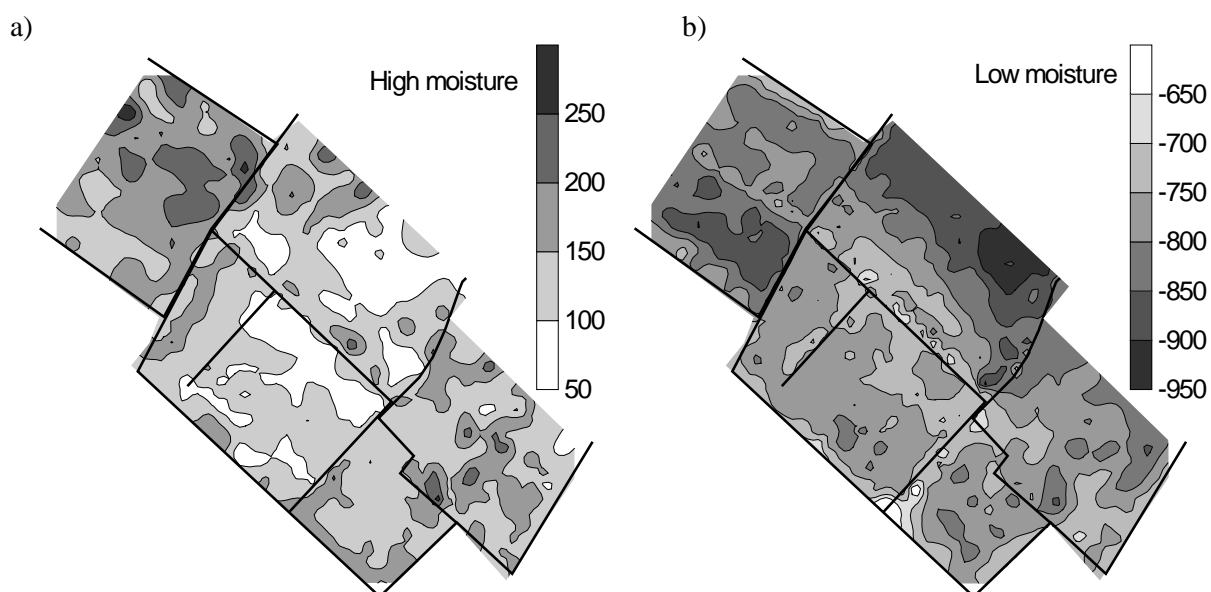
	May-00		Jun-00		Early July-00		Late July-00	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
<i>A. dorsale</i>	0.10	NS	-0.13	NS	-0.06	NS	-0.04	NS
<i>B. lampros</i>	0.14	*	0.03	NS	0.03	NS	-0.14	*
<i>P. cupreus</i>	-0.12	NS	-0.06	NS	-0.12	NS	-0.18	NS
<i>P. madidus</i>	0.01	NS	-0.14	NS	-0.16	NS	-0.17	NS
<i>P. melanarius</i>	0.00	NS	-0.23	NS	-0.26	*	-0.26	**
<i>P. cognatus</i>	0.16	NS	0.14	*	-0.15	NS	-0.15	*

**Table 3.19.** Association indices comparing distribution of insect groups with that of soil moisture in 2000.

(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

	May-00		Jun-00		Early July-00		Late July-00	
	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>	X	P <sub>D</sub>
Total Carabidae	0.04	NS	-0.16	NS	-0.21	*	-0.19	NS
Carabid spp.	0.02	NS	-0.29	NS	-0.11	*	-0.14	**
Boundary Carabidae	0.17	*	-0.01	NS	-0.05	NS	-0.17	***
Total Staphylinidae	0.09	NS	0.13	NS	-0.11	*	-0.14	*
Staphylinid spp.	0.08	NS	0.02	NS	0.10	NS	0.06	NS
Carabid & Staphylinid spp.	0.03	NS	0.03	NS	-0.11	*	-0.11	*
Boundary species	-0.20	**	-0.13	*	-0.01	NS	-0.18	***
Total predators	0.12	NS	-0.07	NS	-0.17	NS	-0.16	NS

**Figure 3.13.** Soil moisture in a) June-July 2000 (Readings are volumetric water content  $\text{m}^3\text{m}^{-3} \times 10^3$ ) and b) November 2001 (Readings are EMI).



**Table 3.20.** Association indices comparing soil moisture in the summer of 2000 with autumn 2001.

(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

2000-02	L1	L2	L3	S1	S2	S3	All fields
X	-0.22	0.15	0.004	0.23	0.19	0.14	0.25
P <sub>D</sub>	0.98	NS	0.48	*	**	0.13	NS

### 3.2.2.7. Effect of cropping and field size on invertebrate community composition

Routines in PRIMER were used to establish the difference in invertebrate community composition between crops. The ANOSIM analysis of the June pitfall data showed that there was an overall difference between crops (Global R = 0.66, significance = 0.2%). The pair-wise tests shown in Table 3.21 confirmed that the differences lay between cereals and legumes. The R value between winter wheat and peas was relatively high; this was the only significant comparison.

**Table 3.21.** Pairwise comparisons showing similarity of crops in June (accounting for year by year differences).

Crops	R Statistic	Significance (%)
Wheat, Barley	0.37	10
Wheat, Peas	0.96	1.3
Barley, Peas	0.58	40

SIMPER was used to quantify the differences in community composition between these crops (Table 3.22). The species which contributed most to the differences were the carabid *P. madidus* and the staphylinid *P. cognatus*, both of which were more abundant in winter wheat and the carabid *P. melanarius* (which was more abundant in peas). Other taxa each contributed <10% to the difference.

**Table 3.22.** The difference in invertebrate community composition between winter wheat and peas in June (all years).

Taxa	Abundance winter wheat	Abundance peas	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>P. madidus</i>	17.61	5.16	13.44	0.75	20.31	20.31
<i>P. cognatus</i>	16.17	12.16	12.75	1.2	19.26	39.58
<i>P. melanarius</i>	0.66	21.85	11.21	0.62	16.94	56.52
Curculionidae	0.17	6.37	7.46	1.05	11.27	67.79
Chrysomelidae	0.4	4.07	4.78	0.84	7.22	75.01
<i>Tachyporus hypnorum</i>	1.12	3.35	2.99	1.32	4.53	79.53
<i>Nebria</i> spp.	3.45	2.4	2.96	0.89	4.47	84
<i>Agonum dorsale</i>	2.72	1.26	2.33	0.92	3.52	87.52
<i>Poecillus cupreus</i>	0.56	2.36	1.48	1.59	2.23	89.75
<i>Bembidion lampros</i>	0.61	2.16	1.36	1.91	2.06	91.81

In July, the Global R value was 0.76 (significance =0.3%) indicating that there were differences in the invertebrate community composition between crops; the pair-wise differences (Table 3.23) showed that, as in June, only the difference between winter wheat and peas was significant at 5%.

These differences were characterised using SIMPER as shown in Table 3.24. In July, the majority of the difference was accounted for by *P. madidus* (43%) which occurred in greater numbers in peas. This was the reverse of the situation in June. The pattern for *P. melanarius* did not change, numbers remained higher in pea fields.

**Table 3.23.** Pairwise comparisons showing similarity of crops in July (accounting for year by year differences).

Crops	R Statistic	Significance (%)
Wheat, peas	0.93	1.3
Wheat, barley	0.85	10
Peas, barley	0.50	20

**Table 3.24.** The difference in invertebrate community composition between winter wheat and peas in July 2002.

Taxa	Abundance winter wheat	Abundance peas	Av.Diss	Diss/SD	Contrib%	Cum.%
<i>P. madidus</i>	34.39	103.92	42.85	1.64	69.88	69.88
<i>P. melanarius</i>	1.77	15.99	7.6	0.67	12.39	82.27
Curculionidae	0.05	2.98	3.17	0.66	5.17	87.44
<i>P. cognatus</i>	3.34	4.93	2.24	0.68	3.65	91.09

### 3.2.3. Conclusions

1. Field boundaries were most important as a source of beneficial insects earlier in the year (May and June), whereas mid-field overwintering species were most numerous in July.
2. Boundary overwintering species remained associated with the boundaries throughout the spring and summer and only penetrated to the centre of the smallest field (4ha).
3. All beneficial invertebrate species and groups were heterogeneously distributed across the six fields and often within fields.
4. The location of the patches and gaps was relatively stable within years for most insect species and groups.
5. The location of the patches and gaps between years varied between species and for the groups.
6. The vegetation cover was heterogeneous across the study area and within fields.
7. Weed cover had a greater influence on the invertebrate distribution than total vegetation cover (crop and weeds). Many of the invertebrate species, total numbers of Carabidae and predators were positively associated with weed cover.

8. The weed patches with 10-14% cover were the most strongly associated with high numbers of predatory invertebrates.
9. In the summer, many of the invertebrate groups were disassociated with areas of high soil moisture.
10. The spatial pattern of soil moisture levels in winter were generally not related to patterns in the summer.
11. The invertebrate species composition differed between the peas and winter wheat, but not the other crops.



### **3.3. INVESTIGATION OF THE LARGE-SCALE, SPATIO-TEMPORAL DYNAMICS OF PREDATORY EPIGEAL INVERTEBRATE EMERGENCE IN ARABLE FARMLAND.**

Pitfall traps have many well known biases but the most important is that they measure a combination of activity and density, the catch being dependent on insects moving and falling into the traps (Adis, 1979). As a consequence, they only catch those species that can fall and be retained by the traps while the insect's activity is determined to some extent by the density of vegetation surrounding the traps. Some of the most numerous beneficial insects that occur within arable fields also overwinter as larvae within the soil, emerging in the spring/summer. Some of these carabid beetle species were shown to disperse little (<55m over 30 days) within arable fields (Thomas et al., 1998), therefore, to verify to what extent the distribution patterns recorded by pitfall trapping were reflecting actual density, their density on emergence was measured. In addition, during the first two years of the spatio-temporal study (section 3.2) the invertebrates were most numerous in those fields containing peas. As soil cultivation was conducted in the spring this may have affected invertebrate overwintering survival. Emergence trapping is the best method of measuring cultivation effects on invertebrates (Holland & Reynolds, 2003).

#### **3.3.1. Materials and methods**

Emergence boxes consisted of a 1m<sup>2</sup> x 0.2m high wooden box covered with an insect proof mesh. The boxes' sides were buried 5cm deep into the soil. Within each box, a 10 cm high guidance plate was placed diagonally, at the end of which was placed a pitfall trap (6 cm diam., partly filled with 50% ethylene glycol and detergent). One emergence box was placed within 3m of the pitfall trap sampling location in early April 2002 prior to the start of emergence. Two hundred emergence boxes were established in two fields (L2, winter wheat and S2, winter barley) along alternate rows. The pitfall traps within each box were opened on 3 May and emptied on 21 May, 30 May, 10 June, 17 June, 4 July and 11 July 2002. Most invertebrates collected were identified to species.

The spatial pattern of emergence was determined using the red/blue SADIE analysis for four beetle species that overwinter within fields (the carabids *Nebria brevicollis*, *Pterostichus madidus*, *P. melanarius* & the staphylinid *Philonthus cognatus*) and for the total number of carabid beetles. This was conducted for each field separately. Their spatial pattern was then compared to that obtained from the pitfall trapping using the SADIE association test and regression for each date when the two sampling methods were conducted simultaneously (weeks beginning 17 June and 11 July). The total numbers of the above species and total Carabidae that were collected were also compared to soil moisture levels measured in the autumn of 2001 (see section 3.2.2.6) using the SADIE association test.

### 3.3.2. Results

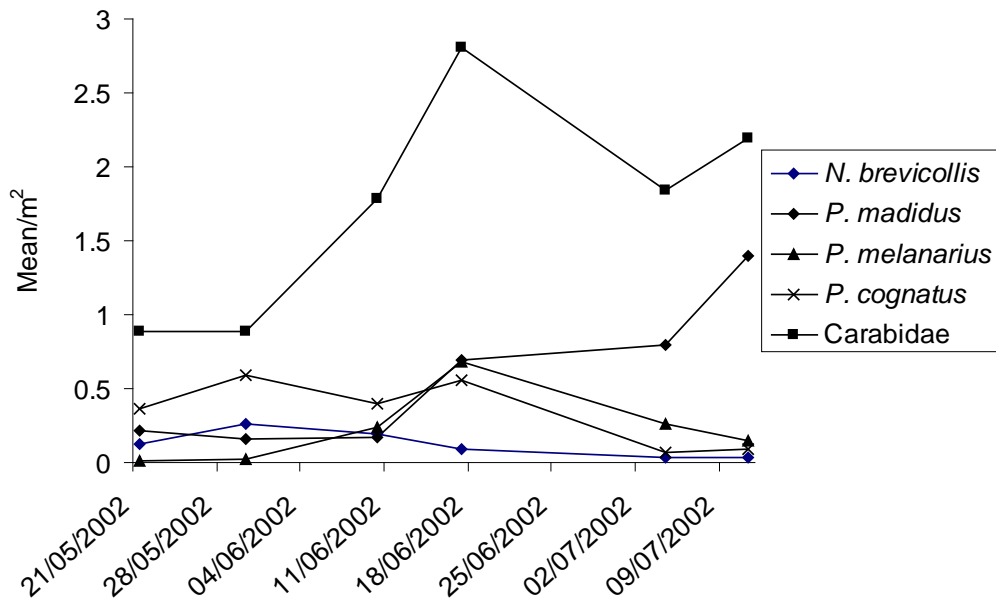
The number of invertebrates known to overwinter within the soil and captured within the boxes was 157 and 86 m<sup>-2</sup> for the small and large field respectively (Table 3.25). This demonstrates the importance of arable soils as an overwintering habitat for beneficial invertebrates. The taxa were comprised of the larger Carabidae and Staphylinidae, e.g. *Pterostichus* spp., *Poecillus cupreus* and *Philonthus cognatus*. The time of emergence varied between species but peaked for Carabidae in mid-June (Fig. 3.14). Data for the many other species were collected but are not presented here.

**Table 3.25.** Total emergence (m<sup>-2</sup>) over 69 days for field overwintering taxa.

<b>Taxa</b>	<b>S2-winter barley</b>	<b>L2-winter wheat</b>
<i>Amara</i> spp.	14.5	6.4
<i>Calathus fuscipes</i>	4.6	7.6
<i>Harpalus affinis</i>	1.4	1.2
<i>Harpalus rufipes</i>	1.6	4.1
<i>Loricera pillicornis</i>	6.6	2.2
<i>Nebria brevicollis</i>	7.9	8.2
<i>Notiophilus biguttatus</i>	2.0	1.3
<i>Poecillus cupreus</i>	10.6	3.1
<i>Pterostichus madidus</i>	26.8	41.9
<i>Pterostichus melanarius</i>	29.0	1.2
<i>Trechus quadristiatus</i>	1.4	1.8
<b>Carabidae</b>	<b>106.4</b>	<b>79.2</b>
<i>Philonthus cognatus</i>	43.6	5.9
<i>Philonthus</i> spp.	6.6	0.7
<b>Staphylinidae</b>	<b>50.1</b>	<b>6.6</b>
<b>Total</b>	<b>156.5</b>	<b>85.9</b>

All four species, total Carabidae in June, and all but *P. cognatus* in July within field S2 showed strong spatial and numerical correlations between abundance in pitfall traps and emergence trap catches (Table 3.26). Strong spatial and numerical correlations were also found in June and July for field L2, except for those species that were captured in too few numbers, although the regressions were often significant. The regression equations were relatively consistent between dates and fields. The strong correlations between the two sampling methodologies confirmed that the pitfall traps were providing evidence of emergence patterns and an indication of actual density.

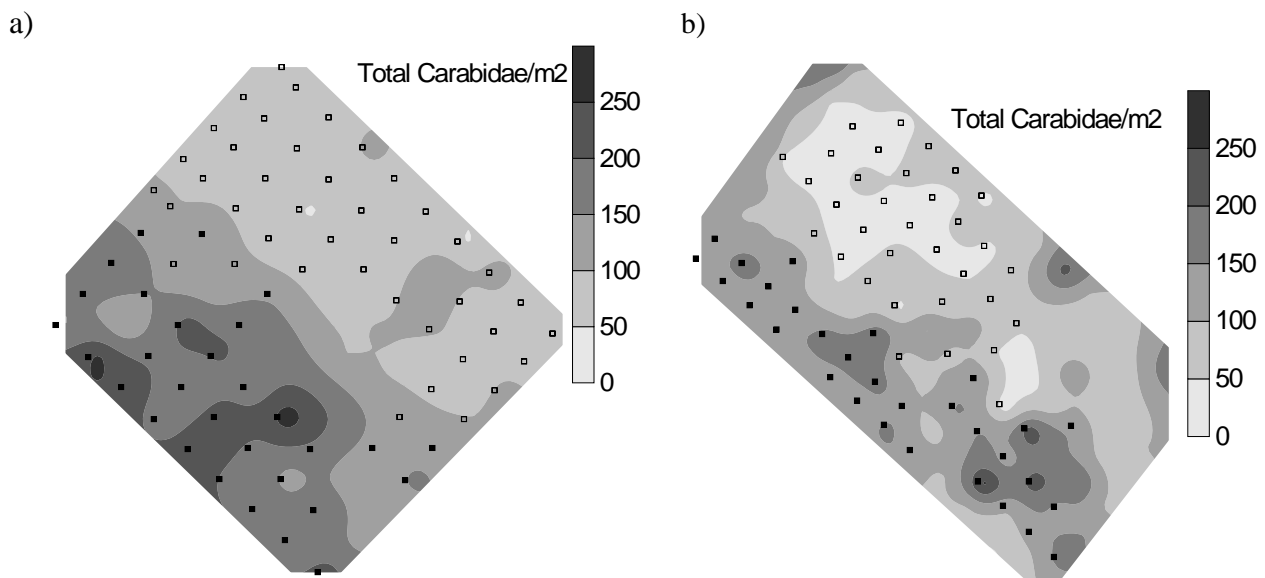
**Figure 3.14.** Emergence period and density of four species of predatory beetle and total Carabidae in June 2002.



In S2 there was a strong association between soil moisture and numbers of the four species tested and of total Carabidae, with emergence being highest in the wet areas (Values of -760 to -800) of the field (Fig. 3.15). In contrast, in L2 there was strong dissociation, although the soil wetness was higher in this field and it was the wettest areas that had the lowest emergence. The emergence of *Pterostichus madidus*, *Philonthus cognatus* and total Carabidae (Fig. 3.15) was highest in those areas of the field that had a moisture level of -750 to -800, as found in S2.

**Figure 3.15.** Pattern for total carabid emergence within fields a) S2 and b) L2 in 2002.

(filled squares indicate  $v_i > 1.5$ , empty squares indicate  $v_j < -1.5$ )



**Table 3.26.** Spatial association indices and regression values comparing beetles collected using emergence traps compared to pitfall traps for sampling conducted in June and July 2002.

(\*\*\*= $P_D < 0.001$  or  $> 0.999$ , \*\*= $P_D < 0.01$  or  $> 0.99$ , \*= $P_D < 0.05$  or  $> 0.975$ )

Field S2	X	P <sub>D</sub>	F ratio	p	Regression	R <sup>2</sup>
<i>Nebria brevicollis</i>						
June	0.05	NS	3.9	0.05	y=0.15x +0.12	0.03
July	-1.0	NS	11	<0.001	y=0.33x+0.04	0.09
<i>P. madidus</i>						
June	0.55	***	32	<0.001	y=0.51x+0.28	0.22
July	0.55	***	142	<0.001	y=0.89x+0.26	0.34
<i>P. melanarius</i>						
June	0.82	***	132	<0.001	y=0.53x-0.004	0.54
July	0.77	***	58	<0.001	y=0.57x+0.03	0.34
<i>Philonthus cognatus</i>						
June	0.48	***	153	<0.001	y=1.0x+0.43	0.58
July	0.04	NS	0.6	0.4	NS	
Carabidae						
June	0.46	***	375	<0.001	y=0.08x+0.09	0.77
July	0.54	***	304	<0.001	y=0.96+0.17	0.73
Field L2						
<i>Nebria brevicollis</i>						
June	Insufficient capture		7.6	0.007	NS	
July	Insufficient capture		13	<0.001	y=0.45x+0.07	0.11
<i>P. madidus</i>						
June	0.68	***	31	<0.001	y=0.61x+0.74	0.22
July	0.59	***	24	<0.001	y=0.71x+0.85	0.17
<i>P. melanarius</i>						
June	Insufficient capture		1.1	0.3	NS	
July	Insufficient capture		16	<0.001	y=0.36x+0.6	0.13
<i>Philonthus cognatus</i>						
June	0.28	**	13	<0.001	y=0.4x+0.53	0.11
July	0.37	***	19	<0.001	y=0.51x+0.32	0.14
Carabidae						
June	0.25	**	0.1	0.7	NS	
July	0.33	***	1.4	0.2	NS	

### 3.3.3 Conclusions

1. Arable soils support high numbers of beneficial invertebrates through the winter.
2. The time of peak emergence varied between species but was highest for Carabidae in June.
3. Strong correlations were found between the spatial pattern of emergence and numbers emerging in the pitfall compared to emergence traps. The pitfall traps were therefore providing an indication of not just activity but also density.
4. The spatial pattern of emergence was related to soil moisture in the previous winter. An optimum soil moisture level that maximised emergence was found for some species and the Carabidae.

**3.4. THE SPATIAL DYNAMICS AND MOVEMENT OF CARABID BEETLES BETWEEN AND WITHIN ARABLE FIELDS.** (Published in *International Journal of Ecology and Environmental Sciences*. Special issue: *Habitat Manipulation and Arthropod Pest Management*)

Understanding dispersal power is essential for a number of reasons. Dispersal is a key mechanism for the exchange of genetic material between local populations. Barriers to dispersal can thus restrict opportunities for gene flow (Frankham, 1995) and may influence fitness (Reed & Frankham, 2003). They may also confine a population to a small area and consequently increase the risk of stochastic extinction of a local population. Species' dispersal ability and landscape permeability also determine the linkage between local populations within a metapopulation structure. This is especially relevant in agricultural landscapes where, for example, the application of a pesticide may result in the severe depletion or extinction of a local population. Evidence from simulation models has demonstrated that population persistence depends to a large extent on the permeability of the field boundaries and the frequency of exposure to pesticides (Sherratt & Jepson, 1993). The speed with which a species can respond to changes in its environment and food supply is also a function of dispersal ability (Wallin, 1986). Those that can respond the quickest are more likely to survive the sudden changes that can occur in such ephemeral habitats as cropped fields. The capacity for population expansion across a landscape, for example by an introduced species, is also dependent on dispersal power and landscape permeability.

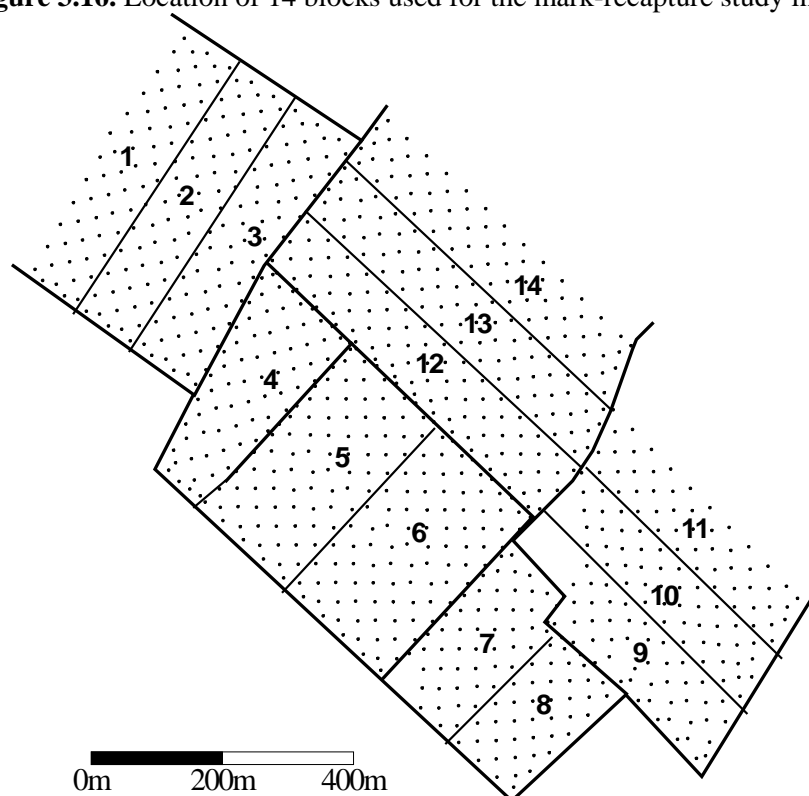
During the first two years of this project, mark-release-recapture experiments were conducted at the farm scale to determine to what extent hedgerows and crop rotations influenced the distribution and movement of *Pterostichus* species carabid beetles. These species were chosen because *Pterostichus* species and especially *P. melanarius* are common and widespread in cultivated fields throughout Europe, North America and Japan (Luff, 2002), while *P. madidus* is common throughout Europe. Both species are considered to be important for biological control since they are known to consume a range of crop pests including cereal aphids and slugs (Sunderland, 2002). *P. melanarius* has also been shown to exhibit a spatial and temporal linkage to cereal aphid distributions (Winder et al., 2001).

**3.4.1. Methodology for Mark-Recapture Studies**

The same 66 ha study area described in section 3.2.1.1. was used along with the grid of pitfall traps. To determine the extent of movement by carabid beetles within the study area, mark-release-recapture studies were conducted in 2000 and 2001. In 2000, the study area was subdivided into 14 blocks of approximately 5 ha each (Fig. 3.16). Beetles were captured using dry pitfall traps left open for two days prior to marking on 15 and 16 June 2000. The two most numerous carabid species, *Pterostichus madidus* and *P. melanarius*, were marked and released at the point of capture. These two species overwinter as larvae in the soil and emerge in the spring as adults. A small proportion of the population may survive between years as adults by seeking refuge in the field boundaries during the winter. Immediately after emergence the males seek out females and may

therefore disperse over greater distances. However, once the females are ready to oviposit they may become more active. Permanent marks were made using a drill to engrave codes on their elytra according to the block in which they were captured, following the method developed by Thomas (1995). The beetles were then released within 2 m of their capture location. The presence of marked beetles and their location in the grid were recorded when the all the contents of the pitfall traps used for examining the beetle's distribution were identified.

**Figure 3.16.** Location of 14 blocks used for the mark-recapture study in 2000.

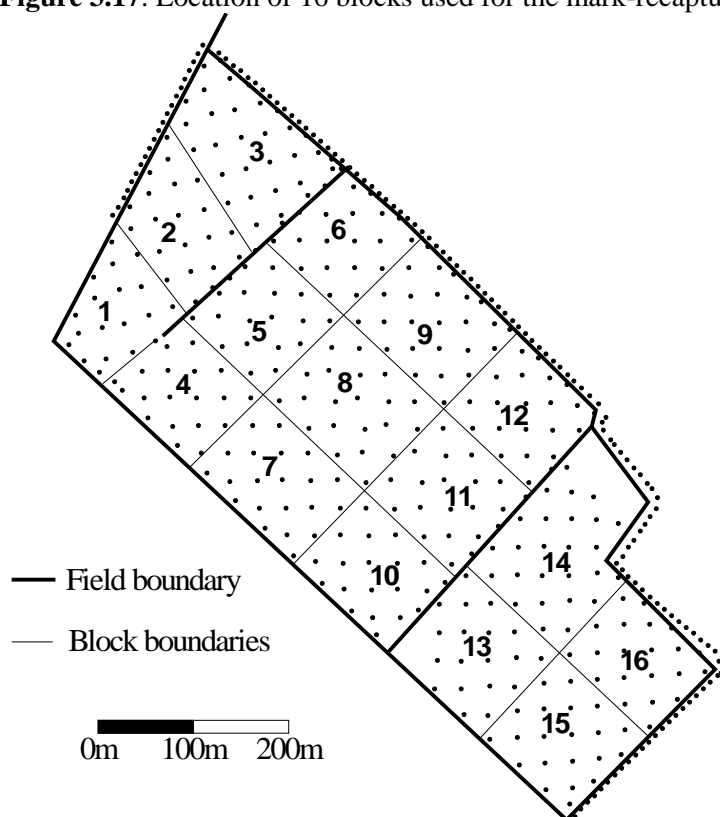


To examine the distribution of beetles across the study site in 2001 the same grid of pitfall traps described above was re-established. Traps were filled with a 50% solution of ethylene glycol and detergent and then opened for three periods (4-11 June; 9-16 July; and 5-12 September).

In 2001, the mark-release-recapture experiment was conducted in the three smallest fields to enable a more intensive study of within-field movement. This area was subdivided into 16 blocks of approximately 1.5 ha each (Fig. 3.17). Beetles of the three most numerous carabid species caught that year, *Pterostichus madidus*, *Nebria brevicollis* and *Poecilus cupreus*, were marked and released as above. Beetles were captured, marked and released on five two-day periods (10-11 May, 24-25 May, 13-14 June, 27-28 June, 2-3 August). Beetles were recaptured in dry pitfall traps between 13-16 August, and in wet traps as described above. In addition, 135 pitfall trap sites, 40 m apart, were established in a line around the outer boundary of the three fields to catch

beetles leaving the fields and so provide an estimate of emigration (Fig. 3.17). At each of these trapping locations, a pair of dry pitfall traps was set 1 m apart and joined by a linear barrier (1 x 0.20 m) to increase the capture rate. These were opened during six periods (23-25 May, 11-13 June, 26-28 June, 10-12 July, 27-30 July, 8-10 August).

**Figure 3.17.** Location of 16 blocks used for the mark-recapture study in 2001.



### 3.4.2. Data Analysis

The distribution of insects was analysed using SADIE analysis as described in section 3.2.1.5. To examine whether the stimulus for dispersal was density-dependent, regression analysis was used to examine the number of each species moving out of a particular block ( $m$ ) in relation to the number captured ( $c$ ) (both transformed  $\log n + 1$ ). The regression was performed both ways ( $\log (m+1) \times \log (c+1)$  and  $\log (c+1) \times \log (m+1)$ ) and a t-test was used to determine whether the slope differed significantly from 1. Density-dependent movement was assumed if both regressions were significant.

Beetles were not individually coded but their capture position was known. Therefore, to obtain an approximate indication of the distance moved by each marked beetle the distance between their capture position and the centroid of the block in which they were marked was calculated within Mapinfo 5.5 using the spherical distance procedure (Mapinfo Corporation, New York, USA). For each species, a t-test was used to determine whether the distance moved differed between the sexes.

In 2001, abundance estimates were calculated using Chapman's small sample adjustment to the Peterson index (Seber, 1982). Each successive pair of dates was treated as a single mark and recapture sequence. The confidence intervals were calculated assuming a Normal distribution for those instances that had recaptures >50 individuals; if <50 then recaptures were assumed to have a Poisson distribution and hence have asymmetric confidence intervals.

### 3.4.3. Results of Beetle Movement Studies

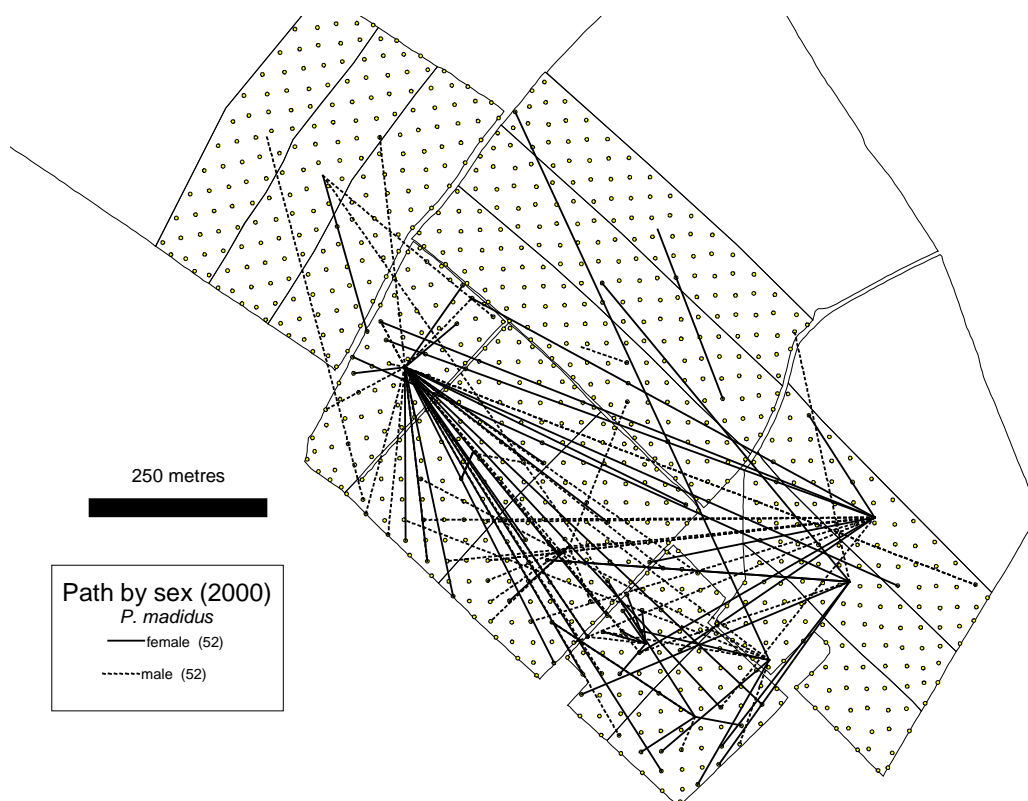
In 2000, a total of 1853 *P. melanarius*, and 1630 *P. madidus* were marked, of which the proportion recaptured was 11.4 and 7.4% respectively. In general, proportionally more of the recaptured *P. madidus* were found to have moved to a different block than remained in the block where they were originally marked and released. However, there were exceptions when all were recaptured inside the block in which they were marked (Table 3.27).

**Table 3.27.** Number and percentage of marked *P. madidus* that remained in the same or moved to a different block or field in 2000.

Marking Field/Block	Recapture Field/Block														Total	% same	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		Block	Field
L1	1			2	1		2								1	0	0
	2			2											4	0	
	3														0	--	
S1	4	1		13	6	6	12	4		3					45	29	29
S2.	5				4										4	100	91
	6					6						1			7	86	
S3	7						11								11	100	82
	8					3		3							6	50	
L1	9			1	2	1	2	3					1		10	0	2
	10			2		2	3	2					1	1	11	0	
	11			3	3	5	3	2		1				3	20	5	
L2	12											1			1	100	100
	13														0	--	
	14												1		1	0	
Total	0	1	0	21	16	23	33	14	0	0	4	2	2	5	121	32	33
	L1		S1		S2		S3		L3		L2						

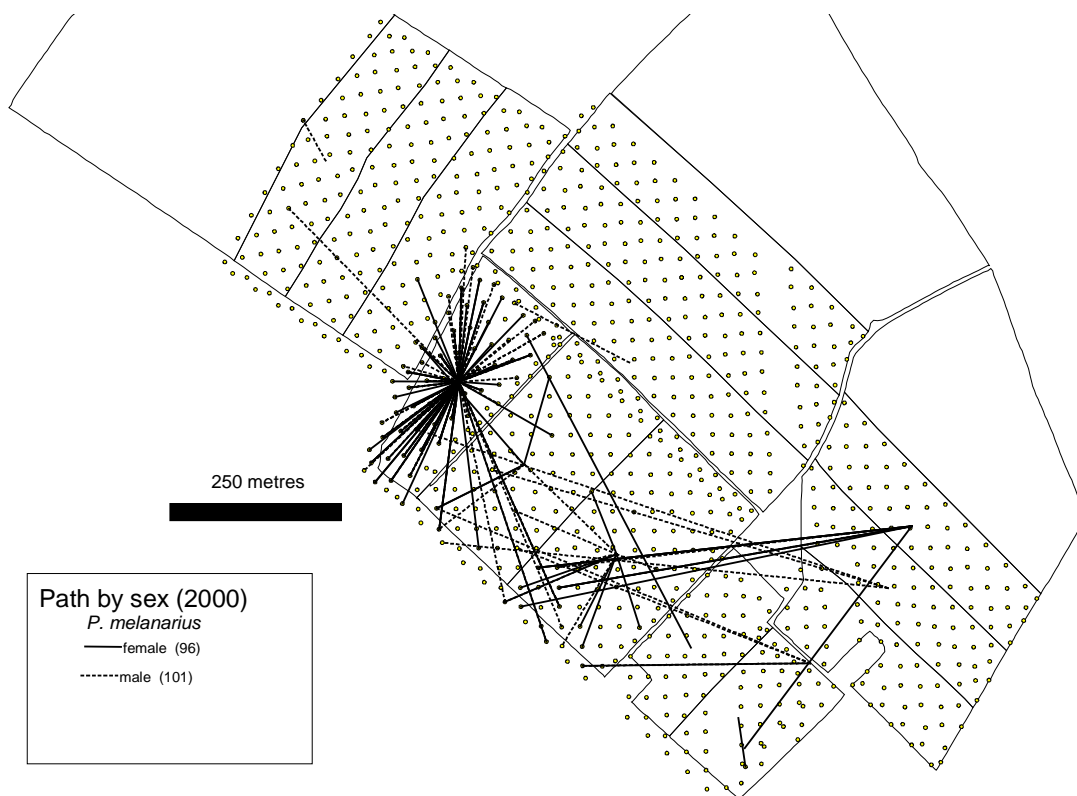


**Figure 3.18.** Movement of marked *P. madidus* males and females between blocks in 2000. A central release point within each block was assumed.



*P. melanarius* appeared to be more static, with a smaller percentage of marked individuals than *P. madidus* being recaptured in a block other than the one in which they were released, although again there were exceptions (Fig. 3.19). The overall proportion of *P. melanarius* that moved to another block and were recaptured was 20% compared to 67% for *P. madidus* (Tables 3.27 & 3.28). Overall a higher proportion of *P. madidus* moved between fields than remained in the field where they were originally released (Table 3.27). However, most movement occurred from those fields where activity-density was lowest and least where it was highest (Fig. 3.18). In contrast, in the fields where *P. melanarius* were most abundant, the majority remained within the same field (Table 3.28). However, the majority of recaptured *P. madidus* (94%) and all *P. melanarius* that were marked in the pea fields were also recaptured in pea fields. Of the 43 *P. madidus* that moved between fields and were recaptured, 74% crossed only one hedgerow, 21% crossed two and only 7% passed through three hedgerows. Of the 23 *P. melanarius* recorded moving between fields 78% crossed one hedgerow and the remainder crossed two hedgerows.

**Fig. 3.19.** Movement of marked *P. melanarius* males and females between blocks in 2000. A central release point within each block was assumed.



The average displacement distance of *P. madidus* was  $12 \text{ m day}^{-1}$  but values as high as  $43 \text{ m day}^{-1}$  occurred (Table 3.29). There was no significant difference between the sexes (t-Test) in the distance moved. Overall 36% moved less than  $5 \text{ m day}^{-1}$ , with the proportion moving greater distances gradually declining (Fig. 3.20). The speed of *P. melanarius* was only half that of *P. madidus*. 49% moved less than  $5 \text{ m day}^{-1}$  and only 4% moved more than  $20 \text{ m day}^{-1}$ , with a maximum of  $29 \text{ m day}^{-1}$  (Table 3.29). Again, there was no significant difference between the sexes. The maximum distance moved by a *P. melanarius* was lower (725 m over 25 days) than that recorded for *P. madidus* (859 over 20 days) and 83% of *P. melanarius* moved less than 200 m. These estimates may, however, underestimate the true dispersal distances because some beetles may have moved outside the pitfall area and consequently they could not be recaptured. In addition, the differences between species may be artefacts of the different distributions of the release points, influencing the potential to detect long-distance movement.

**Table 3.28.** Number and percentage of marked *P. melanarius* that remained in the same or moved to a different block or field in 2000.

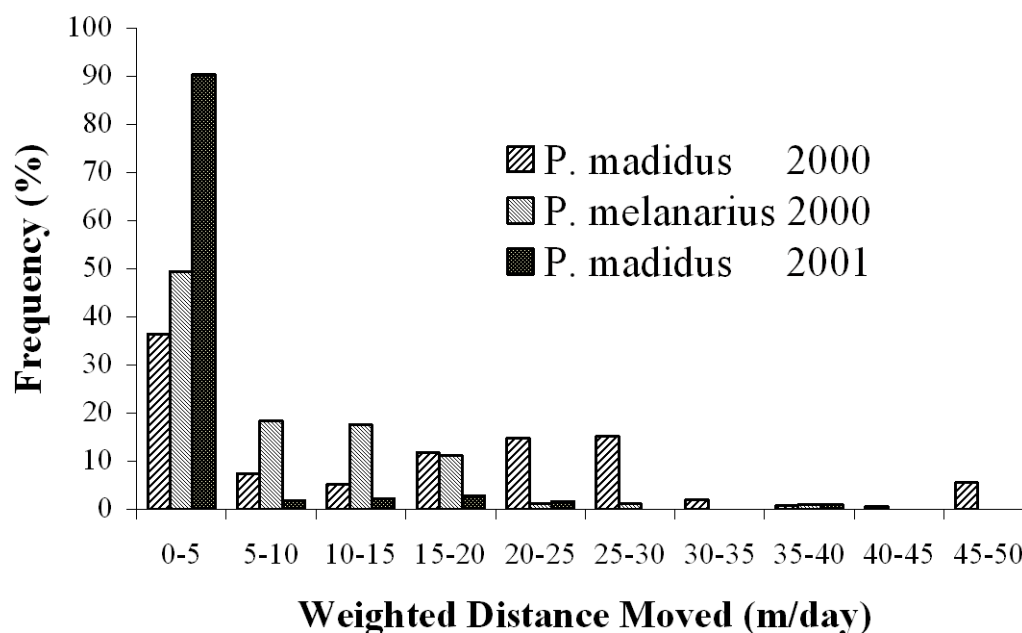
Marking Field/Block	Recapture Field/Block														Total	% same	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		Block	Field
L1	1	1													1	100	100
	2														0	--	
	3														0	--	
S1	4	1	5	14 8	7	7									168	88	88
S2	5			1	5										6	83	94
	6				2	10									12	83	
S3	7			1											1	0	50
	8							1							1	100	
L3	9				2	3								1	6	0	0
	10			1	2									1	4	0	
	11				2	8		1							11	0	
L2	12			1											1	0	0
	13														0	--	
	14														0	--	
Total	2	0	5	26	20	28	0	2	0	0	0	0	0	2	211	78	79
	L1			S1	S2		S3		L3			L2					

**Table 3.29.** Distances moved (m d<sup>-1</sup>) by *P. madidus* and *P. melanarius*.

(SE=standard error, N=number of beetles)

	<i>P. madidus</i>			<i>P. melanarius</i>		
2000	All	Males	Females	All	Males	Females
Mean	12	12	12	5.9	5.5	5.6
SE	0.8	1.3	1.3	0.4	0.5	0.5
Median	10	10	11	4.1	3.7	4.1
Minimum	0.6	0.6	1	0.4	0.4	0.6
Maximum	43	43	38	29	29	26
N	121	52	52	211	101	96
t value	0.28			0.18		
2001						
Mean	4	3.1	2.9			
SE	0.5	0.4	0.7			
Median	2.4	2.3	2.6			
Minimum	0.1	0.4	1			
Maximum	43	16	5			
N	134	66	5			
t value	-0.22					

**Figure 3.20.** Frequency distribution for distance moved by *P. madidus* and *P. melanarius* in 2000 and 2001. Frequencies were weighted by trap density for each distance class.



In 2001, the most frequently captured species was *P. madidus* and a total of 3813 were marked, of which only 3.6% were recaptured. Of the 1567 *N. brevicollis* that were marked 1.1% were recaptured while only one of the 303 marked *P. cupreus* was recaptured. Given the very low recapture rate for these two species, no further data are presented on them. As found in the previous year, a greater proportion of *P. madidus* (66%) had moved between blocks and been recaptured compared to those that stayed where they were marked (Table 3.30). Of the 24 *P. madidus* that moved between fields and were recaptured (Table 3.30), 92% crossed only one hedgerow and 8% crossed two boundaries (Fig. 3.21). Only five female beetles were recaptured of the 72 beetles that were sexed.

In 2001, the average daily displacement distance of *P. madidus* was a third of that found in 2000. 90% of beetles moved  $<5 \text{ m day}^{-1}$  (Fig. 3.20). The maximum distance moved was the same as that found in 2000, but a higher proportion of beetles moved shorter distances, with 99% moving less than 25 m (Fig. 3.20). There was no significant difference between the distances moved by males and females (Table 3.29). These displacement distances may again be an underestimate of the true value because they reflect the scale of the sampling grid.

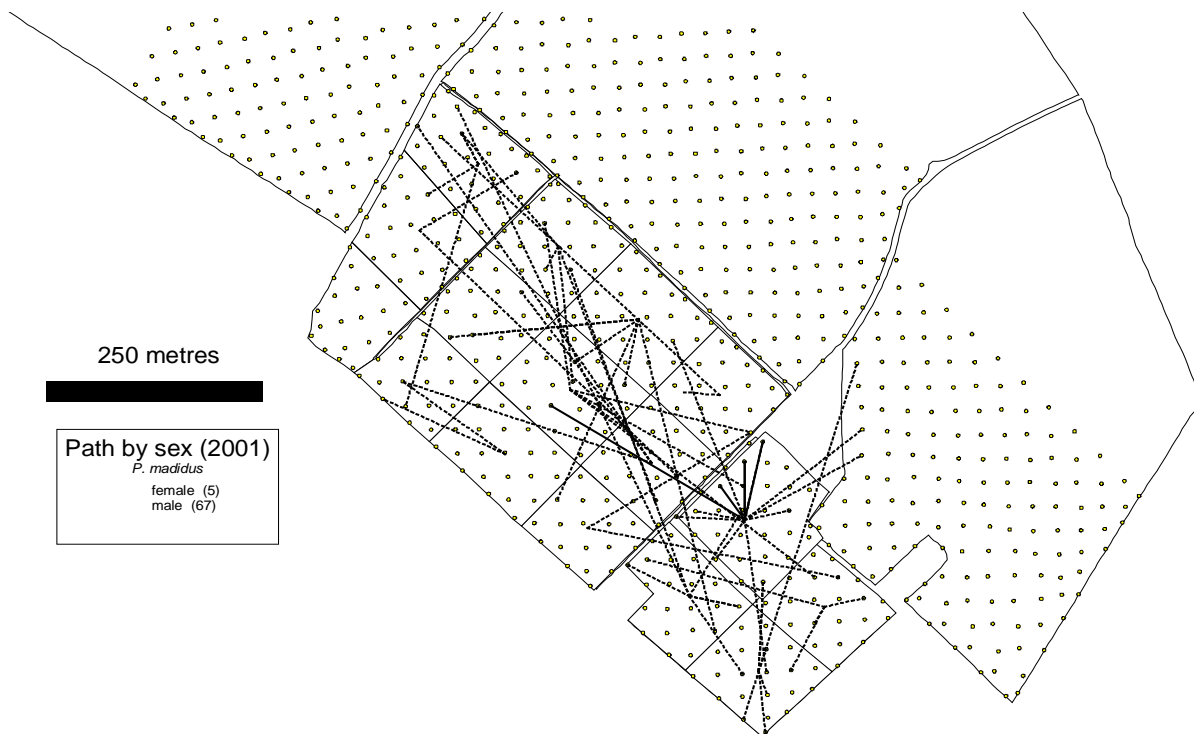
No marked beetles were recaptured in the line of pitfall traps surrounding the three fields.

No significant density-dependent movement was detected for either species in either year.

**Table 3.30.** Number and percentage of marked *P. madidus* that remained in the same or moved to a different block or field in 2001.

Marking Field/Block	Recapture Field/Block																% same		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Total	Block	Field
S1	1		1	3													4	0	68
	2	1	1	2			1	2			1						8	13	
	3		2	3	1	1											7	43	
S2	4				3	2	1					1					7	43	84
	5					1	1	1	1								4	25	
	6			1		1	4	1	2	1		1					11	36	
	7			1	2		1	2									6	33	
	8						1	1	1	1	1	3	1		1		11	9	
	9					2	1		2	2	1			1			9	22	
	10									1	2	1	1			1	7	29	
	11			1							1						7	0	
	12			1					1			3	1			1	8	13	
	13		1	1			1						3	1	1		8	38	80
	14				1			2	1	1				11	1		17	65	
	15								1				1	2	5	1	10	50	
	16												1		3	6	10	60	
Total	1	5	13	8	7	12	9	12	6	5	9	4	6	15	12	10	134	34	81
	S1			S2									S3						

**Figure 3.21.** Movement of marked *P. madidus* males and females between blocks in 2001. A central release point within each block was assumed.



#### **3.4.4. Conclusions**

1. The two carabid species investigated differed in their mobility. *P. melanarius* was relatively immobile with most individuals remaining within the same areas where they emerged. A greater proportion of *P. madidus* moved within and between fields.
2. Both carabid species were shown to cross hedgerows.
3. The average distance moved by *P. madidus* varied between years indicating that movement may vary according to the conditions encountered.
4. No significant density-dependent movement was detected for either species in either year.

### **3.5. QUANTIFYING THE IMPACT OF HABITAT MANIPULATION ON THE ABUNDANCE AND DISTRIBUTION OF GENERALIST PREDATORS AND APHIDS**

Certain non-crop habitats are known to encourage beneficial invertebrates leading to subsequent reductions in pest numbers in adjacent crops, as described in section 3.1. On farmland, however, these areas occupy a relatively small proportion of the land while set-aside typically forms 13% of arable land (Defra Statistics). There is therefore potential to manage set-aside land to benefit biocontrol through provision of overwintering sites and floral resources, as investigated for field margins in section 2. The set-aside strips established within the study area were initially sown with a mixture of kale, millet, *Phacelia*, quinoa, triticale, sunflowers and yellow sweet clover, although a weedy understorey also developed during the course of the study. These provided considerable floral resources that could be utilised by hoverflies and parasitoids, and in the latter years the weedy understorey may have provided overwintering sites for beetles and spiders. To test specifically whether the strips were encouraging beneficial insects and whether aphid abundance was being reduced, an experimental design similar to that used in section 2 was established.

#### **3.5.1. Effect of Set-Aside Strips on Aphid and Predatory Invertebrate Abundance in 2002**

In 2002, a pilot study was conducted to determine whether the set-aside strips were affecting aphid abundance on winter wheat. To test whether the set-aside strips were affecting the numbers of predatory epigeal invertebrates within the crop a subset of the spatial dataset described in section 3.2 was analysed.

##### **3.5.1.1 Materials and methods.**

Four fields of winter wheat were used on the Cranborne Estate, Dorset. Two were large fields that had 24 m wide set-aside strips established along two edges, the third contained a single set-aside strip while the fourth had none. Three sampling transects were established at the same distances (10, 30 & 100 m from the crop edge) as used in section 2. Transects were established next to four set-aside strips and four conventional field margins which acted as controls. The large fields were considered of sufficient size to accommodate transects next to set-aside and conventional margins without interaction between the two, although subsequent results from the hoverfly and parasitoid manipulation studies (section 2) indicate that these insects are capable of dispersing across large fields. Cereal aphids were counted on three occasions (14, 20 & 27 June) *in situ* on 25 tillers chosen at random along each transect. Numbers of each species present on all parts of the plant were recorded along with any natural enemies present and their development stage (e.g. ladybird eggs, larvae, pupae, adults). These included parasitoid mummies and aphids killed by fungal pathogens. The number of rose-grain aphids and grain aphids was analysed after log transformation using regression analysis comparing the effect of distance from the crop edge and treatment (with or without set-aside strip).

A subset of the spatial dataset was analysed to examine whether the numbers of predatory invertebrates was higher next to the margins with, compared to without, the set-aside strips. The mean number of predatory invertebrates for three transects of pitfall traps (7 traps/transect) located in the crop at approximately 10, 30 and 100 m from four standard field boundaries and from four with set-aside strips was calculated. Data were analysed as above for the June and July data separately.

### 3.5.1.2 Results

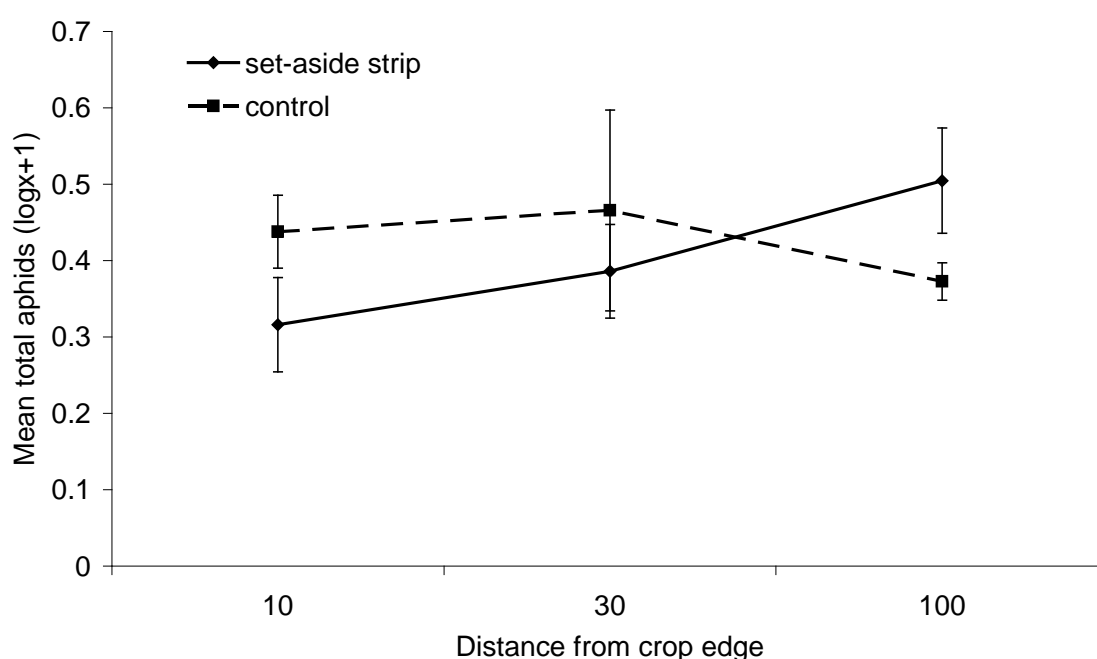
The treatment or distance from the margin had no significant effect on grain aphids (Table 3.31), although the distance from the margin had an almost significant effect on total aphids ( $F=1.98$ ,  $p=0.06$ ). Grain aphids were lower in the fields with the set-aside strips at 10 and 30 m from the crop edge but the reverse occurred at 100 m (Fig. 3.22).

In June there was no significant effect of the set-aside strips on numbers of predatory invertebrates captured by the pitfall traps. However, by July there were significantly more ( $F=5.4$ ,  $p=0.04$ ) in those transects located next to the set-aside strips where there were 1.7 (Mean number transformed  $\log x+1$ ) compared to 1.5 next the field boundaries. The distance of the transect from the crop had no effect.

**Table 3.31.** Regression analysis for aphid numbers comparing winter wheat fields with and without set-aside strips and at three distances from the boundary. Mean for three sampling occasions. (NS=non-significant)

	Field		Treatment		Distance		TxD	
Rose-grain aphid	NS		NS		NS		NS	
Grain aphid	NS		$t=1.8$	$p=0.08$	$t=2.0$	$p=0.06$	$t=-1.9$	$p=0.09$

**Figure 3.22.** Effect of set-aside strips on cereal aphids in winter wheat.





### **3.5.1.3 Conclusions**

1. There was some indication that set-aside strips sown with game cover reduced cereal aphid distribution or abundance for up to 30 m into the field.
2. Numbers of predatory invertebrates captured by pitfall trapping were higher in three transects located next to set-aside strips compared to the field boundary.

### **3.5.2. Effect of Set-Aside Strips on Aphids and Beneficial Invertebrates in 2003**

This study had three aims:

1. To confirm whether the set-aside strips increase beneficial insects and decrease cereal and pea aphid infestation levels.
2. To confirm whether previous differences in predatory beetle captures from pea and wheat fields were due to increased survival over winter in the pea fields.
3. To confirm whether predatory beetles were consuming cereal and pea aphids.

#### **3.5.2.1 Materials and methods.**

In 2003, the effect of the set-aside strips on cereal and pea aphids was tested. Four pea fields and four winter wheat fields were used that contained set-aside strips. The 24 m wide set-aside strips were established in 2001 but by 2003 they contained a mixture of sown plants that had survived through self seeding and also a wide range of weed species. In 2003, half of each strip was re-sown with the original mixture.

Next to each set-aside strip and on the opposite side of the field next to a normal field boundary three sampling transects at 10, 30 and 100 m from the crop edge were established. At each distance there were five sampling locations 20 m apart. At each sampling location the abundance of beneficial and pest species were measured using a range of sampling techniques.

- a) Three standard pitfall traps (2 m apart arranged in a triangle) containing a mixture of water and detergent. Traps were operated continuously from when aphids started to appear and were emptied weekly.
- b) At 30 m only. One 1m<sup>2</sup> emergence trap, as described in section 4.3.1. Traps were set up in early April and emptied fortnightly from mid-April.
- c) Three 6 cm diam. empty pitfall traps (wheat fields only). These were used for collecting *Pterostichus melanarius* and *P. madidus* (Carabidae) from which predation on cereal aphids was determined by analysing their gut contents using PCR. Traps were opened overnight and contents immediately frozen after collection. Sampling was conducted once during the aphid population peak. Gut contents were extracted, weighed and refrozen. Project partners at SAC conducted PCR analysis to determine the proportion that had consumed aphids (section 4).

- d) Two D-vac suction net samples, each of 10 sucks of 10 seconds each (area sampled = 1 m<sup>2</sup>/location). Samples were taken at weekly intervals.
- e) For wheat fields, cereal aphids were counted on 20 tillers/sampling location in a designated aphid counting area. Counts were carried out weekly (9/10 June, 16/17 June), from when aphids first appeared.
- f) For pea fields, pea aphids were counted on 12 plants/sampling location in a designated aphid counting area. Counts were carried out weekly from when aphids first appeared.

In the pitfall trap samples, all relevant arthropods were identified to species, along with wolf spiders (Lycosidae). In the D-vac suction samples, all beetles (Coleoptera) were identified to species, spiders (Araneae) and predatory flies (Diptera) to family and aphids (Aphididae) to species.

To test whether the set-aside strips affected invertebrates within the crop, some predatory invertebrate taxa, those important as bird food and aphids, were analysed from the Dvac suction sampling (Table 3.29). The predatory invertebrates included the total number of spiders and the money spiders (Linyphiidae), the predatory flies from the families Empididae and Dolichopodidae, total predators and number of predatory species. Those invertebrates important for farmland birds included total invertebrates important as food, Heteroptera and Homoptera. From the pitfall traps the following groups were analysed: Boundary overwintering Carabidae, Carabidae, number of carabid species, Staphylinidae, number of staphylinid species, total invertebrates, total predators and number of predatory species. Data were analysed using a repeated measures ANOVA comparing treatment and sampling distance with field as a blocking factor in Genstat.

The number of individuals in each taxon captured using the emergence traps was summed over the whole trapping period and the effect of the set-aside strips compared using one-way ANOVA field as a blocking factor in Genstat. To determine whether invertebrate emergence was greater in wheat compared to pea fields, the results for fields with and without set-aside were tested separately as initial analysis indicated there was a significant interaction effect between cropping and the margin type for all groups tested.

### **3.5.2.2. Results**

The aphid counting revealed that the abundance of cereal aphids on the wheat ears and the total number were significantly higher in the transects next to the set-aside strips (Table 3.32). The aphid counting revealed that there were no significant differences in the numbers of pea aphids between transects with and without the set-aside strips. Pea aphids reached very high numbers in all fields, with almost 100% of plants infested.

All of the taxa or groups tested varied significantly over time as expected. Other significant results are shown in Table 3.31. The abundance of pea aphids sampled by D-vac suction sampling varied with distance and

was highest at 30 m from the field boundary (Table 3.32). Numbers of *Sitobion avenae* collected by suction sampling were significantly higher in the transects next to the set-aside strips.

The pitfall trapping revealed that there were fewer staphylinid beetles in the transects next to the set-aside strips compared to the control areas in the pea fields (Table 3.32). The number of carabid and staphylinid species declined with distance from the crop edge in the pea fields. In the wheat fields the total numbers of invertebrates and predators increased with distance from the crop edge.

The emergence traps showed that the set-aside strips in the wheat fields were decreasing the number of some predatory taxa and overall abundance of predatory invertebrates at 30 m into the field (Table 3.33). In contrast, in the peas the reverse trend was found with greater numbers of some taxa occurring where there were set-aside strips.

More invertebrates of all taxa tested emerged in wheat compared to pea control fields (Table 3.34). However, in the fields with the set-aside strips only higher numbers of *P. cognatus* emerged in the wheat fields.

**Table 3.32.** ANOVA results and means examining effect of set-aside strips on invertebrates within fields. Means are log(x+1). (C=control, S=set-aside strip, \*\*p<0.01, \*p<0.05)

Group	Factor	F value and significance	Means			SE of mean
APHIDS-Wheat						
Total aphids	Treatment	4.9 *	C=0.36	S=0.52		0.05
APHIDS-Peas						
Pea aphid	Distance	3.9 *	10m=0.57	30m=0.62	100m=0.51	0.03
DVAC-Wheat						
S. avenae	Treatment	4.5 *	C=1.15	S=1.39		0.14
DVAC-Peas						
Heteroptera	Distance	5.0 *	10m=0.04	30m=0.01	100m=0.01	0.006
Pea aphid	Distance	4.5 *	10m=1.39	30m=1.37	100m=1.24	0.04
PITFALL-Wheat						
Total insects	Distance	3.9 *	10m=1.11	30m=1.23	100m=1.27	0.12
Predators	Distance	6.2 **	10m=1.05	30m=1.19	100m=1.22	0.04
PITFALL-Peas						
No. carabid spp.	Distance	4.7 *	10m=0.80	30m=0.73	100m=0.71	0.02
No. carabid & staphylinid spp.	Distance	4.2 *	10m=0.89	30m=0.84	100m=0.81	0.03
Staphylinidae	Treatment	8.02 **	C=0.55	S=0.41		0.04

**Table 3.33.** ANOVA results and means examining effect of set-aside strips on invertebrate emergence within fields. Means are log(x+1). (NS=non-significant, \*\*\*=p<0.001, \*\*p<0.01, \*p<0.05)

Wheat	F value and significance	Control	Set-aside strips	SE of mean
<i>Pterostichus</i> spp.	6.59 *	1.18	0.90	0.08
<i>Harpalus</i> spp.	3.09 NS	1.12	1.0	0.05
Carabidae	2.13 NS	1.80	1.70	0.05
No. carabid species	0.7 NS	1.08	1.06	0.02
<i>P. cognatus</i>	0.90 NS	0.40	0.48	0.06
Staphylinidae	13.3 ***	1.37	1.16	0.04
No. staphylinid spp.	14.5 ***	1.09	0.95	0.03
Field overwintering	5.15 *	1.62	1.44	0.06
Predators	13.3 ***	2.10	1.88	0.03
<b>Peas</b>				
<i>Pterostichus</i> spp.	3.0 NS	0.55	0.78	0.09
<i>Harpalus</i> spp.	13.7 ***	0.84	1.09	0.05
Carabidae	14.4 ***	1.34	1.57	0.04
No. carabid species	10.4 **	0.92	1.02	0.02
<i>P. cognatus</i>	1.36 NS	0.14	0.08	0.04
Staphylinidae	1.25 NS	1.01	1.1	0.05
No. staphylinid spp.	0.6 NS	0.93	0.97	0.04
Field overwintering	13.1 ***	1.14	1.40	0.05
Predators	9.08 **	1.59	1.75	0.04

**Table 3.34.** Comparison of invertebrate emergence in peas and winter wheat for with and without the set-aside strips. Means are log(x+1). (NS=non-significant, \*\*\*=p<0.001, \*\*p<0.01, \*p<0.05)

Control fields	t-test	Peas		Winter wheat	
		Mean	SE	Mean	SE
<i>Pterostichus</i> spp.	-3.6 ***	0.55	0.08	1.18	0.15
<i>Harpalus</i> spp.	-2.1 *	0.84	0.07	1.12	0.12
Carabidae	-4.2 ***	1.34	0.06	1.8	0.09
No. carabid species	-3.3 **	0.92	0.03	1.08	0.03
<i>P. cognatus</i>	-3.1 **	0.14	0.04	0.39	0.07
Staphylinidae	-5.1 ***	1.0	0.06	1.4	0.04
No. staphylinid spp.	-2.9 **	0.92	0.05	1.09	0.03
Field overwintering	-3.8 ***	1.14	0.06	1.16	0.11
Predators	-6.4 ***	1.59	0.06	2.05	0.05
<b>Set-aside fields</b>					
<i>Pterostichus</i> spp.	-0.7 NS	0.78	0.1	0.90	0.14
<i>Harpalus</i> spp.	0.7 NS	1.1	0.07	1.0	0.09
Carabidae	-1.5 NS	1.57	0.05	1.71	0.07
No. carabid species	-1.1 NS	1.02	0.02	1.06	1.03
<i>P. cognatus</i>	-4.5 ***	0.07	0.04	0.48	0.08
Staphylinidae	-1.4 NS	1.1	0.04	1.2	0.04
No. staphylinid spp.	0.34 NS	1.0	0.03	1.0	0.03
Field overwintering	-0.3 NS	1.4	0.05	1.44	0.1
Predators	-1.7 NS	1.75	0.05	1.88	0.06

The PCR technique was successfully used to confirm aphid predation in two carabid species (see Section 4). Of the 233 beetles tested for the presence of aphid remains, 21% were found to have consumed aphids. For males and females the percentage that had consumed aphids was 23 and 19% respectively. In the fields without and with set-aside strips 18 and 23% respectively had consumed aphids (Table 3.35). At 10m from the set-aside strip, 10% more of beetles had fed on aphids compared to where there was no set-aside strip.

**Table 3.35.** Number and percentage of *Pterostichus madidus* and *P. melanarius* that tested positive or negative for aphids in fields with and without the set-aside strips.

		10m		30m		100m		Total	
		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Control	No.	5	30	8	28	7	33	20	91
	%	14	86	22	78	18	82	18	72
Set-aside strip	No.	9	29	11	33	8	32	28	94
	%	24	76	25	75	20	80	23	77

### 3.5.2.3. Conclusions

1. The set-aside strips sown with game cover had no affect on the distribution or abundance of pea aphids in peas but increased cereal aphids on winter wheat.
2. The set-aside strips had no affect on the distribution or abundance of most predatory invertebrates within the adjacent crop measured by suction sampling and pitfall traps.
3. The set-aside strips had a negative affect on invertebrate emergence in wheat but a positive affect in pea fields.
4. Invertebrate emergence was higher in wheat compared to peas where there were no set-aside strips.
5. Aphid consumption by the carabids, *P. madidus* and *P. melanarius* was confirmed using the PCR technique and 21% on average were found to have consumed aphids.

### 3.5.3. Effect of Weed Cover on Beneficial Invertebrates.

A positive association between beneficial invertebrates and weed cover was found from analysis of their spatial correlation. To confirm this relationship an experiment was established in which weed cover was manipulated through a reduction in herbicide inputs and the impact on beneficial invertebrates measured. Weed levels within fields of spring barley were increased by omitting/reducing the spring herbicide application.

#### 3.5.3.1 Materials and methods.

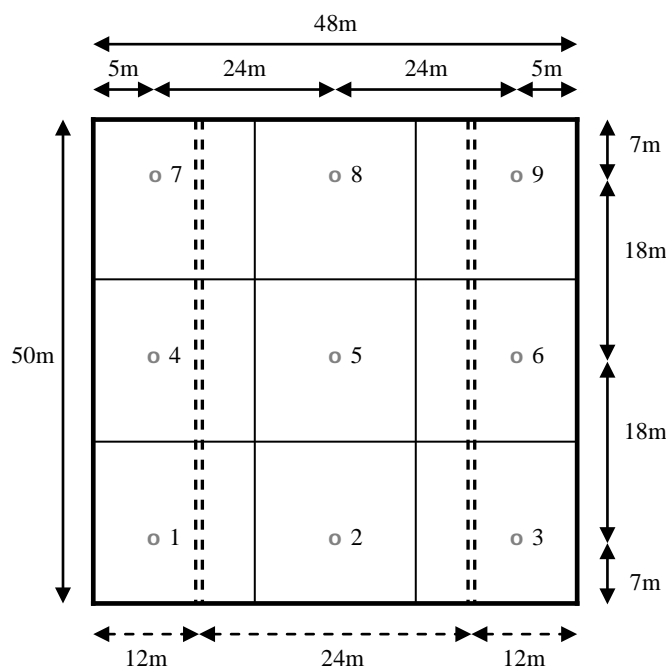
Two fields of spring barley were used on the Cranborne Estate, one located at Cranborne farm and the other at Woodyates farm, both in North-East Dorset. Within each field there were eight 0.5 ha plots (48 m wide x 50 m long) each arranged in a row with 50 m between each plot. The two treatments were randomly

allocated, providing four fully sprayed and four selectively sprayed plots. On Cranborne farm the fully sprayed field received an application of bromoxynil, ioxynil and mecoprop-p on 7/5/03 and tralkoxydim on 29/5/03. The fully sprayed and control plots were treated with metsulfuron-methyl and fluroxypyr on 12/6/03 to control weed growth, particularly of charlock (*Sinapis arvensis*). On Woodyates farm the fully sprayed plots received an application of tralkoxydim on 28/5/03 and metsulfuron-methyl on 12/6/03. The control plots received no herbicide.

Within each plot, beneficial invertebrates were assessed at 9 sampling locations/plot arranged in a 3 x 3 grid with a grid spacing of 18 m (Fig. 3.23). At each sampling location, epigeal invertebrates were measured using a single pitfall trap (6 cm diam.). The pitfall traps were first opened on 28<sup>th</sup> May 2003 and collected after a week and replaced with closed, clean pitfalls. These were again opened a week later. This was repeated for a total of four times. Crop and ground active invertebrates were collected by taking three D-vac suction net samples per plot. Whilst the pitfall traps were open, D-vac suction samples were taken at sample points 1, 5 and 9. At each point the D-vac was placed over the ground for 5x ten second intervals, thereby sampling an area of approximately 0.5m<sup>2</sup> at each post. Due to the weather, D-vac samples were not taken at exactly two week intervals but were sampled on the following dates: 30<sup>th</sup> May, 13<sup>th</sup> June, 1<sup>st</sup> July and 15<sup>th</sup> July, here on referred to as Dates 1, 2, 3 and 4.

**Figure 3.23.** Pitfall layout in each plot.

(○ represents a single pitfall trap, parallel dashed lines indicate location of the tramlines)



Invertebrates collected by the pitfall traps and D-vac suction sampling were counted and placed in the following groups:

*Ground dwelling Invertebrates (obtained by pitfall trapping):* Total number of carabid beetles (excluding larvae); Total number of staphylinid beetles (excluding larvae); All Other Coleoptera (consisting of click beetles (Elateridae), weevils (Curculionidae), ladybird adults and larvae (Coccinellidae) and leaf beetles (Chrysomelidae)); wolf spiders (Lycosidae); Total invertebrates (adults); Species richness (number of different species found); Carabid larvae; Staphylinid larvae; Other Coleoptera larvae.

*Crop dwelling Invertebrate groups (obtained by the D-vac suction sampler):* Aphid adults and nymphs (Aphididae); Carabid adults and larvae; Predatory Invertebrates (consisting of spiders (Araneae), lacewing larvae (Neuroptera), Carabid adults and larvae, Staphylinid adults and larvae, ladybirds (Coccinellidae), predatory flies (Dolichopodidae & Empidae and soldier beetles (Cantharidae); Species Richness (number of different species found); Total Invertebrates and Total Predatory Invertebrates.

A chick food index (CFI) was determined by grouping invertebrates from the D-vac samples according to the following list:

Carabidae (adults) and Elateridae

Symphyta (sawflies), Lepidoptera (adults and larvae) and Neuroptera

Chrysomelidae (adults and larvae) and Curculionidae

Non-aphid Hemiptera (adults and nymphs)

Aphididae (adults and nymphs)

The CFI was then calculated using the following formula:

$$0.1411 * (\text{Carabidae} \ \& \ \text{Elateridae}) \ + \ 0.1199 * (\text{Symphyta}, \ \text{Lepidoptera} \ \& \ \text{Neuroptera}) \ + \\ 0.0832 * (\text{Chrysomelidae} \ \& \ \text{Curculionidae}) \ + \ 0.00614 * (\text{Non-aphid Hemiptera}) \ + \ 0.000368 * (\text{Aphididae})$$

Weed cover and diversity was determined at each sampling point within each plot on 28 May 2003. At each point the weed cover for each species was estimated in four 0.5m<sup>2</sup> quadrats located randomly within 1m of the marker cane.

### **3.5.3.2 Data analysis**

The results for invertebrates and weeds were analysed separately due to the proliferation of weeds at the Cranbourne site that were not present at the Woodyates site. For the invertebrates, a Repeated Measures ANOVA was used to test for treatment effects. Where there was a statistically significant interaction between date and treatment, ANOVA's were carried out to determine when treatment was having a

statistically significant effect for each of the four dates. The percentage cover for each weed species, total percentage cover and total number of weed species was analysed using a one-way ANOVA.

### 3.5.3.3 Results

The weed cover only varied significantly between the control (fully sprayed) and selectively sprayed plots at the Cranborne site (Table 3.36). This was caused by an increased survival of *Sinapis arvensis* (charlock) which accounted for the majority of the weed cover. Very little weed cover developed at the Woodyates site (Table 3.37). Historically this farm has always been less weedy but the dry spring also inhibited weed germination.

**Table 3.36.** Mean percentage cover ( $\pm 1$  SE) and ANOVA results for treatment effect at the Cranborne Site, (\*= $p < 0.05$ ; \*\*= $p < 0.01$ ; \*\*\*= $p < 0.001$ )

Variate	Treatment		F- value	Significance
	Control	Selectively sprayed		
	mean $\pm 1$ se			
Percentage weed cover	12.3 $\pm$ 0.55	97.2 $\pm$ 5.45	739	***
Number of weed species	5.5 $\pm$ 0.29	6.0 $\pm$ 0.41	3.0	NS
<i>Fallopia convolvulus</i>	0.3 $\pm$ 0.25	2.2 $\pm$ 1.44	2.8	NS
<i>Sinapis arvensis</i>	0.5 $\pm$ 0.13	82.3 $\pm$ 5.81	1237	***
<i>Galium aparine</i>	1.0 $\pm$ 0.33	1.25 $\pm$ 0.78	0	NS
<i>Viola arvensis</i>	4.0 $\pm$ 1.65	4.3 $\pm$ 1.31	0.2	NS
<i>Fumaria officinale</i>	5.0 $\pm$ 2.07	5.4 $\pm$ 3.04	0.03	NS
<i>Polygonium aviculare</i>	1.5 $\pm$ 0.58	0.5 $\pm$ 0.21	4.1	NS
<i>Papaver rhoeas</i>	0.2 $\pm$ 0.05	1.5 $\pm$ 0.69	4.1	NS

**Table 3.37.** Mean percentage cover ( $\pm 1$  SE) and ANOVA results for treatment effect at the Woodyates Site. (\*= $p < 0.05$ ; \*\*= $p < 0.01$ ; \*\*\*= $p < 0.001$ )

Variate	Treatment		F- value	Significance
	Control	Selectively sprayed		
	mean $\pm 1$ se			
Percentage weed cover	7.1 $\pm$ 1.07	5.5 $\pm$ 0.53	1.0	NS
Number of weed species	5.0 $\pm$ 0.58	5.5 $\pm$ 0.65	0.02	NS
<i>Fallopia convolvulus</i>	0.4 $\pm$ 0.17	0.4 $\pm$ 0.21	0	NS
<i>Sinapis arvensis</i>	0 $\pm$ 0	0.1 $\pm$ 0.05	1.0	NS
<i>Galium aparine</i>	0 $\pm$ 0	0 $\pm$ 0	-	N/A
<i>Viola arvensis</i>	3.5 $\pm$ 1.54	2.6 $\pm$ 0.54	0.1	NS
<i>Fumaria officinale</i>	0 $\pm$ 0	0.1 $\pm$ 0.05	1.0	NS
<i>Polygonium aviculare</i>	1.3 $\pm$ 0.47	0.8 $\pm$ 0.14	0.9	NS
<i>Papaver rhoeas</i>	0.1 $\pm$ 0.05	0 $\pm$ 0	1.0	NS

At the Cranborne site, of the ground dwelling invertebrates captured by pitfall trapping, only the total number differed between the control and selectively sprayed plots (Table 3.38). No significant differences



were found at the Woodyates site (Table 3.39). Of the crop active invertebrates collected by suction sampling the two bug groups (Homoptera & Heteroptera) and money spiders (Linyphiidae) were all significantly lower in the selectively sprayed plots (Table 3.40). In contrast the predatory Diptera, species richness, staphylinid beetles, total invertebrates and total predators were all higher where the plots had been selectively sprayed (Table 3.40). There was, however, a time.treatment interaction effect and thus for the predatory Diptera, staphylinid beetles, total invertebrates and total predators the differences were only found on certain dates. At the Woodyates site, there were no significant effects on the ground or crop active invertebrates (Table 3.41).

**Table 3.38.** Mean ground active invertebrates ( $\pm 1$  SE) and Repeated Measures ANOVA results for treatment effect at Cranborne site. (\*= $p<0.05$ ; \*\*= $p<0.01$ ; \*\*\*= $p<0.001$ )

Variate	Treatment		F- value	Significance
	Control	Selectively sprayed		
	mean $\pm 1$ se			
Carabidae	26.4 $\pm$ 6.39	20.7 $\pm$ 2.79	0.16	NS
Staphylinidae	12.9 $\pm$ 3.62	11.4 $\pm$ 3.42	0.36	NS
All other Coleoptera	0.7 $\pm$ 0.20	0.6 $\pm$ 0.12	0.02	NS
Lycosidae	0.7 $\pm$ 0.18	0.8 $\pm$ 0.20	0.17	NS
Total invertebrates	42.2 $\pm$ 8.85	34.9 $\pm$ 4.29	7.86	*
Species richness	8.4 $\pm$ 0.48	7.7 $\pm$ 0.45	1.60	NS
Carabid larvae	0.1 $\pm$ 0.12	0.5 $\pm$ 0.28	1.47	NS
Staphilinid larvae	0.8 $\pm$ 0.49	0.1 $\pm$ 0.10	2.20	NS
Other Coleopteran larvae	0.7 $\pm$ 0.58	0.5 $\pm$ 0.37	0.62	NS

**Table 3.39.** Mean ground active invertebrates ( $\pm 1$  SE) and Repeated Measures ANOVA results for treatment effect at Woodyates site. (\*= $p<0.05$ ; \*\*= $p<0.01$ ; \*\*\*= $p<0.001$ )

Variate	Treatment		F-value	Significance
	Control	Selectively sprayed		
	mean $\pm 1$ se			
Carabidae	1.9 $\pm$ 0.45	2.9 $\pm$ 0.66	3.6	NS
Staphylinidae	2.9 $\pm$ 0.59	1.9 $\pm$ 0.35	1.2	NS
All other Coleoptera	0.6 $\pm$ 0.11	0.9 $\pm$ 0.11	2.1	NS
Lycosidae	1.8 $\pm$ 0.51	0.1 $\pm$ 0.60	0.9	NS
Total invertebrates	7.4 $\pm$ 0.92	7.6 $\pm$ 0.87	0	NS
Species richness	3.1 $\pm$ 0.22	3.3 $\pm$ 0.15	1.3	NS
Carabid larvae	0.1 $\pm$ 0.06	0.1 $\pm$ 0.04	0.01	NS
Staphilinid larvae	0.2 $\pm$ 0.08	0.5 $\pm$ 0.21	1.8	NS
Other Coleopteran larvae	0.1 $\pm$ 0.06	0.1 $\pm$ 0.06	0.2	NS

**Table 3.40.** Mean crop active invertebrates ( $\pm 1$  SE) and Repeated Measures ANOVA results for treatment effect at Cranborne site. (\*= $p<0.05$ ; \*\*= $p<0.01$ ; \*\*\*= $p<0.001$ )

Variate	Treatment		F-value	Significance
	Control	Selectively sprayed		
	Mean $\pm 1$ se			
Aphidae	62.3 $\pm$ 4.47	81.5 $\pm$ 13.8	0.7	ns
CFI	0.6 $\pm$ 0.15	0.9 $\pm$ 0.16	4.8	ns
Carabidae	0.5 $\pm$ 0.14	0.3 $\pm$ 0.09	1.1	ns
Heteroptera	0.5 $\pm$ 0.17	0.2 $\pm$ 0.36	12.8	*
Homoptera	3.8 $\pm$ 0.75	2.3 $\pm$ 0.45	14.3	**
Linyphiidae	5.1 $\pm$ 0.69	2.7 $\pm$ 0.52	12.0	*
Predator species richness	4.0 $\pm$ 0.30	3.8 $\pm$ 0.31	0.6	ns
Predatory Diptera	7.2 $\pm$ 1.61	11.4 $\pm$ 1.85	5.7	*
Species Richness	7.9 $\pm$ 0.34	9.8 $\pm$ 0.26	42.8	***
Staphylinidae	5.4 $\pm$ 1.3	9.5 $\pm$ 1.78	8.9	*
Total invertebrates	94.4 $\pm$ 6.74	255 $\pm$ 47.9	74.0	***
Total Predators	20.0 $\pm$ 2.77	25.8 $\pm$ 3.47	6.2	*

**Table 3.41.** Mean crop active invertebrates ( $\pm 1$  SE) and Repeated Measures ANOVA results for treatment effect at Woodyates site(\*= $p<0.05$ ; \*\*= $p<0.01$ ; \*\*\*= $p<0.001$ )

Variate	Treatment		F-value	Significance
	Control	Selectively sprayed		
	Mean $\pm 1$ se			
Aphidae	34.8 $\pm$ 2.49	33.4 $\pm$ 2.38	0.1	ns
CFI	0.39 $\pm$ 0.07	0.3 $\pm$ 0.06	3.5	ns
Carabidae	0.38 $\pm$ 0.2	0.3 $\pm$ 0.09	0.6	ns
Heteroptera	0.44 $\pm$ 0.18	0.5 $\pm$ 0.20	0.03	ns
Homoptera	5.7 $\pm$ 0.80	6.5 $\pm$ 0.79	1.1	ns
Linyphiidae	3.8 $\pm$ 0.53	3.9 $\pm$ 0.54	0.1	ns
Predator species richness	3.1 $\pm$ 0.29	3.2 $\pm$ 0.38	0.1	ns
Predatory Diptera	9.6 $\pm$ 2.53	11.8 $\pm$ 3.50	1.9	ns
Species Richness	6.5 $\pm$ 0.32	6.9 $\pm$ 0.51	0.3	ns
Staphylinidae	1.8 $\pm$ 0.36	1.8 $\pm$ 0.43	0.01	ns
Total invertebrates	61.1 $\pm$ 4.79	62.1 $\pm$ 5.88	0.01	ns
Total Predators	17.4 $\pm$ 3.31	19.5 $\pm$ 4.30	0.6	ns

### 3.5.3.4. Conclusions

1. The reduced herbicide inputs did not increase weeds at one site because dry weather inhibited weed germination.
2. Where weed cover was higher, this did not increase numbers of ground dwelling invertebrates but increased densities of predatory Diptera, Staphylinidae, total invertebrates, total predators and species richness on some occasions.
3. The higher weed cover reduced the density of some of the invertebrates groups important as bird food.

### 3.6 SPATIAL DISTRIBUTION OF PEA APHIDS AND THEIR PREDATORS

The spatial distribution of cereal aphids has been much studied recently (Winder et al., 1999) and this revealed that cereal aphids had an ephemeral distribution within cereal fields with patches appearing and disappearing through the summer. Examination of the spatio-temporal association between the carabid beetle *P. melanarius* and two aphid species revealed a strong correlation (Winder et al., 2001). These spatially coupled dynamics were sufficiently strong for this beetle predator to have a negative effect on the intrinsic rate of aphid increase. This was the first time that generalist predators were confirmed as impacting on aphid population dynamics. Research on the dynamics of cereal aphids and their prey has continued with BBSRC funding and therefore was not investigated here; instead the spatial relationships between pea aphids and generalist predators were examined. Pea aphids exhibit a similar behavioural response to cereal aphids in the presence of predators; to escape predation they drop to the ground (Clegg & Barlow, 1982). This also makes them vulnerable to ground active predators. In alfalfa, ground active predators worked synergistically with foliage active ones to make a significant contribution to aphid suppression (Losey & Denno, 1998a). The value of ground active predators for pea aphid control in pea crops has not been investigated.

#### 3.6.1 Materials and Methods.

In 2001, the distribution of pea aphids and epigeal invertebrates was monitored within one of the study fields (L1) during the period of pea aphid infestation. Pea aphid abundance was measured at each sampling point within the field by counting the number of aphids on 10 plants on 18, 21, 25 and 29 June and 3 July. The number of epigeal predators was measured using the grid of pitfall traps as described in section 3.2.1.2., and data was collected on three sampling occasions (Table 3.1).

The distribution of pea aphids and the total number of invertebrate predators collected in the pitfall traps was analysed using the Sadie red/blue method. The spatial association between pea aphids on five sample dates and total number of aphid predators on four occasions, giving 20 possible combinations over the five lagged sample occasions, was calculated as described in section 3.2.1.5.. The spatial correlation value  $X$  was plotted in two dimensions, as carried out in Winder et. al. (2001).

#### 3.6.2. Results

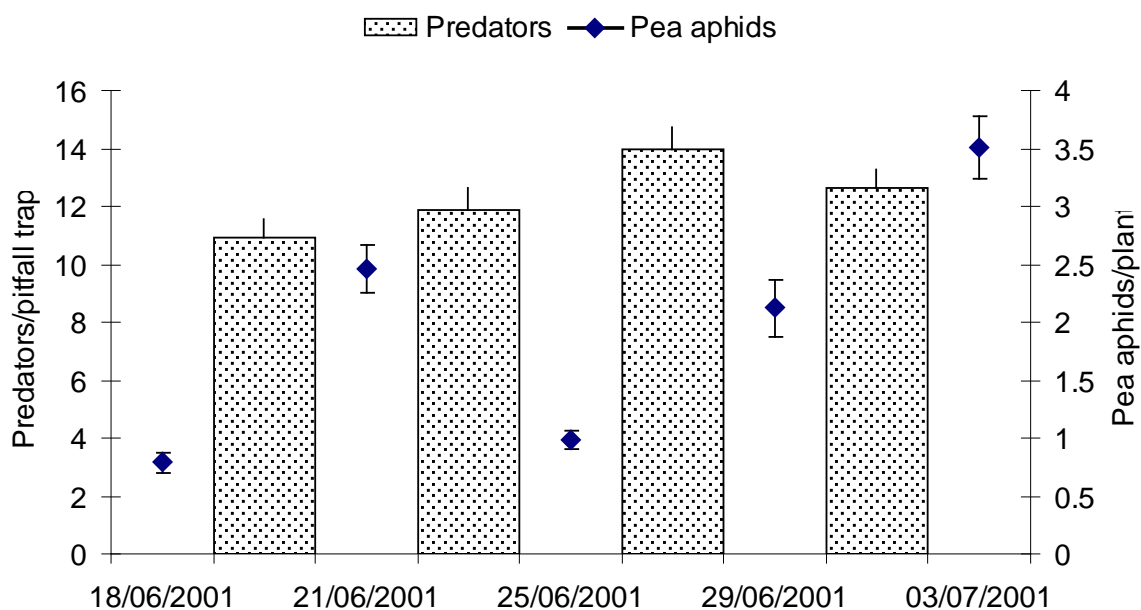
Pea aphid numbers showed an initial increase followed by a decrease then built further towards the last sample date (Fig. 3.24). They exhibited evidence of significant clustering into patches with gaps between on the 2-5 sampling occasions (Table 3.42), but the location of the patches and gaps was ephemeral (Fig. 3.25). The size of the patches and gaps also varied during the infestation.

The number of predators peaked between 25 and 29 June, but was relatively consistent over the pea aphid infestation period (Fig 3.24). The predator numbers were highest in the bottom corner of the field, with a small patch mid-field (Fig. 3.26).

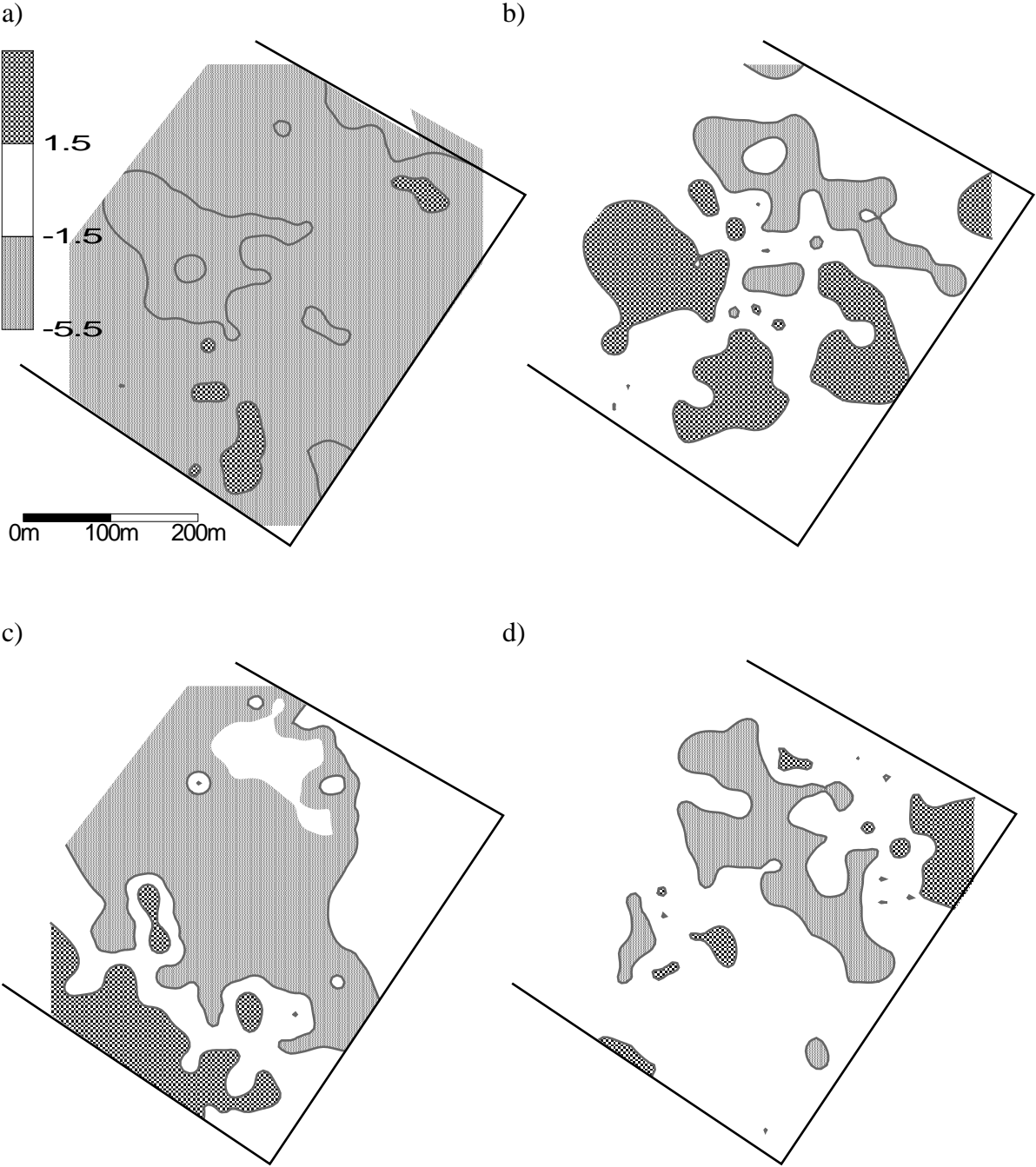
**Table 3.42.** Degree of clustering into 'patches' using overall index  $\bar{v}_i$  and associated probability  $P_i$ , or of 'gaps' using overall index  $\bar{v}_j$  and associated probability  $P_j$  for pea aphids and the total number of predatory arthropods in field L1 in 2001. (\*\*\*=P<0.001, \*\*= P<0.01, \*= P<0.05).

	Sample dates	Pea aphids	Sample dates	Total predators
$\bar{v}_j$	18/06/01	-1.05	18-21/6/2001	-1.86
$\bar{v}_i$		0.99		1.85
$P_j$		NS		***
$P_i$		NS		***
$\bar{v}_j$	21/06/01	-1.25	21-25/6/2001	-2.23
$\bar{v}_i$		1.28		2.32
$P_j$		NS		***
$P_i$		NS		***
$\bar{v}_j$	25/06/01	-1.62	25-29/6/2001	-1.63
$\bar{v}_i$		1.74		1.78
$P_j$		***		***
$P_i$		***		***
$\bar{v}_j$	29/06/01	-2.72	29/6-3/7/2001	-2.85
$\bar{v}_i$		2.72		2.82
$P_j$		***		***
$P_i$		***		***
$\bar{v}_j$	03/07/01	-1.47		
$\bar{v}_i$		1.45		
$P_j$		*		
$P_i$		*		

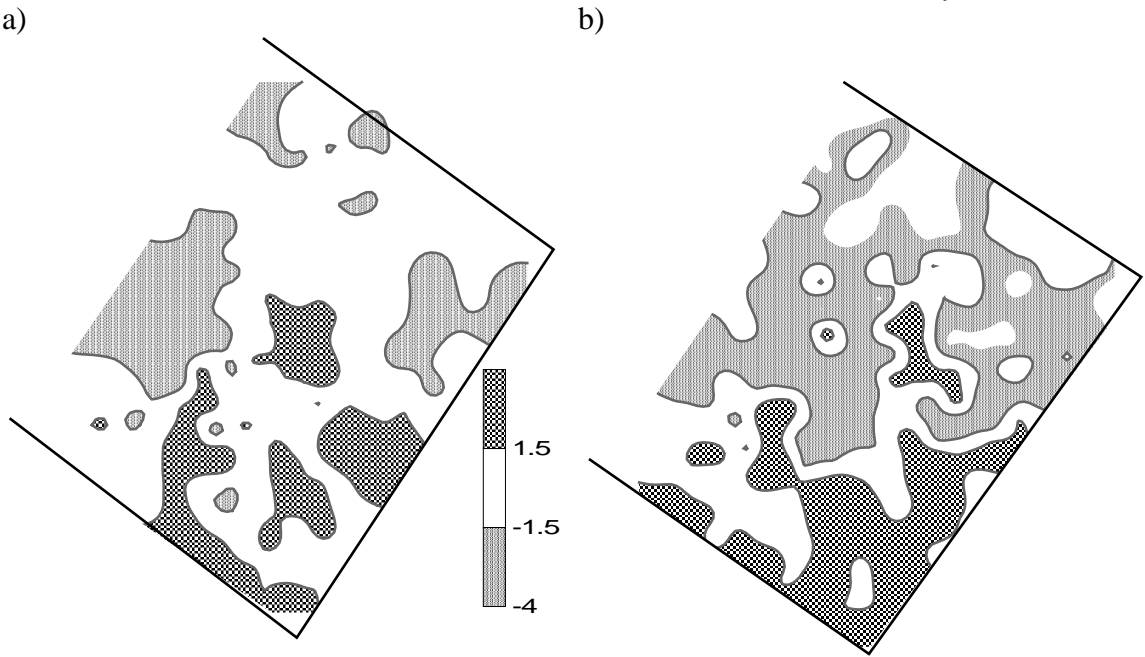
**Figure 3.24.** Abundance of pea aphids and predatory invertebrates.



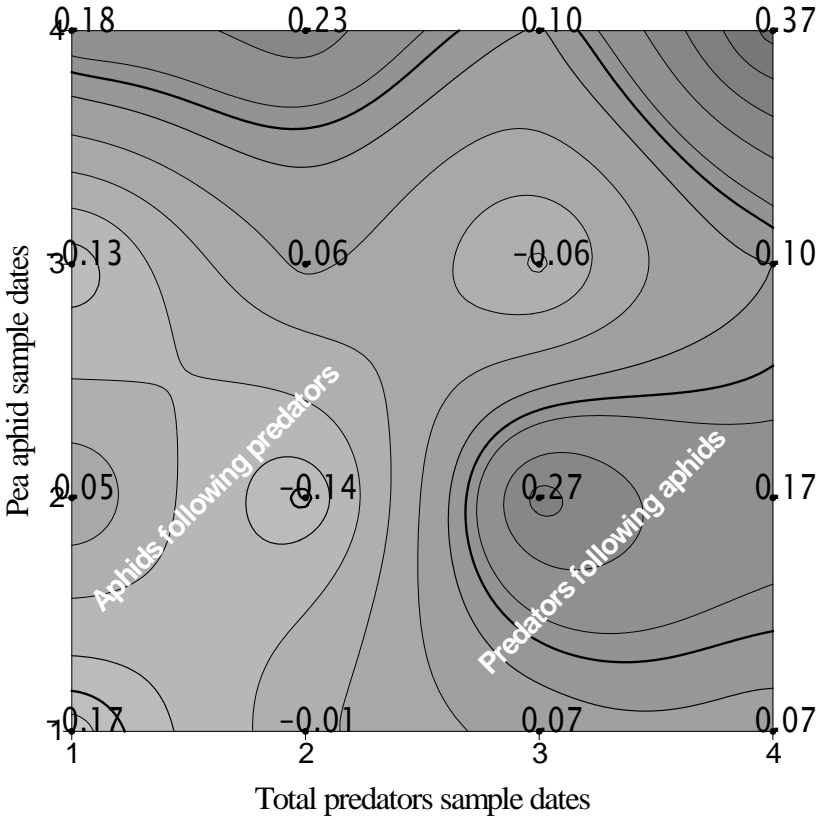
**Figure 3.25.** Spatial clustering for pea aphids on a) 21/6, b) 25/6, c) 29/6 and d) 3/7/2001. The maps indicate clusters of relatively high counts ( $v_i > 1.5$ ) and small counts ( $v_j < -1.5$ ).



**Figure 3.26.** Spatial clustering for predatory invertebrates in pitfall traps on a) 25-29/6 and b) 29/6-3/7/2001. The maps indicate clusters of relatively high counts ( $v_i > 1.5$ ) and small counts ( $v_j < -1.5$ ).



**Figure 3.27.** Spatio-temporal association between predator and prey. Interpolated surface of unlagged (main diagonal) and lagged (above and below main diagonal) associations between pea aphids (y-axes) and predatory invertebrates (x-axis).



The contour map depicting the spatio-temporal correlation between predators and pea aphids for all possible sampling combinations indicates that for those samples taken simultaneously there was negative then positive association (Fig. 3.27). The lagged associations revealed that the predators were positively correlated with the preceding aphid spatial pattern while the aphids were negatively correlated with the preceding predator spatial pattern. Much of this relationship was likely to be driven by what occurred in the bottom corner of the field, where a large predator patch developed, in which pea aphids were largely absent.

### **3.6.3. Conclusions**

1. Pea aphids were highly aggregated with patches of high density, but the location of these varied through the infestation period.
2. The location of the patches containing the highest number of predators was consistent through the pea aphid infestation period.
3. There was evidence that the ground active predators were influencing the abundance and distribution of pea aphids.

### 3.7 THE INFLUENCE OF FIELD MARGINS ON INVERTEBRATES WITHIN FIELDS

Many species of beneficial invertebrate overwinter either in the hedgebase or in the hedge vegetation. In addition, the field margins provide food resources such as pollen, nectar, vegetation and seeds, along with prey for predatory and parasitic species. As a consequence, they are a rich source of beneficial invertebrates, some of which colonise the cropped areas. The field margins also act as a refuge from otherwise damaging crop production practices such as cultivation and insecticide spraying. Field margins, however, vary enormously in terms of their structure and species composition. Around the six fields in which the spatial studies were conducted there was a range of different field margin types including hedges with a hedge base, grassy strips and farm tracks that varied in the diversity of their vegetation and structure. This may have influenced the abundance and diversity of invertebrates within the nearby field area. To assess whether there was any such impact, the structure and vegetational diversity of each field margin was surveyed in 2000 and 2002 and relationships sought to the within-field invertebrate community.

#### 3.7.1 Materials and Methods.

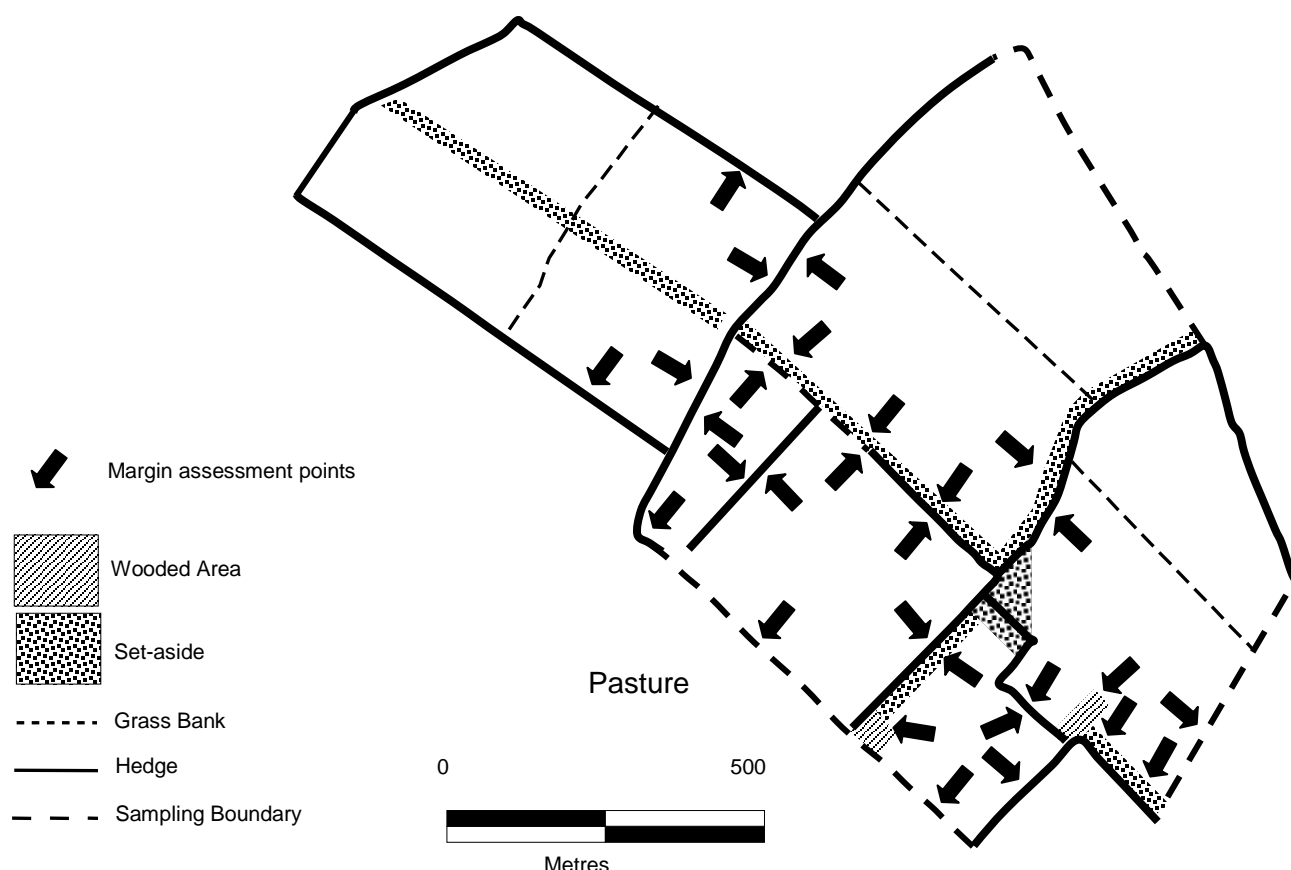
The field boundary vegetation was assessed in 30 field boundary lengths from the six study fields (Fig. 3.28). Boundary lengths were separated on the basis of aspect and major change in boundary structure, e.g. post and wire fences were split from hedgerow lengths along the same field edge. The higher plant species present in each field boundary length were recorded as part of the ground flora (0-1m), the shrub layer (1-4m) or the tree layer (>4m) in June 2000 and 2002. Each species present was given a score (0-9), based on a modified Braun-Blanquet cover-abundance score (Table 3.43) (Westhoff & Maarel, 1973).

**Table 3.43.** Cover-abundance scores (0-9) used to assess the flora of Cranbourne field boundaries, based on modified dominance scores (Westhoff & Maarel, 1973).

Score	Description; % ground cover by eye	% cover used for formal analyses
1	Rare; 1 or 2 plants	0.25
2	Sparse; 3 – 10 small plants	0.5
3	Frequent, <4% cover	1
4	Abundant, 5% cover	2
5	5 - 12.5%	5
6	12.5 - 25%	12.5
7	25 - 50%	25
8	51 - 75%	50
9	76 - 100%	75



**Figure 3.28.** Location of field margin assessment points across study area.



To determine whether there was any relationship between the field boundary characteristics and the invertebrates, a subset of the spatial distribution data for June 2000 and 2002 was used. Data was summed from a block of eight pitfall sampling locations (3 in the first row, 2 in the second and 3 in the third row in from the boundary) adjacent to each boundary monitoring position. This gave a figure that was matched with the data point provided in the vegetation survey data sets. Regression analyses were run to establish if there was a linear relationship between species richness of the herb layer and species richness or abundance of the invertebrates adjacent to each boundary position in June. Invertebrate data were log transformed and the analysis was run in GENSTAT 7. There was no significant relationship in either 2000 or 2002.

An RDA analysis was used to: 1) establish whether characteristics of the shrub layer in hedges were related to the composition of the invertebrate community and 2) to establish whether species richness of the herb layer was related to the composition of the invertebrate community. The RDA was run in CANOCO; invertebrate data were used as the species data (response variable) and were log transformed before analysis. The shrub and herb data were used as environmental (predictive) variables as follows: hedge width, hedge height, percentage gaps in hedge, abundance of grass in the herb layer, number of grass species in the herb layer, abundance of forbs in the herb layer, number of forb species in the herb layer and total species richness of the herb layer. Data from 2000 and 2002 was analysed separately.

The extent to which the vegetation composition of the hedge base influenced the invertebrate community composition sampled using the pitfall traps was examined by matching the similarity matrices for invertebrate and ground flora data using RELATE in PRIMER.

### 3.7.2. Results

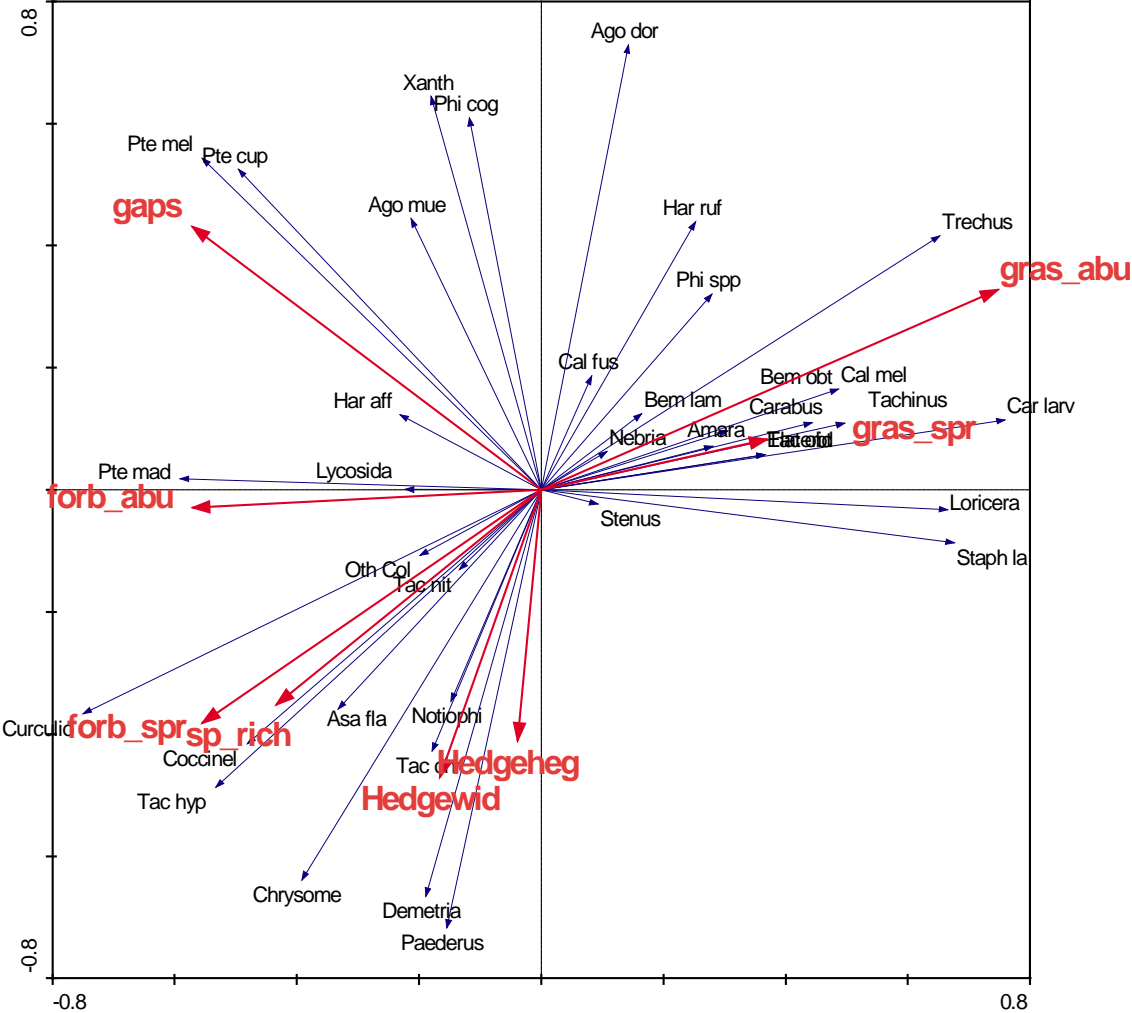
In 2000 canonical axes 1 and 2 were significant (Fig. 3.29). The results suggest that swards dominated by grass supported a different community than that supported by forbs. Grasses were associated with *Bembidion* spp. and *Carabus* spp. (carabid beetles), *Tachinus* spp. (staphylinid beetles) and both staphylinid and carabid larvae. In contrast, forb species richness was associated with ladybirds (Coccinellidae) and weevils (Curculionidae). The carabid *P. madidus* was associated with forb abundance. Species such as the carabids *P. melanarius* and *Poecillus cupreus* were associated with gaps in the hedge. In general, grass abundance was more influential than grass species richness.

General species richness was associated with the same species as forb species richness, probably because this amounts to the same thing (i.e. where there is increased species richness it is due to an increase in the numbers of forbs, not grasses). *Demetrias* spp., and *Notiophilus biguttatus* (carabid beetles) and *Paederus* spp. (staphylinid beetles) were associated with taller, thicker hedges.

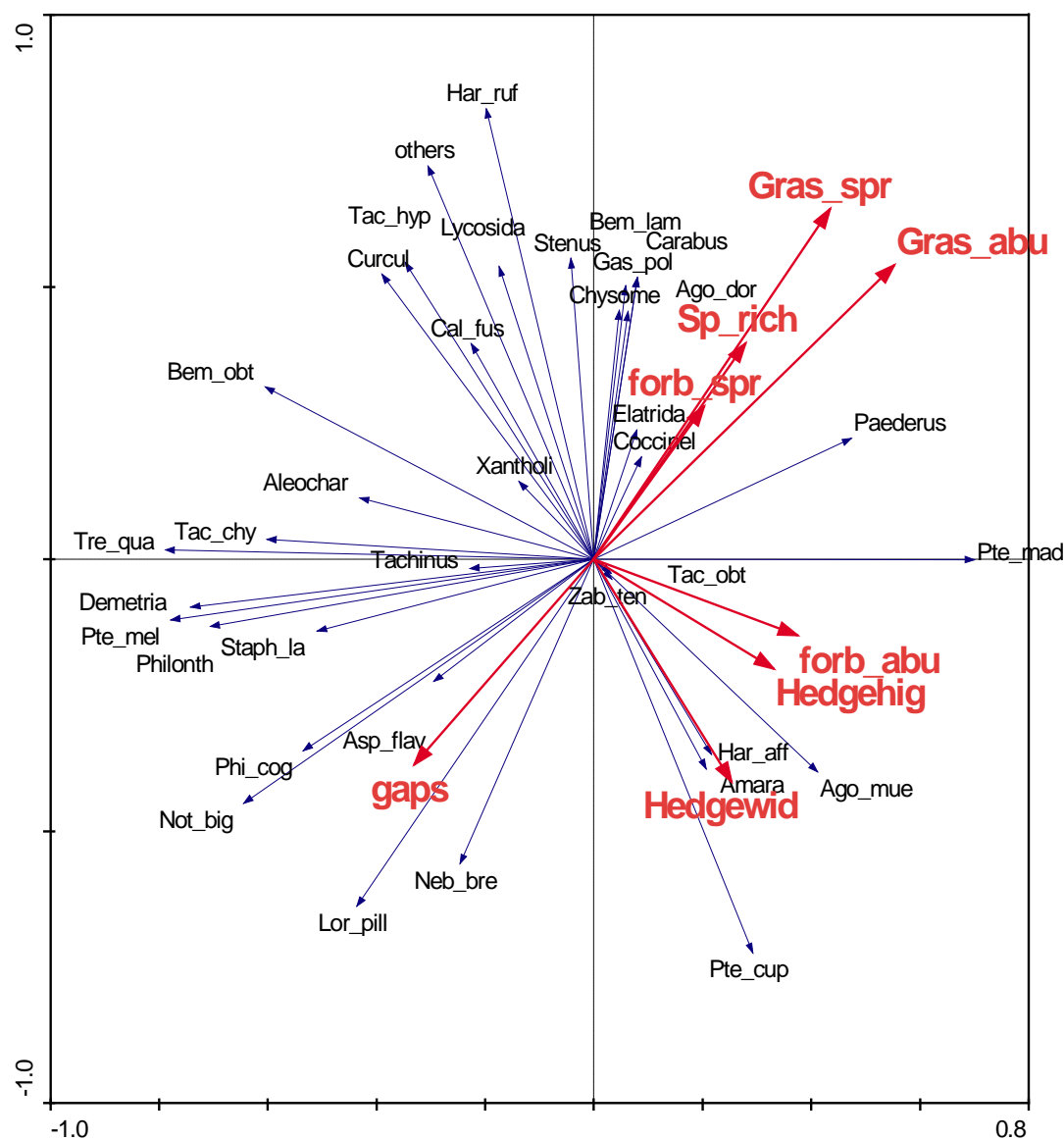
In 2002, canonical axis was significant (Fig. 3.30). However, the relationships were not quite so clear in 2002. Much of the community composition was not influenced by the environmental variables and it is likely that there was some other factor that was important in determining species distribution. There was less differentiation in the community composition according to the species richness of grass and forbs, only forb abundance was separated on the graph (Fig. 3.28). However, some of the associations hold true. *Bembidion* spp., total carabid beetles and elaterid beetles were found in grassy areas and ladybirds were associated with forb species richness.

The similarity matrices for invertebrate and herb layer data were matched using RELATE in PRIMER, but no relationship was established in either 2000 or 2002.

**Figure 3.27.** Ordination plot showing the relationship between vegetation characteristics and invertebrate communities in June 2000. Key: hedgewid = hedge width, Hedgeheg = hedge height, gaps = percentage of gaps in hedge, Gras\_abu = grass abundance, Gras\_spr = grass species richness, Forb\_spr = forb species richness, Forb\_abu = forb abundance, , Sp\_rich = herb layer species richness.



**Figure 3.28.** Ordination plot showing the relationship between vegetation characteristics and invertebrate communities in June 2002. Key: hedgewid = hedge width, Hedgehig = hedge height, gaps = percentage of gaps in hedge, Gras\_abu = grass abundance, Gras\_spr = grass species richness, Forb\_spr = forb species richness, Forb\_abu = forb abundance, , Sp\_rich = herb layer species richness.



### 3.7.3 Determining the cost of establishing flower-rich field margins

One of the outcomes of the knowledge transfer meeting held in the last year of the 3DF project was a decision to find a way to communicate to farmers the costs and benefits of introducing margins on to farms. It was decided that this would be best presented as a 'margin calculator'. The aim of the margin calculator was to provide a user-friendly system for farmers to compare income generated on their own farm both with and without margins. **A prototype of the calculator has been developed. Using the 'margin calculator' a farmer will be able compare potential income from incentive schemes with current income based upon only a few essential facts about farm income.**

Calculations, based on the current Countryside Stewardship and ESA agreements, are embedded in the spreadsheet. The user has a choice of whether to use imperial, metric or a screen that allows one to enter in imperial and have the calculations converted into metric. These calculations can be easily updated when the Entry Level and Higher Tier Scheme is in place.

Information on how margins will benefit wildlife has been added on separate sheets within the workbook. It is envisaged that the information on wildlife benefits is enlarged but kept straightforward. Links to more detailed information can be added. The aim is to refine the calculations, adding an option to put in margins tailored inline with the findings of the 3D Farming project. This is dependent on the final costings being passed on.

It is possible to put this spreadsheet on the web, however the front end is not immediately attractive. **It is recommended that a small sum is invested in employing a software designer to produce a simple clean system to present the calculator as user friendly.**

### 3.7.4 Conclusions

1. The margin structure and vegetation influenced the invertebrate species composition in the adjacent crop.
2. More associations were found for 2000 than for 2002, but there were some consistent trends. Carabids were more strongly associated with grassy margins and Coccinellidae with the forbs.

### 3.8 DISCUSSION

#### 3.8.1 Investigation of the Large-Scale, Spatio-Temporal Dynamics of Predatory Epigeal Invertebrates in Arable Farmland.

The spatial dynamics of predatory invertebrates was examined across a contiguous block of six fields through a cereal-cereal-pea rotation. **The spatial scale and extent of the trapping grid used in this study surpassed all previous investigations of this type anywhere in the world.** Previous studies have focussed on either parts of fields, whole single fields or pairs of neighbouring fields. In addition, no previous study has ever examined the spatial dynamics of predatory invertebrates between years. As a consequence it has been possible to answer some key questions regarding the spatio-temporal dynamics of predatory invertebrates and thus to understand their ecology better and be able to inform farmers and agronomists on how best to encourage the natural biocontrol provided by these generalist predators. Some of the key questions were:

1. To what extent are predatory invertebrate distributions aggregated across a contiguous block of fields?
2. How stable are predatory invertebrate aggregations within and between years?
3. What is the scale of the predatory invertebrate distribution patterns across the study area?
4. What factors (biotic and abiotic) influence predatory invertebrate distribution patterns in the field?
5. How important are different field boundary types to invertebrate abundance, distribution and diversity within the fields?

The answers to these questioned are addressed below.

The discussion is focussed towards the findings that are likely to be of most relevance to agricultural advisors, scientists and policy makers, however, there is also a wealth of key ecological information that has not been discussed. Moreover, in order to keep the text as short and accessible as possible the results have not been fully discussed with respect to the published scientific literature. This will be achieved when the findings are compiled for scientific journals.

#### **1. To what extent are predatory invertebrate distributions aggregated across a contiguous block of fields?**

In other words are patches of higher than average density isolated within fields or do they extend across field boundaries? This is important because it can affect the potential for population recovery following adverse agricultural practices, such as an insecticide application; invertebrates reinvade treated areas from adjacent unsprayed crops and habitats. In addition, this information will allow us to determine to what extent field boundaries act as sources of invertebrates or as barriers to their distributions. Species with populations that are isolated within single fields are more at risk of becoming locally extinct within that field compared to species with more widely dispersed populations. The level and size of population aggregations will also influence the extent of pest control. If the total predatory effort is relatively evenly distributed across arable fields then likewise pest control may be similarly even. However, if there is an uneven distribution, for

example, a higher predatory effort near to field margins, then management plans are needed to encourage a more even distribution.

In this study, pitfall trapping was used and therefore the invertebrates collected were predominantly ground-active, generalist predators. **Early in the year (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 started to emerge as adults and these then dominated the species composition, while also being very numerous. The boundary overwintering species remained close to the field margins (<100 m) throughout the summer in 2000,** as found previously in studies of single fields (Holland et al., 1999). Only in the smallest field did they penetrate to the field centre, where the distance to the centre was only 60 m. In 2001 and 2002 the boundary overwintering species penetrated further into the larger fields. Why this occurred in these years and fields cannot be ascertained from the data yet analysed. Set-aside strips and a beetle bank were established around these fields in the autumn of 2000 and may have contributed to the more widespread dispersal of field boundary species, although this was not apparent from the detailed investigations in which the impact of the set-aside strips on within field invertebrate abundance was examined (section 3.5.2). In 2002, cereal aphids were very abundant in the winter wheat fields L1 and L2, where the boundary overwintering species were highest and this may have encouraged a more widespread dispersal across the fields. Previous studies employing transects of pitfall traps also found that boundary overwintering species moved rapidly across fields in the spring and were fully dispersed across the fields in late May-June (Coombes & Sotherton, 1986; Jensen et al., 1989; Kromp & Nitzlander, 1995; Thomas et al., 2000), although some species remained associated with the field boundaries (Kromp & Nitzlander, 1995).

**The mid-field overwintering species were, as expected, found spread across fields.** For some species the patches 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 but they can extend across field boundaries.** Likewise, a study examining the distribution of six carabid species in parts of two adjacent fields revealed that each species restricted its distribution to certain parts of the fields (Thomas et al., 1998; Fernández García *et al.*, 2000; Thomas et al., 2001). A further study comparing invertebrate distributions in two fields of 4 and 16 ha also confirmed that many of the species present had heterogeneous distributions, although some of the more mobile species showed no evidence of spatial pattern (Holland et al., 1999). Consequently, if maximum biodiversity is to be maintained then broad-scale management treatments (eg. cropping and insecticide applications) should be avoided across groups of fields, otherwise there is a risk of reducing species diversity within individual fields. Reinvasion from untreated fields is also likely to be faster if these are in close proximity to the treated ones.

**Data from these spatial studies could be used to design sampling strategies that are more accurate at predicting the within field numbers of beneficial invertebrates while also reducing the labour inputs needed to make such measurements.** This would be of benefit when designing protocols to estimate the effects of plant protection products for registration purposes or when introducing changes in crop management such as the use of GM crops. Likewise, more efficient sampling protocols can improve the value of ecological studies. Such an approach was tried using data on the spatial distribution of cereal aphids and revealed considerable differences in the accuracy of the various sampling regimes currently in use (Alexander et al. submitted).

## **2. How stable are predatory invertebrate aggregations within and between years?**

**The species and groups analysed had a consistent distribution pattern within each year, the patches and gaps remaining in the same parts of the study area.** This is in agreement with most other studies that have examined within-year distribution (Thomas et al., 1998; Holland et al., 1999; Fernández García *et al.*, 2000; Thomas et al., 2001), although one of the species, *P. melanarius* has been shown in another study to be more mobile within the year, moving in response to the distribution of aphids (Winder et al., 2001) and slugs (Bohan et al., 2000). Redistribution of carabids from June to August was likewise found using a grid sampling approach that encompassed two pairs of fields (Brown, 2000; Thomas et al., 2002). The difference in cropping was considered to be important, with a preference being shown for a bean compared to a wheat field. **In this study the total predatory effort as indicated by their numbers was stable within years but not between years.** There were exceptions for some species, for example *P. melanarius*, which remained in the same location over the three years, and some other species and groups that persisted in broadly the same place for two years. Thus, for some species, notably one of the most common and widespread predatory carabids, a great deal of affinity is shown for particular locations. This is likely to be due to microclimatic, edaphic and hydrological factors which, if determined, may make excellent predictors of parts of farmland where care should be taken to preserve the area from damage. Such locations are likely to act as sources for seeding population spread across wider areas of a farm when optimum conditions prevail. For other species whose population distributions appear less stable between years, these findings suggest that either: a) population patches are continually being destroyed and created, giving the appearance of a dynamic redistribution; or b) that the invertebrates are moving around the landscape and re-aggregating in different areas in response to changing conditions; or c) that both these mechanisms are to some extent responsible to various degrees for different species. Consequently, **the level of bio-control 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.

## **3. What is the scale of the predatory invertebrate distribution patterns across the study area?**

**All of the species studied showed heterogeneous distribution patterns across the study area indicating that certain areas provided more attractive conditions and they were dispersing to congregate in these**



**areas or the areas favoured their population growth.** The importance of movement is considered in section 3.8.2. **The size of patch within each field and across the site varied between species.** Some species appeared to be restricted to particular areas of the study site, for example the patches of *P. melanarius* covered approximately 10 ha, whereas those of *P. madidus* extended across several fields and covered 23 ha in July 2000. This is much larger than previously found for these species (Thomas et al., 1998; Holland et al., 1999; Brown, 2000), but reflects the scale of this study compared to previous ones. For some of the smaller species the patches were also much smaller. The heterogeneous distributions patterns, especially early in the year, indicate that for invertebrates arable fields are far from uniform in their suitability for colonisation, despite our attempts to create homogeneous monocultures. 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 had much less. **The extent of coverage varied most between May and June, the extent of the patches being relatively consistent between June and July each year. The between year differences showed that the number of predators was inconsistent within a field between years.** To some extent this may have been a result of changes in cropping as discussed below. However, there is potential to manipulate the environment to reduce such variation and ensure more consistent, predictable and dependable levels of biological control.

#### **4. What factors (biotic and abiotic) are influencing predatory invertebrate distribution patterns in the field?**

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. Thus factors such as previous methods of tillage, agrochemical inputs and cropping will have influenced soil structure and the weed seedbank. The subsequent weed and crop growth will then determine cover and humidity, which will, in turn, affect which invertebrate species find the environment most suitable. Current management will likewise be influential and it is likely that this may change through the year and between years. For invertebrates, the factors that are already known to determine the suitability of a particular area include: a) presence of food, which includes pest species, b) microclimatic conditions, most importantly humidity and c) the physical structure of habitat. The ability of a species to locate and colonise suitable areas will depend on their life strategy: those with good dispersal abilities and high reproductive rates being the most able. For most predatory species found in arable fields we know little about their movement, fecundity and factors controlling this; neither do we know much about survival rates.

In this study we examined whether the crop, weed cover or soil moisture influenced the predatory invertebrate distribution patterns. The importance of these and other factors on the spatial distribution of Carabidae was reviewed by Thomas et al. (2002). The importance of abiotic and biotic factors for Carabidae was reviewed by Holland & Luff (2000) but little information exists for Staphylinidae.

### ***Crop type***

The type of crop will influence many factors that are important to beneficial invertebrates including: the type and timing of cultivations; extent and timing of vegetation cover; abundance of prey; environmental conditions; agrochemical inputs and time of harvest. It is therefore likely that particular crops will favour particular species according to their phenology, environmental requirements and diet. This will determine the species composition and dominance ratios as found previously (Holland, 2000; Hance, 2002: reviewed for Carabidae). Overall, root crops were found to have a negative impact on ground-active invertebrates whilst there was little difference between cereals and legumes. In this study the species composition varied between winter wheat and peas, probably because of marked differences in their physical characteristics, management and sowing dates. As conditions change within a crop this may also affect its suitability and encourage immigration or emigration. The invertebrate distribution patterns were relatively stable within-years suggesting that there was little movement between crop types prior to harvest, as confirmed in the mark-release-recapture studies. In contrast, the distribution of *P. melanarius* has been shown to shift between bean and wheat fields during summer (Brown, 2000; Thomas et al., 2002). The large differences in the location of patches between the years indicated that either movement was occurring or survival was different between fields.

High numbers of predators were captured in the pea fields in 2000 and 2001 indicating that this crop may have favoured the survival of some species, especially *P. madidus* which was the numerically dominant species. The comparison of community composition between the crops showed that there were only differences between winter wheat and spring-sown peas. It was often just a few species that were responsible for most of the differences, notably the large species that overwintered within the fields as larvae, e.g. *P. melanarius*, *P. madidus* and *P. cognatus*. There were also some differences between the results for June and July. In June *P. madidus* was more abundant in wheat than peas but the reverse was found in July, but this can be explained by examining the age of the captured beetles. In June the adults that had survived the winter dominated the catch, whereas by July most of the beetles caught were teneral. This may have also held true for *P. cognatus*. The pitfall traps also only provide a snapshot measurement of the invertebrate community whereas the emergence traps provided season-long activity. When they were used in these two 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 (Fadl et al. 1996; Purvis & Fadl, 1996).

Diversity declined as the crop matured and the fauna became dominated by just a few species. This is typical for arable fields and the decline of other species may occur for a range of reasons. Many of these species are active early in the year and activity naturally declines through the summer following breeding. The conditions within the crop may become unsuitable, they may be predated by, for example, the larger species

that emerge later, prey may become less abundant or they may succumb to disease and parasitism. A more diverse fauna is considered to improve biological control because the pests are controlled through a variety of mechanisms. Nevertheless, the large carabid beetles that dominated the ground fauna in July were shown to actively focus on cereal aphid patches and contribute to their demise (Winder et al., 2001). Further studies on these species using marked beetles are currently underway, funded through BBSRC.

Long-term investigations of invertebrates, in which the same fields were repeatedly sampled, showed that particular fields can consistently have high numbers of an individual species, but densities of other species were highly variable between years (Thomas et al., 2002). There was, however, always some variation between years. In this study, high numbers of predators were always captured in field L3, despite the changes in cropping. The community composition analysis also revealed that differences between the fields were always greater than between the crops. **These results and those from studies of dispersal suggest that it is changes in invertebrate reproduction, survival and mortality that are driving population change rather than mass dispersal.** Moreover, the impact of cropping and the associated management were less influential than those factors that make a particular field most suitable, e.g. soil type and moisture levels. There will always be species specific response to management inputs because each species differs in its susceptibility to change. For example, the impact of deep cultivation will depend on the species phenology and lifecycle. However, we know very little about the comparative impact of management inputs. Partly this is because many of the factors that influence invertebrate populations interact with each other, as depicted for Carabidae in Holland (2002).

In 2000 and 2001 the insecticide 'pirimicarb' was applied for pea aphid control in mid-June. Pirimicarb is considered to be a selective insecticide with a low toxicity to beneficial invertebrates. The toxicity to ground and crop-active predators has been examined in a number of laboratory (Çilgi et al., 1996), semi-field (Kennedy et al., 2001) and field trials (Vickerman et al., 1987). In the laboratory trials the full application rate of pirimicarb (as used in the peas) caused between 5-26% mortality of four carabid species, although lower mortality would be expected in the crop because only a proportion of the spray penetrates to ground level. The semi-field trials were a rigorous test of mortality because enclosures were used to prevent immigration or emigration, yet under field-like conditions. In these trials, pirimicarb had no impact on Carabidae or Staphylinidae. In full field trials, the reductions in the density of Carabidae and Staphylinidae were 27% and 26% respectively, possibly due to emigration. There were, however, differences between species; the crop-active species were more susceptible than those at ground level. The abundance of some species may have been reduced by the pirimicarb applications in 2000 and 2001, but as there was only one untreated pea field, the effect cannot be statistically tested. The total predatory effort was unlikely to be much effected because the most abundant species were the large, predominantly ground-active Carabidae and Staphylinidae, as found in the semi-field trials.

### *Crop and weed cover*

Analysis of the spatial association between vegetation cover and invertebrates captured in the pitfall traps revealed many significant findings. **Weed cover was more strongly associated with the invertebrates than total vegetation cover that included crop cover.** The proportion of ground covered by vegetation can influence humidity, shading, soil moisture and architectural complexity at ground level, and these are known to be important in habitat selection for a number of taxa (Tutin et al., 1991; Rypstra et al., 1999). Hydrophilic species will choose dense crops or areas of high weed cover whereas hydrophobic species will choose those with a more open canopy and less weed cover. It is likely that the low growing weeds would have a larger impact on humidity than the taller crop which has most of the leaves well above the ground. Indeed, the application of a herbicide reduced the numbers of carabid larvae (Powell et al., 1985) possibly because the weedy areas had a higher humidity that was attractive to gravid females. In addition, the weeds may support a variety of phytophagous invertebrates that could attract the generalist predators (Speight & Lawton, 1976) and provide seed for spermophagous and generalist species (Tooley & Brust, 2002). Generalist predators were also found to be better fed in areas of higher weed density and this may lead to higher populations through improvements in their fecundity and survival (Chiverton & Sotherton, 1991). **Whatever, the mechanism the results show that weed cover could be manipulated to encourage predatory invertebrates.** The attractiveness of weedy crops for generalist predators has been shown previously (Speight & Lawton, 1976; Purvis & Curry, 1984; Powell et al., 1985; Kromp, 1989; Pavuk et al., 1997) but no attempt was made to determine what percentage of weed cover is needed to maximise usage by invertebrates. **In this study between 10 and 14% was optimal when the total number of predators was considered, however, this could vary according to the species composition.** The patchy distribution of weeds may also be beneficial as greater habitat diversity may occur. The weedy areas create a dense humid environment in contrast to the more open, drier areas where no weeds are present. Indeed, invertebrate species richness was also found to be higher where weeds were allowed to develop (Pavuk et al., 1997).

### *Soil moisture*

The soil moisture levels within a field are governed by the soil type and drainage, along with the type and amount of vegetation and can be expected to change considerably within the year. Consequently, a wet area in winter may not necessarily persist through the summer. Previous studies examining carabid distributions within cereal fields showed that for some carabid species soil moisture was a key factor (Hengeveld, 1979). Adults may seek out particular humidity conditions, but the location of the preferred soil moisture requirements are likely to be more important to the less mobile, soil-bound larval stages as this may affect survival and development (Thomas et al., 2002). This was confirmed in this study. **The soil moisture levels in summer were less important to the distribution of active adults than their emergence densities earlier in the season. Indeed, there appeared to be a particular range of soil moisture conditions that were optimal for several carabid and one staphylinid species.** The measurements provided by the Magnascan cannot be converted to a soil moisture content so further work would be needed to identify the

optimal conditions. 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, but again would require more detailed investigations.

## **5. How important are different field boundary types to invertebrate abundance, distribution and diversity within the fields?**

For cereal aphids, natural enemy impact early in the infestation period is considered important if an outbreak is to be prevented (Ekbohm *et al.*, 1992 & see section 2 of this report), and the evidence collected here and in other studies (Chiverton, 1986; Coombes & Sotherton, 1986) indicated that the boundary overwintering species of ground-dwelling predators are more likely to provide aphid control at this time. Having suitable off-crop overwintering habitats has been identified as one key factor influencing invertebrate survival through the winter (eg. Desender, 1982; Sotherton, 1984; Andersen, 1997), the type of habitat controlling the species present and their abundance (Griffiths *et al.*, 2000) and subsequently numbers reinvading the crop in the spring (Thomas *et al.*, 2000). The margins also provide a different habitat to that of the crop and so support additional species, some of which may also move into the crop. In addition, the field edges are often weedier, providing better foraging resources. Indeed, practical ways of encouraging predatory arthropods for pest control through the provision of additional non-crop habitat have been developed. These techniques include the reintroduction of hedges, beetle banks, weedy strips and wildflower margins and various headland management schemes. In addition, practices that help prevent damage to boundaries by cultivation and misapplication of fertilisers, herbicides and insecticides should also be beneficial e.g. buffer zones and Conservation Headlands. Although, it has been demonstrated that margin habitats support high densities of beneficial insects *per se*, the benefits for pest control in the adjacent crop remain, on the whole, to be demonstrated. There is some evidence that beetle banks, a technique that improves overwintering conditions, can help reduce cereal aphid infestations (Collins *et al.*, 2002) but other overwintering habitats have not been investigated. Landscape evaluations have also revealed that invertebrate reproduction and diversity can be higher in areas with greater complexity and a larger proportion of non-crop areas (Bommarco, 1999). Landscapes with more abundant field margins and perennial crops were also associated with lower aphid establishment and population growth (Östman *et al.* (2001). **We would therefore recommend that management practices that improve, increase and protect the boundaries and allow the tussock forming grasses that provide the most suitable overwintering habitat for the survival of these ground-dwelling predators should be encouraged.**

### **3.8.2. Invertebrate Emergence Patterns within Arable Fields**

Entomological field experiments using pitfall traps are often criticised, sometimes justifiably, on the grounds that activity-density can be difficult to interpret (Thomas *et al.*, 1998). This can be true, particularly in cases where sampling is conducted in small, within-field plots, such as are often employed in pesticide trials, when

treatments under comparison may have sub-lethal effects including direct and variable effects on activity. However, in this study we have used a grid of pitfall traps to take repeated ‘snapshots’ of invertebrate activity-density at an extremely large spatial scale. Since locomotory activity in natural populations is influenced principally by temperature (Honek, 1997), pitfall trap data from our experimental design describe very well the relative variation in absolute density over a wide area. It is still not possible, however, to state from these data the absolute population density at any particular location; although the mark-recapture data does give an overall measure of population density. **Our deployment of emergence traps over two of the fields had four main functions. First, to allay the type of criticisms mentioned above, it allowed a correlate of activity-density to be taken and a direct comparison of the two sampling methods to be made. Second, it allowed an absolute measure of population density to be made over part of the farm. Third, it allowed the timing of the appearance in the field of adults of different species to be made. Fourth, from the spatial distribution of adult emergence it allowed us to infer the locations that were optimal or preferred by different species for oviposition or larval development and survival.** These latter data were also spatially correlated with environmental factors, notably soil moisture.

**Little need be said about the correlation between pitfall trap data and emergence trap data, other than it was good and highly significant on most dates.** There were insufficient data on *Nebria brevicollis* and *P. melanarius* in field L2 for SADIE analyses and in June the regressions between emergence and pitfall trap data were not significant. This is probably due to the progressive disappearance of *N. brevicollis* into aestivation sites at this time, and simply too low numbers of *P. melanarius* in an unfavoured field. In field S2, *N. brevicollis* and *Philonthus cognatus* showed no spatial patterning into significant aggregations. Generally, the emergence trap data give good justification of the use of pitfall traps for this type of study.

The absolute measures of population density are given in the results section for 15 taxa. Few data on absolute population density exist elsewhere in the literature for most of these taxa. Where data do exist they have been derived from mark-release-recapture data on the active population of the large and frequently most abundant carabid *P. melanarius*. These types of study have generally determined population density of that species to be of the order of 1 m<sup>-2</sup>, occasionally a little higher (Ericson, 1978; Hance et al., 1990; Thomas et al., 1998; section 3.5). Our emergence trap data show the accumulated population density of emerging beetles of all species to be at least 1 m<sup>-2</sup>, while *P. melanarius* and *P. madidus* emerged at densities of nearly 30 m<sup>-2</sup> in field S2. In L2, *P. madidus* emerged at over 40 m<sup>-2</sup> with *P. melanarius* at very low density. **Overall, carabids together with staphylinids emerged at population densities of 86 m<sup>-2</sup> in L2 and almost double that density at a massive 157 m<sup>-2</sup> in S2. These results highlight the important productivity of arable soils for these invertebrates.** Considering that population persistence, in its simplest terms, only requires the survival of two individuals m<sup>-2</sup> to maintain a population at that density and that the females of many of these species produce several hundred eggs, clearly **the greater biomass of these invertebrates serves not only to contribute to pest population suppression but also represents a major food resource for farmland**

**birds and small mammals, and in some cases each other.** Indeed, the Carabidae, which were the most abundant taxa emerging from the soil, were identified as one of the most important families of invertebrates in the diet of farmland birds (Wilson et al., 1999). Their contribution to ecosystem function is thus critical and these data emphasise the importance of maintaining invertebrate diversity in farmland. Of all the farming practices, intensive soil cultivations and inversion tillage are probably the most damaging to invertebrates overwintering within the soil (Fadl et al. 1996; Purvis & Fadl, 1996; Holland & Reynolds, 2003). However, alternatives to these are now available e.g. non-inversion tillage and direct drilling, and offer a means by which invertebrate survival can be improved while also providing additional environmental benefits (Holland, 2004).

Further emphasis on the importance of invertebrate diversity for ecosystem function can be seen in the data of the timing of emergence. Although these are given for only a few selected species it can be seen that the timing of peak emergence and the breadth of the emergence curve, varies among the different species. **Thus, a diversity of species helps to provide a continuous supply of predators for pest control throughout the season, and a reliable and reasonable duration of potential food items for farmland birds.**

**One of the most interesting observations revealed by the emergence trap data was the spatial association of emergence with soil moisture.** Soil with good moisture retention properties has often been considered to be a key factor in determining the distribution of epigeal invertebrates, though rarely observed (Hengeveld, 1979). Initially, our results seemed contradictory, as emergence was highest in the wettest area of S2 and in the driest area of L2. However, further analysis showed that the soils of the two fields were generally different in moisture content. Closer analysis showed that highest carabid emergence in both fields was associated with areas where soil moisture levels were in the range between -750 and -800 EMI units. Not only does this confirm our hypothesis that soil moisture is probably a key factor for oviposition and larval development but the observation offers important potential for management and manipulation of populations. **Soil moisture is a factor that can be measured, and may be done so as routine in precision agriculture. Areas of a farm important for sustaining populations of epigeal invertebrates could therefore be identified and protected.** Furthermore, soil moisture is a factor that could be manipulated by drainage and the addition of organic material such as farm yard manure. Further experimental work would be required to refine the detail of our knowledge of invertebrate moisture requirements and test the ease with which this factor could be economically manipulated by simple management procedures at the field and farm scale.

### **3.8.3 The Spatial Dynamics and Movement of Carabid Beetles between and within Arable Fields**

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 most natural unit of management since it is

generally under the control of a single farmer. It 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 populations. Previous studies by Thomas et al. (1998), Holland et al. (1999), Winder et al. (2001) and others, although limited to single or paired fields, have determined the vagility of some of the dominant *Pterostichus* carabid species with either mean daily dispersal rates or diffusion coefficients. Some of these studies and others in small experimental plots (e.g. Mauremooto et al., 1995) have also demonstrated that field boundaries (such as hedgerows) are not impenetrable barriers to movement. Taken together these studies have conclusively demonstrated the potential for some individuals in populations of these key species to disperse across areas as large as a whole farm within a single season or generation. However, none has demonstrated the actual extent of this movement at the farm-scale.

The snapshot views of species' spatial distributions across the grid, within and between years were discussed in detail in the previous section and more fully in other papers published elsewhere (reviewed by Thomas et al., 2002). The temporal stability of these distributions gives some indication of the dynamics of population distributions and has shown that there are fairly consistent differences between the two dominant *Pterostichus* species. *P. melanarius* existed as a highly stable patch in S1 and the lower half of S2. This stability was apparent both within and between years. *P. madidus* was more varied in its behaviour. In the first year (2000) its distribution appeared quite unstable with populations aggregating successively in different fields. However, between July 2001 and July 2002 the population was consistently aggregated in a large patch spread across the majority of L2 and L3 with a further, smaller, stable patch in L1. The snapshot views of population distributions also showed that *P. melanarius* existed in a much smaller sized patch than *P. madidus*.

These results suggested *P. madidus* to be a more vagile species than *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. The execution of the two large-scale mark-release-recapture experiments within the extensive trapping grid in 2000 and 2001 did, however, enable some questions concerning movement of individuals within populations to be addressed.

The first experiment was conducted over the whole 66 ha grid. Sex was noted during marking and for analysis, but no significant differences between the dispersal behaviour of males and females were found. Previous smaller scale studies at finer levels of resolution have found small differences in the activity of male and female carabids but these are probably irrelevant at the spatial and temporal scale of the present study. Males and females were therefore combined for most analyses here. The overall recapture rate of 11.4% and 7.4% for *P. melanarius* and *P. madidus* respectively were similar to other comparable studies



(Brown, 2000; Thomas & Brown, unpublished). Although the recapture rate for *P. madidus* was lower than that for *P. melanarius*, suggesting lower dispersal power, this may have been due to more *P. madidus* emigrating from the system, since other results (see below) clearly show higher dispersal power for that species. The results confirmed that *P. madidus* was the more mobile of the two *Pterostichus* species. 67% of recaptured *P. madidus* had moved to a different block from their original release area compared with only 20% of *P. melanarius*. In some fields, the populations of *P. madidus* were small at the time of marking (e.g. L1 and L2) and it is difficult to draw firm conclusions about the dispersal behaviour of this species since in L1 100% of recaptured beetles were found in other fields, while in L2 none were. Both these fields were winter cereals. In field L3, however, there appears to have been a mass emigration of *P. madidus*. Of over 40 recaptured individuals originally marked in that field only one was recaptured there. The greater majority of the others had migrated to fields S1, S2 and S3. This must have been unrelated to crop type as all these fields were planted to peas in 2000. A similar process occurred with *P. melanarius*.

Analysis of (mean) release and (actual) recapture positions showed the daily displacement distances of *P. madidus* in 2000 to be approximately twice that of *P. melanarius* with mean daily displacement distances of 12 and 5 m per day, respectively, and maxima of 43 and 29 m per day, respectively. This provides further evidence confirming the greater dispersal power of *P. madidus* compared with *P. melanarius*. Previous studies on the dispersal power of *P. melanarius* conducted at smaller spatial scales also gave values of approximately 5 m per day (Thomas et al., 1998; Brown, 2000). In the following year, 2001, there were too few *P. melanarius* for mark-recapture and the experimental work focussed on *P. madidus*. Conducted at a smaller spatial scale, the three small fields S1, S2 and S3 were divided into more and smaller blocks. As in 2000, more beetles moved between blocks than remained where they were. However, more beetles stayed within their original field than moved to another. This is also reflected in an overall lower mean daily dispersal rate for *P. madidus* in 2001 compared with 2000 at 4 m per day compared with 12 m per day. When viewed at the larger scale, *P. madidus* population aggregations were also more stable in 2001 than 2000 as noted above. These results together with data from other studies suggest that *P. madidus* is generally more mobile than *P. melanarius* but that its dispersal rates are also more variable between years. It is difficult to state why this should be so. We found no evidence of density dependent dispersal, although further analysis of our data and meteorological records may reveal new insights into the environmental cues that drive movement behaviour in these species.

Two further observations of interest were firstly, that in the fields and blocks where *P. melanarius* were most abundant, emigration was least; and vice versa – emigration was highest from fields where abundance was lowest. This indicates that, at least at the population densities found here, crowded neighbourhoods are not an incentive to disperse and corroborates the lack of density dependent dispersal observed. It further 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. The fact that the

optimum environmental parameter values for each of these are likely to differ, and the associated behaviour is likely to be exhibited at different seasons, and all of these are likely to vary among species, may go some way to explaining why some species' aggregations appear more or less static whilst others change as the optimum conditions are sought. The other observation of interest was that all the *P. melanarius*, and virtually all the *P. madidus*, that were marked in pea fields and that moved between fields, only moved to another pea field. This may indicate an important behaviour that could be used to manage population distribution and spread at the farm scale – for example by using strips of favoured vegetation as corridors linking areas where natural pest control was most required. However, these results may be an artefact as the numbers of beetles involved were small. The disposition of the crop types and field boundaries may have made all the pea fields most accessible to each other. Further manipulative studies would be required in a geometrically symmetrical layout of crop types in an otherwise uniform environment to eliminate the potential biases from the natural farm situation used in the present study.

Movement between blocks within fields appeared to occur with no pattern, indicating free mixing at that spatial scale; although there were some areas of S2 in 2001 within which *P. madidus* remained after marking. In the same year, approximately 80% of recaptures were made in the same field. Thus, **the 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.** As mentioned in the introduction, there is likely to be an optimum level of boundary permeability for survival of carabid metapopulations at the farm-scale where local populations within fields are exposed to lethal insecticide sprays at different times and frequencies throughout a season. So far, such scenarios have only been addressed in theoretical models (Sherratt & Jepson, 1993) as the field data at an appropriate spatial scale required to parameterise such models have been wanting. Our study for the first time goes some way to providing such data.

Where recaptured beetles were found to have moved between fields there was, as expected, a declining percentage of migrants with distance to the field in which they were recaptured. For *P. madidus* 74%, 21% and 7% must have crossed at least one, two or three hedgerows, respectively, to reach their destination. Similarly, although less dispersive, 78% and 22% *P. melanarius* must have moved through one or two hedgerows, respectively. Thus, migration reduces by a factor in the order of approximately two thirds with each hedgerow crossed into an adjacent field. Although derived from the most extensive field experiment yet conducted, these data still represent movement over a fairly small scale and the numbers of beetles involved are necessarily small. Nevertheless, these data provide the most extensive direct field evidence of carabid movements at the farm scale. Their value and importance will be enhanced in future modelling work. It will now be possible to build a simulation model of the farm system at Cranborne including the fields and field boundaries. Beetle movements within the system would then be parameterised with speed of movement, turning angle and frequency etc. These parameters would then be manipulated to simulate accurately the

dispersal of *P. melanarius* and *P. madidus* observed within fields in this and other studies. Boundary permeabilities (probability of a beetle passing through a hedgerow when encountered), can then be varied to simulate the between field movements observed in this study. More generalised farm-scale (and larger) metapopulation models could then be constructed using the parameter values so determined. **These models could then ultimately be used to test the outcome of various farm-scale and landscape-scale management scenarios including the effects of corridors for movement, crop types and cropping patterns, pesticide regimes, organic or low input areas, cultivations, beetle banks and other non-crop areas as refugia and agri-environment schemes.**

#### **3.8.3.1. Summary and Conclusions**

The mark-release-recapture experiments investigating carabid movements at the field- and farm-scale focussed on two *Pterostichus* species: *P. melanarius* and *P. madidus*. Despite their similarity in size, general morphology, habits and habitat, they have been shown to differ in their behaviour, with *P. madidus* being generally more dispersive than *P. melanarius*, although its higher mobility is probably not consistent between years. Both species move freely within fields yet exist in discrete aggregations, with those of *P. melanarius* being more spatially stable both within and between years. Both species are also capable of longer distance movements between adjacent fields and further involving the crossing of one or more hedgerows or other potential barriers to movement. Approximately 20% of *P. madidus* emigrate from their original 'home' field. Because *P. melanarius* appears to remain in more localised and stable population aggregations it may be more vulnerable to the detrimental effects of intensive farming. Such an instance may have been observed on a farm in Hampshire (Thomas et al., 2002) where over a large extent of farmland *P. melanarius* appears to have been replaced by *P. madidus* over a period of approximately 15 years. These differences between species mean that it is unlikely that all species can be managed together under one general management plan.

#### **3.8.4 Quantifying the Impact of Habitat Manipulation on the Abundance and Distribution of Generalist Predators and Aphids**

##### **3.8.4.1 Effect of set-aside strips on aphid abundance in 2002**

**Grain aphids on wheat were higher at 10 and 30 m from the set-aside strips compared to the field boundary indicating that set-aside strips were encouraging biological control.** The set-aside strips contained a high proportion of flowering plants at this time, including sown species such as *Phacelia tanacetifolia* and flowering weeds, and thus may have boosted numbers of hoverflies and parasitic wasps leading to higher levels of aphid predation or parasitism. There was some evidence that predatory invertebrates were encouraged by the set-aside strips, possibly through a diversification of food resources and overwinter cover. This was not apparent from the distribution maps (section 3.2.2), but a more detailed analysis may be needed to identify such differences.

#### 3.8.4.2 Effect of set-aside strips on aphids and beneficial invertebrates in 2003

**The set-aside strips had the reverse effect on cereal aphids from that in 2002, with higher numbers occurring in the transects adjacent to them.** This indicated that levels of biological control were lower in the areas adjacent to the strips. There was no evidence that this occurred from the suction and pitfall sampling, but not all predatory species are collected using these techniques. Hoverfly and lacewing larvae were not sampled, and parasitoids were not identified in the suction samples. Alternatively, the set-aside strips may have acted as a sink habitat, attracting these invertebrates from the adjacent crop. There was some evidence from the emergence traps that this was occurring for Staphylinidae because emergence at 30 m from the strips was lower compared to those traps next to the field boundaries. Further analysis of the spatial dataset is needed to determine to what extent the set-aside strips were acting as a sink habitat.

There was some change in the vegetation within the strips between 2002 and 2003 that may have accounted for the changes observed. In 2002, the floral diversity was greater, and the vegetation was overall much shorter compared to 2003. In 2003, the yellow sweet clover was more abundant and the ground cover was much denser. In addition, half of some strips had been resown with the original mixture, but because of the dry weather establishment and growth was poor. Consequently, the overall abundance of floral resources was lower in 2003 compared to the previous year.

**The per studies confirmed that aphid predation could be successfully evaluated using this approach, thus providing us with an accurate tool for the extensive evaluation of cereal aphid predation (see section 4). In this study, the average proportion of *P. melanarius* and *P. madidus* that had consumed aphids was 21%.** This was very similar to the levels found by gut dissection for *P. melanarius* but a higher proportion of *P. madidus* (34%) had consumed aphids (Holland & Thomas, 1996). A higher proportion of these beetles contained aphid remains at 10 m from the set-aside strip and as aphid numbers were also higher at this distance from the crop edge, this would suggest a density-dependent feeding response was occurring. Although these two species are generalist predators feeding on a wide range of prey, *P. melanarius* has been shown to respond spatially to aphid patches and this subsequently had an impact on the aphid's intrinsic rate of increase (Winder et al., 2001).

**In the pea fields the set-aside strips had no effect on the abundance of pea aphids.** Numbers of pea aphids were very high, reaching spray threshold levels in all fields. When aphid populations increase rapidly, biological control is often insufficient to prevent outbreaks occurring, especially if natural enemy impact doesn't occur early enough in the aphid population development, and this may explain why no difference was detected. Alternatively, the set-aside strips may not have increased predator abundance or could have acted as a sink habitat. The number of staphylinid beetles sampled by pitfall trapping was lower next to the strips. Many of the most abundant staphylinid beetle species typically found in arable crops (eg. *Tachyporus*

species) overwinter in tussocky grasses before flying into the crop in the spring, therefore it is unlikely that the set-aside strips acted as a barrier to dispersal. The density of several taxa emerging at 30 m from the strips was also lower compared to those emerging near to the field boundaries. This could occur if the adults in the previous year had chosen to oviposit in the strips rather than the crop, thus the set-aside strips could have acted as a sink habitat for oviposition.

**Fewer field overwintering invertebrates emerged in the pea compared to winter wheat fields, but only in the fields without the set-aside strips**, with the exception of *P. cognatus* which was also lower in the pea fields with set-aside strips. Differences in emergence between the crops are discussed above. The absence of any difference where the set-aside strips were present may have been because the set-aside strips increased the number of adults in the previous crop and subsequent oviposition was higher, increasing the chance of survival in the pea crop and reducing the difference compared to the wheat field.

**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 not to act as a sink or to have no affect.**

#### **3.8.4.3. Effect of weed cover on beneficial invertebrates.**

The trials to manipulate weed cover were disrupted by the dry weather. This inhibited weed emergence and as a consequence at the Woodyates site there was no difference between the fully sprayed and unsprayed plots. At the other site, the weed cover was dominated by charlock, but because this is a relatively tall species, the differences in cover and humidity at ground level may have been relatively small. Nevertheless, some differences were detected, with **higher numbers of predators occurring where there was higher weed cover**. This confirms what was found from the spatial associations, namely that weed cover effects the distribution of predatory invertebrates. The possible mechanisms behind this are discussed in section 3.8.1.

To enable us to investigate the impact of higher weed cover on aphids this study was repeated in a field of spring oats in 2004 by The Game Conservancy Trust and will be reported elsewhere.

#### **3.8.5. Spatial Distribution of Pea Aphids and their Predators**

**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 to measure the extent of an infestation. The highly ephemeral nature of their infestation, with little relationship to field boundaries, also precludes the use of patch spraying because an infestation could appear anywhere across the field.

**Ground-active predators were exerting a noticeable level of control, with fewer pea aphids occurring where they were present.** In the USA, pea aphids are known to be predated by a wide range of generalist and aphid-specific species (Frazer et al., 1981), with the foliage active species causing the release of an alarm pheromone that induces dropping behaviour (Clegg & Barlow, 1981). Once on the ground they will be predated by ground active predators such as beetles and spiders. A similar range of predators are present in the UK and likewise pea aphid control would be expected. The ground-active predators were more effective at reducing pea aphid numbers when foliage active predators, such as ladybirds were present (Losey & Denno, 1998a), thus a strategy to improve pea aphid control should aim to improve the abundance of ground- and crop-active predators. This may be achieved by ensuring that crop management causes the minimum disruption, for example, avoidance of broad-spectrum insecticides and a switch to reduced cultivations. In this study 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. This would be expected to 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 ground- and crop-active predators and parasitoids.

#### **3.8.6. The Influence of Field Margins on Invertebrates within Fields**

**In 2000 and to a lesser extent in 2002, the type of field margin influenced the ground-active invertebrate community in the adjacent crop. The grasses encouraged the beetle species that had used the margin as an overwintering habitat, along with beetle larvae. Presumably, the undisturbed nature of the margins increased larval survival. The herbaceous forbs increased numbers of ladybirds and weevils. Overall, the presence of grass was more important for the ground-dwelling predatory invertebrates and further supports the evidence that beetle banks are an appropriate way to encourage biological control.** Indeed, numbers of predators emerging from grass margins was higher compared to mature hedgerows (Griffiths et al., 2000). Moreover, the different boundary types had their own unique invertebrate fauna and therefore a subset of all field boundary types is required for complete species representation at the farm-scale. Ladybirds were associated with the forbs here because the most abundant forb within the margins was stinging nettle and this supports large numbers of aphids on which ladybird adults and larvae specialise. Two carabid species were associated with gaps in the hedge. They may have been using gaps to cross through the field boundary or the absence of a hedge and thereby shading, may have created more suitable environmental conditions within the crop.

#### **3.8.7. General Discussion**

Wratten and Thomas (1990) list five main spatially dynamic processes of relevance to developing integrated control programmes. These are:

- seasonal movements between crops and non-crop habitats;
- movement between phenologically asynchronous crops;

- colonisation of new habitats;
- recolonisation of areas previously depopulated by insecticides;
- aggregative movement to areas of high prey density.

The detection of spatially dynamic processes is a difficult feat to accomplish in the field (Thomas et al., 2002) but 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 habitats but the extent of this can vary between fields and years. The reluctance of the boundary overwintering, generalist predator species to disperse across fields has implications for the extent and reliability of their contribution to pest control within fields.** Species that appear early have a greater impact on aphid population regulation than those that appear later (Ekbohm et al., 1992). Dispersal across fields may not occur if for example the environmental conditions within the crop are unsuitable or if insufficient prey is present. The density overwintering within the surrounding margins may also be too low to ensure sufficient coverage. Large fields with a low margin:field ratio would be expected to have lower densities of boundary overwintering species and further analysis of the data will be conducted to test for this. There are ways in which the early dispersal of predatory invertebrates could be encouraged and densities increased. Crops could be manipulated to provide more favourable environmental conditions and in this study weed cover was identified as one key factor. Alternative prey can be increased through the application of organic manures (Purvis & Curry, 1984). Field margins may be improved and the margin:field ratio increased. Avoiding intensive soil cultivations may also increase the survival of field overwintering species.

Movement between phenologically asynchronous crops is only likely for the more mobile species, eg. hoverflies and parasitoids, but few studies have investigated the extent of this, partly because of the difficulties in marking and recapturing highly dispersive species. However, data from this project has provided good evidence that both hoverflies and parasitoids are capable of spreading into and across large fields very rapidly (see section 2). Many generalist predators are also capable of flight and dispersal but the extent to which this occurs has not been investigated. In this study, one carabid species, *P. madidus*, that disperses primarily by walking was shown to move between fields, although the proportion that moved between fields was relatively low and declined according to the number of boundaries that had to be crossed. Overall, the invertebrate distribution patterns were relatively stable within each year indicating that little between-field movement was occurring. Consequently, reinvasion following disruptive agricultural practices is always going to be limited and dependent on recolonisation from the margins, as has been shown to occur (Holland et al., 2000) or from unsprayed refuges within the crop including emergence from the soil.

The colonisation of new habitats and recolonisation of areas previously depopulated by insecticides was not investigated in this study. Recolonisation following insecticide application has been the subject of a number of studies (Duffield & Aebischer, 1994; Thomas et al., 1990; Thomas & Jepson, 1997; Holland et al., 2000).

The occurrence of aggregative movement by predators to areas of high cereal aphid density has been the subject of two BBSRC funded projects and further studies on other pests have been completed or are underway. In this study we demonstrated that ground-active predators were regulating pea aphids, but this was not as a consequence of aggregative movement. Instead, pea aphids were regulated where a relatively stable patch of predators occurred. **This indicates that adequate pest control may therefore be achieved by ensuring sufficient predators are present across fields rather than relying on them to respond to the ephemeral prey patches.**

This study has enabled us to further understand some of the factors influencing the spatial dynamics of generalist predators and some recommendations are made based upon the results. We have also contributed to the evidence supporting the benefits of generalist predators for pest control. In this study we showed that the predators were very abundant, originating early in the season from boundaries and later emerging from the soil, ensuring continuity in the overall predator abundance. This ability to rapidly colonise fields in the spring is one of the key abilities required if a natural enemy is to be effective (Symondson et al., 2002). There were however, gaps in their distribution within some fields, especially the larger ones, indicating that some form of enhancement is needed. Moreover, the relative stability of the patches indicated that movement was not as dynamic as shown in some previous studies of individual species (Winder et al., 2001). The persistence of patches through the season would, however, ensure continuity of control, another important attribute for natural enemies to possess (Symondson et al., 2002). Overall, the findings further support the evidence that generalist predators can contribute to pest control whether acting alone or synergistically with specialists as has been shown to occur (Losey & Denno, 1998b). There remains uncertainty regarding the numbers of invertebrates needed to ensure adequate levels of control and this will vary with both crop and target pest. This may not be achievable for some pest situations given the diversity of species and the interactions that can occur between them. However, it may be possible to identify the proportion of the landscape needed to ensure pest levels remain below damage thresholds and this will form part of a new RELU funded project, involving some of the contributors to this Link project.

The value of set-aside strips sown with a range of plants that provide floral and seed resources for pest control was evaluated. These impacted cereal aphids in the adjacent crop in one of the study years but not the other. Moreover, they had few effects on numbers of ground-active predators. Other plant mixtures, specifically designed for this purpose, as studied in section 2, were able to contribute to pest control through the encouragement of beneficial invertebrates. Such mixtures, however, can be costly to establish and only suitable for non-rotational set-aside or margins established in agri-environment schemes. Further studies are



needed if mixtures for non-rotational set-aside are to be developed. In particular, annual mixtures 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. The spatial arrangement of such habitats is also important if maximum field coverage is to be achieved. The concentration of ground-active predators around the field margins found here indicate that non-crop habitats should split the larger fields ensuring that distance to the nearest habitat is 100 m or less.

**Only the key data relevant to the end user is presented here, however, a more extensive dataset was compiled containing information on a larger number of species. Moreover, we accept that there are many other ways in which the data could be analysed and interpreted, not only for applied studies relevant to pest control and farming systems, but also to answer key ecological questions. For example, the data could be used to greatly enhance our knowledge of individual species and how they interact with farming practices, but also to explore the factors driving their tempo-spatial dynamics. Every effort will therefore be made to fully utilise this most valuable resource and ensure that the full findings from the study are published in scientific journals and the popular press.**

### **3.9. ACKNOWLEDGEMENTS**

Sincere thanks to all those involved with the field and laboratory work, especially Tom Birkett and Matt Begbie who carried out much of the identification work. Studies were organised and analysed by John Holland, Christina Reynolds, Sue Thomas, Sue Southway, Heather Oaten and Barbara Smith. Field and laboratory assistance was also provided by placement students (Sam Bishop from Reading University, Vicky Carter from Harper Adams and Catherine Holley from Cardiff University) and data was used for their student projects. The studies were designed by John Holland and George Thomas. We gratefully thank Lord Cranborne for permission to use Cranborne and Woodyates farms and much appreciated the assistance provided by the staff of Cranborne Estates and especially the farm manager Giles Blatchford.

#### **4. ASSESSMENT OF APHID PREDATION BY LINYPHIID SPIDERS AND CARABID BEETLES USING PCR TECHNIQUES**

##### **4.1. INTRODUCTION**

The objective of this part of the project was to develop and use a molecular (PCR) technique to detect the presence of aphid prey within the guts of polyphagous arthropod predators, specifically linyphiids (money) spiders and carabid beetles, collected within crops and field margins. The wider objective was to quantify aphid predation in relation to field margin management treatments that were aimed at encouraging natural predators and parasitoids for the biological control of aphids. Field margins are important habitats for polyphagous predator groups and the diversification and management of margin habitats on farms (e.g. in arable stewardship schemes) will affect these groups (Coombes & Sotherton, 1986; Holopainen, 1995). Previous HGCA research has indicated that these predator groups contribute to cereal pest control, including aphids (Holland, 1997 - HGCA Report No. 148). Because polyphagous predators, by definition, eat a range of prey, there is no guarantee that increasing their numbers through diversifying and managing field margin habitats will automatically increase predation of pests such as aphids within the crop. It was therefore important to provide evidence that predatory spiders and beetles foraging in the crop alongside field margins were feeding to a significant degree on aphids, and thereby demonstrate the additional value of margins being managed to enhance the more specific natural enemies of aphids such as parasitoids and hoverflies.

Money spiders (Linyphiidae) are numerically dominant spider species in UK agroecosystems and feed on aphids either directly or via web catches when aphids become dislodged from the plant. The small size of these spiders (less than 5mm) and the fact that they ingest partially digested food means that direct dissection of their gut to examine for aphid contents is not possible. Predation of cereal aphids by linyphiid spiders has been demonstrated in the past using both direct observation in the field and serology techniques to detect aphid remains in field collected spiders (Sunderland et al., 1986, 1987b). Although chemical methods to detect prey in the guts of their predators have been useful in the past (eg chromatography, electrophoresis, radiolabelling of prey, serology), PCR is a particularly attractive means of detection which offers new opportunities to improve the efficiency and accuracy of prey detection in field collected predators. PCR is now routine in many labs, efficient DNA extraction kits are commercially available, candidate target DNA sequences are known for many insects, oligonucleotide primers are cheap to make and reproducible to use, and real-time PCR offers the possibility of quantification.

In this project we developed, tested and applied PCR primers for detecting aphids eaten by spiders and although the test was developed primarily for spiders, it was also successfully applied to carabid beetles collected by project partners at the Game Conservancy Trust as part of their study on the effects of set aside on aphids and beneficial invertebrates (section 3.5.2).

## 4.2. MATERIALS AND METHODS

### 4.2.1. Development of a PCR Test for Detecting Aphids in Predator Guts

#### 4.2.1.1. DNA extraction

DNA was extracted using commercially-available kits: Genome Star (Hybaid) or Purgene (Gentra/Flowgen). DNA was extracted from single whole aphids and spiders, which were stored at  $-80^{\circ}\text{C}$ , using the kit protocol for extraction from a single *Drosophila melanogaster*. Carabid beetle guts that had been removed from the beetles and stored at  $-80^{\circ}\text{C}$  were extracted using the kit protocol for 100-200mg solid tissue.

#### 4.2.1.2. Primer design

Primers were designed to the aphid mitochondrial COII gene as described in Chen et al. (2000). As a mitochondrial gene, it occurs in multiple copies per cell, increasing the chance of successful amplification in predator guts. Although Chen et al. (2000) described the design of primers to North American aphid species, some of which are common to the UK, in order to ensure success with UK aphids we designed primers to amplify the common UK species by aligning their sequences as found in Genbank with the COII gene sequence. A primer pair was chosen that amplified a number of common UK species but did not amplify DNA from predators, other insects or microbial contaminants found on predator surfaces.

#### 4.2.1.3. PCR cycling conditions and electrophoresis

PCR was carried out in 25ul volumes:

PCR mix

DNA.....	150ng
SDW.....	x for $V_t=25\mu\text{l}$
10x Buffer .....	2.50 $\mu\text{l}$
MgCl <sub>2</sub> .....	1.50 $\mu\text{l}$
10mM dNTPs .....	1.00 $\mu\text{l}$
Primers .....	1.00 $\mu\text{l}$
<hr/>	
Total Volume .....	25.00 $\mu\text{l}$

Thermocycler program

Cycle 1: 1X	Denaturation.....	94 $^{\circ}\text{C}$ for 15 min
Cycle 2: 35X	Denaturation.....	94 $^{\circ}\text{C}$ for 30 sec
	Annealing.....	51 $^{\circ}\text{C}$ for 30 sec
	Extension.....	72 $^{\circ}\text{C}$ for 30 sec
Cycle 3: 1X	Extension .....	72 $^{\circ}\text{C}$ for 2 min

The thermocycler was an Applied Biosystems Genamp PCR System 9700

PCR reactions were electrophoresed in 1.5% agarose in 0.5x TBE. Gels were run at 100V for 2h. Marker used was a 1kb ladder.

#### **4.2.1.4. Spider feeding studies**

Aphids were fed to spiders (*Lepthyphantes tenuis*), which were then sampled at various times after feeding and subjected to PCR testing to determine a) if aphid DNA could be detected in the gut, and b) the length of time following ingestion after which aphid DNA was still detectable. The aphids used in the tests were the grain aphid *Sitobion avenae*, the rose-grain aphid *Metopolophium dirhodum*, the bird cherry-oat aphid *Rhopalosiphum padi* and the peach-potato aphid *Myzus persicae*. All feeding experiments were carried out in an insectary at a temperature of 20°C +/- 5°C. Spiders were collected from the field, housed individually in small Petri dishes with a plug of moist cotton-wool and left without food for five days. Aphids (approx. 50% body size of the spider) were dropped onto the sheet webs of the spiders after the five day starvation period. Digestion time was recorded from when the spider released the aphid remains after feeding. Spiders were then stored at -80°C until DNA extraction and PCR.

### **4.2.2. PCR Detection of Aphids Eaten by Linyphiid Spiders and Carabid Beetles Collected within Crops**

#### **4.2.2.1. Linyphiid spiders**

Linyphiid spiders were collected within spring barley crops at West Fenton Farm, East Lothian, during summer 2001 and 2002, within winter cereals at Unilever's Colworth Farm, Bedfordshire, during summer 2001 and within a crop of vining peas at Muirton Farm, Drem, East Lothian, during summer 2003. Spiders were collected using either a D-Vac or Vortis suction net sampling machine by sweeping along a 100m length within the field margin, and by sweeping along the crop base for 100m at 10, 30 and 100m distances into the crop. These samples were therefore taken along the four sampling transects used in the aphid parasitoid and hoverfly manipulation studies reported in Section 2 above. Spiders were sampled from each of the three field treatments set up in those studies (flower-rich margin, aphid sex pheromone deployment and untreated control) at the selected sites. Spiders were not sampled when the crop was wet because the suction net samplers do not work efficiently in wet conditions. Immediately after collection, linyphiid spiders were picked out of the sampling net using an entomological pooter or forceps and placed in Eppendorf tubes, one spider per Eppendorf, 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, consisting of several species, were then transported to the laboratory at SAC where they were transferred into a -80°C freezer and kept at that temperature until used for PCR.

#### **4.2.2.2. Carabid beetles**

The carabid beetles *Pterostichus melanarius* and *Pterostichus madidus* were collected alive, using 6 cm diam. empty pitfall traps, from wheat crops alongside margins with and without set aside strips at the Cranborne study site (see section 4.5). Traps were opened overnight and contents immediately frozen after collection.

Sampling was conducted once during the aphid population peak. Gut contents were extracted, weighed and refrozen and PCR analysis was done to determine the proportion of beetles that had consumed aphids.

### 4.3. RESULTS AND DISCUSSION

#### 4.3.1. Development of a PCR Test for Detecting Aphids in Predator Guts

Primer sequences were derived from the aphid COII gene following alignments of the same gene from collembolan, dipteran, coleopteran, hymenopteran and arachnid sequences within Genbank (Fig. 4.1). The derived primer sequences were:

Forward SWA/F: ATAGATGAAATTAAATGTCCAATT  
Reverse SWA/R: TAGTTTTATTATCTACTTCAATTAA

These primers were tested initially against DNA extracted from aphids (*S. avenae*, *M. dirhodum* and *M. persicae*) and starved linyphiid spiders (Fig. 4.2) and later against other aphids (*R. padi*, *Acyrtosiphum pisum*), carabid beetle species, a panel of other invertebrates, and bacterial and yeast isolates obtained from the surfaces of *L. tenuis* (results not shown). **No amplification was produced from non-aphid DNA.** Aphid DNA produced a band of 180bp when amplified with the primers (Fig. 4.2). This compares to products of 79 to 386bp from the primers designed by Chen et al (2000) against the COII gene; these authors carried out DNA half-life detection studies in the guts of predators using a 198bp product, which was the reason we designed primers which gave an amplicon of approximately the same size.

**Figure 4.1.** 5' and 3' sequences of the aphid cytochrome oxidase (COII) gene (bold type) which were used to derive aphid-specific primer sequences, designated SWA/F (forward primer) and SWA/R (reverse primer). These sequences are aligned with selected non-aphid species (non-bold type); bases that differ are shown in red and underlined.

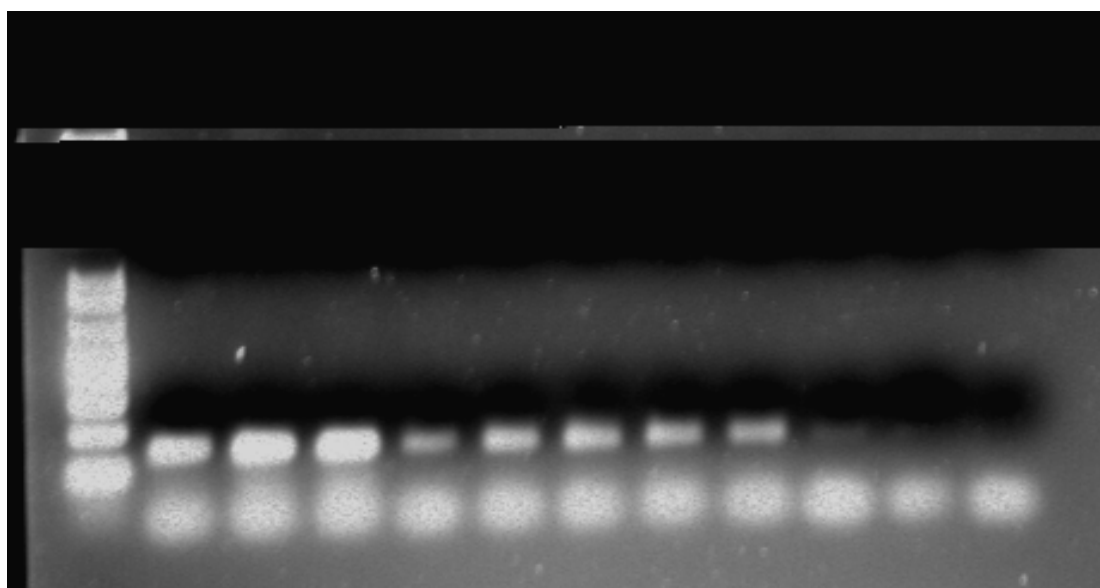
a). 5' end

	5' Forward primer 3'
SWA/F	ATAGATGAAATTAAATGTCCAATT
R.maidis	ATAGATGAAATTAAATGTCCAATT
S.graminum	ATAGATGAAATTAAATGTCCAATT
R.padi	ATAGATGAAATTAAATG <u>C</u> CCAATT
D.noxia	ATAGATGAAATTAAATGTCC <u>T</u> ATT
S.avenae	ATAGATGAAATTAAATGTCC <u>C</u> ATT
M.persicae	ATAGATGAAATTAAATGTCC <u>T</u> ATT
Culex	<u>T</u> TAGATGAAATTAA <u>T</u> <u>T</u> <u>C</u> TCCT <u>T</u> <u>T</u> <u>A</u>
Chironomus	ATAGATGAAATTAA <u>T</u> <u>G</u> <u>A</u> <u>A</u> CC <u>T</u> <u>T</u> <u>C</u> <u>T</u>
Drosophila	<u>T</u> TAGATGAAATTAA <u>T</u> <u>G</u> <u>A</u> <u>A</u> CCAT <u>C</u> <u>T</u>
Coccinella	AT <u>T</u> GATGAAATT <u>C</u> <u>G</u> <u>T</u> <u>A</u> ATCCATT <u>G</u>
Drusilla	<u>T</u> TAGATGAAATTAA <u>T</u> <u>A</u> <u>A</u> <u>C</u> CC <u>T</u> <u>T</u> <u>T</u> <u>G</u>

Heliconus	<u>TTAGAC</u> <u>CGAAC</u> <u>CTTAATAATCCTTTA</u>
Neanura	<u>TTAGATGAAGTATACACC</u> <u>CCAGCT</u>
Billobella	<u>TTAGATGAAGTTTATAACC</u> <u>CTTCC</u>
Isotomurus	<u>TTAGATGAAGTTTATAACC</u> <u>CTGCA</u>
Harbron	<u>ATAGAAGA</u> <u>GTCT</u> <u>GAGTCTTATGAT</u>
b). 3' end	
	5'                      Reverse primer                      3'
SWA/R	TAGTTTTATTATCTACTTCAATTAA
R.maidis	TAGTTTTATTATCTACTTCAATTAA
S.graminum	TAGTTTTATTATCTACTTCAATTAA
R.padi	TAGTTTTATTATCTACTTCAATTAA
D.noxia	TAGTTTTATTATCTACTTCAATTAA
S.avenae	TAGTTTTATT <u>G</u> TCTACTTCAATTAA
M.persicae	TAGTTTTATTATCTACTTCAATTAA
Culex	TA <u>ATT</u> <u>CG</u> ATTATCA <u>AAC</u> ATCTATTGA
Chironomus	<u>CA</u> ATT <u>CG</u> ATTATCA <u>AAC</u> ATCTATTAA
Drosophila	<u>CT</u> ACT <u>CGG</u> TTATCA <u>AAC</u> ATCTATTAA
Coccinella	<u>CAG</u> TT <u>CG</u> ATTATCA <u>AACT</u> CTATTAA
Drusilla	<u>CA</u> ATT <u>CG</u> ATTATCA <u>AAC</u> ATCTATTAA
Heliconus	<u>CA</u> ATT <u>CG</u> ATTATCTAC <u>ATC</u> TATTAA
Neanura	<u>AG</u> AT <u>GT</u> <u>G</u> GTT <u>GTC</u> <u>AGT</u> GTCATTA <u>AAG</u>
Billobella	TAGT <u>GCG</u> ATTATCA <u>AAC</u> ATCTATTAG
Isotomurus	<u>CT</u> GT <u>ACG</u> ATT <u>GTC</u> <u>GAC</u> GTCTAGGAG

**Figure 4.2.** PCR of the COII gene using primers SWA/F and SWA/R applied to DNA from aphids and spiders. Each lane shows the pcr product from a single individual aphid or spider. A 180bp product is produced from aphid DNA but not from spider DNA. M = marker; SA = *Sitobion avenae*; MD = *Metapolophium dirhodum*; MP = *Myzus persicae*; L = *Lepthyphantes tenuis*; C = control (water, no DNA).

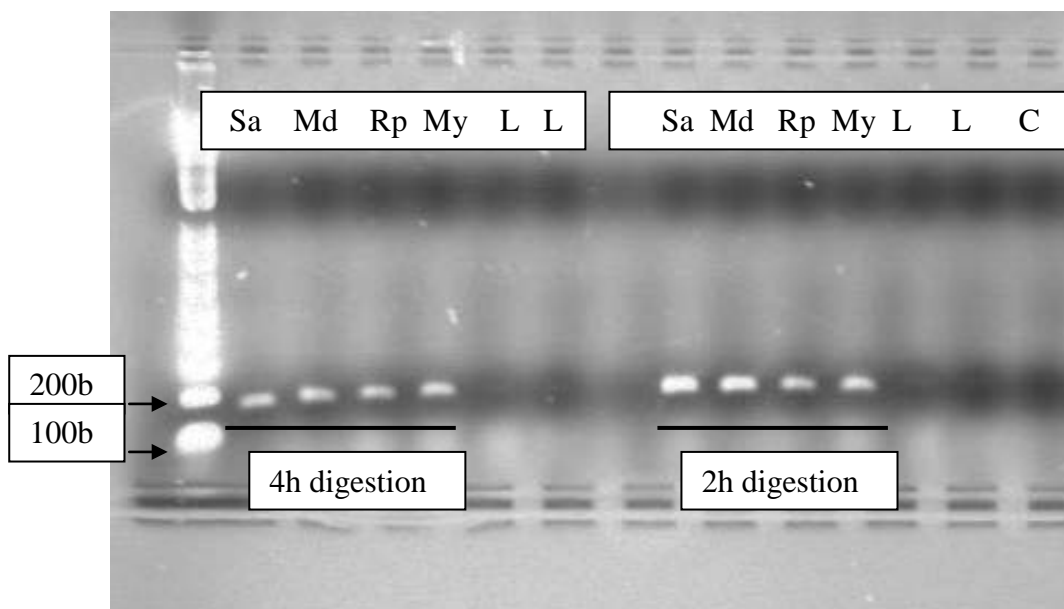
M SA1 MD1 MP1 SA2 SA3 MD2 MD3 MP2 L L C



Single aphids were fed to spiders (*L. tenuis*) and the pcr test applied to the spiders up to 8h following ingestion. **The aphid-specific band was still detectable after 8h**, but we were unable to determine the extinction point owing to the difficulty of coercing spiders to take aphids: many refused to eat in captivity. Fig. 4.3 shows the detection of aphid DNA in the guts of spiders up to 4 hours following ingestion. In the feeding studies of Chen et al (2000), the half-life of aphid DNA in lacewings and lady beetles was determined as 4h and 8.8h respectively, meaning the DNA could no longer be detected in ladybird beetles after 17.6h.

**At the present state of this technology, it is only possible to determine whether or not a predator has eaten prey; it is not possible to determine how many have been eaten, or the specific developmental stages of the prey consumed.** Although the density of the pcr band could be quantified by real-time pcr, this density is a function of the size of the prey, how many have been eaten, and the time since consumption and it is not possible to separate the effects of these different parameters.

**Figure 4.3.** Detection of aphid DNA in spiders following 2 and 4 hours digestion. Individual linyphiid spiders (*Lepthyphantes tenuis*) were fed single aphids then total DNA was extracted after 2 and 4 hours and subjected to pcr assay for aphid detection using primers SWA/F and SWA/R. Sa = spider fed *Sitobion avenae*; Md = spider fed *Metapolophium dirhodum*; Rp = spider fed *Rhopalosiphum padi*; My = spider fed *Myzus persicae*; L = unfed spider; C = no DNA



### 4.3.2. PCR Detection of Aphids Eaten by Linyphiid Spiders and Carabid Beetles Collected within Crops

#### 4.3.2.1. Linyphiid spiders

Spiders were caught and subjected to pcr assay for aphid content within spring barley at West Fenton in 2001 and 2002, winter barley at Colworth 2001 and peas at Drem 2003. Results are shown in Tables 4.1-4.3. Results are not tabulated for West Fenton 2002 because numbers of spiders caught, and aphid counts, were very low for that year, with only 27 spiders being caught during the whole of the sampling period (24/6/02 – 30/7/02), of which 4 (15%) contained aphid DNA. A gel showing detection results for field-caught spiders is shown in Fig. 4.4.

**Table 4.1.** PCR detection of aphid DNA within the guts of spiders caught in spring barley at West Fenton, E Lothian, in 2001. Total numbers of spiders caught at each location, in a 100m sweep with a suction net sampler, over the sampling period (four sampling occasions from 3/7/01 – 31/7/01). Numbers in brackets are spiders giving a positive result for aphid DNA.

Location in field	Field margin treatment			Total
	untreated	flower	pheromone	
Margin	22 (5)	27 (4)	25 (4)	74 (13)
Crop at 10m	14 (4)	14 (4)	10 (3)	38 (11)
Crop at 30m	6 (2)	8 (4)	11 (3)	25 (9)
Crop at 100m	4 (1)	9 (3)	3 (0)	16 (4)
Total	46 (12)	58 (15)	48 (10)	153 (37) = 24%

In general, more spiders were found in the field margins irrespective of crop, and irrespective of treatment (untreated, flower or pheromone). This is presumably due to the denser, undisturbed vegetation and the greater diversity of plant species found within the field margins. **The numbers of spiders caught declined with distance into the crop, but spiders were feeding on aphids with equal efficiency up to 100m into the crop, the maximum distance sampled. At Colworth and West Fenton in 2001, 26% and 24% of spiders, respectively, were positive for aphid DNA.** At West Fenton in 2002, 15% of spiders were positive, but numbers of spiders and aphids were both very low that year, perhaps due to the cool, wet weather conditions prevailing through the summer. Aphid species recorded in the cereal crops from which the spiders were collected at West Fenton and Colworth were *S. avenae*, *M. dirhodum* and *R. padi*. **In the pea crop at Drem in 2003, 88% of spiders caught had eaten aphids (exclusively the pea aphid, *Acyrtosiphum pisum*).** Pea aphid numbers increased rapidly during August, encouraged by favourable temperatures, and



local clusters of aphids (20-30 individuals in some groups) on pea shoots provided spiders with an abundant and accessible food supply.

**Table 4.2.** PCR detection of aphid DNA within the guts of spiders caught in spring cereals at Colworth, Bedfordshire, in 2001. Total numbers of spiders caught at each location, in a 100m sweep with a suction net sampler, over the sampling period (four sampling occasions from 5/6/01 – 26/6/01). Numbers in brackets are spiders giving a positive result for aphid DNA.

Location in field	Field margin treatment			Total
	untreated	flower	pheromone	
Margin	15 (2)	14 (6)	17 (3)	46 (11)
Crop at 10m	2 (2)	0	5 (2)	7 (4)
Crop at 30m	4 (1)	3 (2)	3 (0)	10 (3)
Crop at 100m	3 (0)	3 (0)	0	6 (0)
Total	24 (5)	20 (8)	25 (5)	69 (18) = 26%

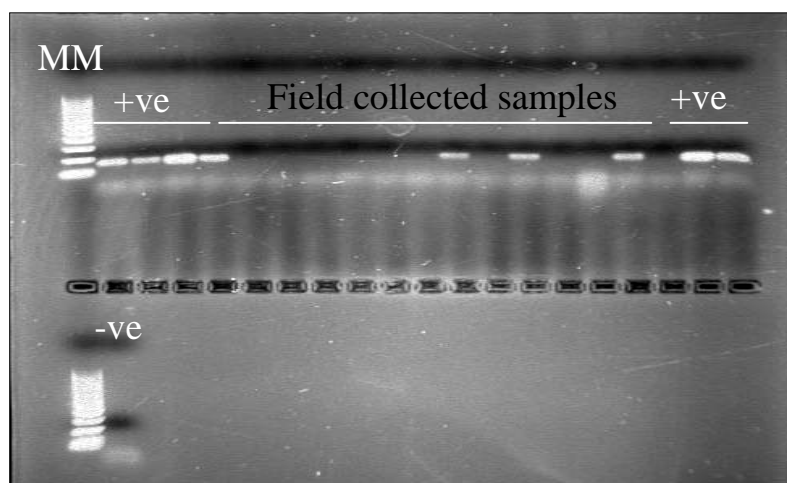
**Table 4.3.** PCR detection of aphid DNA within the guts of spiders caught in vining peas at Drem, E Lothian, in 2003. Total numbers of spiders caught at each location, in a 100m sweep with a suction net sampler, over the sampling period (four sampling occasions from 2/7/03 – 1/8/03). Numbers in brackets are spiders giving a positive result for aphid DNA.

Location in field	Field margin treatment			Total
	untreated	flower	pheromone	
Margin	30 (23)	27 (24)	8 (8)	65 (55)
Crop at 10m	3 (2)	10 (8)	10 (10)	23 (20)
Crop at 30m	3 (3)	7 (5)	3 (3)	13 (11)
Crop at 100m	5 (5)	11 (11)	4 (4)	20 (20)
Total	41 (33)	55 (48)	25 (25)	121 (106) = 88%

**These results provide evidence that Linyphiid spiders are 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 is influenced by aphid abundance, but even at low aphid**

**densities in cereal crops spiders are functioning as important aphid predators.** The much higher proportion of spiders detected feeding on aphids in the pea crop, compared with the cereal crops, is almost certainly due to the much greater aphid density in the former. Establishment of diverse field margins that provide valuable habitats for linyphiid spiders would increase the overall density of spider populations in arable ecosystems that could impact on pest populations as they develop on adjacent crops. The ability of these small spiders to disperse rapidly by ‘ballooning’ on silk threads ensures rapid colonisation of crop areas.

**Figure 4.4.** An example of pcr results from field-sampled linyphiid spiders. “+ve” and “-ve” are controls consisting of DNA extracted from a single aphid per lane (*Sitobion avenae*, four lanes on left of gel; *Metapolophium dirhodum* two lanes on right of gel) and of water respectively. MM = molecular marker. Band shown is 180bp.



#### 4.3.2.2. Carabid beetles

A total of 233 carabid beetles (*Pterostichus madidus* and *Pterostichus melanarius* combined) from the Cranborne study site were tested for the presence of aphid remains. Of these, 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 (Table 4.4). 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. These results are discussed further in section 3.5.2.

**Table 4.4.** Number and percentage of *Pterostichus madidus* and *P. melanarius* than tested positive or negative for aphids in fields with and without the set-aside strips.

		10m		30m		100m		Total	
		+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Control	No.	5	30	8	28	7	33	20	91
	%	14	86	22	78	18	82	18	72
Set-aside strip	No.	9	29	11	33	8	32	28	94
	%	24	76	25	75	20	80	23	77

#### 4.4. ACKNOWLEDGEMENTS

We gratefully acknowledge the help of Coll Hutchison, Jeannette Taylor, Irawan Tan and Sabrina Reignoux of SAC Edinburgh in conducting the PCR analyses.

## **5. HOVERFLY BEHAVIOUR STUDIES**

### **5.1. HOVERFLY FLORAL PREFERENCES**

#### **5.1.1. Introduction**

The amount of nectar and pollen available to adult hoverflies can have a significant effect on the egg load of the females (Scholz and Poehling, 2000) and thus also on the potential of hoverfly populations in agricultural fields to reduce cereal aphid numbers. When insufficient food sources are available, fewer eggs are laid near aphid colonies.

Adult hoverflies can be divided into two groups, those that are polyphagous and those that are highly specific to a small range of flowers (section 2.1.2). The former group change their feeding behaviour to ensure that the flowers that currently provide most resources are visited most often (Cowgill *et al.*, 1993). Ensuring that floral margins provide a range of host plants that offer suitable resources for aphidophagous hoverflies throughout their period of activity in agricultural fields is therefore of primary importance if the full potential of these natural enemies is to be realised in the conservation biological control system investigated in this project.

When establishing floral margins, the use of a diverse seed mixture that includes carefully selected species, is therefore a prerequisite of any attempt to manipulate populations of hoverflies for natural control. However, the relative value of candidate native perennial wild flowers to the most common species of hoverflies was poorly understood and required further investigation as part of this project. Existing published data were used to design the initial seed mixture (sown at 15 Kg/acre; 80% grass; 20% flowering plants) used in the trials at Manor Farm, which contained a range of different flowering types including umbellifers, compositae, caryophyllaceae and other taxonomic groups (Table 5.1). This diversity of flower types ensured that hoverfly species with different preferences, either based on nectar or pollen quality or determined by morphological characteristics of the flowers (e.g. exposed nectaries), were considered. In addition, it incorporated a range of species that had hitherto not been tested as a hoverfly resource but were known to be of importance for general farmland biodiversity.

To provide a wider range of plants known to be effective in providing resources for hoverfly adults and which will grow in a range of soil types within the UK, a method of screening their efficacy was developed as part of this study. This was used to test native perennial wildflowers (including some selected from the initial seed mix) that had not been previously investigated and others that were not considered initially. As *Episyrphus balteatus* is the most common aphidophagous hoverfly species in arable habitats and its larvae are known to feed on cereal aphids, this species was selected for use in the floral preference experiments and investigation of the effects on egg load.

**Table 5.1.** Plant species sown in the flowering margin at Manor Farm, North Yorks.

<b>Common Name</b>	<b>Species</b>
Betony	<i>Betonica officinalis</i>
Birdsfoot	<i>Ornithopus perpusillus</i> = Least; or <i>Lotus angustissimus</i> = Long fruited
Common meadow buttercup	<i>Ranunculus acris</i>
Cornflower	<i>Centaurea cyanus</i>
Cowslip	<i>Primula veris</i>
Crested dog's tail	<i>Cynocurus cristatus</i>
Red fescue / Creeping fescue	<i>Festuca rubra</i> subsp. <i>commutata</i>
	<i>Festuca rubra</i> subsp. <i>pruinosa</i>
	<i>Festuca rubra</i> subsp. <i>rubra</i>
Field scabious	<i>Knautia arvensis</i>
Hoary plantain	<i>Plantago media</i>
Kidney vetch	<i>Anthyllis vulneraria</i>
Knapweed	<i>Centaurea nigra</i>
Lady's bedstraw	<i>Galium verum</i>
Meadow barley	<i>Hordeum secalinum</i>
Meadow fescue	<i>Festuca pratensis</i>
Meadow foxtail	<i>Alopecurus pratensis</i>
Musk mallow	<i>Malva moschata</i>
Oxeye daisy	<i>Chrysanthemum leucanthemum</i>
Ragged robin	<i>Lychnis flos-cuculi</i>
Red campion	<i>Silene dioica</i>
Ribwort plantain	<i>Plantago lanceolata</i>
Rough hawkbit	<i>Leontodon hispidus</i>
Salad burnet	<i>Sanguisorba minor</i>
Self heal	<i>Prunella vulgaris</i>
Smooth meadow grass	<i>Poa pratensis</i>
Sorrel	<i>Rumex acetosa</i>
Timothy	<i>Phleum pratensae</i>
White campion	<i>Silene alba</i>
Wild carrot	<i>Daucus carota</i>
Yarrow	<i>Achillea millefolium</i>
Yellow oat grass	<i>Trisetum flavescens</i>
Yellow rattle	<i>Rhinanthus minor</i>

### 5.1.2. Hoverfly Flower Preference and Egg Load - Pilot Study

#### 5.1.2.1. Materials and methods

To establish a screening method, initial studies investigated the feeding preferences of female hoverflies and their resultant egg load on three species of flowering plant (*Centaurea cyanus* – cornflower; *Calendula officinalis* - pot marigold; and *Phacelia tanacetifolia*), each selected for ease of production but not intended for eventual use in margins.

A circle of twelve plants (all at the flowering stage) was arranged in flight cages (1m<sup>3</sup>) such that each was equidistant from the centre of the cage and from its neighbours. The cage consisted of a wooden frame with mesh sides that was lit from above and maintained at 22°C ±1°C throughout the experiments. A single newly emerged adult female hoverfly (*E. balteatus*) 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. The experiment was replicated 20 times, using different hoverflies (to avoid problems of flower constancy) and different plants. Three experimental arrangements were used

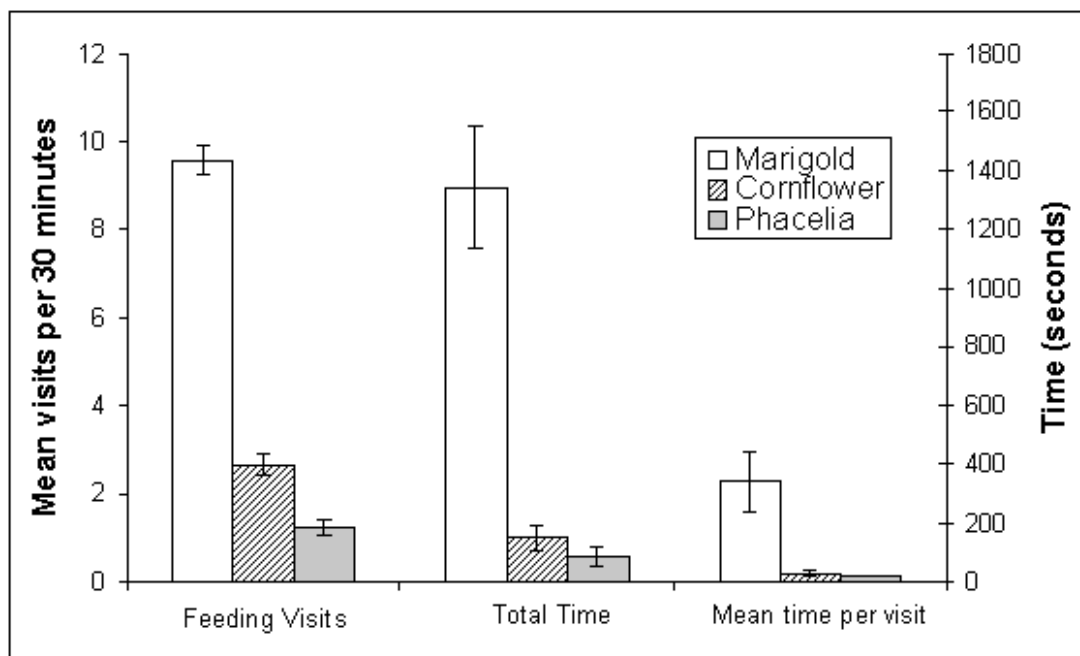
- (i) No-choice: All 12 plants within the set up were from one of the three plant species.
- (ii) Two plant species choice: Two plant species were presented simultaneously (6 plants each).
- (iii) Three plant species choice: All three plant species were presented simultaneously (4 plants each)

To investigate the oviposition rates of female hoverflies feeding on these species, flight cages were set out with a circle of six plants, each equidistant from its nearest neighbour. Cages were lit from above and maintained at 22°C with a 16:8 Light:Dark daylength regime. Four wheat plants that had been infested with a similar number of *Sitobion avenae* 7 days previously, were placed over a tray of water and detergent (to prevent escape of aphids) and positioned in the centre of the circle of plants in the flight cage to act as oviposition sites. Two, newly eclosed, 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 this period, 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.

#### **5.1.2.2. Results**

In no-choice tests, in which only one plant species was offered to adult hoverflies, there was a significant difference ( $P < 0.001$ ) between the three plant species tested in the number of feeding visits recorded in 30 minutes (Fig. 5.1). The highest feeding activity was recorded on pot marigold, followed by cornflower. *Phacelia*, a plant that has been widely cited in the literature as being particularly attractive to hoverflies (Hickman & Wratten, 1996), was the least effective in these experiments with a mean of only 0.5 feeding visits. Similar differences between plants were recorded when the mean length of individual feeding visits and total time spent feeding on each plant were compared. In each case strong preferences ( $P < 0.001$ ) for pot marigold compared to either cornflower or *Phacelia* were recorded.

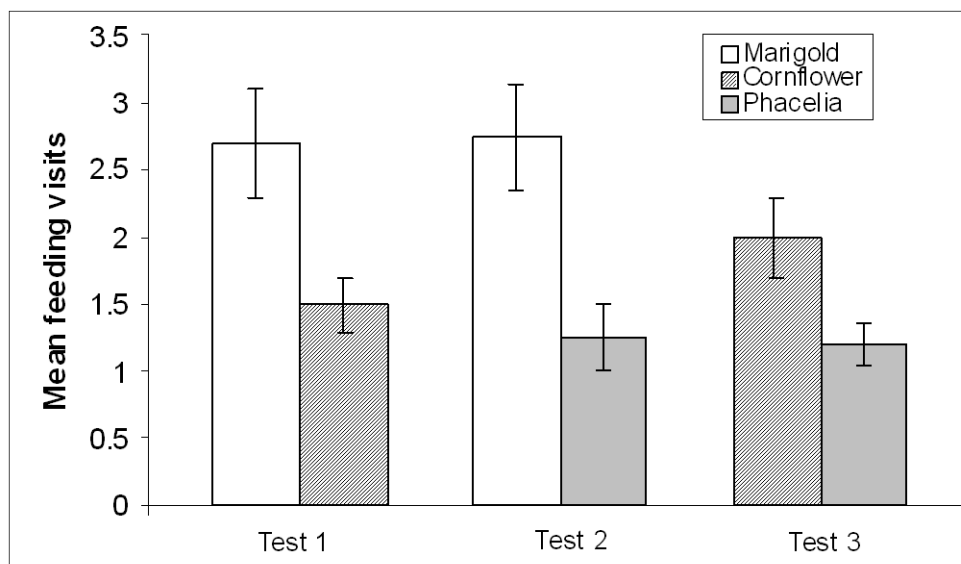
**Figure 5.1.** Mean ( $\pm$  standard error) number of feeding visits in 30 minutes, the total time spent feeding in 30 minutes and the mean length of each feeding visit, when host plants were offered in no-choice tests to adult hoverflies (*Episyrphus balteatus*).



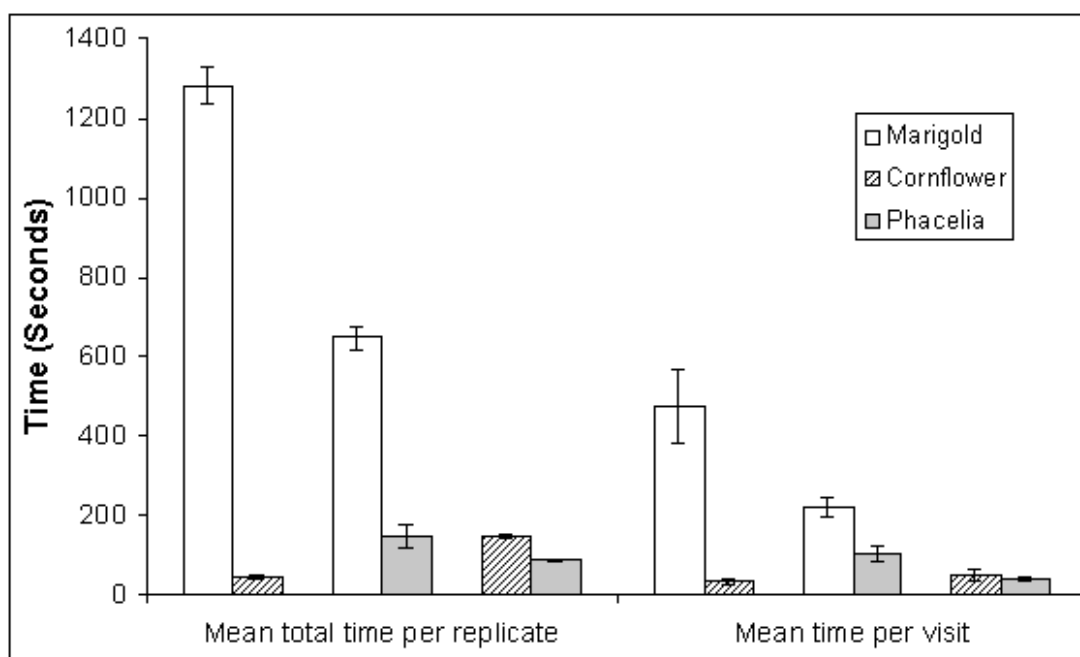
When the hoverflies were provided with a choice of host plants, a preference pattern consistent with the no-choice results was recorded. Pot marigold was visited more frequently than both cornflower ( $P < 0.01$ ) and *Phacelia* ( $P < 0.001$ ) and cornflower was visited more frequently than *Phacelia* ( $P < 0.05$ ; Fig 5.2). Analysis of data describing mean length of feeding visits and total time spent feeding confirmed the preference for pot marigold in comparison with both cornflower and *Phacelia* (Fig 5.3). However, the data from the two-plant choice experiment should be interpreted with care as they were conducted late in the flowering cycle of the cornflower plants, which consequently displayed reduced pollen and nectar production. As a result, the differences between *Phacelia* and cornflower that were recorded in both of the other experiments were not so clearly apparent in this trial series, whereas those between cornflower and marigold were magnified.

When all three host plants were offered simultaneously, the hoverfly preference pattern was again evident. Both mean number of visits to each plant and the time spent visiting the plant was significantly ( $P < 0.001$ ) greater for pot marigold than for either cornflower or *Phacelia*, and a greater preference was shown for cornflower than *Phacelia* ( $P < 0.05$ ) (Fig 5.4).

**Figure 5.2.** Mean ( $\pm$  standard error) number of feeding visits in 30 minutes, when host plants were offered to adult hoverflies (*Episyrphus balteatus*) in two plant species choice tests. Test 1 = Pot marigold offered together with cornflower; Test 2 = Pot marigold offered with *Phacelia*; test 3 = Cornflower offered with *Phacelia*.

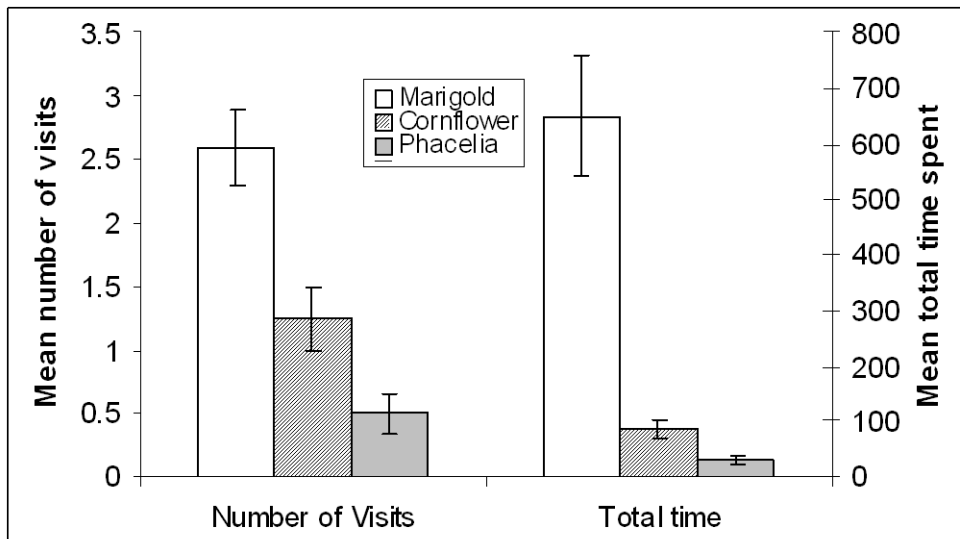


**Figure 5.3.** The total time spent feeding in 30 minutes and the mean length of each feeding visit when host plants were offered to adult hoverflies (*Episyrphus balteatus*) in two plant species choice tests. Bars =  $\pm$  1 standard error.



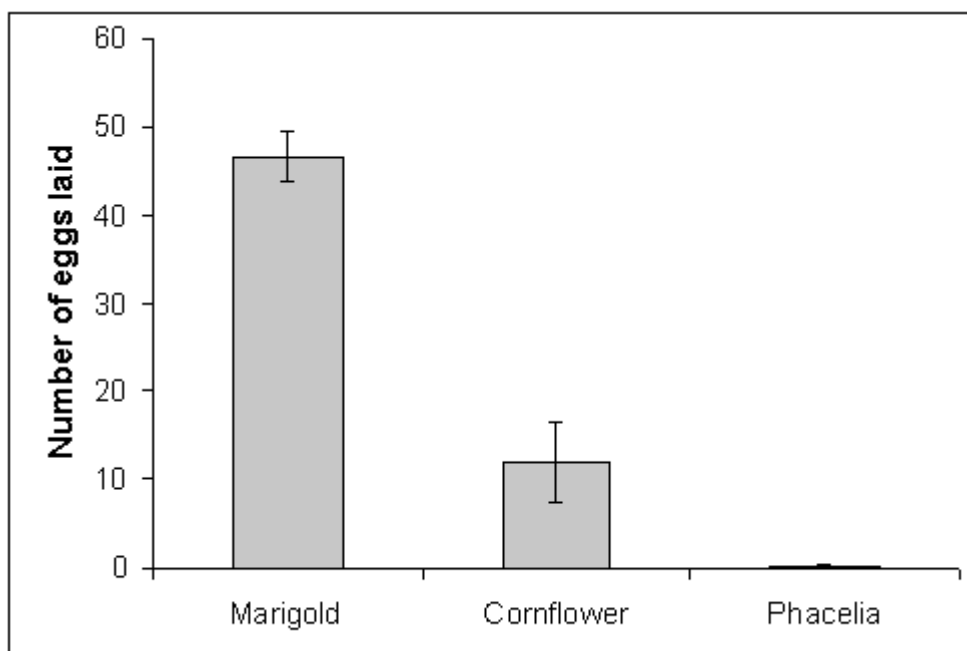


**Figure 5.4.** Mean ( $\pm$  standard error) number of feeding visits in 30 minutes, and the total time spent feeding in 30 minutes, when host plants were offered to adult hoverflies (*Episyrphus balteatus*) in three plant species choice tests.



The number of eggs laid by adult hoverflies on cereal seedlings infested with *S. avenae* after both 12 ( $P < 0.001$ ) and 14 ( $P < 0.001$ ) days varied with the nectar and pollen source available. Hoverflies offered pot marigold laid significantly ( $P < 0.001$ ) more eggs than those offered cornflower, which in turn laid more eggs ( $P < 0.01$ ) than those offered *Phacelia* (Fig. 5.5).

**Figure 5.5.** Mean ( $\pm$  standard error) number of eggs laid after 14 days when adult hoverflies were offered pollen and nectar from pot marigold, cornflower and phacelia flowers.



### 5.1.3. Hoverfly Flower Preference

#### 5.1.3.1. Materials and methods

Modifications of the no-choice and two-plant species choice tests above were used to screen UK native perennial wildflower species.

As before, a circle of twelve plants (all at the flowering stage) was arranged in flight cages (1m<sup>3</sup>) such that each was equidistant from the centre of the cage and from its neighbours. The cage consisted of a wooden frame with mesh sides that was lit from above and maintained at 22°C ±1°C throughout the experiments. A single newly eclosed adult female hoverfly (*E. balteatus*) 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 recorded. The experiment was replicated 20 times, using different hoverflies (to avoid problems of flower constancy) and different plants.

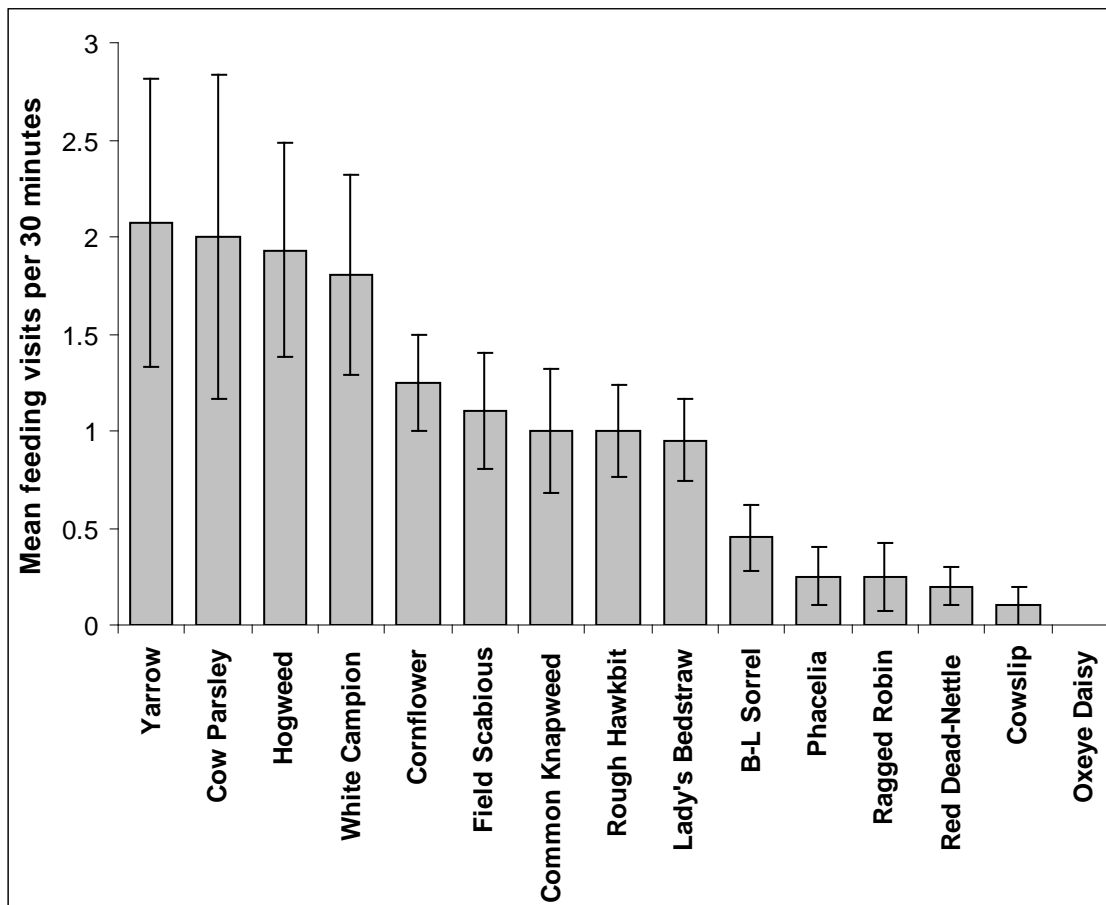
Each plant species screened was subjected to two tests. In the first, plants were offered in a no-choice experiment in which 12 plants of the same species were offered in a screen cage. In the second, 6 plants of the test species were offered in conjunction with 6 plants of a standard. *Phacelia tanacetifolia* was used as the standard in all experiments, as it is widely cited in the scientific literature and trade press as a useful nectary plant for the attraction of hoverflies. No-choice tests of the standard were also conducted.

The revised protocol enabled the rapid screening of a range of candidate wild plant species within the financial and time resources available.

#### 5.1.3.2. Results

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 (Fig. 5.6). Three groups of plants were identified. The most frequent plants on which hoverflies fed were species with umbelliferous or umbel-like flowers (yarrow (*Achillea millefolium*), cow parsley (*Anthriscus sylvestris*) and hogweed (*Heracleum sphondylium*)) and white campion (*Silene alba*). The second grouping consisted of three members of the daisy family with similar flower structures (cornflower, (*Centaurea cyanus*) common knapweed (*Centaurea nigra*) and rough hawkbit (*Leontodon hispidus*)), as well as field scabious (*Knautia arvensis*) and lady's bedstraw (*Galium verum*). Hoverflies fed on the third group, which included *Phacelia tanacetifolia*, ragged robin (*Lychnis flos-cuculi*), red dead-nettle (*Lamium purpureum*), cowslip (*Primula veris*) and ox-eye daisy (*Chrysanthemum leucanthemum*), least often.

**Figure 5.6.** Mean number of feeding visits by hoverflies (*Episyrphus balteatus*) to flowers of different plant species during a 30 minute exposure period in no-choice experiments.

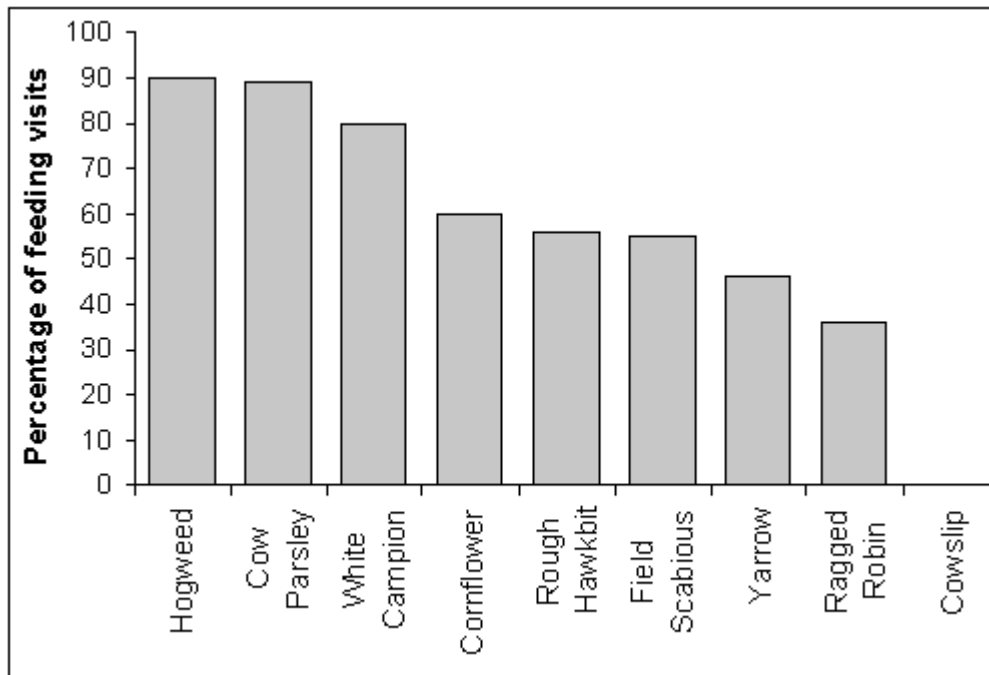


The two plant choice tests largely confirmed the preferences identified by no-choice tests (Fig 5.7). When offered a choice of *Phacelia* (the standard) or one of the plant species from the first group identified by no-choice experiments, more than 80% of feeding visits made were to the test species. For example, when *Phacelia* and hogweed were offered simultaneously, 90% of feeding visits were to hogweed, compared with 89% to cow parsley and 80% to white campion in equivalent tests. Another first group plant from no-choice experiments (yarrow) appeared to be slightly less attractive to hoverflies than predicted by no-choice tests, with only 45% of feeding visits compared to 55% on *Phacelia*.

The second group identified by no-choice tests, were also found to be slightly less attractive than white campion, cow parsley and hogweed, but still preferred by hoverflies. For example, in comparative tests with *Phacelia*, 60% of feeding visits were made to cornflower, and 58% to each of rough hawkbit and field scabious (Fig 5.7).

Those plant species identified by no-choice tests as being relatively unattractive to hoverflies were also confirmed by the two plant choice tests. For example, ragged robin received only 35% of the feeding visits when compared with *Phacelia*, and cowslip received none.

**Figure 5.7.** Percentage of the total number of hoverfly (*Episyrphus balteatus*) feeding visits observed that were made to selected test plants when offered in two plant choice tests with the standard, *Phacelia*.



## **5.2. PLANT STRUCTURAL CUES FOR HOVERFLY OVIPOSITION**

### **5.2.1. Introduction**

Aphidophagous hoverfly (Syrphidae) species have been classified into two categories, those that oviposit in response to aphid presence/density ('aphidozetic' species) and those that oviposit in response to plant species irrespective of aphid presence; ('phytozetic' species) (Chandler, 1968). Aphidozetic species such as the marmalade hoverfly *Episyrphus balteatus* are able to search out and oviposit close to small isolated aphid infestations and are therefore considered most significant in terms of biological control (Chambers, 1991). Searching involves a sequence of stages, during which aphid mediated cues such as honeydew concentration, volatile chemicals and aphid presence appear to be important in determining whether a plant has sufficient aphids to stimulate oviposition (Bargen *et al.*, 1998; Scholz & Poehling, 2000; Budenberg & Powell, 1992). Initially, focused hovering occurs during which the adult inspects the plant, looking for aphids or signs of aphid infestation (Dixon, 1959). The adult then lands and may either rest, or walk on the plant surface probing with the labella (mouthparts) while searching for aphids. The ovipositor will then be extended and dragged, or used to probe the plant surface. Following selection of a suitable site, the eggs are laid. Soon after eggs hatch, the larvae begin to seek out aphid prey. At emergence they are unable to travel large distances (about 1 metre; Chandler, 1968), and therefore understanding the stimuli that are significant in determining where adults oviposit is important if hoverflies are to be manipulated effectively as components of integrated pest management (IPM) systems.

Previous research investigating oviposition behaviour in the field (Smith 1969, 1976; Pollard 1971) has shown that plant species is a significant factor in the selection of a suitable egg laying site. However, cues leading to the selection of an appropriate plant or small patch of plants for the initial focused hovering inspection have not been investigated in detail. It has been proposed that female hoverflies may utilise olfactory cues, from aphids or plant semiochemicals (Bargen *et al.*, 1998). However, in this case aphid derived volatiles appear to work only over short distances and it has been suggested that cues detectable over longer distances, such as plant structure, may also be important (Scholz & Poehling, 2000). The effect of plant structure (e.g. size and shape) has received little attention and little information on this factor is available.

This component of the study investigated whether plant size/structure was influential in hoverfly searching, to determine if cereal plants at the growth stages present in the field during the period in which hoverflies seek egg laying sites will result in significant focussed hovering and subsequently egg laying.

### **5.2.2. Materials and Methods**

#### **5.2.2.1. Experimental insects**

A stock culture of *Sitobion avenae* (Fabricus) was maintained on 1-3 week old barley seedlings in cages (60 x 60 x 60cm) maintained in a controlled environment (CE) room at a 16:8 L:D daylength regime and 20±1°C using the method of Huggett *et al.* (1999).

A stock culture of *E. balteatus* was maintained in purpose built flight cages (60 x 60 x 120cm). Barley plants infested with *S. avenae* were provided as oviposition sites for the adult hoverflies. Eggs were allowed to hatch and larvae to feed and develop within the flight cages, but pupae were removed and placed on dampened filter paper in rearing cages before adult emergence. Sources of pollen were provided for adults, to facilitate egg development. Cultures were maintained in a CE room at 22±1°C, and with a 16:8 L:D daylength regime.

For experiments, standard aged cohorts of gravid female adults were reared from pupae taken from the stock culture, in 1m<sup>3</sup> cages in a CE room at 22±1°C, and with a 16:8 L:D daylength regime. Emerging adults were provided with tree pollen, sugar cubes and water (offered on cotton wool). Each adult was used only once in experiments (to avoid opportunities for rapid associative learning as described for Lepidoptera by Rausher (1978)) and was transferred directly from the rearing cage to the experimental arenas.

#### **5.2.2.2. Hoverfly searching behaviour**

Experiments were conducted in a 1m<sup>3</sup> screen cage, in a CE room at 22±1°C and with a 16:8 L:D daylength regime. Experimental plants were arranged in a triangle, equidistant from each other and from a central take off platform.

Individual female *E. balteatus* were placed in a Petri dish, which was positioned on the central take off platform and left undisturbed for 1h prior to the experiment. After this period, each fly was released by removing the lid, 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 focussed hovering, resting, walking, labella probing, or ovipositor probing, and the number of eggs that were laid, was noted separately for each plant (Table 5.2).

The plants used were grown in 9cm diameter pots in John Innes No 1 potting compost and fell into three categories. 'Large infested' plants were at the booting stage and were infested with 0.6g (± 0.04g) of *S. avenae*. 'Small infested' plants comprised 10 cereal seedlings with only one leaf between 6 cm and 7 cm long infested with 0.2g (± 0.04g) of *S. avenae*. These infestations provided an aphid density equivalent to 8-10 aphids per leaf. All plants were infested the evening prior to use in experiments. 'Large un-infested' plants were also at the booting stage but were not infested with aphids.

**Table 5.2.** Classes of hoverfly behaviour recorded during hoverfly searching behaviour experiments

Behaviour	Description
Focused hovering	Hovering behaviour associated with particular plant/pot
Resting	Resting on a plant surface
Walking search	Walking across the plant surface - may involve occasional labella probing.
Labella probing	Proboscis protruded repeatedly tasting the plant surface whilst remaining stationary
Ovipositor Probing	The extended ovipositor used to probe the plant surface
Number of eggs laid	Eggs laid on the plants (in each pot) at the end of the 30 minute observation.

All possible combinations of plant categories were presented to the female hoverflies (Table 5.3). (A) In three-choice cage designs one plant of each of the three categories was offered simultaneously to female *E. balteatus*; (B) In two choice cage designs two categories were offered, in a ratio of 2:1; (C) In single treatment cage designs three pots of a single category were offered within the cage. In all experiments the positions of the pots within the cage were randomised. There were 20 replicates of each experiment.

**Table 5.3.** Cage designs used in hoverfly searching behaviour experiments

Cage design	Treatments			N°. replicates
	Large uninfested	Small infested	Large infested	
(A) 3 - Choice	x 1	x 1	x 1	20
(B) 2 - Choice	x 2	x 1	-	20
	x 2	-	x 1	20
	x 1	x 2	-	20
	x 1	-	x 2	20
	-	x 2	x 1	20
	-	x 1	x 2	20
(C) Single treatment	x 3	-	-	20
	-	x 3	-	20
	-	-	x 3	20

### 5.2.2.3. Hoverfly oviposition behaviour

Oviposition experiments were conducted in 1 m<sup>3</sup> screen cages, in a CE room at 22±1°C, and with a 16:8 L:D daylength regime. Single female *E. balteatus* were released into cages using the method described above and allowed 30 hours for egg laying. Plants were offered using the single treatment and three choice cage designs described above. Pollen, sugar cubes, and water soaked cotton wool were provided throughout the experiment. The total number of eggs laid on each plant was recorded. There were fifteen replicates of each experiment.

### 5.2.2.4. Statistical analysis

Kruskal-Wallis tests were used to compare pooled data between treatments describing the total time engaged in each behaviour category or the number of eggs laid on each plant category.

## 5.2.3. Results

### 5.2.3.1. Hoverfly searching behaviour

Adult female hoverflies spent significantly more time engaged in focused hovering in front of large infested and large uninfested plants than small infested plants (Table 5.4). No significant difference was recorded between time spent engaged in focused hovering in front of large infested and large un-infested plants (Table 5.4).

**Table 5.4.** Hoverfly searching behaviour directed at three plant size/aphid combinations (Plants at the booting growth stage infested with aphids (LI); Plants at the booting growth stage without aphids (LUI) and seedling plants infested with aphids (SI)). Significance values are based on a Kruskal-Wallis test performed on data across all cage designs separated by plant and behaviour category: df =1 and n=200.

Behaviour	Total seconds			Significance P		
	LUI	SI	LI	LUI Vs SI	LUI Vs LI	LI Vs SI
Focused hovering	2668	875	3377	<0.001	0.085	<0.001
Resting	9109	6075	52903	0.984	<0.001	<0.001
Walking Search	787	687	12049	0.106	<0.001	<0.001
Labella probing	145	339	3747	0.586	<0.001	<0.001
Ovipositor probing	86	1626	7722	0.002	<0.001	<0.001
No. eggs laid	0	54	261	<0.001	<0.001	<0.001



Adults spent significantly more time engaged in walking search, resting on a plant surface, and labella probing on large infested plants compared with both small infested and large un-infested plants (Table 5.4).

Significantly more time was spent engaged in ovipositor probing on both large and small infested plants compared to large un-infested plants (Table 5.4). Significantly more time was spent engaged in ovipositor probing on large infested plants compared to small infested plants and on small infested plants compared to the large uninfested plants. Significantly more eggs were laid on large than on small infested plants, and on both infested treatments compared with un-infested plants.

### 5.2.3.2. Hoverfly oviposition behaviour

When all three plant size/aphid combinations were offered to adult *E. balteatus* simultaneously, no eggs were laid on large un-infested plants during any of the replicates. Significantly more eggs were laid on large than on small infested plants, and on large and small infested plants than on large un-infested plants (Table 5.5).

**Table 5.5.** The number of eggs laid on three plant size/aphid combinations (Plants at the booting growth stage infested with aphids (LI); Plants at the booting growth stage without aphids (LUI) and seedling plants infested with aphids (SI)). Figures = mean number of eggs per plant or pot of seedlings. Significance values are based on a Kruskal-Wallis test performed on pooled data across all cage designs.

Cage design	Mean total eggs/treatment			Significance P		
	LUI	SI	LI	LUI Vs SI	LUI Vs LI	LI Vs SI
Three choice	0	129	617	<0.001	<0.001	0.001
Single treatment	0	296	328	<0.001	<0.001	0.404

When the plant size/aphid combinations were offered to adult *E. balteatus* individually, the mean total egg number per replicate was significantly greater on both large infested and small infested plants when compared with large un-infested plants. No eggs were laid on large un-infested plants during the 15 replicates. No significant difference was observed between mean number of eggs laid per replicate on small infested and large infested plants (Table 5.5).

### 5.3. DISCUSSION

#### 5.3.1. Hoverfly Flower Preference and Egg Load – Pilot Study

**The pilot study showed that the attractiveness of flowering plants to hoverflies is positively associated with the number of eggs that females subsequently develop and lay,** supporting the hypothesis that female hoverflies select plant species that currently offer high quality food resources, which will result in increased egg load. *Phacelia tanacetifolia*, a plant that has been widely cited in the literature as being particularly attractive to hoverflies (Hickman & Wratten, 1996), was the least effective in these experiments with a mean of fewer than 5 eggs per female. Cornflower, a once common arable wildflower, provided a better resource, indicating that other **UK indigenous wild plants, which unlike *Phacelia* can be considered for inclusion in field margins, may be equally or more effective at promoting hoverfly predatory impact.**

Hoverfly preferences remained consistent both when plant species were presented individually and when a choice of species was offered, supporting earlier studies (Cowgill *et al.*, 1993), and offering the potential for developing a cost effective laboratory technique for establishing the relative effect of different perennial wildflower species on hoverfly predatory impact. This method was then used to identify important UK perennial wildflowers, as a basis for recommendations for improved species composition of seed mixes used to establish field margins that enable simultaneous promotion of biodiversity and enhancement of naturally occurring pest control agents.

#### 5.3.2. Hoverfly Flower Preference

Adult *Episyrphus balteatus* feed on nectar and pollen from a range of flowering plant species. Previous studies (e.g. Cowgill *et al.*, 1993, Gilbert, 1981) have shown that polyphagous hoverflies are selective in their use of available resources, developing transient flower constancies to ensure that as spring/summer progresses a sequence of plants that offer hoverfly populations the highest quality nectar and pollen are exploited. **The current study indicated that when hoverflies were offered a choice of flowers from different plant species, those selected for feeding most frequently were from plants that were subsequently associated with the development of the highest egg load in females.** These eggs give rise to the aphidophagous stages of the hoverfly, and 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 the approach to conservation bio-control developed in this study.

*Phacelia tanacetifolia* is often cited in the literature as being highly attractive to hoverflies and a source of high quality pollen and nectar (MacLeod, 1999). This species, however, is not native in the UK and therefore cannot be considered for use in field margins and conservation headlands, but few native species have been tested for suitability as pollen and nectar sources. In the current study, candidate perennial flowering plants native to the UK were compared with *Phacelia* to identify suitable species for inclusion in florally enriched

field margins established in agri-environment schemes and as a component of a conservation biocontrol approach to the control of aphids in arable crops. The objective was to determine if alternative species could be found that offered as high quality resources as *Phacelia*, which has been shown in field trials to encourage hoverfly populations and consequently to contribute to the depression of aphid populations in winter wheat (Hickman and Wratten, 1996).

**A range of UK native plant species were shown to be equally or more attractive to hoverflies when compared to *Phacelia*. In particular, a range of umbellifer species, 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 (P. A. S. Mason *pers comm.*). Field observations have also confirmed that hoverfly species other than *E. balteatus* are also attracted by these flower species (e.g. *Metasyrphus corollae*). A second group of plants were also found to show high potential as components of flower-rich margins for hoverflies, including cornflower, field scabious, common knapweed, rough hawkbit and lady's bedstraw. With one exception (yarrow), a close correlation was obtained between the relative attractiveness of the different plant species, when assessed using no-choice experiments and by comparisons with *Phacelia*. No-choice experiments indicated that yarrow was more attractive to hoverflies than was suggested by experiments comparing yarrow with *Phacelia*. However, no-choice experiments were conducted at the mid-flowering period, whereas resource constraints resulted in choice experiments being conducted towards the end of the plants flowering period, and thus the lower level of attraction recorded may be the result of reduced nectar and pollen availability at this plant growth stage.**

These results support and extend the findings of Cowgill *et al.* (1993), in which two of the four species they found to be consistently preferred by *E. balteatus*, during 8 weekly observational sessions in the field, were umbelliferous (fool's parsley and wild carrot), the third was white campion and the fourth was autumn hawkbit (a species from the same genus, *Leontodon*, as rough hawkbit). One apparent difference from the findings of the earlier study and the current work was that yarrow, a preferred species in no-choice tests described above, was only a preferential flower in one week of the four week flowering period. This may indicate that yarrow has a very short period in which it offers high quality nutrition to hoverflies. Colley & Luna (2000) have also reported that umbelliferous flowers (including coriander, fennel), as well as yarrow, were highly attractive to hoverflies, although *E. balteatus* was not one of the species investigated.

**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 (Keble Martin, 1974). 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 (see section 5.2), 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.

### 5.3.3. Plant Structural Cues for Hoverfly Oviposition

The characteristic sequence of behaviour displayed by adult female hoverflies searching for aphids or oviposition sites progresses from focused hovering, to walking and labella probing searches on the plant, and culminates in ovipositor probing and egg laying. This study has shown that plant size/structure is an important stimulus, in addition to aphid infestation, for initiation of focused hovering. However, an increasing importance of aphid mediated cues can be traced through the behaviour sequence. Small infested plants stimulate less focused hovering attention than large plants irrespective of aphid infestation, suggesting that size/structure acts as a primary cue in the early stages of searching.

In herbivorous insects, behaviour sequences leading to host selection are not fully prescriptive. Insects will omit steps in the sequence if the relevant environmental cues are not present and proceed with behaviours characteristic of subsequent steps (Kennedy & Fosbrooke, 1973; Kennedy, 1974). In the current study, when only small infested plants were offered to adult females, and hence the large plant structure which usually stimulates focused hovering was missing, random encounter of aphid mediated cues on small plants resulted in a greater incidence of labella and ovipositor probing and egg laying than was recorded on large uninfested plants. Thus females did not reject small infested plants when they were encountered. Where a choice was available the hoverflies responded to the large infested plants and laid more eggs than on the small infested plants suggesting a clear preference for the larger plants. However, when no choice was available, as many eggs were laid on the small infested plants as on the large infested plants. This suggests that **although the preference is for large infested plants when available, the presence of aphids is enough to ensure oviposition on the smaller plants.**

The principal larval mortality factor for aphidophagous hoverflies is starvation, and insufficient food in the larval stage results in decreased fecundity (Cornelius & Barlow, 1980). The adaptive advantage to responding to large plants may be associated with plants large enough to sustain high aphid population growth. Studies of aphid population growth and plant growth stage interaction, indicates that larger cereal plants can sustain maximum aphid population growth rates (Watt, 1979) and are selected preferentially by aphids, which settle less readily on cereals at early growth stages (Walters & Dixon, 1982). Also, large plants

have more complex structures and thus present more refuges from predators and parasitoids for hoverfly larvae. It has been suggested that predation of hoverfly larvae is low (Chambers, 1988), but recent research looking at intraguild predation between aphidophagous predators, found that *E. balteatus* eggs and first and second instar larvae are highly vulnerable to larger aphidophagous predators such as ladybirds and lacewing larvae (Hindayana *et al.* 2001).

Recent work into the effect of egg load (Sadeghi & Gilbert, 2000a), presence of conspecific eggs (Scholtz & Poehling, 2000), female hoverfly age (e.g. Chandler, 1968) and aphid species preference (Sadeghi & Gilbert 1999, 2000b), suggest that all of these factors are also important in oviposition site selection by *E. balteatus*.

In the UK, the large growth stages of the autumn/winter sown cereals coincide with the arrival of *E. balteatus* in the crop. 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 some control of aphid populations as part of a natural predator complex. However, it is possible that late spring sown cereals may be at a disadvantage, particularly if there are larger, aphid infested plants in the area.** 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.

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## **6. OVERALL CONCLUSIONS AND KEY MESSAGES**

Natural populations of beneficial invertebrates are capable of controlling aphid pests on arable field crops, especially cereals, but this natural biological control can break down, particularly as a result of annual variability in climatic conditions. This project has demonstrated that this natural control can be boosted and therefore made more resilient by management of the agricultural environment and manipulation of key components of the natural enemy fauna. Two essential factors are 1) the maintenance of natural enemy diversity, including parasitoids, specialist predators such as hoverflies and generalist, ground-dwelling predators such as carabid beetles and spiders and 2) the enhancement of early season activity, particularly of parasitoids and carabid beetles. Data has clearly shown that field margins, including those established within agri-environment schemes, can play a valuable role in promoting these two factors. Also, commercially-produced aphid sex pheromones deployed in cereal crops to coincide with summer aphid invasions can significantly enhance the impact of parasitoids at this critical time for control. Native wild flower species that provide essential food resources for hoverflies have also been identified for incorporation into field margin seed mixes and/or conservation in other non-crop habitats around the farm. Important within-crop factors that can help to conserve and boost ground-dwelling insect predators, such as optimum levels of weed cover, have also been identified.

Not surprisingly, there is no single field margin vegetation type that will benefit all components of the natural enemy community, including aphid-pathogenic fungi, which have been shown in a companion Sustainable Arable Link project to benefit from appropriate field margin design and management. It is therefore beneficial to establish and maintain a variety of field margins, including set-aside strips within the farming landscape. It is also proposed that composite margins, comprising a strip of uncut vegetation containing tussocky grasses next to the field boundary bordered by a more botanically diverse strip incorporating key wild flower species and cut annually in late summer, would provide the greatest benefits.

Natural biological control of aphids on other field crops, particularly peas, brassicas and salad crops is more challenging but data collected during this project has highlighted potential approaches that could prove profitable but require further research and development. The success of our approaches in the cereal cropping system offers encouragement to pursue the development of conservation biological control and natural enemy manipulation in other field crops.

The following key messages have arisen from the extensive work done in the 3D Farming project:

- 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 the most appropriate 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.



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## APPENDIX 1: COMMUNICATION AND TECHNOLOGY TRANSFER

### Scientific Publications

- Holland J.M., Begbie M., Birkett T., Reynolds C.J.M. & Thomas C.F.G. (2001) The influence of hedgerows on coleopteran distributions: results from a multi-field sampling study. In: *Hedgerows of the world: their ecological functions in different landscapes*, Eds. C. Barr & S. Petit. Proceedings of the 2001 Annual IALE (UK) Conference. IALE (UK), pp. 167-176.
- Holland J.M., Begbie M., Birkett T., Reynolds C.J.M. & Thomas C.F.G. (2002) The distribution of carabid beetles across the arable landscape: results from a 70ha sampling study. In: *How to protect or what we know about carabid beetles*. Eds. Szyszko, J. et al. Warsaw Agricultural University Press, Warsaw, pp. 117-131.
- Holland J.M., Birkett T., Begbie M., Southway, S. & Thomas C.F.G. (2003) The spatial dynamics of predatory arthropods and the importance of crop and adjacent margin habitats. In: *Landscape management for Functional Biodiversity*, Eds W.A.H. Rossing, H-M. Poehling & G. Burgio. *IOBC/wprs Bulletin*, 26 (4), 65-70.
- Holland J.M., Begbie M., Birkett T., Southway, S., Thomas, S.R., Alexander, C.J. & Thomas C.F.G. 2004. The spatial dynamics and movement of *Pterostichus melanarius* and *P. madidus* (Carabidae) between and within arable fields in the UK. *International Journal of Ecology and Environmental Sciences* (In Press)
- Powell, W. (2000) The use of field margins in the manipulation of parasitoids for aphid control in arable crops. *Proceedings The BCPC Conference -Pests and Diseases 2000*, 2, 579-584.
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- Powell, W. & Pickett, J.A. (2003) Manipulation of parasitoids for aphid pest management: progress and prospects. *Pest Management Science*, 59, 149-155.
- Powell, W., Walters, K., A'Hara, S., Ashby, J., Stevenson, H. & Northing, P. (2003) Using field margin diversification in agri-environment schemes to enhance aphid natural enemies. In: *Landscape Management for Functional Biodiversity*, Eds. W.A.H. Rossing, H-M. Poehling & G. Burgio. *IOBC/wprs Bulletin*, 26(4), 123-128.
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- Walters, K.F.A., Young, J.E.B., Kromp, B. & Cox, P.D. (2003) Management of oilseed rape pests. In: *Biological control of pests of oilseed rape*, Ed. D.V. Alford. Blackwell Science, pp. 43-71.

## **Other Publications**

- Holland, J.M. 2000. 3D Farming- making biodiversity work for the farmer. Farmland Research section. The Game Conservancy Trust Review of 2000. pp.75-76. The Game Conservancy Trust, Fordingbridge.
- Holland, J.M. 2001. 3D Farming- making biodiversity work for the farmer. Farmland Research section. The Game Conservancy Trust Review of 2000. pp.77-79. The Game Conservancy Trust, Fordingbridge.
- Holland, J.M. 2002. Making biodiversity work for the farmer (3D Farming). Farmland Research section. The Game Conservancy Trust Review of 2000. pp.83-84. The Game Conservancy Trust, Fordingbridge.
- Powell, W. (2001) Increasing beneficial insects in field margins for aphid control. *ARIA Newsletter*, August 2001.
- Powell, W., Pell, J. & Williams, I. (2004) Pest control for free. Rothamsted Research Association Newsletter, Issue 14, June 2004.

## **Articles about the Project**

- ‘Make use of margins for natural aphid control.’ *Farmers Weekly*, May 2001.
- ‘Marginal action.’ *HGCA Research in Action, Crops*, April 2002.
- ‘Marginal success against aphids.’ *HDC News*, October 2002.
- ‘Biodiversity to boost pest control.’ *Agriculture and the Environment, Defra R&D Newsletter*, June 2003.

## **Presentations at Scientific Meetings**

2000

- Platform presentation at the BCPC Pests & Diseases Conference in Brighton, UK.

2001

- Platform presentation at the Annual International Association of Landscape Ecologists (UK) Conference in Birmingham, UK.
- Platform presentation at the 10<sup>th</sup> European Carabidologist meeting in Poland.
- Poster presentation at the 10<sup>th</sup> European Carabidologist meeting in Poland.
- Platform presentation at an International Biological Control Symposium in Brazil.
- Platform presentation at an International Pest Control Conference in Cuba.

2002

- Platform presentation at the European Congress of Entomology in Greece

2003

- Platform presentation at EWRS Working Group - Weeds and Biodiversity, Discussion meeting on “Weeds in the food chain” in Bristol, UK
- Two platform presentations at the 1<sup>st</sup> meeting of the IOBC/WPRS Study group “Landscape Management for Functional Biodiversity” in Italy.

2004

- British Ecological Society, Agroecology group meeting on “The Spatial Distribution of Invertebrates in Agroecosystems” in Fordingbridge, UK.
- Platform presentation at the XXII International Congress of Entomology in Australia.

### **Presentations at Farming Industry/Environmental Events**

2000

- Poster presentation at the Cereals Event.

2001

- Presentation to Chadacre Trust trustees and Defra staff during open day at Allerton Educational Trust, Loddington.

2002

- Poster presentation at Unilever Sustainability Workshop.
- Field demonstration to farmers and advisors at HGCA/LEAF farm day in Cambridgeshire.
- Poster presentation at the Cereals Event.
- Poster presentation and demonstration at Great Yorkshire Show.
- Poster presentation at the HGCA R&D Workshop.
- Talk given to the Vegetable Agonomists Association at PGRO.

2003

- Talk at Unilever Workshop for European field site managers.
- Talk at ADAS Workshop for Lincolnshire Vegetable Growers.
- Field demonstrations to farmers and advisors at HGCA/LEAF farm days in Suffolk and Lincolnshire.
- Site demonstration of project to farmers and advisors at Unilever Colworth.
- Poster presentation at the Cereals Event.
- Poster presentation at the Environment Research Funders Forum at CSL
- Talk to Lincolnshire Brassica Growers.
- Talk and poster presentation at HGCA Roadshow, Oadby Lodge Farm, Leicestershire.
- Poster presentation on the ‘Grain Trail’ at the Royal Show.
- Project presented at Sustainable Arable LINK workshop at Rothamsted Research
- Talk at joint Rothamsted Research Association/HGCA Workshop in Leicestershire.

2004

- Talk given at HDC Workshop for brassica and salad growers at HRI Wellesbourne.
- Poster presentation at PGRO member’s day.
- Poster presentation at the Cereals Event.
- Poster presentation and demonstration at CSL Science Day



In January 2003, prior to the start of the final year of the project, a valuable project workshop was held at Unilever Research Colworth to discuss communication strategies for the project outputs. This was facilitated by Pete Stephenson of 'The Falling Apples Centres Ltd.' This helped to identify key messages, target audiences and routes for dissemination of information. A meeting was also held with Adrian Bell of 'The Mistral Group Ltd.' and Chrissie Davies of Unilever to discuss technology transfer, following which Adrian Bell gave a presentation to a project management group meeting and helped with the design and production of display material for incorporation in the Grain Trail display at the Royal Show.

## **APPENDIX 2. MINUTES OF A MEETING TO ARRIVE AT A CONSENSUS ON SEED MIXES FOR AGRICULTURAL MARGINS**

In recent years, a number of other research projects have looked at the potential value of field margins on arable farmland for a variety of different benefits. Consequently, this is in danger of generating a range of contrasting recommendations for seed mixes to be used when establishing margins, creating confusion for the farmer. Obviously, no single margin design can fulfil all environmental, biodiversity and pest management objectives but it was necessary to collate available information and consider the most appropriate options. Therefore, on May 6<sup>th</sup> 2004 a meeting was held at the Game Conservancy Trust in Fordingbridge, Hampshire to discuss seed mix options and arrive at a consensus view. The 3D Farming project was represented by John Holland. The following is a copy of the minutes of that meeting.

### **PREAMBLE**

Over the last 20 years there has been a great deal of research into developing seed mixes for agricultural margins with the aim of increasing biodiversity and controlling pests. The work done over this period has informed current farm-scale, countrywide experiments including 3D Farming, SAFFIE, Entomopathogenic fungi and BUZZ. These projects are differentiated by the detail of their objectives but address aspects of a common question; frequently both researchers and funding bodies are part of two or more projects. It would be valuable if those involved could arrive at a consensus, together with those who have worked on such projects in the past and those who are developing policy right now. The concern is that these projects will each arrive at a 'best' seed mix but that the knowledge gained remains fragmented and that those seeking guidance from the results of these projects will receive conflicting messages.

It is worth bearing in mind that at Cereals 2003, farmers were asking three questions of those presenting the 3D Farming and SAFFIE projects:

1. What seed mix shall I use?
2. Where can I buy it?
3. What will it do for me?

The aim of the meeting was not to formulate material which would suggest that expert advice is no longer necessary, but to discuss the extent to which a consensus on the critical components of seed mixes can be reached and how much flexibility there is in their design.

There were also concerns from HGCA, which funds several of these projects. HGCA would like to ensure that the guidelines being produced by HGCA funded projects (3D Farming, SAFFIE, Entomopathogenic

fungi) and projects funded by other organisations are pulled together so as to be practically applicable. They are also concerned that field margin guidelines are used to influence, and are not at odds to, policy, for example the Entry Level Scheme.

## **MEETING STRUCTURE**

The following questions were circulated for discussion at the meeting:

### **What are the main objectives of field margin mixes?**

What BAP, HAP and conservation targets are relevant?

Should we consider start points, end points and succession?

How can we balance prescription with diversity?

### **How many mixes are needed to achieve these objectives?**

Can we arrive at a set of very basic seed mixes with key species for each objective?

### **What should be in these mixes?**

Which species are performing well?

Which are failing to establish and why?

How important are species in these two categories (good and poor performers) in terms of the objective of the mixes?

Are there any species which are critical to all mixes?

### **Are there any cost-benefit issues?**

Does the cost of any of the mixes, or their essential management, outweigh the benefit in terms of meeting the objectives of the field margin? (are they worth it?)

### **HGCA Concerns**

Can the guidelines being produced by HGCA funded projects (3D Farming, SAFFIE, entomopathogenic fungi) and projects funded by other organisations be pulled together so as to be practically applicable?

### **Policy and Regulation**

What are the current requirements of AE schemes and how do they sit with any guidelines?

Is it possible to ensure that field margin guidelines are used to influence, and are not at odds to, policy, for example the Entry Level Scheme?

## **OUTCOME FROM MEETING**

Attendees

Chair: Jon Marshall (JM)

Organiser: Barbara Smith - GCT (BS)

Alex Ramsey - CAER (AR)

Andrew Sherrat - DEFRA (AS)

Clive Edwards - HGCA (CE)

David Sheppard - EN (DS)

David Smallshire - DEFRA (DSm)

Donald MacIntyre - Emorsgate (DM)

Duncan Westbury - CAER (DW)

John Holland - GCT (JH)

Judith Pell - Rothamsted Research (JP)

Marek Nowakowski - FE (MN)  
 Paresh Shah - Rothamsted Research (PS)  
 Peter Street - DEFRA (PSt)  
 Peter Thompson - GCT (PT)  
 Richard Brown - Emorsgate (RB)  
 Richard Pywell - CEH (RP)  
 Richard Snow - MOD (RS)  
 Shona Campbell - HGCA (SC)  
 Trevor West - Marshall Agro-ecology (TW)

Apologies from: Keith Walters, Mike Edwards, Paul Toynton, Simon Potts.

Types of margin were identified as :

Uncropped wildlife strip (natural regen)
Conservation headlands
Game cover
Wild bird seed
Pollen & nectar mixes
2m grass margin
6m grass margin
Tussocky grass strip
20m set-aside (sown)
Beetle Banks
20m set-aside (natural regen)
Flower-rich margin strips
Differential mowing on grass margins
Scrub margins
Riparian strips + willow strips
Hedge base flora, inc. re-creation
Poplar planted margins

### **Opportunities for maximising the value of margins**

- Unsprayed margins can benefit species such as cornflower.
- A hedge-based margin can be harvested to increase margin value.
- The cropped area can also be useful, incorporating annual weeds into the crop.
- Restoration of existing, but derelict, margins by over-sowing.
- Diversity can be increased by introducing more than one margin type.
- Manipulating the margins of pasture may increase seed food and invertebrates for birds.
- Flower margins could be useful around silage fields.
- Reducing nitrogen leads to a more flowery sward.

### **The objectives for the uncropped margin**

Rare arable plants.  
 Pollen and nectar sources.  
 Game cover and food.

Weediness can be seen as 'good' thing. It is possible to sow unsprayed margins and increase the weediness of the field in a managed fashion (e.g cornflower, corn marigold). However increased biodiversity may reduce yield and species such as cornflower may be difficult to eradicate. There is resistance by farmers to a weedy crop as they prefer to keep production and annual plants separate. Experience shows that even using a low seed rate, 8 years on annual plants are well established.

Arable weeds may be threatened by hedge bottom damage, if the hedge bottom is reinstated then arable weeds will recover. A 1m margin is appropriate for rare arable weeds, as they survive in and tend to 'hug' the edges; this may be because this is the only part if the field missed by the spray.

It is possible to sow arable species in a wild strip but winter crops are frequently sown and access to cultivate the wild strip is a problem.

Structure is important. For species such as grey partridge, cover is essential. For game species the strips are designed to provide winter seed for adult birds and in the summer, insects for chick food.

Diversity of approach is important.

#### **The objectives for stewardship and flower mixes.**

- Grass and flower margins
- Buffer zone – no inputs
- Soil conservation
- Bio-control
- Landscape connectivity

Pollen and nectar strips should be sown separately from the crop but managed as a crop. Inclusion of these species has huge environmental delivery. The strips should be managed for accessibility for the birds. The margins also serve as buffers - a no input zone – which benefits soil conservation. This will interest farmers who might be thinking of buffering watercourses.

The system may be steering farmers in the wrong direction because it is tempting to choose cheap and easy. Heterogeneity is important for biodiversity. From the farmers perspective, margins must be managed for each objective on a field by field basis. A farmer can have as many margins types as he can manage but good advice will be essential. One problem associated with the Entry level scheme is the lack of advice, although DEFRA feel confident about advice at the Higher Tier. European legislation is aimed at achieving: Full crop establishment, sustainable seed production, patches of bare ground with undesirable species controlled.

There is a general fear of fungal passage. A margin that isn't perceived as a fungal vector would be successful.

There is a trade-off between quality, quantity and dispersion, all of which are important.

### **Other types of margin**

- Poplar planted margins
- 20m buffer strips, unmanaged for 20 years in stewardship.

### **Succession**

Although beetle banks were planted with cocksfoot and yorkshire fog, over 15 years they became similar to the margin. Much depends on location, soil type and management. These are the most influential factors. Management is critical in the first two years; in different years there are different drivers of the community. The sown community also keeps developing over time; Terry Wells experimental plots are bringing up 'sown' species 20 years after sowing.

Nutrient management is very important. Many margins have high nutrients due to run-off. Difficult to reduce it.

It is not possible to re-create semi-natural communities. These are 'new' or gardened communities and should be treated as such. Management must be tailored to these communities.

Some endangered species are hedge bound, hedge garlic and red campion are examples of species that are reliable and will improve hedge bottoms. The improvement of degraded hedge bottoms is likely to be variable, depending on location.

### **General principles**

- The location of a margin may influence its success.
- Aspect will influence colonising species.
- Even distribution of margins over a farm is important.
- Strategic placement is beneficial (near rare species for example)
- An internal margin may maximise refuges for bio-control

Farmers do tend to choose less profitable places to locate margins (e.g. watercourses, north facing slopes, shaded areas). Sometimes this may be useful as nutrient poor land supports diverse wild flowers and beneficial insect-pathogenic fungi like damper/shaded areas. Advisors are needed and DEFRA will be introducing guidelines in the future.

There are some benefits of an edge margin for within field treatments but these are difficult to maximise. An internal 'margin' may be an answer. Yield tends to be poor at the centre of a field and so it is possible to have a centre flower patch, buffered by grasses.

### **Selecting plants species**

- Rare annual species can be included but rare perennials should be avoided.
- Rare species should be restricted to special projects as they are localised.

### **Provenance**

It is hard to generalise sufficiently to create localised mixes. Generally, using seeds of local provenance is not a priority in agricultural margins, especially as agricultural varieties are often used. These are a good pollen source and don't tend to persist. Broad regional seed resources are currently being developed.

Both grass and clover have been widely traded so provenance may be irrelevant. The native range of plants is being changed.

- A native mix should only be used within the native range.

An alternative is to use green hay which can be very high quality although the quantity is likely to be limited. Best practice should be implemented in buffer zones around SSSI etc. Other agricultural areas are less important.

### **Performance of plants**

- Generalists do well
- Predictive traits for good performers: colonisation ability, vegetative growth and seed bank persistence.
- Stress tolerators perform poorly
- Grass/flowers = 80/20 split is a good ratio
- Management is critical to maintain diversity

### **Successful species**

*Achillea millefolium*

*Anthyllis vulneraria*

*Centaurea nigra*

*Cynosurus cristatus*

*Echium vulgare*

*Festuca rubra*

*Leucanthemum vulgare*

*Lotus corniculatus*

*Prunella vulgaris*

*Rumex acetosa*.

## Poor performers

*Sanguisorba officinalis*

*Thymus vulgaris*

*Rhinanthus* may be a useful management tool and could be tested at a field scale. Introduction should be cautious as it has an effect on other species and establishment is inconsistent. 1000 seeds/m<sup>2</sup> is a useful rate for controlled productivity. The majority of failure is associated with mowing or mowing at the wrong time.

In general, it is necessary to have enough representation in the seed mix to allow species development. The proportions are not really important the sward will find a natural balance over time.

As long as there is a nearby seed source to provide good colonising species, a simple mix can be used.

*Festuca rubra* can form dense swards which lodge, preventing native forbs to colonise; this can be prevented by adding *Cynosurus* and mowing correctly.

## Legumes

Legumes should not be added to wildflower mixes

When sown with ryegrass, legumes will dominate in the initial years but become less dominant in time. It has been observed that areas which are low in N but high in P and K will have a pulse of legumes first followed by grasses.

Legumes must be used with caution, for example, a mixture of black medic and vetch will be dense and unlikely to recruit other species.

Legume species can be important for encouraging beneficial organisms such as parasitoids and aphid-pathogenic fungi.

## Grasses

A tussocky grass mix should include *Holcus*, *Dactylis* and *Deschampsia*. The management is very important, for example, tussock mixes are good for spiders, so a low cut will not be beneficial. It is the structure of the tussock that is important rather than the species of plant, a tussock and an understory are necessary for cover to benefit many invertebrate species.

It is not necessary to have a big block of tussock grass, hedge bound grass may be sufficient.



Research has shown:

- Predatory beetle diversity can be greater in fine grass plots when compared to tussocky plots.
- In comparison: Grass by wire fences = greater abundance of predatory beetles while Grass in hedge bottoms = greater predatory beetle diversity.
- Including a nectar source nearby will be beneficial.
- *Holcus* and *Dactylis* is good for aphids and also a reservoir for their natural enemies, including fungus.
- *Sitobion* spp. (e.g. *S. avenae* a cereal pest and *S. fragariae*, a non-pest) use grasses in the summer.
- Aphids are not necessarily moving out from the margin – some species exist only on the wild plants in the margin.
- Pest effects are difficult to predict.

### Flower mixes

- A grassless flower mix would need so much seed it is impractical.
- What ever is done, the proportion of grasses and flowers will end up the same. Proportions will be controlled by soil type and management.
- It is important to have a long period of nectar production for all bee species.

Standardizing is dangerous, as species are not reliable in all regions.

Some species should be included for specific invertebrates or mammal species. For example, long tongued bees need plants with deep corollas and these may also provide food for bats. 3D Farming has shown that umbellifers are useful for beneficials such as the hoverfly and should be included. Umbelliferae, compositae and rosacea are useful species for bees

Diversity is the best option and serendipity will adjust the mix.

### Cost

The costs of some of these mixes may not be tolerated by farmers. The aim of the Entry level scheme is to draw in farmers to Higher Tier where they could recoup costs. It is important to discourage farmers using very low sowing rate as this may lead to disappointment and discouragement. Linking economic benefits directly to plants (e.g. by showing that beneficial fungi are supported by legumes for example) may help encourage farmers to invest as they get added benefits.