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APHID SEX PHEROMONES TO ENHANCE PARASITOID EFFICIENCY

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APHID SEX PHEROMONES TO ENHANCE PARASITOID EFFICIENCY

by

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Contents

SUMMARY

Insectary cultures of the major aphid pests of cereals, legumes and brassicas were established, together with cultures of seven aphid parasitoid species which attack these aphids in the U.K. The parasitoids chosen included specialist species relevant to the three crop types as well as generalists, including species that had previously been caught in field traps baited with aphid sex pheromones. These cultures were used to provide insects for laboratory studies of parasitoid responses to synthetic aphid sex pheromones.

Prior to the start of this project, only generalist parasitoids of the genus *Praon* had been caught in aphid sex pheromone field traps but electrophysiological studies had shown that other, more specialised species had antennal receptors that detected the pheromones. Therefore, wind tunnel studies were done to determine whether or not these other parasitoids also responded behaviourally to the pheromones. The cereal aphid specialist *Aphidius rhopalosiphi* and the pea aphid specialists *Aphidius ervi* and *Aphidius eadyi*, as well as the generalists *Praon volucre, Praon myzophgum* and *Ephedrus plagiator* were all strongly attracted to synthetic aphid sex pheromones in the wind tunnel. The brassica aphid specialist *Diaeretiella rapae* did not fly well in the wind tunnel due to its small size but this species was strongly attracted to pheromone traps placed in oilseed rape crops during the summer. Aphid sex pheromones, therefore, appear to act as foraging cues for a wide range of aphid parasitoids including the important species associated with cereal, pea and brassica aphids.

Laboratory experiments also established that exposure of female parasitoids to synthetic aphid sex pheromones did not hinder their searching behaviour on plants or their willingness to attack any suitable aphid hosts that they encountered. On the contrary, the results indicated

that the sex pheromones act as searching stimulants that would potentially retain parasitoids in crops when aphids were scarce, as is usually the case at the beginning of a crop infestation.

Aphid parasitoids are also strongly attracted to volatile plant chemicals that are emitted by plants in response to aphid feeding damage. In wind tunnels the presence of aphid sex pheromones did not interfere with this response but rather increased the proportion of parasitoids responding to these plant volatile foraging cues. Therefore, the presence of aphid sex pheromone lures is likely to enhance parasitoid attraction into aphid-infested areas.

An extensive series of field experiments, using aphid populations on potted 'trap' plants, clearly demonstrated that, when deployed at the appropriate time in the late summer/autumn, the presence of pheromone lures can greatly increase rates of parasitization by both specialist and generalist parasitoid species. The number of aphids that were parasitized on trap plants in the autumn indicated that there was still enough parasitoid activity around the margins of harvested fields to generate significant overwintering populations if appropriate aphid hosts were available. It was particularly encouraging that parasitization by the specialist *Aphidius* species was greatly increased in the vicinity of pheromone lures, even though these parasitoids are not caught in pheromone traps. This indicates a difference in foraging behaviour compared with the generalist *Praon* species and the brassica specialist *D. rapae*, which tend to fly directly to the source of the pheromone whilst the specialist *Aphidius* species appear to forage intensively in the vicinity of the pheromone source without flying directly to it. This latter behaviour is a positive advantage for our proposed parasitoid manipulation strategy. In the case of the cereal aphid specialist *A. rhopalosiphi*, the timing of pheromone lure deployment is important because this species enters a summer diapause or resting period in August and early September.

Field plot trials in winter wheat crops in 1996 and 1997, designed to investigate the potential of aphid sex pheromone lures for attracting parasitoids back into crops in spring/early summer, gave inconclusive results. Despite poor aphid populations and consequent small parasitoid numbers, the 1996 results were encouraging in that more aphids were parasitized and parasitization was better synchronized with aphid invasion when pheromone lures were present in the centre of plots. Furthermore, the effect of the pheromone lures was evident throughout the 6 m x 6 m plots and not just around the lures. Disappointingly, these results were not repeated in the following year. Similar field trials in legume and brassica crops had to be abandoned due to lack of insects in these crops in 1996 and 1997.

The potential for parasitoid manipulation using synthetic aphid sex pheromones was greatly supported by the results of the laboratory studies and by the use of trap plants in the field. However, the final testing of the manipulation strategy in the field was considerably hindered by the climatic conditions prevailing during the study. The combination of the hot dry summer of 1995 followed by the late cold spring of 1996 resulted in very late and poor colonisation of crops by aphids which in turn led to few parasitoids, preventing robust field experimentation. The conditions also hindered the establishment of experimental plots designed to test different vegetation mixes as potential field margin habitats for overwintering parasitoids. Consequently, the effectiveness of the manipulation strategy as an aphid control method remains to be tested in a season with sufficiently large aphid infestations. Based on our experimental results, this should involve the deployment of nepetalactone lures in field margins in September/early October. Field trials should be done in field margins already under development, such as wildlife strips, conservation headlands, grassland marginal strips, set aside strips to determine the potential of these different management options for providing the appropriate field margin conditions for different crop types.

KEY FINDINGS

1. A wide range of aphid parasitoids are attracted to synthetic aphid sex pheromones.
2. Both generalist species, which attack a range of aphid pests, and species which specialize on cereal aphids, pea aphids and brassica aphids potentially can be manipulated using aphid sex pheromones.
3. Aphid sex pheromones act as arrestants and searching stimulants as well as attractants for foraging female parasitoids.
4. Aphid sex pheromones do not interfere with the plant searching or aphid attack behaviour of parasitoids.
5. Aphid sex pheromones enhance parasitoid responses to plant volatiles.
6. Parasitization rates are greatly increased in aphid populations in field margins in the vicinity of aphid sex pheromone lures.
7. Some specialist parasitoid species are stimulated to forage on plants near to pheromone lures, without flying directly to the pheromone source.
8. There is sufficient parasitoid activity in the margins of harvested fields in late summer/ autumn to generate significant overwintering reservoirs if suitable aphid hosts are available.
9. Aphid sex pheromones should be deployed as discrete lures rather than applied to field margins as sprays.
10. Aphid sex pheromone lures should be deployed in September and early October in arable field margins.

RECOMMENDATIONS

1. Aphid parasitoids should be encouraged on farmland by providing a diversity of semi-natural habitats, especially in field boundaries, and by avoiding the use of broad-spectrum insecticides whenever possible.

2. The management of field margins as wildlife strips, conservation headlands, set aside margins or grassland margins should be developed to provide suitable marginal habitats in which overwintering parasitoid populations can be established using pheromone lures.

3. The ability of pheromone lures to establish high overwintering parasitoid densities in such field margins needs to be tested in commercial farming situations, involving a variety of crops.

4. The manipulation strategy needs to be evaluated in a commercial farming environment over several consecutive seasons as the benefits are likely to be cumulative over time.

5. The commercial production of synthetic aphid sex pheromones needs to be developed, including investigation of the feasibility of growing commercial crops of catmint for this purpose.

1 GENERAL INTRODUCTION

Aphids are one of the most important groups of agricultural and horticultural pests in Britain, causing direct damage to a variety of crops and acting as vectors of virus diseases. Aphids are attacked by a range of natural enemies, including insect predators, parasitic wasps (parasitoids) and fungal pathogens, which help to keep their populations in check but sometimes fail to prevent them from reaching economically damaging levels on crops. Parasitoids are an important component of this natural enemy complex, and are now recognized as the most effective natural enemy group causing mortality of plant-feeding insects (Hawkins *et al.*, 1997). Indeed, several species such as *Aphidius colemani, Aphidius matricariae, Aphidius ervi* and *Praon volucre* are reared commercially for the control of aphids in glasshouses. Other species have been introduced into parts of the world where they didn't previously occur to help control exotic aphid pests, with some success.

Extensive research on arable crops such as cereals has highlighted the importance of early-season synchronization between parasitoid populations and their aphid hosts in preventing pest outbreaks (Wratten & Powell, 1991). High rates of parasitism during the early stages of an aphid infestation slow down the pest population's rate of growth, allowing other natural enemies within the system (such as ladybirds and hoverflies) to maintain the aphids below damaging levels. Without this early check to their growth rate, aphid populations can quickly outpace their predators, often requiring insecticide treatment to prevent yield losses. Unfortunately, this early-season synchronization between aphids and their parasitoids is often disrupted by natural factors (e.g. weather conditions in winter) or by farming operations (e.g. pesticide applications).

In agricultural ecosystems, adult parasitoids are forced to leave crop fields when their aphid hosts disappear due to crop ripening and harvest in late summer/autumn. Many disperse to semi-natural habitats, such as hedgerows, woodland, road verges, the margins of water courses and permanent meadows, where they find other aphids to attack before hibernating for the winter, usually as pupae inside the dead bodies of their hosts. In the following spring, they emerge as adult wasps in these semi-natural habitats and attack any suitable aphids in the immediate vicinity before spreading into neighbouring crops. This can result in a significant delay between aphid colonisation of the crop and subsequent parasitoid arrival, i.e. poor synchronization.

Although aphids exist as all-female populations for most of the year, reproducing without mating (parthenogenesis), many species produce a sexual generation in the autumn in response to falling temperatures and shortening daylength. The sexual females release sex pheromones to attract the winged males, with which they mate to produce overwintering eggs. The sex pheromones of a range of pest aphid species have been successfully isolated and the main chemical components identified as the two compounds nepetalactone and nepetalactol (Pickett *et al.*, 1992). Some aphids only release one of these compounds as the pheromone, usually nepetalactone, whilst others release both, but in specific ratios according to species. Chemists at IACR-Rothamsted are now able to synthesize these sex pheromone components in the laboratory, starting with a chemical extracted from catmint plants (*Nepeta cataria*).

During collaborative work between scientists at IACR-Rothamsted and Imperial College (Silwood Park), to investigate the ability of synthetic aphid sex pheromones to attract male aphids in the autumn, it was discovered that female parasitoids belonging to several species of the genus *Praon* were highly attracted to the pheromones (Hardie *et al.*, 1991). One of these, *Praon volucre*, attacks a wide range of aphid species, including cereal aphids, and when pheromone traps were placed in winter wheat crops in autumn, large numbers of female *P. volucre* were caught and appeared to be particularly attracted by the compound nepetalactone (Powell *et al.*, 1993; Hardie *et al.*, 1994).

The Link project was set up to investigate the attraction of parasitoids to aphid sex pheromones in more detail and to try to develop an aphid pest management strategy based on the manipulation of parasitoids using the synthetic aphid pheromones produced in the laboratory. The main aim of such a manipulation strategy would be to facilitate aphid/parasitoid synchronization within crops in spring, thereby promoting natural biological control and reducing the need for insecticide use. The proposed approach is to concentrate parasitoids in field margins in autumn by using pheromone lures to intercept them as they disperse from crop fields,. Field margins would be selected and managed to provide hosts and shelter for the parasitoids, thereby establishing an overwintering reservoir population readily able to re-invade crops rapidly in the following spring. It was viewed as desirable to develop a strategy applicable to a range of crops and so studies have involved parasitoids of cereal aphids (*Sitobion avenae*), pea aphids (*Acyrthosiphon pisum*) and cabbage aphids (*Brevicoryne brassicae*).

The scientific targets set at the beginning of the project were:

1. To establish overwintering parasitoid populations in field margins alongside the crop. Suitable aphid hosts in which the parasitoid can overwinter need to be identified and the management of the field margin vegetation needs to be tailored to support these hosts.
2. To achieve high rates of parasitization of these field margin aphid populations by attracting parasitoids to lures containing aphid sex pheromones. Rates of parasitization in the vicinity of the lures need to be measured and appropriate spacing of lures determined.

3. To ensure good overwintering survival of parasitized aphids in the field margin habitat, leading to high parasitoid emergence rates in the spring. Vegetation management strategies for the field margins need to be tested.
4. To enhance the impact of emerging parasitoids on crop aphid populations in adjacent fields. The dispersal of parasitoids which emerge from field margin reservoirs needs to be investigated, in relation to early spring aphid infestation of adjacent crops.

2 INSECT CULTURES AND HOST SELECTION

2.1 Insect Cultures

To provide insect material for experimental work, a range of aphid and parasitoid cultures were established in the insect rearing unit at Rothamsted. Cultures of the three main target pests, *Sitobion avenae, Acyrthosiphon pisum* and *Brevicoryne brassicae* were supplemented by cultures of the rose-grain aphid *Metopolophium dirhodum* and the peach-potato aphid *Myzus persicae*. The cereal aphids were reared on winter wheat or oats, the pea aphid on *Vicia faba* beans and *B. brassicae* and *M. persicae* on chinese cabbage.

In addition, cultures of the following parasitoids were set up:

- *Aphidius rhopalosiphi* on *S. avenae* and *M. dirhodum*
- *Aphidius ervi* and *Aphidius eadyi* on *Ac. pisum*
- *Diaeretiella rapae* on *B. brassicae* and *M. persicae*
- *Praon volucre* on *S. avenae*
- *Praon myzophagum* on *Ac. pisum* and *M. persicae*
- *Ephedrus plagiator* on *S. avenae*

A. rhopalosiphi is a specialist, only attacking cereal aphids; *A. ervi* and *A. eadyi* are the dominant parasitoids attacking pea aphids, although *A. ervi* will also attack cereal aphids; *D. rapae* specializes on aphids feeding on brassicas; whilst *P. volucre*, *P. myzophagum* and *E. plagiator* are generalists, attacking a wide variety of aphid species. These parasitoids all occur naturally on arable farmland.

2.2 Host Selection

To establish overwintering reservoirs of aphids in field margin strips, the margins need to contain suitable aphid hosts and so need to be managed or designed to provide appropriate food plants for those aphids. Suitable aphid hosts could either be the pest species themselves or alternative, non-pest hosts feeding on wild plant species, and there are obvious advantages and disadvantages to both options. Previous research at Rothamsted on host preferences amongst aphid parasitoids has shown that they are often reluctant to switch from one host species to another, especially if the first host is still available, and this could obviously hinder the recolonisation of crops in the spring. On the other hand, growers are likely to be concerned about maintaining pest species in field margins, even though this generally occurs naturally. Also, the more specialised parasitoid species are usually the more efficient biological control agents within the crop but are also the least likely to readily switch between different host species. On the other hand, the generalist *Praon* species were the only ones attracted in large numbers to the pheromone traps in previous field trials, even though it had been demonstrated, by attaching electrodes to parasitoid antennae (electroantennograms), that the specialist species had antennal receptors which responded to the pheromone chemicals. Therefore, the best theoretical option would be a field margin that provided food plants for more than one aphid host.

3 LABORATORY STUDIES OF PARASITOID RESPONSES TO APHID SEX PHEROMONES

3.1 Introduction

At the start of the project, the only parasitoid species that had been caught in significant numbers in simple field traps baited with synthetic aphid sex pheromones were several *Praon* species, notably the polyphagous *P. volucre* (Hardie *et al.* 1991, 1994; Powell *et al.* 1993). However, electroantennogram studies had revealed that a number of other species, including cereal aphid, pea aphid and brassica aphid specialists, had antennal receptors which gave physiological responses to the pheromones. If these other species also responded behaviourally to the pheromones, showing that it is a widespread phenomenon amongst aphid parasitoid species, this would offer greater opportunities for implimentation of the manipulation strategy in a variety of crops. Therefore, the flight responses to synthetic aphid sex pheromones of seven parasitoid species were investigated using a wind tunnel that was already being used successfully to study parasitoid attraction to other semiochemicals such as plant volatiles (Du *et al.* 1996). A few studies were also done using a four-arm olfactometer.

3.2 Flight Responses to Aphid Sex Pheromones by Female Parasitoids

3.2.1 *Methods*

A wind tunnel was specially designed and built at IACR-Rothamsted to study aphid parasitoid flight behaviour (Du *et al.* 1996). It consists of a rectangular Plexiglas chamber (90 x 30 x 30 cm) through which an airflow is created by means of a fan placed at one end (Fig. 3.1). An airstream is pulled through the tunnel after passing through glass wool and charcoal filters to remove contaminating smells. Both wind speed and light intensity in the tunnel can be adjusted.

Aphid sex pheromones were released into the airstream from filter paper targets (1 x 2 cm) which were attached to a vertical glass column near the upwind end of the tunnel. The

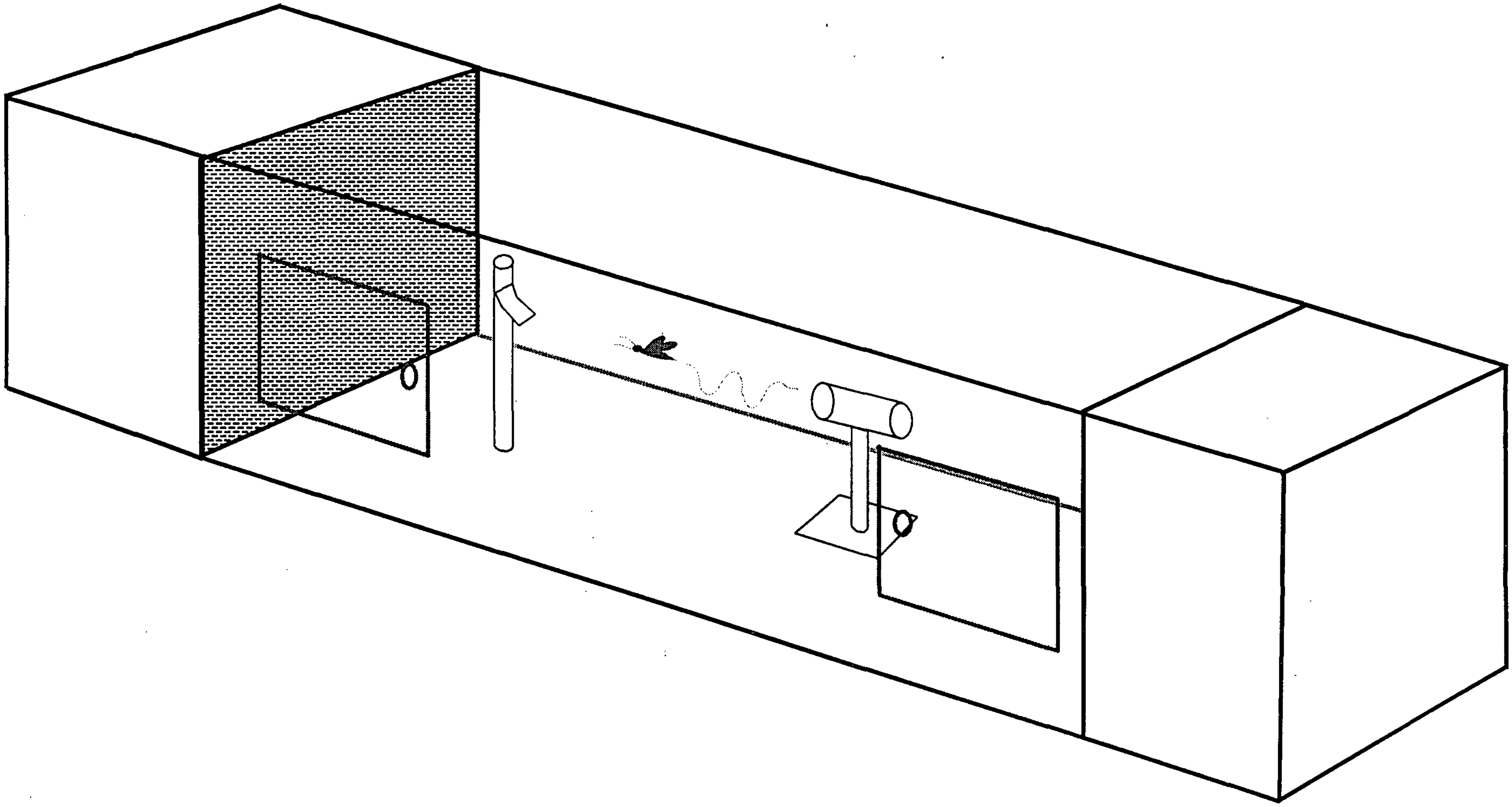

Fig. 3.1. *Diagram of the wind tunnel used to study parasitoid flight responses to aphid sex pheromones.*

pheromone components, nepetalactone and nepetalactol (1 mg/ml in hexane solvent) were applied to the filter paper using a microcapillary dispenser and each application, whether of a single component or a mixture of both, amounted to 10µl of solution. The hexane solvent alone was used as a control treatment.

All the female parasitoids used in these experiments were naive, meaning that they emerged from their cocoons in isolation and had had no contact with either aphids or plants before being flown in the wind tunnel. Parasitoids were released individually from an open ended glass tube (5 cm long x 1.5 cm diameter) placed horizontally in the airstream, 20 cm downwind from the filter paper odour source. Each parasitoid was allowed 3 minutes in which to take off from the release tube, and each was used only once. In most experiments, between 40 and 60 parasitoids were tested for each treatment. A positive response to the pheromone odour was recorded if the parasitoid flew directly upwind towards the filter paper target in one continuous flight (oriented flight response). Unresponsive parasitoids flew in random directions in the tunnel, often landing on the sides of the tunnel. The percentage of parasitoids responding was recorded for each treatment and the data were subjected to a logit transformation before being analysed using analysis of variance (ANOVA).

Nepetalactone, nepetalactol and a 1:1 ratio mixture of the two compounds were used in tests with the cereal aphid parasitoid *A. rhopalosiphi,* the pea aphid parasitoids *A. ervi* and *A. eadyi* and the generalist parasitoid *E. plagiator*, whilst the brassica aphid parasitoid *D. rapae* was tested against nepetalactone only. In further tests, *P. myzophagum* females reared from *Ac. pisum* and from *M. persicae* were tested against 1:1 and 1:2 ratios of nepetalactone:nepetalactol as well as against both compounds individually, whilst *P. volucre* females reared from *Ac. pisum* and from *M. persicae* were tested against nepetalactone and a 1:1 ratio of the two compounds.

3.2.2 *Results*

The percentage of female parasitoids showing oriented flights to the various wind tunnel treatments are shown in Tables 3.1 and 3.2. Females of *A. rhopalosiphi*, *A. ervi*, *A. eadyi* and *E. plagiator* were all strongly attracted to the synthetic pheromones in the wind tunnel (Table 3.1), demonstrating obvious behavioural responses despite the fact that none of these species had been caught by aphid sex pheromone traps in the field. The only species not to show strong flight responses was *D. rapae* (Table 3.1), although a significantly greater percentage took off from the release tube ($P<0.01$) and they took off significantly more quickly ($P<0.05$) when nepetalactone was present compared with controls.

Table 3.1. *Percentage of female parasitoids making oriented flights to synthetic aphid sex pheromone components in a wind tunnel (n = 40-60). Values in the same row are not significantly different from each other (P>0.05) if they are followed by the same letter.*

Parasitoid	Control	Nepetalactone	Nepetalactol	1:1 Mixture
A. rhopalosiphi	2.5 a	46.8 c	12.5 b	9.3 b
A. ervi	10.0 a	60.1 b	46.7 b	70.3 b
A. eadyi	0.0 a	27.6 b	8.1 a	31.8 b
E. plagiator	0.0 a	15.7 b	37.8 c	27.7 bc
D. rapae	4.8 a	8.1 a	not tested	not tested

Although the cereal aphid specialist *A. rhopalosiphi* gave a significant response to all the pheromone treatments its response was significantly stronger to nepetalactone than to nepetalactol or a mixture of the two compounds (Table 3.1). In contrast, the two pea aphid specialists *A. ervi* and *A. eadyi* responded equally well to nepetalactone and to the 1:1 ratio mixture of nepetalactone and nepetalactol, whilst *A. ervi* also responded to nepetalactol (Table

3.1). The generalist *E. plagiator* responded most strongly to nepetalactol and the 1:1 mixture whilst showing a smaller but significant response to nepetalactone (Table 3.1).

Praon volucre females were strongly attracted to both nepetalactone and a 1:1 mixture of nepetalactone and nepetalactol regardless of which host they had been reared on: their response to nepetalactol on its own was not tested (Table 3.2). *Praon myzophagum* females were strongly attracted to the 1:1 mixture of the two pheromone components, regardless of which host they had been reared on, but only those reared on *M. persicae* gave a significant response to the 1:2 mixture of nepetalactone:nepetalactol (Table 3.2). This parasitoid did not appear to respond to either nepetalactone or nepetalactol when these were tested singly.

Table 3.2. *Percentage of female parasitoids reared on two alternative hosts making oriented flights to synthetic aphid sex pheromone components in a wind tunnel (n = 40-60 in the case of* P. myzophagum *and 25-35 in the case of* P. volucre*). Values in the same row are not significantly different from each other (P>0.05) if they are followed by the same letter.*

Parasitoid (Host)	Control	Nepetalactone	Nepetalactol	1:1 Mixture	1:2 Mixture
P. myzophagum (Ac. pisum)	23.9 a	27.0 a	35.4 a	68.8 b	31.0 a
P. myzophagum (M. persicae)	20.6 a	24.6 a	22.0 a	41.4 b	44.0 b
P. volucre (Ac. pisum)	12.2 a	37.7 b	not tested	43.0 b	not tested
P. volucre (S. avenae)	9.0 a	45.7 b	not tested	40.7 b	not tested

3.2.3 *Discussion*

The results of the wind tunnel studies demonstrate that behavioural responses to aphid sex pheromones are widespread amongst both specialist and generalist aphid parasitoid species; all the species tested, except *D. rapae*, being strongly attracted to pheromone components in the tests. This means that, if successful, the proposed parasitoid manipulation strategy for aphid pest management is potentially applicable to a variety of agricultural and horticultural crops. Since the adult parasitoids used in the experiments had not been exposed to aphids or plants prior to their release in the wind tunnel and they had all been reared on asexual aphid populations, their responses to the sex pheromones must be innate.

The tests revealed interesting differences between the responses of different parasitoid species. The most common host of *A. rhopalosiphi* is the cereal aphid *S. avenae*, whose sex pheromone only contains nepetalactone (Pickett *et al.* 1992) and this compound elicited the strongest response from *A. rhopalosiphi*. However, the sex pheromone of the pea aphid *Ac. pisum* consists of a 1:1 mixture of nepetalactone and nepetalactol (Pickett *et al.* 1992) and both *A. ervi* and *A. eadyi*, for whom the pea aphid is the preferred host, responded strongly to this mixture. However, their response does not appear to be very specific to this pheromone blend since both species also responded to nepetalactone when tested alone and *A. ervi* responded similarly to nepetalactol. Interestingly, *A. ervi* is known to attack several other aphid species, including *S. avenae* which only emits nepetalactone. The generalist *P. volucre* responded equally well to synthetic pheromone blends appropriate to both *S. avenae* and *Ac. pisum*, regardless of which of these two hosts it was reared on. The other generalist *P. myzophagum* also responded to the 1:1 pheromone blend typical of *Ac. pisum*, even when reared on *M. persicae* but only responded to the 1:2 blend of *M. persicae* when reared on that species.

In summary, six of the seven parasitoid species tested flew directly towards filter paper targets releasing synthetic aphid sex pheromones in the wind tunnel. Most species responded to single pheromone components, particularly nepetalactone, but some were most strongly attracted to pheromone blends associated with their most preferred host species or the species on which they had been reared. This is encouraging since single compound lures or lures releasing simple pheromone blends are likely to attract several parasitoid species in the field and, if necessary, key species could be targetted using pheromone blends most closely mimicking the sex pheromone of their preferred host. In the case of cereals, for example, lures releasing only nepetalactone would be the most appropriate.

3.3 Effect of Aphid Sex Pheromones on Searching and Attack Behaviour of *Aphidius ervi.*

3.3.1 *Introduction*

The wind tunnel flight responses of the parasitoids indicated that aphid sex pheromones function as a host location cue in the early stages of the foraging process, when the parasitoid is searching for appropriate aphid-infested habitats. Once an appropriate habitat has been found the parasitoid intensively searches individual plants while walking on them, using other cues such as the presence of aphid honeydew to locate the aphids themselves (Budenberg 1990, Budenberg *et al.* 1992, Powell *et al.* 1998). When an aphid is encountered, still further cues, both chemical and physical, are used to recognise the aphid as the right species to attack (Powell *et al.* 1998). It was important, therefore, to establish that exposure to synthetic aphid sex pheromones, either prior to or during both plant searching and encounters with aphids, did not interfere with parasitoid host location and attack behaviour. Searching responses of *A. ervi* were investigated using an olfactometer and aphid attack behaviour was assessed in a simple Petri dish bioassay; both techniques had been used successfully in previous studies of aphid parasitoid foraging behaviour (Powell & Zhang 1983, Powell & Wright 1988, 1992).

3.3.2 *Methods*

3.3.2.1 Searching behaviour

A four-arm 'Petterssen' olfactometer was used to investigate the effect of synthetic aphid sex pheromone on the searching behaviour of *A. ervi*. The olfactometer chamber had the shape of a four-pointed star and a pump was used to draw air out of the chamber through a central hole, thereby creating an air flow into the chamber through each of the four arms. Before entering the olfactometer the air was passed through an activated charcoal filter to remove any contaminating organic odours. A 1:1 ratio mixture of the aphid sex pheromone components nepetalactone and nepetalactol (in hexane solution) was introduced into the airstream entering one of the four olfactometer arms, at a rate of 0.6 µl/min by evaporation from a microcapillary tube. Parasitoids were placed into the chamber individually and their behaviour observed for 8 minutes. The number of entries into, and time spent in, each arm of the olfactometer was measured. The parasitoids used were either naive or were exposed to one of the following treatments before testing:

- synthetic aphid sex pheromone
- pea aphid hosts + hexane
- pheromone and pea aphids simultaneously
- pea aphids followed separately by pheromone

Female parasitoids were exposed to these pre-testing treatments in 9 cm diameter glass Petri dishes and 5 parasitoids were treated together in each dish. Exposure to aphids involved 20 pea aphid nymphs, and a 1:1 ratio mixture of nepetalactone and nepetalactol was released into appropriate dishes at the rate of 0.3 µl/min by evaporation from microcapillaries. All pre-testing treatments lasted for 30 minutes and when parasitoids were exposed to aphids and then pheromone separately, they were exposed to each for 30 minutes. After pre-testing treatment, parasitoids were given access to food and kept at 18 °C for two hours before testing, to minimize any effect of reduced egg load on their responses.

The number of entries into, and total time spent in each of the four arms of the olfactometer were expressed as percentages of the total entries into and the total time spent in all four arms.

the percentage data for the three control arms were meaned to give a single value, which was compared with the value for the pheromone-treated arm by a Chi-squared (χ^2) test.

3.3.2.2 Attack behaviour

In the attack rate bioassays, female parasitoids were released individually into plastic Petri dishes (9 cm diameter) containing 20 aphid nymphs (*Ac. pisum* or *S. avenae*) and observed for 10 minutes. The number of attacks made on the aphids during that time was recorded. The female *A. ervi* used in the experiments were either naive or had been exposed to a 1:1 ratio mixture of nepetalactone and nepetalactol (as described in section 3.3.2.1) prior to release into the Petri dishes. In the first experiment, attack rates against the preferred host *Ac. pisum* and against the alternative host *S. avenae* were measured, whilst in the second experiment attack rates against *Ac. pisum* with and without pheromone present in the Petri dish were measured. Attack rate was quantified as the number of oviposition stabs that made contact with an aphid during the observation period. In the first experiment, 22-24 parasitoids per treatment were tested whilst 17 parasitoids per treatment were tested in the second experiment.

3.3.3 *Results*

3.3.3.1 Searching behaviour

In the olfactometer, naive female parasitoids and those that had been exposed to aphids, to aphids and pheromone simultaneously or to aphids followed by pheromone entered the arm containing synthetic aphid sex pheromone significantly more often and spent significantly more time in that arm than they did in each of the clean air arms ($P< 0.01$ - $P< 0.001$) (Fig. 3.2). However, when they had been exposed to the pheromone without being able to attack aphids before testing, their response to the pheromone in the olfactometer was greatly reduced and the data for the pheromone-treated and clean air arms were not significantly different (Fig. 3.2).

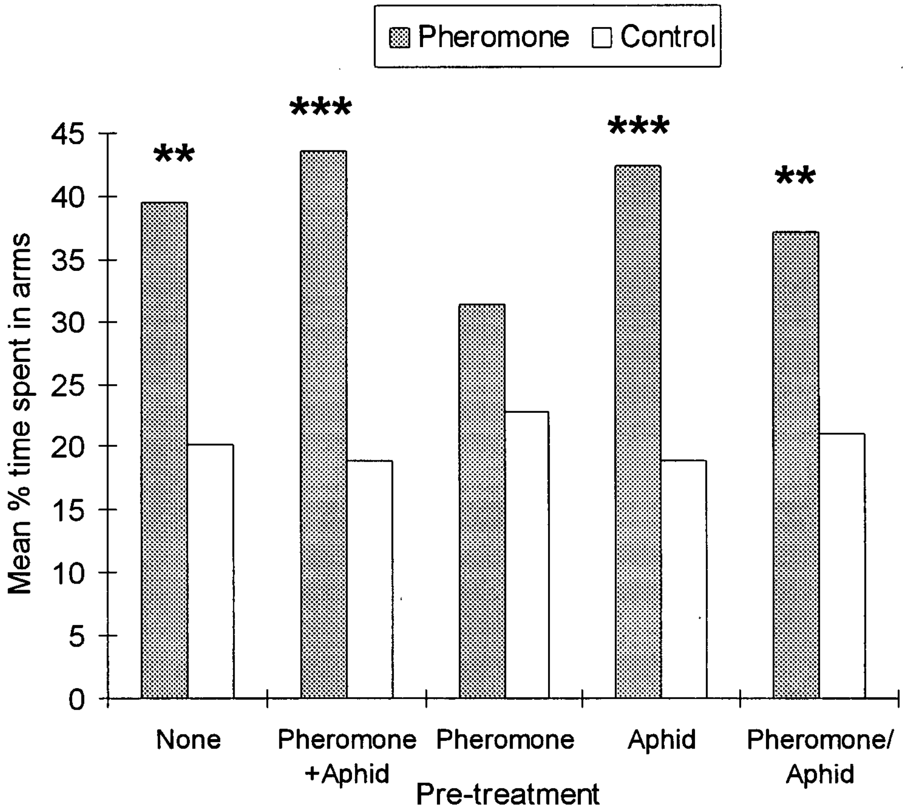

Fig. 3.2. *Mean % time spent in aphid sex pheromone-treated and untreated control arms of an olfactometer by female parasitoids* (Aphidius ervi) *which had been exposed to various aphid and pheromone pre-treatments before being tested in the olfactometer.*

3.3.3.2 Attack behaviour

Exposure of female *A. ervi* to synthetic aphid sex pheromones immediately before they were presented with aphids did not affect (t-test, P> 0.05) their attack rate against either their preferred host *Ac. pisum* or the alternative host *S. avenae* (Fig. 3.3). Similarly, attack rates against *S. avenae* were not affected (ANOVA, P> 0.05) by the presence of pheromone in the Petri dish arena during aphid encounters, whether or not the parasitoids had been exposed to the pheromone prior to testing (Fig. 3.4).

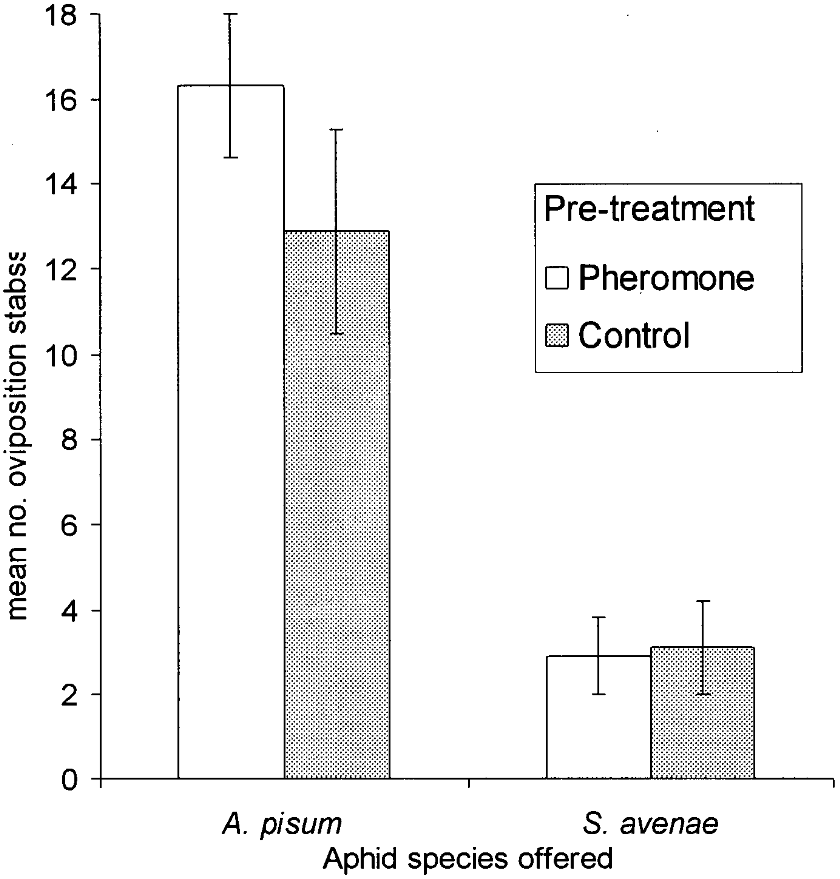

Fig. 3.3. *Mean number of oviposition attacks made within 10 minutes by female parasitoids* (Aphidius ervi), *which had been exposed or not to aphid sex pheromones prior to testing, against pea aphids* (A. pisum) *or cereal aphids* (S. avenae) *in Petri dish arenas.*

3.3.4 *Discussion*

Aphid sex pheromones appeared to act as a searching stimulant for parasitoids after they have been attracted to the habitat and are searching for aphids on plants. At this stage of the foraging process, the pheromone probably serves as an arrestant, lengthening the time spent searching for aphids in the area and so increasing the probability that any aphids that are present will be found. Aphid honeydew is known to act as an arrestant and searching stimulus in the same way (Budenberg 1990, Budenberg *et al.* 1992).

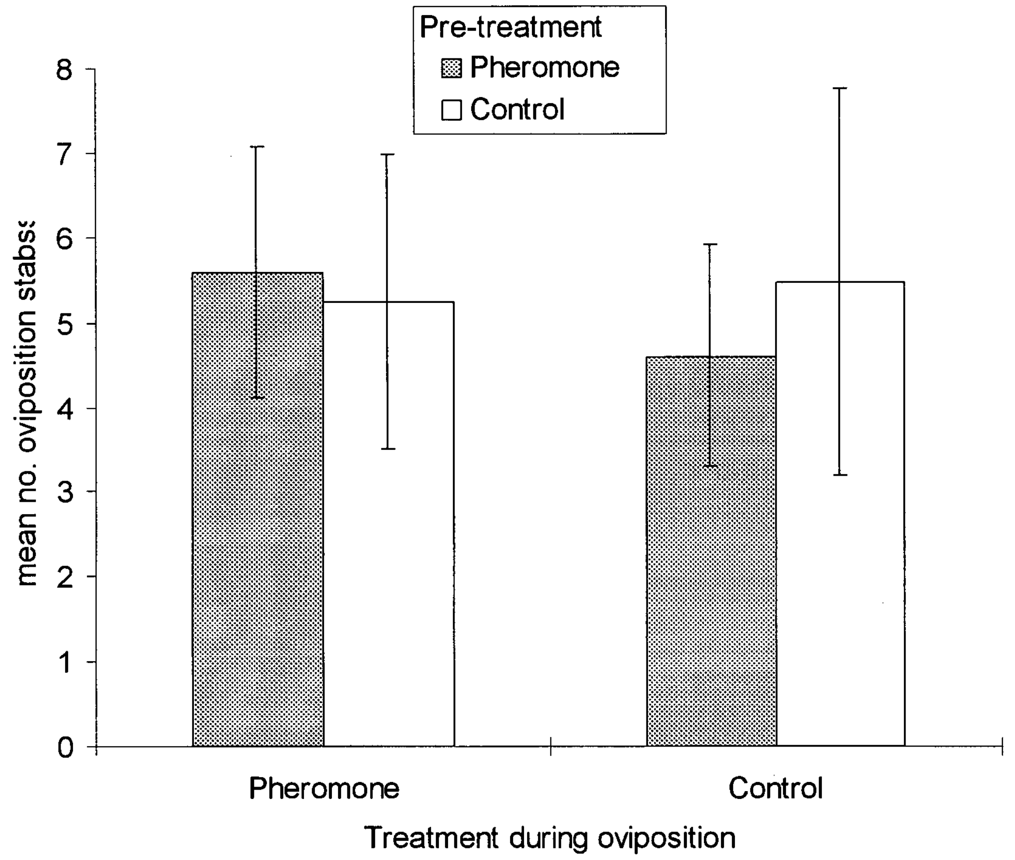

Fig. 3.4. *Mean number of oviposition attacks made within 10 minutes by female parasitoids* (Aphidius ervi), *which had been exposed or not to aphid sex pheromones prior to testing, against cereal aphids* (S. avenae) *in the presence or absence of aphid sex pheromone.*

There was also evidence that, in the absence of any aphids to attack, the response of the parasitoid to the pheromone gradually declined if the latter was constantly present. This is a process known as habituation. Parasitoids also become habituated to honeydew as a searching stimulus (Budenberg 1990) and this process serves to prevent them from continually searching an unprofitable area or plant which may contain a searching stimulus but no aphid hosts. However, if aphids are found, the pheromone does not interfere with aphid attack and the response to the pheromone is reinforced, thereby counteracting habituation.

The results of these experiments are very encouraging as they show that pheromone lures are unlikely to hinder the host location and attack behaviour of parasitoids, once they have been attracted to field margins by the pheromones. Furthermore, there was evidence that the pheromones acted as positive searching stimuli, arresting parasitoids in the area and thereby increasing the likelihood of them finding any aphids that might be present. The habituation mechanism would prevent parasitoids from remaining in the immediate vicinity of a pheromone lure if no aphids were available, allowing them to widen their search area. In view of this, the use of discrete pheromone lures in field margins rather than extensive pheromone sprays is likely to be the more efficient strategy.

3.4 Interaction between Aphid Sex Pheromones and Plant Volatiles as Parasitoid Attractants

3.4.1 *Introduction*

Plant volatiles play an important role as attractants for foraging aphid parasitoids. Recent work at IACR-Rothamsted has shown that pea aphids feeding on broad bean plants cause changes in the plant volatiles released by the plant and that female *Aphidius ervi* use these 'aphid-induced' plant volatiles as host location cues (Du *et al.* 1996). The release of these attractant plant volatiles appears to be a function of the number of aphids present and the length of time they have been feeding on the plant. For example, an infestation of 40 aphids for 3 days causes a strong parasitoid response, as does an infestation of 100 aphids for 2 days. Other research work has shown that certain plant volatiles released by brassica plants are strongly attractive to the parasitoid of brassica aphids *Diaeretiella rapae* (Read *et al.,* 1970) and the cereal aphid specialist *Aphidius rhopalosiphi* also uses plant-derived cues during host location (Powell & Wright, 1992; Wickremasinghe & van Emden, 1992). If synthetic aphid sex pheromone lures were to be used in the field, these plant volatile attractants would sometimes also be present in the same location. The wind tunnel was used to investigate possible interactions between these two different attractant foraging cues; synthetic aphid sex pheromones and aphid-induced plant volatiles.

3.4.2 *Methods*

Two separate experiments were done using naïve female *A. ervi* which were known to make oriented flights in the wind tunnel both towards aphid-infested bean plants and towards the synthetic aphid sex pheromone component, nepetalactone. In the first experiment, parasitoids were flown to uninfested and to *Ac. pisum*-infested plants, with and without nepetalactone present. Aphid-infested plants had been infested with 100 mixed age *Ac. pisum* for a period of three days before testing. In the second experiment, bean plants that had been infested with 100 pea aphids either for 24 hours or for 48 hours were tested with and without nepetalactone. In both experiments, the pheromone was placed on a small piece of filter paper and attached to plants immediately before testing. Naïve female parasitoids were released individually into the wind tunnel and the percentage making oriented flights to the plants was recorded for each treatment, as described in section 3.2.1. Between 35 and 40 parasitoids were flown for each treatment.

3.4.3 *Results*

In the first experiment, significantly more parasitoids flew to aphid-infested plants than to uninfested plants (Fig. 3.5). However, the presence of nepetalactone significantly increased responses both to uninfested and to aphid-infested plants. There was no significant difference in the number of oriented flights made to uninfested plants with nepetalactone and to aphid-infested plants without nepetalactone (Fig. 3.5).

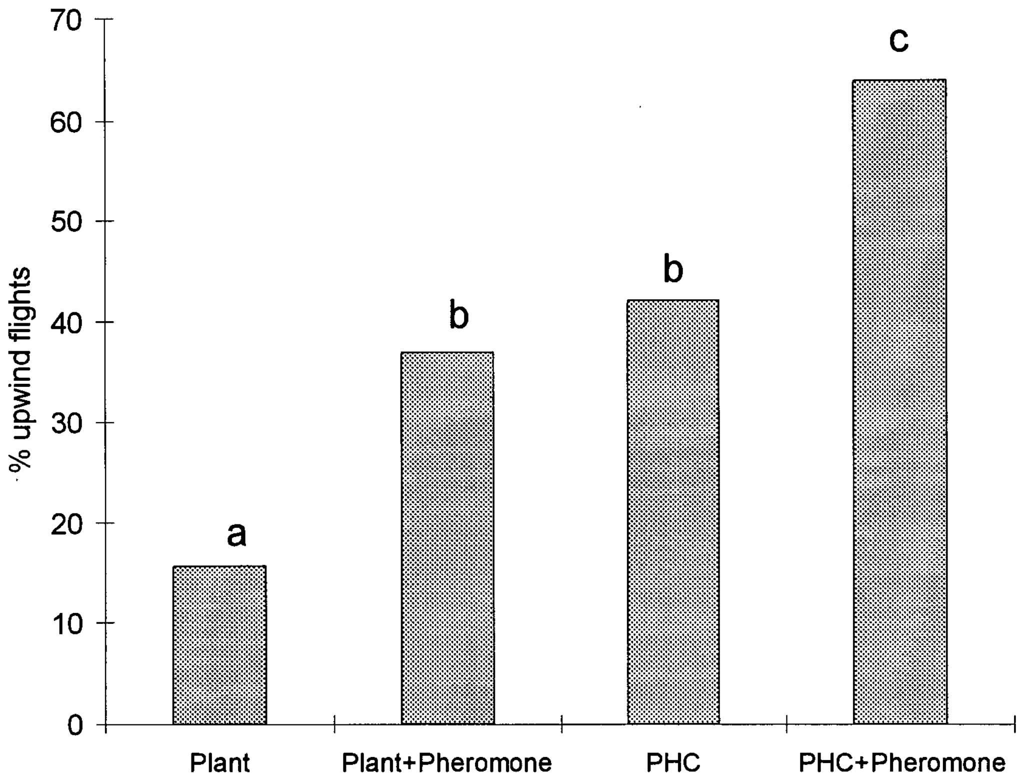

Fig. 3.5. *Percentage of female parasitoids* (Aphidius ervi) *making direct flights in a wind tunnel towards uninfested broad bean plants, with or without an aphid sex pheromone lure, and towards bean plants infested with pea aphids* (A. pisum), *with and without an aphid sex pheromone lure attached. PHC = Plant/host complex.*

In the second experiment, significantly more parasitoids flew to plants that had been infested for 48 hours than to plants infested for only 24 hours (Fig. 3.6). Again, the presence of nepetalactone significantly increased the percentage of parasitoids responding both to 24 hour-infested and to 48 hour-infested plants (Fig. 3.6).

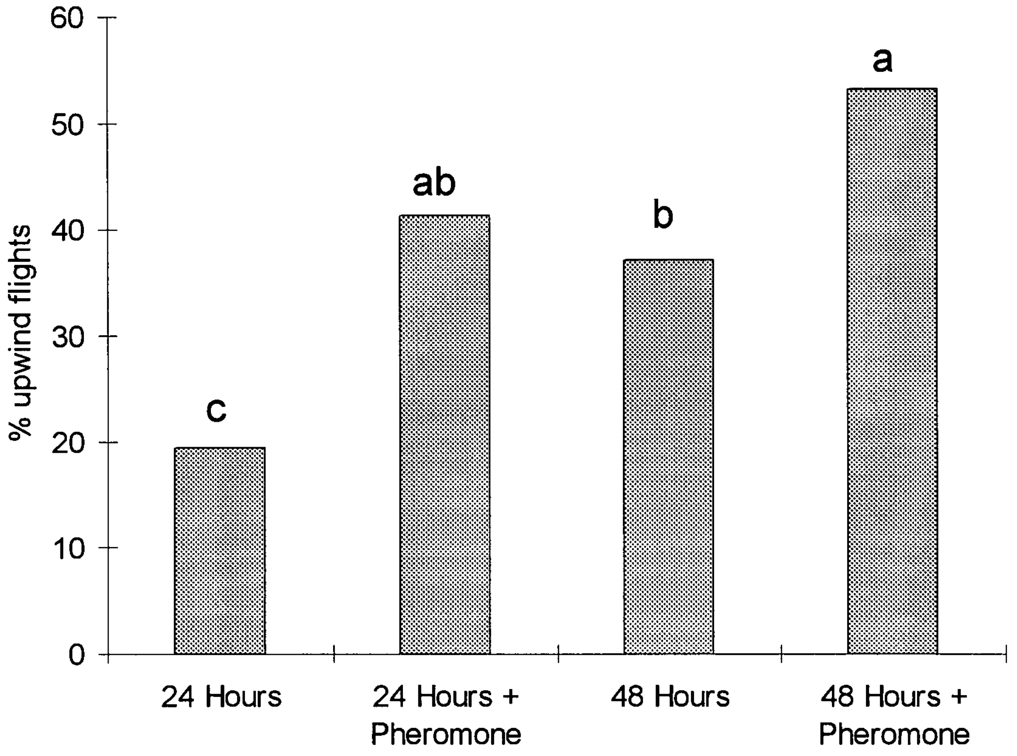

Fig. 3.6. *Percentage of female parasitoids* (Aphidius ervi) *making direct flights in a wind tunnel towards bean plants infested with pea aphids* (A. pisum) *for either 24 hours or 48 hours, with or without an aphid sex pheromone lure attached.*

3.4.4 *Discussion*

Both synthetic aphid sex pheromones and aphid-induced plant volatiles appear to be equally attractive to female aphid parasitoids. However, when both foraging cues are present at the same time, the response of the parasitoids is heightened, showing a positive interaction between the two stimuli. Therefore, the presence of aphid sex pheromone lures is likely to enhance parasitoid attraction into aphid infested areas.

4 FIELD STUDIES OF PARASITOID RESPONSES TO APHID SEX PHEROMONES

4.1 Introduction

In previous studies, simple Petri dish water traps baited with synthetic aphid sex pheromone lures caught large numbers of female aphid parasitoids of several *Praon* species when deployed in cereal fields or at the edge of woodland (Hardie *et al.* 1991, Powell *et al.* 1993). However, wind tunnel studies revealed that a number of other parasitoid species were attracted towards aphid sex pheromone components, although these species had not previously been caught in the pheromone traps in the field. For the proposed aphid pest management strategy to work, it was important to demonstrate that the presence of pheromone lures in the field could not only attract a range of parasitoid species but could increase levels of parasitization in nearby aphid populations.

4.2 Pheromone Trapping of the Brassica Aphid Parasitoid *Diaeretiella rapae*

4.2.1 *Introduction*

The only parasitoid species tested in the wind tunnel that did not make strong oriented flights towards synthetic aphid sex pheromones was *D. rapae*, a species which specializes in attacking aphids on brassica plants. Paradoxically, this was the only species not belonging to the genus *Praon* that had previously been caught in pheromone-baited water traps, when these were used in the field by Polish collaborators just prior to the commencement of this project (Gabrys *et al.* 1997). In 1995, very large populations of *D. rapae* occurred in oilseed rape crops in the U.K., attacking a heavy infestation of the cabbage aphid, *B. brassicae*. This gave us an opportunity to confirm the Polish results.

4.2.2 *Methods*

Pheromone traps baited with nepetalactone, nepetalactol or a 1:1 ratio mixture of the two pheromone components were placed, together with unbaited control traps, in three fields of oilseed rape on Rothamsted farm in July 1995. The traps consisted of plastic Petri dishes (14 cm diameter) containing water with a detergent added. Traps were emptied daily over a period of four weeks and all aphid parasitoids caught were identified, sexed and counted. The experiment was repeated in 1996 when the 1:1 ratio treatment was replaced by a 1:2 ratio of nepetalactone:nepetalactol.

4.2.3 *Results*

In 1995 large numbers, predominantly females, of *D. rapae* were caught in the traps. All three pheromone treatments caught significantly more female parasitoids than did the control traps, but there were no significant differences between the numbers caught by the three different pheromone treatments (Table 4.1). Catches of male parasitoids were much smaller, but significantly more were caught in the traps baited with nepetalactone than in the control traps (Table 4.1).

In 1996, aphid and parasitoid populations were much smaller than in the previous year, but again the pheromone traps caught more female parasitoids than did the control traps; however, the difference was not statistically significant in the case of traps baited with the 1:2 ratio mixture (Table 4.1). The nepetalactone traps again caught the greatest number and caught significantly more than those baited with the 1:2 ratio mixture (Table 4.1). Very few males were caught in 1996 and there were no significant differences between treatments (Table 4.1).

Table 4.1. *Mean number (per trap) of* D. rapae *caught in pheromone-baited and unbaited water traps placed in oilseed rape crops for 4 weeks in July 1995 and 1996. (n = 3). Values in the same column are not significantly different from each other (P>0.05) if they are followed by the same letter.*

Treatment	1995 Females	1995 Males	1996 Females	1996 Males
Control	36.6 a	2.8 a	2.3 a	1.3 a
Nepetalactone	65.8 b	5.2 b	9.3 c	1.8 a
Nepetalactol	48.3 b	3.5 ab	6.2 bc	1.5 a
1:1 Mixture	52.9 b	3.8 ab	not tested	not tested
1:2 Mixture	not tested	not tested	4.4 ab	1.0 a

4.2.4 *Discussion*

The results confirmed the previous observations made in Poland that *D. rapae* females are attracted to synthetic aphid sex pheromones in the field, despite the lack of an obvious flight response in the wind tunnel experiments. It is possible that the pheromone concentration used in the wind tunnel was not appropriate for *D. rapae*, but it is more likely that the parasitoids tested, which were very small, were unable to fly properly in the wind tunnel conditions. Although few oriented flights were achieved by *D. rapae* in the wind tunnel (see Table 3.1), significantly more females took off from the release tube and attempted to fly when nepetalactone was present and they took off significantly more quickly than did females in the control tests.

In the 1995 field experiment, significantly more male *D. rapae* were caught in nepetalactone-baited traps than in control traps. However, this did not occur in the following year, when populations were much smaller, and no other male parasitoids have responded to synthetic aphid sex pheromones during either field trapping or laboratory studies. Because parasitoid densities were unusually high in oilseed rape crops in 1995, it is likely that the males were being attracted to the large numbers of female parasitoids that were aggregating around the pheromone traps. It is known that male aphid parasitoids are strongly attracted in the field to sex pheromones released by virgin female parasitoids (Decker *et al.* 1993).

4.3 Effect of Synthetic Aphid Sex Pheromone Lures on Parasitization of Aphids on Trap Plants

4.3.1 *Introduction*

The proposed aphid pest management strategy involves the establishment of overwintering reservoirs of parasitoids in field margin strips, using aphid sex pheromone lures to concentrate foraging female parasitoids in the field margins in autumn. For this to work, it is essential that attracted parasitoids should actively find and attack any aphid hosts in the vicinity of the lures. To test this, aphid populations on potted 'trap' plants were used in a series of field trials to measure the effects of pheromone lures on parasitization rates. In the later trials, done in 1996 and 1997, we also began to assess the distance of influence of a lure on parasitization rates.

4.3.2 *Methods*

Trap plants consisted of young winter wheat or broad bean plants, heavily infested with *S. avenae* and *Ac. pisum* respectively, growing in 13 cm diameter plastic plant plots. In autumn 1993, a preliminary trial was done, involving trap plants placed at the edges of two cereal stubble fields (*S. avenae* plants) and one field of white clover (*Ac. pisum* plants). In each field, two pairs of trap plants were placed 30 metres apart; pheromone lures releasing nepetalactone were attached to one of the pairs of plants in each field. The trap plants were replaced twice weekly over a six week period (October 7 to November 18) and the surviving aphids kept until parasitoid mummies formed. In autumn 1994, a slightly larger trial was done, involving trap plants in two cereal stubble fields and three clover fields during September and October. In the cereal stubble fields, plants infested with cereal aphids, with and without nepetalactone-releasing lures, were used whilst in the clover fields plants infested with pea aphids, without lures or with lures releasing nepetalactone or nepetalactol or a 1:1 ratio mixture of the two compounds, were used.

In autumn 1996, two spacing trials were done using cereal trap plants infested with *S. avenae*. In the first trial, four plants were placed along a cereal stubble field margin, the first of which had attached to it a pheromone lure releasing nepetalactone. The second plant was placed 20 cm away from the pheromone plant, the third plant was placed a further 15 m along the field margin, with the fourth plant 20 cm beyond that (Fig. 4.1a). Plants were left in the field for three days and the experiment was repeated six times between August 26 and September 12.

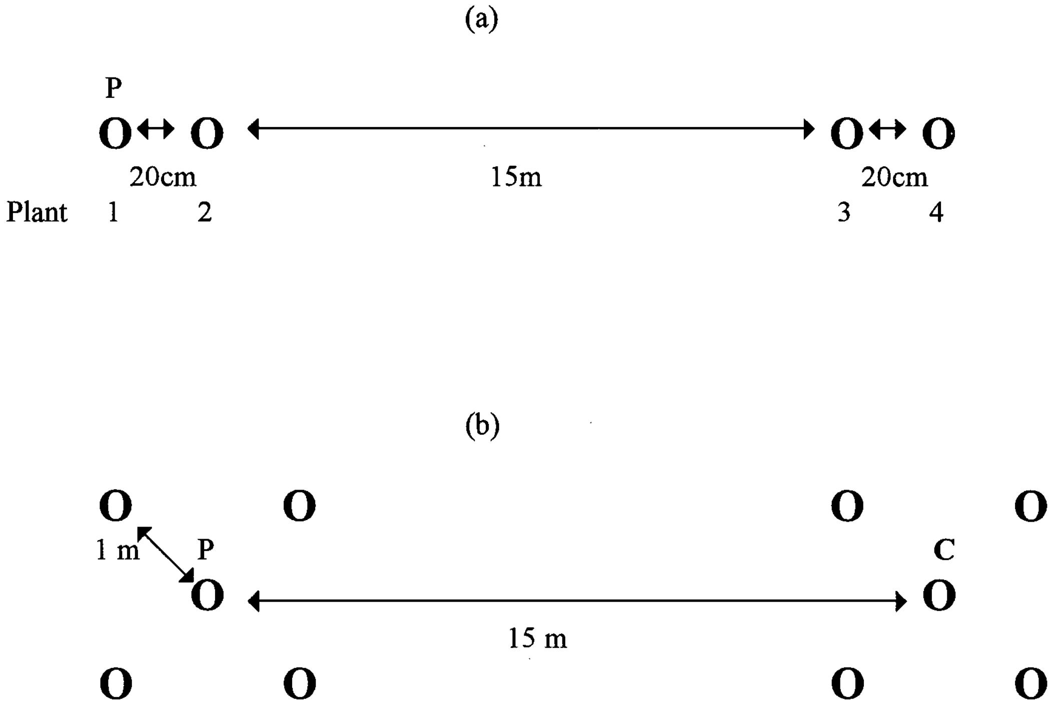

Fig. 4.1. Spatial arrangement of aphid trap plants used in the field spacing trials done in 1996 aand 1997. P = plant with pheromone lure attached. C = control group.

In the second trial, two sets of five plants were placed 15 m apart along the margin of a cereal stubble field. Each set of five plants was positioned with four plants forming a square around the central fifth plant (Fig. 4.1b). A pheromone lure releasing nepetalactone was attached to the central plant of one of the two groups of five. The plants were left in the field for three days and the experiment was repeated four times between September 23 and October 25.

In 1997, the second 1996 trial (Fig. 4.1b) was repeated and a further trial was done, which used the same spatial arrangement of trap plants but with the distance between the central plant and the four surrounding plants in each group increased to 3 m. The 1 m spacing trial was repeated six times between July 9 and August 6, followed by the 3 m spacing trial which was repeated five times between August 15 and September 26.

4.3.3 *Results*

There was no difference in the survival rates of aphids, while the trap plants were in the field, between those on plants with pheromone lures attached and those on plants without lures, in any of the trap plant trials. In the 1993 trial, a total of 111 parasitoid mummies were recovered from the plants in the three fields, 82% of which formed on plants with pheromone lures attached (Table 4.2). The most dramatic effect occurred at one of the cereal sites, where only a single mummy was recovered from control plants during the whole exposure period, compared with 32 from pheromone plants. All the parasitoids which emerged from the cereal aphids were *Praon volucre*, whereas those emerging from the pea aphids were *Aphidius ervi*.

Table 4.2. *Number of parasitized aphids recovered from trap plants, with and without aphid sex pheromone lures, placed in field margins in autumn 1993.*

Crop	Aphid	Control Plants	Pheromone Plants
Cereal Stubble	*S. avenae*	1	32
Cereal Stubble	*S. avenae*	13	39
Clover	*Ac. pisum*	6	20

In the 1994 trials, more parasitoid mummies were again formed on plants with pheromone lures than on control plants. A total of 108 mummies formed on the cereal plants infested with *S. avenae* (Table 4.3). Thirty-seven of these were *P. volucre*, only one of which occurred on the control plants. The remainder were *Aphidius rhopalosiphi*, the cereal aphid specialist, which appeared to respond positively to the pheromone at one of the sites but not at the other (Table 4.3).

Table 4.3. *Number of parasitized cereal aphids recovered from trap plants, with and without aphid sex pheromone lures, placed in cereal stubble field margins in autumn 1994.*

Site	Parasitoid	Control Plants	Pheromone Plants
1	*P. volucre*	0	28
	A. rhopalosiphi	33	16
2	*P. volucre*	1	8
	A. rhopalosiphi	1	21

Only 34 mummies formed on the bean plants placed in clover field margins in 1994, but all except one of these formed on plants with pheromone lures attached (Table 4.4). Site one was an old, established clover crop and all the parasitoids recovered from plants at this site were the pea aphid specialist *Aphidius eadyi*, which showed a distinct preference for lures releasing the 1:1 ratio of nepetalactone and nepetalactol (Table 4.4).

Table 4.4. *Number of parasitized pea aphids recovered from trap plants, with and without pheromone lures, placed in clover field margins in autumn 1994.*

Site	Parasitoid	Control Plants	Nepetalactone Plants	Neptalactol Plants	1:1 Mixture Plants
1	*A. eadyi*	1	1	1	12
2	*P. volucre*	0	0	3	0
	A. ervi	0	4	2	1
3	*P. volucre*	0	5	1	3

In the first 1996 trial, a total of 368 *P. volucre* were recovered from the cereal trap plants, of which 87% formed on plants 1 and 2 (see Fig. 4.1a), i.e. the plant with the pheromone lure and the plant placed only 20 cm away from it (Table 4.5). There was no significant difference between the numbers recovered from plants 1 and 2. Only six *A. rhopalosiphi* were recovered, five of which were on plants 1 and 2 (Table 4.5).

Table 4.5. *Number of parasitized cereal aphids recovered from trap plants placed at the margin of a cereal stubble field in autumn 1996. A pheromone lure releasing nepetalactone was attached to plant 1 (see Fig. 4.1a).*

Plant	Distance from Lure	*P. volucre* Recovered	*A. rhopalosiphi* Recovered
1	0	154	1
2	0.2 m	165	4
3	15.2 m	18	0
4	15.4 m	31	1

In the second 1996 trial, a total of 202 *P. volucre* were recovered from the trap plants, of which 169 (84%) occurred on the pheromone-baited group of plants. Similarly, a total of 112 *A. rhopalosiphi* were recovered, of which 95 (85%) occurred on the pheromone-baited group of plants. However, there was a difference between the two parasitoid species in their pattern of distribution amongst the individual plants. In the case of *P. volucre,* more parasitoids were recovered from the central plant of each group of five than was recovered from each of the outer four plants, regardless of the presence of a pheromone lure (Table 4.6). In the case of *A. rhopalosiphi*, there was no significant difference between numbers recovered from the central plant and from each of the outer plants in either the pheromone-treated or control groups (Table 4.6).

This trial was repeated in 1997, when a total of 324 *P. volucre* were recovered from the cereal aphid trap plants, of which 229 (71%) occurred on the pheromone-baited group. A total of 387 *A. rhopalosiphi* were recovered from the same plants, of which 300 (78%) occurred on the pheromone-baited group. Significantly more *P. volucre* were recovered from the central plant of the pheromone-baited group (i.e. the plant to which the pheromone lure was attached) than from each of the outer plants (Table 4.6). However, in 1997 there was no significant effect of plant position on the number of *P. volucre* recovered from the control group of plants, and plant position had no effect on the number of *A. rhopalosiphi* recovered from either group of plants (Table 4.6).

In the second 1997 experiment, in which the outer plants were placed 3 m away from the central plant of each group, a total of 597 *P. volucre* were recovered, of which 372 (62%) occurred on the pheromone-baited group. However, the numbers recovered from the outer four plants, placed 3 m away from the pheromone-baited central plant, were not significantly different from those caught on plants in the unbaited control group (Table 4.6). Unfortunately, no *A. rhopalosiphi* were recovered from the cereal aphid trap plants in this trial.

Table 4.6. *Mean number per plant of parasitized cereal aphids recovered from trap plants, arranged in two groups of five, and placed in cereal stubble field margins in autumn 1996 and 1997. A pheromone lure releasing nepetalactone was attached to the central plant in one of the two groups in each experiment (see Fig. 4.1b), and the outer four plants were positioned 1 m or 3 m away from the central plant.*

Plant	Distance from Lure	Mean No. *P. volucre* Recovered / Plant	Mean No. *A. rhopalosiphi* Recovered / Plant
1996			
P (centre)	0 m	13.5	5.8
P (4 outer)	1 m	7.2	4.5
C (centre)	15 m	4.5	0.8
C (4 outer)	14-16 m	0.9	0.9
1997 (1st experiment)			
P (centre)	0 m	16.8	13.7
P (4 outer)	1 m	5.3	9.1
C (centre)	15 m	4.5	2.8
C (4 outer)	14-16 m	2.8	2.9
1997 (2nd experiment)			
P (centre)	0 m	30.0	0
P (4 outer)	3 m	11.1	0
C (centre)	15 m	9.0	0
C (4 outer)	12-18m	9.0	0

4.3.4 *Discussion*

The presence of synthetic aphid sex pheromone lures greatly increased the numbers of aphids parasitized in all the trap plant trials. The number of parasitized aphids developing on these plants in the autumn, especially in the later, more extensive trials was particularly encouraging as it indicated that there was still enough parasitoid activity around harvested fields to generate significant overwintering populations if appropriate aphid hosts were present. The trials also demonstrated that parasitoids attracted to or arrested by pheromone lures would actively search for and attack any suitable hosts in the immediate vicinity. These results support the laboratory observations that the presence of the pheromone does not interfere with parasitoid host searching and attack behaviour on the plant (Section 3.3).

It was also encouraging that all four parasitoid species recovered from aphids during the trials showed a positive response to the pheromone, even though the more specialist *Aphidius* species had never been caught in pheromone-baited water traps during previous studies. A possible explanation for this emerged during the 1996 and 1997 trials involving groups of five cereal trap plants. The generalist *P. volucre* parasitized more aphids on the central plant, to which the pheromone lure was attached, than on the surrounding four plants, even when these were placed only 1 m away from the lure. In contrast, the cereal aphid specialist *A. rhopalosiphi* attacked aphids to the same degree on all five plants in the group. This suggests a difference in foraging behaviour between the two parasitoid species. *Praon volucre* appears to fly directly to the source of the semiochemical stimulus (the pheromone lure) before it begins to search local plants for aphid hosts, whereas *A. rhopalosiphi* appears to be stimulated to start searching plants in the vicinity of the lure without flying to the source of the pheromone itself. This latter behaviour would result if landing and active plant searching was stimulated when the flying parasitoid encountered a critical concentration of the pheromone stimulus as it approached the lure, and would explain why *A. rhopalosiphi* was not caught in pheromone-baited traps in previous studies. Alternatively, *A. rhopalosiphi,* being a cereal specialist, is likely to respond to plant volatiles emitted by aphid infested cereal plants. MAFF-funded work at IACR-Rothamsted has demonstrated that the closely related parasitoid *Aphidius ervi* is strongly attracted by aphid-induced plant volatiles (Du *et al.*, 1996). Therefore, as the attracted *A. rhopalosiphi* females approached the infested trap plants, plant volatiles may have become the dominant cue, diverting them onto the plants before they reached the pheromone lure. Whatever the mechanism involved, the behavioural response shown by the *Aphidius* species would be an advantage in the manipulation strategy that we are developing, as it would lead to a more even distribution of parasitized aphids in pheromone-treated field margins.

The results of these trials also provide valuable information on the timing of pheromone lure deployment, particularly with regard to effects on the cereal aphid specialist *A. rhopalosiphi*. It was noticeable that trials with cereal aphid trap plants conducted in the latter half of August and the first half of September in both 1996 and 1997 attracted very few *A. rhopalosiphi*, although large numbers of *P. volucre* were recovered from the plants. *Aphidius rhopalosiphi* is known to undergo a summer diapause in August/September (thought to be triggered by declining host nutritional quality as a result of crop ripening), which could explain the lack of activity recorded in the trials done during this period. Trap plant trials done before (July) and after (October) this period attracted large numbers of *A. rhopalosiphi*. The generalist aphid parasitoid *P. volucre*, which can attack a range of aphid species, does not seem to require a summer diapause.

The spacing trials done in 1996 and 1997 demonstrated that a single pheromone lure can positively influence parasitization rates of aphids up to 1 metre away. Effects on *P. volucre* parasitization rates were not detected in aphid populations 3 metres from pheromone lures but, because *A. rhopalosiphi* apparently has a different foraging behaviour, it is possible that lures could influence the parasitization rate of this species over a greater area. Unfortunately, *A. rhopalosiphi* was not active during the period when the 3 m spacing trial was done, although results from a cereal plot trial done in 1996 support this hypothesis (see Section 4.4).

4.4 Effect of Synthetic Aphid Sex Pheromone Lures on Parasitization of Aphids in Field Plots

4.4.1 *Introduction*

The main aim of the parasitoid manipulation strategy being developed is to promote early-season synchronization between parasitoids and invading aphids in crop fields. It is hoped to achieve this by using synthetic aphid sex pheromone lures in late summer/autumn to establish overwintering concentrations of parasitoids in field margins, which will then be available to move into adjacent crops early in the following season. To encourage this movement into the crop, it may be useful to deploy aphid sex pheromone lures for a short period during aphid invasion in spring/early summer. To determine whether or not pheromone lures could influence parasitization rates in crop fields in summer, a replicated plot trial was designed and laid out in cereal and oilseed rape crops at IACR-Rothamsted in May 1996. At the same time, pea crops at the RASE farm at Sacrewell were monitored for aphids and parasitoids with a view to establishing a third trial.

Aphid populations were very small in 1996 and were not sufficient to allow the experiment to proceed in either the oilseed rape crop at Rothamsted or the pea crop at Sacrewell. However, aphids did eventually arrive in the winter wheat crop, although much later than normal, and an expanded experiment was done in this crop. In response to the results obtained in 1996, a modified replicated plot experiment was also done in winer wheat in the summer of 1997.

4.4.2 *Methods*

4.4.2.1 1996 cereal plot trial

The experiment was done in a crop of winter wheat sown in October 1995. Individual plots measured 6 m x 6 m and four replicate plots were used for each treatment. The treatments were:

- Untreated control
- 1 pheromone lure placed at the centre of the plot
- 2 pheromone lures placed at the centre of the plot
- 5 pheromone lures placed at the centre of the plot

All the lures used in the trial contained the aphid sex pheromone component nepetalactone. The plots were laid out in a neighbour-balanced latin square design, and adjacent plots were separated by 6 m paths. Every week between June 1 and August 6, aphids and parasitoid mummies were counted on six wheat plants at each of 16 sampling positions in each plot. Four sampling positions were evenly spaced along each of four straight lines running between the lure at the centre of the plot and each of the four corners of the plot (Fig. 4.2).

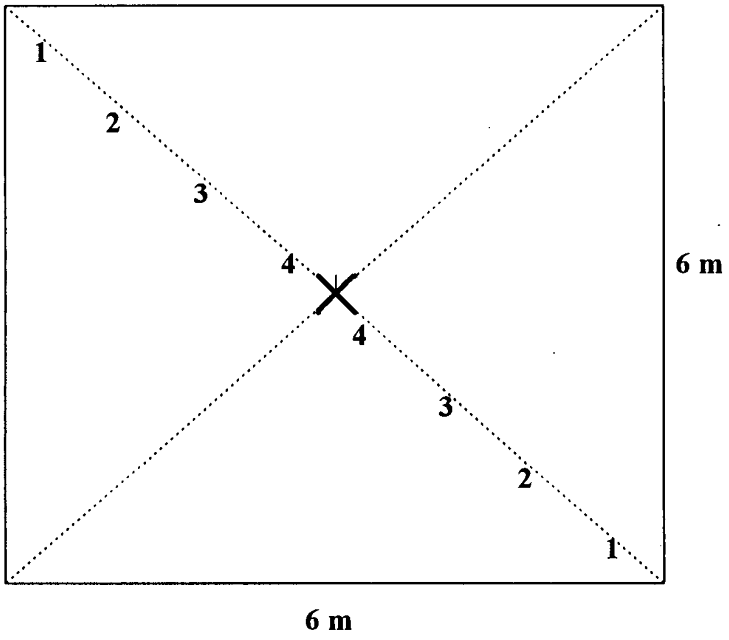

Fig. 4.2. *Positions of sampling points along the four diagonals of the winter wheat plots used for the 1996 field plot trial.* **X** *= position of aphid sex pheromone lures.*

4.4.2.2 1997 cereal plot trial

Because there were no significant differences in parasitization rates between the different pheromone treatments (1, 2 or 5 lures) in the 1996 trial, only the single pheromone lure treatment and the untreated controls were used in the 1997 experiment. The trial was again done in winter wheat and plot size was the same as in 1996, but in 1997 each treatment was replicated five times. Lures were placed in plots in May and, at weekly intervals between June 10 and July 22, aphids and parasitoid mummies were counted on five wheat plants at each of 12 sampling positions in each plot (three sampling positions evenly spaced along each line between the centre and each corner of the plot).

4.4.3 *Results*

4.4.3.1 1996 cereal plot trial

Aphids colonised the crop unusually late and were present in extremely small numbers until late June, only reaching a peak density of 10 aphids per tiller in late July (Fig. 4.3). Significantly more parasitoid mummies were counted in all the pheromone-treated plots than in the control plots, but the number of lures used did not significantly affect the number of mummies present (Table 4.7). Parasitised aphids appeared earlier and increased more rapidly in the pheromone-treated plots than in the control plots; mummy density being significantly greater in treated plots than in controls in the second half of July (Fig 4.3). The presence of pheromone lures appeared to increase mummy densities at all sampling positions throughout the plots (Table 4.8). Also, parasitoids were synchronised much more closely with aphid populations in the pheromone-treated plots than they were in the control plots (Fig. 4.3). However, because of the late arrival of aphids, parasitoids were scarce throughout the crop, and no significant effects of the treatments on aphid populations were detected. The parasitoids involved were predominantly *A. rhopalosiphi*, although some *P. volucre* were also recorded (Table 4.7).

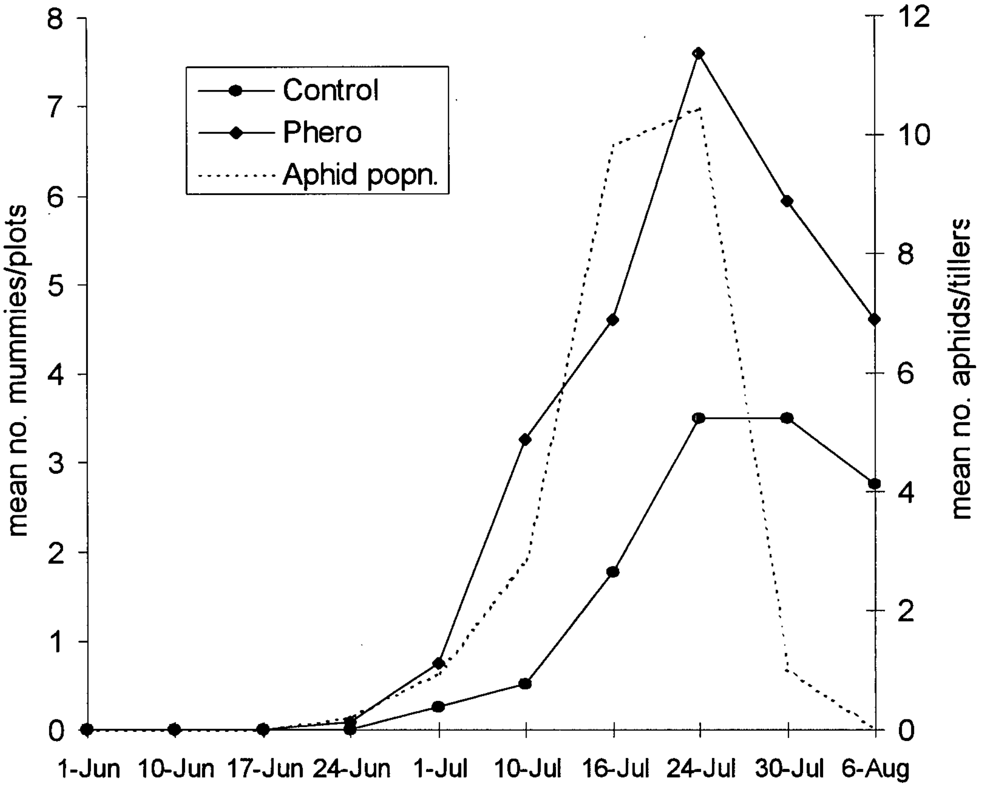

Fig. 4.3. *Mean number of parasitized aphids (mummies) recorded at sampling points in winter wheat plots with and without aphid sex pheromone lures in 1996, together with the overall mean aphid density.*

Table 4.7. *Parasitized aphids (mummies) recorded in winter wheat plots containing different numbers of synthetic aphid sex pheromone lures placed at the plot centre and in untreated control plots in June and July 1996 (see text for sampling details).*

Treatment	Mean No. *Praon* mummies / plot	Mean No. *Aphidius* mummies / plot	Total No. Mummies
Control	0.8	11.5	49
1 Pheromone lure	1.5	25.0	106
2 Pheromone lures	3.0	21.8	99
5 Pheromone lures	3.0	25.3	113

Table 4.8. *Mean number of parasitized aphids (mummies) recorded at four sampling distances from the centre of winter wheat plots, with and without synthetic aphid sex pheromone lures placed at the plot centre. Sampling position 1 was furthest away from and position 4 was nearest to the lure.*

Treatment	Sampling Position 1	Sampling Position 2	Sampling position 3	Sampling Position 4
Control	3.3	3.5	2.8	3.0
Pheromone lures	5.8	5.8	7.0	7.8

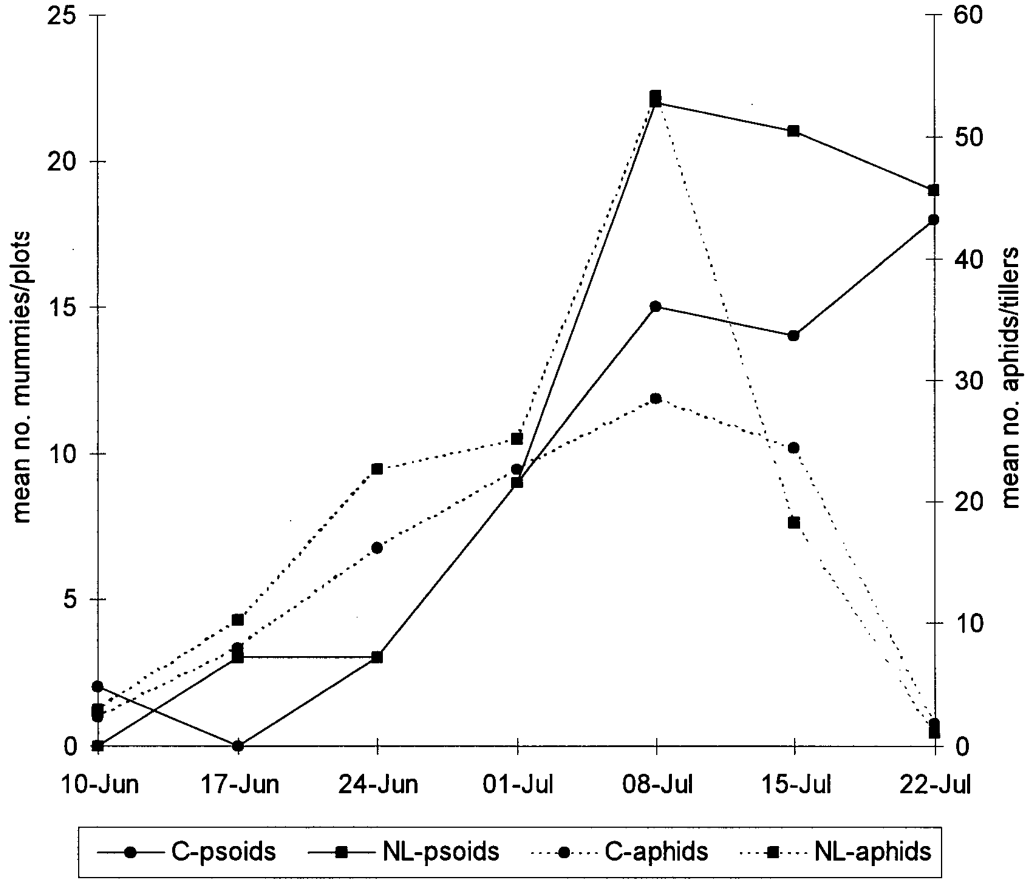

Fig. 4.4. *Mean number of parasitized aphids (mummies) and mean aphid density recorded at sampling points in winter wheat plots with and without aphid sex pheromone lures in 1997. C = control plots; NL = pheromone (nepetalactone) plots.*

4.4.3.2 1997 cereal plot trial

The presence of single pheromone lures in the centre of the 6 m x 6 m plots had no significant effect on either aphid or parasitoid mummy densities (Fig. 4.4). Over the seven week period of the experiment, means of 61 and 77 mummies per plot were recorded for the untreated control and pheromone-treated plots respectively.

4.4.4 *Discussion*

Due to a combination of the hot dry summer of 1995 and the cold, late spring of 1996, aphid populations were either unusually late in arriving in crops in 1996 or failed to arrive at all. In crops where aphids did arrive, such as cereals, numbers remained very low. This had a knock-on effect on natural parasitoid populations which couldn't find hosts in crops early in the season. Consequently, naturally-occurring parasitoid populations were small in 1996 and numbers remained small through into 1997. This meant that planned experiments in oilseed rape and pea crops had to be abandoned due to lack of insects, but cereal trials went ahead.

The results of the 1996 trial were encouraging, despite the small aphid and parasitoid populations present in the crop. The deployment of aphid sex pheromone lures improved the synchronisation between parasitoids and aphids and parasitoid densities built up more rapidly in the early stages of the aphid infestation. However, as aphid populations grew, the initial differential in parasitoid densities between treated and control plots was maintained but did not increase, suggesting that the pheromone ceased to have an effect after the first 2-3 weeks. Based on our knowledge of parasitoid foraging behaviour, gained from other MAFF-funded research at IACR-Rothamsted, we hypothesize that once aphid numbers began to build up on individual plants, female parasitoids then used aphid-induced plant volatiles as their main foraging cue. Therefore, to have the desired effect of attracting parasitoids into crops in spring, pheromone lures would need to be deployed immediately before and/or coincident with aphid colonisation. The timing of this could be determined from suction trap monitoring data. The lack of any detectable effect of the pheromone in the 1997 trial, however, was disappointing and casts doubts on the value of deploying lures in crops in spring and early summer. Ideally, the use of aphid sex pheromone lures early in the season needs to be invesigated in years when aphids and parasitoids are more abundant.

In the 1996 trial, increased densities of *A. rhopalosiphi* mummies were observed at all sampling points throughout the plot, even though the lures were only placed at the plot centre. This reinforces the results previously obtained with in the cereal aphid trap plant experiments (section 4.3), supporting the hypothesis that this specialist parasitoid speciesis stimulated by the pheromone to search plants in the vicinity of a lure without flying directly to the lure itself (see section 4.3.4).

4.5 Field Margin Design

The results of the trap plant experiments (section 4.3) indicate that aphid sex pheromone lures could be successfully deployed to attract/arrest dispersing aphid parasitoids in field margins in late summer/autumn. However, to establish a useful overwintering reservoir of parasitoids in field margin strips, the vegetation may need to be managed to provide the necessary host aphids and winter shelter. As part of this study, a replicated plot experiment was designed to investigate the suitability of different vegetation types for providing these resources. Three simple plant mixtures were sown in 10 m x 9 m plots, each replicated four times. A fourth treatment consisted of ploughing and allowing the natural seedbank to form the vegetation. The three sown plant mixtures were:

- cocksfoot grass (*Dactylis glomerata*) and annual meadow-grass (*Poa annua*)
- clover (*Trifolium* sp.) and annual meadow-grass

- oilseed rape (*Brassica napus*) and annual meadow-grass.

These were chosen to provide appropriate overwintering resources for the parasitoids of cereal aphids, pea aphids and brassica aphids, respectively.

The plots were sown in early May 1995 at IACR's Woburn Farm but, due to the hot, dry conditions that year, establishment was very slow and it was not possible to commence the experiment in the autumn of 1995, as originally planned. Indeed, the poor establishment of these plots within the life of the project prevented any meaningful data on aphid and parasitoid overwintering from being collected and the trial had to be abandoned.

In retrospect, a better approach may have been to monitor insect overwintering success in existing field margins and/or conservation headlands and set aside areas rather than trying to establish new experimental plots.

5 GENERAL DISCUSSION

Knowledge of the ecology and behaviour of beneficial insects has increased greatly over recent years and this is stimulating the development of novel methods for enhancing the biological control of insect pests through the manipulation of their predators and parasitoids. Parasitic wasps in particular offer exciting opportunities for manipulation because of their host finding behaviour and their importance as biological control agents. However, the success of parasitoid manipulation strategies will depend upon our ability to combine our understanding of their behaviour with our knowledge of the ecological constraints on their efficiency in agricultural systems. The manipulation strategy conceived as the basis of this project is a unique attempt to overcome an ecological constraint (the disruption of aphid-parasitoid synchronisation by climatic and agricultural husbandry factors) by behavioural manipulation using a semiochemical attractant (synthetic aphid sex pheromones).

This project has made considerable progress in confirming the potential of this approach for aphid control in field crops. The research has established that a wide range of aphid parasitoids are attracted to and/or arrested by synthetic aphid sex pheromones, and that pheromone lures can be used to greatly increase parasitization rates in aphid populations under field conditions. Most of the results obtained from both laboratory and field work support the theoretical concept behind the strategy and have helped to clarify a number of essential details for its development. We have demonstrated that there is sufficient autumn activity by parasitoids around the margins of arable fields to ensure the establishment of healthy overwintering populations if appropriate resources are available within those margins. Knowledge gained about the foraging patterns of specialist parasitoid species such as the cereal aphid specialist *Aphidius rhopalosiphi* suggests that they could be manipulated as readily and possibly more efficiently than the generalist species that were originally targeted. Furthermore, the simple lures containing the single pheromone component nepetalactone may be sufficient to influence a number of parasitoid species. The field results also indicate that parasitization rates can be significantly increased in aphids within at least a 1 metre radius of a lure, and probably over a greater area in the case of the specialist parasitoids.

Some of the initial scientific targets were not achieved as a direct result of the climatic conditions during the project which prevented the establishment of vegetation field trials and also resulted in poor field populations of both aphids and parasitoids, which were not sufficiently abundant to provide viable field trial results. The ultimate test of the strategy, under commercial farming conditions, therefore still needs to be done. It is proposed that the most appropriate approach at this stage would be to deploy lures in a range of field margins, choosing those already managed as wildlife strips, conservation headlands, set aside or grassland buffer strips and monitoring aphid and parasitoid populations in both the field margins and adjacent crops. Similar margins without lures could act as controls. Because the strategy involves the manipulation of natural parasitoid populations, the beneficial effects are likely to be cumulative over several years as local populations build up. Management of field margins for other purposes such as the conservation of carabid beetles or the enhancement of food resources for farmland birds is likely to be entirely compatible with the deployment of pheromone lures for parasitoid manipulation and could feasible aid its success.

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8 PUBLICATIONS AND PRESENTATIONS CONNECTED WITH THE PROJECT

8.1 Publications

Glinwood, R.T. (1998) Laboratory and field responses of parasitoids to aphid sex pheromones. *Unpublished Ph.D. Thesis*, University of Nottingham.

Hardie, J., Hick, A.J., Höller, C., Mann, J., Merritt, L., Nottingham, S.F., Powell, W., Wadhams, l.J., Witthinrich, J. & Wright, A.F. (1994) The responses of *Praon* spp. parasitoids to aphid sex pheromone components in the field. *Entomologia Experimentalis et Applicata* 71, 95-99.

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Smart, L.E., Pickett, J.A. & Powell, W. (1997) "Push-pull" strategies for pest control. *Grain Legumes* 15, 14-15.

8.2 Poster Presentations

Poster presentations were given at :

- 10th International Entomophagous Insects Workshop
  Vancouver, Canada - Sept. 1994
- MAFF Link Publicity Day - June 1994
- Gordon Conference on "Chemical/Biological Synergies to Reduce Inputs for Pest Control"
  Oxnard, California, USA - Feb. 1995.
- The Royal Show - July 1995

- 12th Annual Meeting of the International Society of Chemical Ecology
  Santiago, Chile - Oct. 1995
- The Smithfield Show - Nov. 1995
- Cereals 96 - June 1996
- PGRO Members Day - June 1996
- XX International Congress of Entomology
  Florence, Italy - Aug. 1996

8.3 Talks

Talks were presented to:

- Vegetable Agronomists Association (at PGRO) - Nov. 1994
- Suffolk Farmers Group - January 1995
- Herts Organic Gardeners Society - June 1995
- Chinese Agricultural Academy (Beijing, China) - Aug. 1995
- Vegetable Agonomists Association (at PGRO) - Jan. 1997
- Birds Eye Growers - Feb. 1997
- Society of Chemical Industries - May 1997
- Royal Entomological Society - May 1997
- Japanese Ministry of Agriculture (Tsukuba, Japan) - June 1997