Responses of Aphid Parasitoids to Aphid Sex Pheromones: Laboratory and Field Studies

by

Robert Thomas Glinwood $BSc_{(Hons)}$

Thesis submitted to The University of Nottingham for the degree of Doctor of Philosophy

January 1998

Entomology & Nematology Department
IACR Rothamsted Experimental Station
Harpenden
Hertfordshire AL5 2JQ

School of Biological Sciences
The University of Nottingham
University Park
Nottingham NG7 2RD

Contents

Abstract vi

Acknowledgements vii

List of Figures viii

List of Tables xii

Chapter 1 LITERATURE REVIEW AND INTRODUCTION

1.1 Aphids and Parasitoids in Agroecosystems 1

1.1.1 Aphids as Pests of Agricultural Crops 1

1.1.2 Aphid Life Cycles 3

1.1.3 Biology of Aphid Parasitoids (Braconidae: Aphidiinae) 5

1.1.4 Role of Aphidiinae in Aphid Population Regulation 7

1.2 Host Selection Process in Aphid Parasitoids 11

1.2.1 Habitat Location 13

1.2.2 Host Location 16

1.2.3 Host Acceptance 20

1.3 Aphid Sex Pheromones and Their Role as Kairomones in Parasitoid Host Location 22

1.3.1 Discovery, Identification and Biology of Aphid Sex Pheromones 22

1.3.2 Role of Aphid Sex Pheromones in Parasitoid Host Location 28

1.4 Responses of Other Parasitoids and Predators to Host Pheromones 31

1.5 Use of Aphidiinae for Biological Control of Aphids in The Field 36

1.5.1 Introduction and Inundative Release of Aphidiinae 36

1.5.2 Enhancement of Parasitoid Activity by Habitat Manipulation with Reference to Aphidiinae 39
1.6 Introduction to the Current Work 43
1.7 General Methods 44
1.7.1 Insects 44
1.7.2 Aphid Sex Pheromones 45

Chapter 2 FLIGHT RESPONSES OF APHID PARASITOIDS TO APHID SEX PHEROMONES

2.1 Introduction 47
2.2 Methods 48
2.2.1 Insects 48
2.2.2 Wind Tunnel Bioassay 48
2.2.3 Statistical Analysis 50
2.3 Flight Response of Four Previously Uninvestigated Aphid Parasitoid Species 51
2.3.1 Introduction
2.3.2 Results 52
2.3.3 Discussion 56
2.4 Flight Responses of Parasitoid Species Reared on Two Different Host Aphids 61
2.4.1 Introduction 61
2.4.2 Results 62
2.4.3 Discussion 65
2.5 Flight Responses of *Praon volucre* to Synthetic and Plant-Extracted Nepetalactone 67
2.5.1 Introduction 67
2.5.2 Results 68
2.5.3 Discussion 69

Chapter 3 LABORATORY INVESTIGATIONS INTO THE NATURE OF THE PARASITOID RESPONSE TO APHID SEX PHEROMONES

3.1	**Introduction**	71
3.2	**Effect of Experience on Aphid Parasitoid Response to Aphid Sex Pheromones in a Four-Way Olfactometer**	72
3.2.1	Introduction	72
3.2.2	Methods	73
3.2.3	Results	77
3.2.4	Discussion	80
3.3	**Effect of Pheromone Pre-Exposure on *Aphidius ervi* Attack Rate Against Two Aphids**	86
3.3.1	Introduction	86
3.3.2	Methods	87
3.3.3	Results	88
3.3.4	Discussion	90
3.4	**Effect of Experiencing Aphid Sex Pheromone at Parasitoid Emergence on Response of *Praon myzophagum***	92
3.4.1	Introduction	92
3.4.2	Methods	92
3.4.3	Results	93
3.4.4	Discussion	94
3.5	**Effect of Aphid Sex Pheromone on Retention of Parasitoids on Cereal Plants in the Laboratory**	96
3.5.1	Introduction	96
3.5.2	Methods	97
3.5.3	Results	98
3.5.4	Discussion	99

Chapter 4 INTERACTIONS BETWEEN APHID SEX PHEROMONE AND THE PLANT-HOST COMPLEX IN *Aphidius ervi*

4.1 Introduction 105

4.2 Methods 107

4.2.1 Insects 107

4.2.2 Wind Tunnel Bioassay 107

4.2.3 Statistical Analysis 108

4.3 Results 109

4.3.1 Experiment I 109

4.3.2 Experiment II 110

4.4 Discussion 111

Chapter 5 FIELD RESPONSES OF APHID PARASITOIDS TO PHEROMONE-BAITED PLANTS AND TRAPS

5.1 Introduction 114

5.2 Active Distance of Aphid Sex Pheromone Lures, Measured by Parasitisation Rates on Aphid-Infested Plants 114

5.2.1 Introduction 114

5.2.2 Methods 115

5.2.3 Results 118

5.2.4 Discussion 123

5.3 The Response of Aphid Parasitoids to Synthetic and Plant-Extracted Nepetalactone in the Field 127

5.3.1 Introduction 127

5.3.2 Methods 128

5.3.3 Results 129

5.3.4 Discussion 129

5.4 Responses of *Diaeretiella rapae* to Aphid Sex Pheromone-Baited Water Traps in the Field 132

5.4.1 Introduction 132

5.4.2 Methods 133

5.4.3 Results 134

5.4.4 Discussion 136

Chapter 6 FIELD TRIAL: EFFECT OF APHID SEX PHEROMONE ON PARASITISATION RATES IN A WINTER WHEAT CROP

6.1 Introduction 138

6.2 **Methods** 140

6.2.1 Experimental Sites 140

6.2.2 Experimental Treatments 141

6.2.3 Experimental Design 142

6.2.4 Assessment of Aphid Numbers and Parasitisation Levels 143

6.2.5 Statistical Analysis 145

6.3 Results 146

6.3.1 1996 Field Trial 146

6.3.2 1997 Field Trial 153

6.4 Discussion 159

6.4.1 1996 Field Trial 159

6.4.2 1997 Field Trial 161

6.4.3 Conclusions and Future Directions 162

Chapter 7 GENERAL DISCUSSION 164

References 171

Appendix 194

Abstract

The behavioural responses of aphid parasitoids (Hymenoptera: Braconidae: Aphidiinae) to aphid sex pheromones were investigated in the laboratory and field. In a wind tunnel bioassay, *Aphidius eadyi, Aphidius rhopalosiphi, Diaeretiella rapae, Ephedrus plagiator, Praon myzophagum* and *Praon volucre* responded to the aphid sex pheromone components nepetalactone and nepetalactol. *P. myzophagum* reared on two different host aphid species showed different responses to combinations of nepetalactone and nepetalactol in the wind tunnel, indicating that long term laboratory rearing may influence parasitoid responses to aphid sex pheromones. The ability of two aphid parasitoids to learn aphid sex pheromones through prior exposure in the presence of host aphids was investigated. The generalist *E. plagiator* showed evidence of associative learning, whereas the specialist *Aphidius ervi* did not. When *A. ervi* was exposed to the pheromone without contact with host aphids, the parasitoid response was reduced by habituation. Exposure to aphid sex pheromone during laboratory host attack trials had no effect on the host attack behaviour of *A. ervi*. In laboratory cage experiments, aphid sex pheromone lures increased the retention of *A. rhopalosiphi,* but not by *Praon volucre*, on aphid-infested plants. In a wind tunnel bioassay, aphid sex pheromone enhanced the attraction of *A. ervi* to a plant-host complex. In the field, aphid sex pheromone lures increased parasitisation rates by *A. rhopalosiphi* and *P. volucre* on aphid-infested potted plants. A series of potted plant experiments indicated that the pheromone may increase parasitisation of aphids by *A. rhopalosiphi*, but not *P. volucre*, at a distance of 1m away from the lure. The effect of baiting plots of winter wheat with aphid sex pheromone was investigated in two field experiments. In 1996, the number of parasitoid mummies was higher in baited plots than in unbaited plots, and the synchrony between aphid and parasitoid populations was closer in baited plots. In 1997, aphid sex pheromone had no effect on parasitisation levels. The results are discussed in the context of developing a novel aphid control strategy based on the use of aphid sex pheromones to manipulate parasitoid populations.

Acknowledgements

This project was funded under the MAFF-LINK programme 'Strategies for sustainable farming systems', and was jointly funded by the Ministry of Agriculture, Fisheries and Food, the Home-Grown Cereals Authority, the Horticultural Development Council and the Processors and Growers Research Organisation.

I would firstly like to acknowledge the support and guidance of my supervisors Dr Wilf Powell and Dr Lester Wadhams at Rothamsted, and Dr Francis Gilbert at Nottingham.

I would like to thank Dave Brooks for supplying aphids and parasitoids, and maintaining the laboratory cultures, and Linda Merrit and Diane Smiley for the synthesis, extraction and formulation of aphid sex pheromones. Thanks to Suzanne Clark for statistical analysis and advice, and to Albert Callewaert and Malcolm Hockham for assistance with glasshouse facilities.

I would like to thank the following colleagues who have provided assistance and advice during the project; Professor John Pickett, Dr Guy Poppy, Dr Diane Smiley, Dr Jon Marshall, and especially to Lesley Smart and Janet Martin.

A big thank you to all the friends I've made during my time at Rothamsted, especially to Jon and Dave.

Finally I would like to extend my warmest appreciation to my mother, father and sister, and to Sarah for all their help and support.

List of Figures

Figure 1.1 Generalised lifecycle of a holocyclic aphid. 4

Figure 1.2 Chemical structures of aphid sex pheromone components. (+)-(4aS,7S,7aR)-nepetalactone I; (-)-(1R,4aS,7S,7aR)-nepetalactol II; (4aR,7S,7aS)-nepetalactol III. 25

Figure 3.1 Four-way olfactometer showing the four arm regions. 74

Figure 3.2a Effect of aphid sex pheromone on retention of female *Praon volucre* on caged barley plants infested with 200 *Sitobion avenae*. 100

Figure 3.2b Effect of aphid sex pheromone on retention of female *Praon volucre* on caged barley plants infested with 20 *Sitobion avenae*. 100

Figure 3.3a Mean % parasitisation (± standard error) by *Praon volucre* on caged barley plants infested with 200 *Sitobion avenae*. 101

Figure 3.3b Mean % parasitisation (± standard error) by *Praon volucre* on caged barley plants infested with 20 *Sitobion avenae*. 101

Figure 3.4a Effect of aphid sex pheromone on retention of female *Aphidius rhopalosiphi* on caged barley plants infested with 200 *Sitobion avenae.* 102

Figure 3.4b Mean % parasitisation (± standard error) by *Aphidius rhopalosiphi* on caged barley plants infested with 200 *Sitobion avenae.* 102

Figure 5.1 Experimental layout of potted plant experiments in the field. 117

Figure 5.2 Mean number of *Praon* mummies recorded on aphid-infested plants. 119

Figure 5.3 Mean number of *Praon* and *Aphidius* mummies recorded on aphid-infested plants in 1996. 121

Figure 5.4 Mean number of *Praon* and *Aphidius* mummies recorded on aphid-infested plants in 1997. 122

Figure 5.5 Mean number of *Praon* mummies recorded on aphid-infested plants. 123

Figure 5.6 The mean number of *Praon* species mummies recorded from aphid-infested plants baited with synthetic or plant-extracted nepetalactone, after exposure in the field for 3 days. 130

Figure 5.7 Capture of male and female *Diaeretiella rapae* in pheromone-baited water traps placed in oilseed rape fields in the summers of 1995 and 1996. 135

Figure 6.1 4x4 neighbour balanced Latin square design used in 1996. 142

Figure 6.2 5x5 neighbour balanced Latin square design used in 1997. 143

Figure 6.3 Layout of experimental plot and sampling protocol in 1996 trial. 144

Figure 6.4 Layout of experimental plot and sampling protocol in 1997 trial. 145

Figure 6.5 Percentage parasitisation of aphids in pheromone-treated and control winter wheat plots in 1996 field trial. 148

Figure 6.6 Aphid population (mean no. aphids/tiller) in pheromone-baited and control winter wheat plots in 1996 field trial. 149

Figure 6.7 Numbers of mummies in pheromone-baited and control winter wheat plots, and the aphid population in 1996 field trial. 150

Figure 6.8 Mean number of mummies/sample occasion (± standard error) at different positions within pheromone and control winter wheat plots in 1996 field trial. 151

Figure 6.9 Total numbers of *Praon* and *Aphidius* mummies recorded from pheromone and control winter wheat plots in 1996 field trial. 152

Figure 6.10 Percentage parasitisation in winter wheat plots treated with semiochemicals in 1997 field trial. 155

Figure 6.11 Aphid populations (mean no. aphids/tiller) in winter wheat plots treated with semiochemicals in 1997 field trial. 156

Figure 6.12 Mean number of mummies/sampling occasion (± standard error) at different positions within winter wheat plots in 1997 field trial. 157

Figure 6.13 Total numbers of *Praon* and *Aphidius* mummies recorded from winter wheat plots treated with semiochemicals in 1997 field trial. 158

List of Tables

Table 1.1 The sex pheromones of some economically important aphid species. 26

Table 1.2 Details of parasitoid cultures used in the experimental work. 45

Table 2.1 Wind speed and lighting conditions for aphid parasitoid species used in wind tunnel bioassays. 49

Table 2.2 Responses of female *Aphidius rhopalosiphi* to neptalactone and nepetalactol, a 1:1 ratio and control in a wind tunnel bioassay. 53

Table 2.3 Responses of female *Aphidius eadyi* to neptalactone and nepetalactol, a 1:1 ratio and control in a wind tunnel bioassay. 54

Table 2.4 Responses of female *Ephedrus plagiator* to neptalactone and nepetalactol, a 1:1 ratio and control in a wind tunnel bioassay. 55

Table 2.5 Responses of *Diaeretiella rapae* to nepetalactone and control in a wind tunnel bioassay. 56

Table 2.6 Parasitoid species responding to aphid sex pheromones in a wind tunnel bioassay, the aphids which they attack and the crops in which they are active. 61

Table 2.7 Responses of female *Praon myzophagum* reared on two host species to nepetalactone and nepetalactol, a 1:1 ratio, a 1:2 ratio and control in a wind tunnel bioassay. 63

Table 2.8 Responses of female *Praon volucre* reared on two host species to nepetalactone, a 1:1 ratio and control in a wind tunnel bioassay. 64

Table 2.9 Responses of *Praon volucre* to synthetic and plant-extracted nepetalactone and control in a wind tunnel bioassay. 68

Table 3.1 Response of *Aphidius ervi,* subjected to various pre-treatments, to aphid sex pheromone in a four-way olfactometer. 78

Table 3.2 Response of *Ephedrus plagiator*, subjected to various pre-treatments, to aphid sex pheromone in a four-way olfactometer. 79

Table 3.3 The effect of pre-exposure to aphid sex pheromone on various aspects of the host acceptance behaviour of *Acyrthosiphon pisum*-reared *Aphidius ervi* against *A. pisum* and *Sitobion avenae.* 89

Table 3.4 The effect of pre-exposure to aphid sex pheromone on various aspects of the host acceptance behaviour of *Acyrthosiphon pisum*-reared *Aphidius ervi* against *Sitobion avenae*, with and without pheromone present during the bioassay. 90

Table 3.5 Responses of female *Praon myzophagum,* which experienced aphid sex pheromone during emergence, to aphid sex pheromone in a wind tunnel bioassay. 94

Table 4.1 Responses of female *Aphidius ervi* to uninfested plants and plant-host complex in combination with aphid sex pheromone. 109

Table 4.2 Responses of female *Aphidius ervi* to plant-host complex with 24 and 48 hours of aphid feeding in combination with aphid sex pheromone. 110

Table 6.1 Details of experimental winter wheat crops in which aphid sex pheromone field trials were performed. 140

Table 6.2 Semiochemical treatments with which experimental plots were baited in aphid sex pheromone field trials. 141

Table 6.3 Results of ANOVA on the number of aphids/tiller for comparisons between three levels of pheromone treatment, and between the mean for the pheromone treatments and the control in the 1996 field trial. 146

Table 6.4 Results of ANOVA on the number of mummies/plot for comparisons between three levels of pheromone treatment, and between the mean for the pheromone treatments and the control in the 1996 field trial. 147

Table 6.5 Grain yields from winter wheat plots treated with 1, 2 or 5 pheromone vials, and from control plots in 1996 field trial. 153

Table 6.6 Results of ANOVA on the number of aphids/tiller for comparisons between treatments in the 1997 field trial. 154

Table 6.7 Results of ANOVA on the number of mummies/plot for comparisons between treatments in the 1997 field trial. 154

Table 6.8 Grain yields from winter wheat plots treated with pheromone, pheromone + methyl salicylate, and from control plots. 158

Chapter 1

Literature Review And Introduction

1.1 Aphids and Parasitoids in Agroecosystems

1.1.1 Aphids as Pests of Agricultural Crops

Aphids (Homoptera: Aphididae) are one of the major insect pests of agricultural crops in temperate regions of the world. In Britain, they attack most economically important arable crops, often causing losses in yield. In cereals the most damaging species are the English grain aphid *Sitobion avenae* (F.), the bird cherry-oat aphid *Rhopalosiphum padi* (L.) and the rose grain aphid *Metopolophium dirhodum* (Walker). Leguminous crops are attacked by the pea aphid *Acyrthosiphon pisum* (Harris) and the black bean aphid *Aphis fabae* Scopoli. The cabbage aphid *Brevicoryne brassicae* (L.) and the peach-potato aphid *Myzus persicae* (Sulzer) are pests of brassica vegetables and oilseed rape.

Aphids damage crops in three ways; (1) by removing nutrients from the phloem, (2) through the toxic effects of their saliva, (3) by transmitting plant viruses. Although a large colony of feeding aphids can drain plants of vital nutrients, aphids probably do more harm due to what they put in, rather than take out of the plant. During feeding, saliva is discharged into the plant, and the toxic substances which it contains may have damaging effects, including local tissue senescence, leaf curling and gall formation (Miles, 1989). There is evidence that these substances can affect the whole plant via translocation, causing for example, reduced root growth, chlorosis and increased susceptibility to wilting (Miles, 1989). The most important role of aphids in agriculture is as vectors of plant viruses, which are readily acquired and transmitted between plants by the aphid and can have a major impact on crops (Vickerman and Wratten, 1979; Sylvester, 1989). Examples of important aphid-borne viruses are barley yellow dwarf virus (BYDV), vectored by *Rhopalosiphum padi* (Plumb, 1983), and cauliflower

mosaic virus (CaMV), which is transmitted by *Myzus persicae* (Sylvester, 1989).

In Britain, the most common aphid in cereals is usually *Sitobion avenae* (Carter *et al.,* 1980), which can cause yield losses of up to 12.5% in winter wheat (Tatchell, 1989). *Rhopalosiphum padi* causes yield losses of up to 15% in cereals by direct feeding (Kieckhefer and Kantack, 1986), but large populations of this species rarely occur in Britain and its major impact is through the transmission of BYDV, with losses of up to 86% having been reported (Doodson and Saunders, 1970; Plumb, 1976). Reviews of cereal aphids as pests are given by Vickerman and Wratten (1979) and Carter *et al.* (1980). In peas, direct feeding damage by *Acyrthosiphon pisum* can cause yield reductions of over 12% (Maiteki and Lamb, 1985). *Brevicoryne brassicae* may cause feeding damage to spring oilseed rape (Alford *et al.,* 1991), but is more important as a vector of cauliflower mosaic virus (CaMV) (Winfield, 1992). In brassica vegetables, although serious yield losses do not usually occur, visual damage caused by *B. brassicae* infestation may result in reduced market values (Anon., 1984). Although *Myzus persicae* is not generally considered a major pest of oilseed rape, it is a vector of beet western yellows virus (BWYV) (Alford *et al.*, 1991), and also carries viruses injurious to vegetables (Anon., 1983).

Because aphids can cause economic losses, they are heavily targeted with insecticides. In 1994, 57% of the total UK area of wheat was treated with insecticides, an area amounting to 1.3 million Ha (Anon., 1994). The treated areas of peas and oilseed rape were 66% and 73% respectively. Of all the treatments applied to wheat in 1994, only 10% was with the selective aphicide pirimicarb, and the figures for pirimicarb use in peas and oilseed rape were of a similar order. Therefore a large area of crop was treated with broad spectrum insecticides which can seriously damage natural enemy populations (Jepson, 1989). Extensive use of insecticides has resulted in the development of insecticide-resistance by some aphid species. Devonshire (1989) reports over 20 resistant species worldwide, with resistance being particularly severe and widespread in *M. persicae*.

1.1.2 Aphid Life Cycles

Many aphids which attack crops in Britain exploit two different host plants during their life cycle and are called heteroecious or host-alternating aphids. The overwintering host is usually a woody tree or shrub, and is sometimes referred to as the primary host, since it is often the plant on which the aphids are thought to have evolved (Shaposhnikov, 1987). Secondary hosts are generally short lived herbaceous plants, including annual crops. Host-alternating aphids usually produce a sexual form which gives rise to overwintering eggs, and this is referred to as the holocyclic life cycle.

Eggs hatch on the primary host plant in spring or summer and the initial generations (fundatrices and fundatrigeniae) give rise to alate (winged) emigrants which fly to the secondary host, where several female parthenogenetic generations occur (virginoparae). These are apterous (wingless), although alate virginoparae may be produced and fly to other secondary hosts if colonies become overcrowded. In the autumn, alate females (gynoparae) and males develop and fly to the primary host plant, where the gynoparae produce sexual females, the oviparae. It is the oviparae which release the aphid sex pheromone to attract males for mating. Eggs are laid on the primary host, and the developing embryos enter diapause until the following spring.

Some aphids have abandoned their primary hosts and complete their development entirely on a secondary host plant. These are referred to as autecious aphids. Some autecious aphids are holocyclic, but others can exist as parthenogenetic morphs throughout the year and are said to display the anholocyclic life cycle. Some species retain the ability to form holocyclic or anholocyclic clones depending on the severity of winter weather. A generalised holocyclic aphid life cycle is presented in figure 1.1.

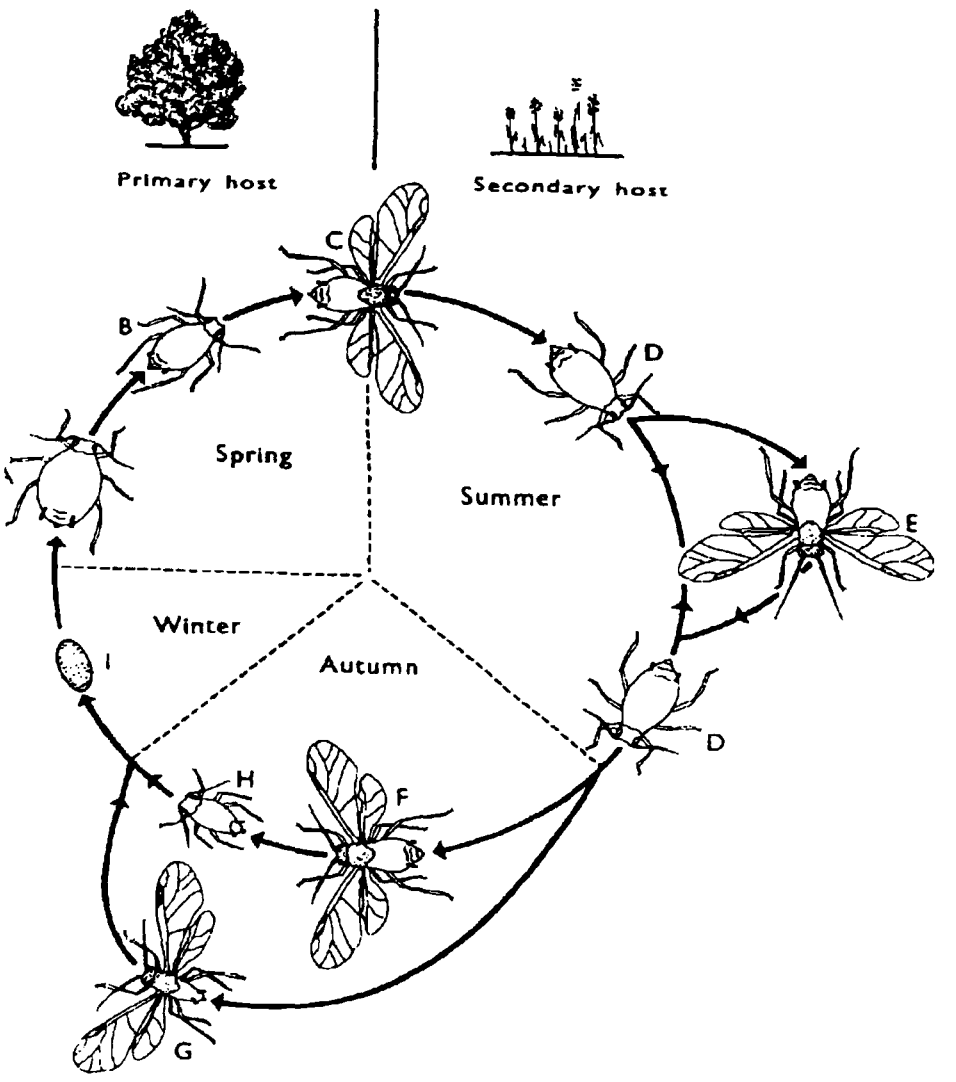

Figure 1.1 Generalised life cycle of a holocyclic aphid (A, fundatrix; B, fundatrigenia; C, emigrant; D, apterous virginopara; E, alate virginopara; F, gynopara; G, male; H, ovipara; I, egg) (modified from Dixon, 1973).

In Britain, aphids display a range of lifecycles. *Sitobion avenae* spends the entire year on grasses, and passes the winter either holocyclically or anholocyclically, depending on the weather (George, 1974; Hand, 1989). *Rhopalosiphum padi* may also overwinter in the parthenogenetic form (George, 1974; Hand, 1989). *Myzus persicae* has the capacity to adopt either life cycle, depending on the weather (Blackman, 1974; Leather, 1993), whereas *Brevicoryne brassicae* is commonly holocyclic in Britain (Chua, 1977), both species remaining on cruciferae. The pea aphid *Acyrthosiphon pisum* may be either holocyclic or anholocyclic in this

country (Leather, 1993), and feeds year-round on leguminous plants and crops. The black bean aphid *Aphis fabae* is holocyclic and alternates between beans and its primary host, spindle (*Euonymus europaeus* L.) (Blackman, 1974).

1.1.3 Biology of Aphid Parasitoids (Braconidae: Aphidiinae)

Aphids are attacked by two groups of hymenopterous parasitoids, the Aphidiinae (Ichneumonoidea: Braconidae) and the Aphelinidae (Chalcidoidea). However, it is the Aphidiinae which are more abundant and active in aphid population regulation. All members of this subfamily are parasitic exclusively upon aphids, whereas only a few aphelinids have become associated with aphids. The biology of the aphid-attacking Aphelinidae is reviewed by Starý (1988a).

Aphidiines are small black or brown wasps, ranging from one to several mm in size. They are all solitary endoparasitoids of aphids, each host supporting a single wasp. More than 400 species are known worldwide, and the distribution closely follows that of their aphid hosts, with the greatest diversity occurring in the temperate and subtropical belts of the northern hemisphere (Starý, 1988b). Although they are generally regarded as a subfamily, Aphidiinae, within the Braconidae, some authors place them within a separate family, Aphidiidae (Chow and Mackauer, 1992; Polgar *et al.*, 1995; Reed *et al.*, 1995).

Starý (1987) gives a subject bibliography of the Aphidiidae up to 1982, and comprehensive reviews dealing with many aspects of their biology are provided by Starý (1970, 1988b), Mackauer and Chow (1986) and Hågvar and Hofsvang (1991).

1.1.3a Life Cycle

During oviposition, the female parasitoid curls the abdomen forward, underneath the thorax and between the legs. She then advances towards the aphid and stings it, though an egg is not necessarily released. The duration of oviposition is species dependent, typically lasting 1-9 seconds (t' Hart *et al.*, 1975; Shirota *et al.*, 1983;

Hofsvang and Hågvar, 1986), although some species require up to one minute (Starý, 1988b).

On hatching, the membrane surrounding the egg disintegrates into separate cells, known as teratocytes, on which the developing larva initially feeds. Four larval instars are usually recognised, with only the final instar feeding on the host's tissues, ultimately causing death (Polaszek, 1986). Other parasitogenic effects on the host include the cessation of embryogenesis, slowed development and changes in behaviour (Starý, 1988b). The aphid is killed on formation of the parasitoid prepupa and only the aphid skin remains uneaten. This is attached to the substrate by a secretion from the parasitoid's silk glands and forms the distinctive aphid 'mummy'. The prepupal and pupal stages are passed inside the cocoon, which is spun inside, or in the case of *Praon*, beneath the mummy. In temperate regions, aphidiines often overwinter as diapausing prepupae within mummies (Mackauer and Chow, 1986; Starý, 1988b; Polgar *et al.*, 1995). This strategy improves synchrony with the host's life cycle, an important factor in the effectiveness of parasitoids in controlling pest populations (Gilbert and Gutierrez, 1973).

The adult parasitoid emerges from the pupa and leaves the mummy via a circular hole which it cuts in the abdomen. The adult food source is thought to consist entirely of aphid honeydew (Starý, 1988b). Reproduction is usually biparental and arrhenotokus (unfertilised eggs produce males, fertilised eggs produce females). Males of several aphidiine species have been shown to respond to sex pheromones released by their females (Askari and Alishah, 1979; Powell and Zhi-Li, 1983; Decker *et al.,* 1993; McNeil and Brodeur, 1995; Nazzi *et al.*, 1996), and these could prove valuable as monitoring tools in integrated control strategies (Decker *et al.,* 1993).

1.1.4 Role of Aphidiinae in Aphid Population Regulation

Aphid parasitoids do not by themselves regulate aphid populations in the field, they usually act in conjunction with a range of other natural enemies (Bode, 1980; Wratten and Powell, 1991). These include aphid specialist predators; ladybirds (Coleoptera: Coccinellidae) (Frazer, 1988), hoverflies (Diptera: Syrphidae) (Chambers and Adams, 1986), anthocorid bugs (Hodgson and Aveling, 1988) and lacewings (New, 1988), as well as more polyphagous groups; carabid and staphylinid beetles, linyphiid spiders (Edwards *et al.*, 1979; Sunderland, 1988) and certain gall midges (Dipt: Cecidomyiidae) (Nijveldt, 1988). Aphids are also infected by fungal pathogens (Dean and Wilding, 1973; Latgé and Papierok, 1988). Evidence suggests that the Aphidiinae play a critical role in this enemy complex, especially during the initial phase of aphid population increase (Dean, 1974; Carter *et al.*, 1980; Fougeroux *et al.*, 1988; Wratten and Powell, 1991).

In cereals, the dominant parasitoids are species of the genus *Aphidius* (Powell, 1982; Vickerman, 1982), and parasitisation rates in cereal aphids of up to 30% or even 50% have been recorded in some years (Dean, 1974; Powell, 1983; Giller *et al.*, 1995). The 1979 cereal aphid outbreak has been partly attributed to the low numbers of natural enemies, including parasitoids, which were present in that year (Dean *et al.*, 1980). By limiting the number of virus vectors, parasitoids may help to reduce virus spread between fields and from non crop plants to crops (Mackauer and Chow, 1986).

Diaeretiella rapae (McIntosh) is the major parasitoid of the cabbage aphid *Brevicoryne brassicae* in Europe (Nemec and Starý, 1984; Kelm, 1988) and North America (Sheehan and Shelton, 1989a; Elliot *et al.,* 1994). According to Lopez and van Driesche (1989), *D. rapae* has only limited effectiveness in controlling *B. brassicae* on brassica vegetables. However, Láska (1984) found that, although other natural enemies were more important overall, *D. rapae* arrived in the crop earlier, and became influential during periods when predator numbers dropped during the season. *D. rapae* may be more effective in oilseed rape. Kelm (1988)

found that *D. rapae* successfully limited the *B. brassicae* population increase and almost eliminated some colonies. In 1995, an unusually hot summer resulted in large populations of *B. brassicae* on oilseed rape in the UK. These populations supported huge numbers of *D. rapae* which subsequently colonised cauliflowers and other brassicas, effectively controlling aphids on these vegetable crops (Anon., 1995).

There is little information on the impact of Aphidiinae in other crops in Britain and Europe. However, the pea aphid *Acyrthosiphon pisum* has been controlled by parasitoids imported into California (Hagan and Schlinger, 1960) and New Zealand (Cameron *et al.*, 1981). By using gel electrophoresis to detect parasitised aphids, it has been shown that measurements based on mummy counts may underestimate rates of parasitism in the field by a factor of ten (Walton *et al.*, 1990; Giller *et al.,* 1995). Therefore the relative importance of parasitoids as aphid enemies may be greater than previously reported.

1.1.4a Importance of Early-Season Activity

In simulations based on field data, Carter *et al.* (1980) showed that when parasitoids were present, the rapid early-season aphid population increase was slowed. Field data also showed that parasitoid effectiveness was dependent upon their early arrival in the crop. In French cereal fields, Fougeroux *et al.* (1988) found a strong relationship between early parasitism and the maximum subsequent aphid population. In fields with 20% parasitism early in the season, aphid populations never exceeded the economic threshold, whereas in fields with little early parasitism, the threshold was greatly exceeded.

To fulfil their potential as control agents, aphid parasitoids must be present in or around the crop when the aphids begin to colonise it. Dean (1974) found that parasitoids were more likely to achieve this synchrony with initial aphid populations than other enemies which became more important later in the season. Chambers and Adams (1986) showed that parasitoids often arrived in winter

wheat fields before other predator groups, before dispersing from the crop later in the season, and Langer *et al.* (1997) found an early season peak in parasitoid activity.

To achieve this early-season synchrony, parasitoids must be able to overwinter successfully. Large numbers of parasitoids may build up on overwintering populations of aphids in winter crops sown early enough to receive an input of aphids and parasitoids in the autumn. Vorley and Wratten (1987) estimated that a single early-sown winter wheat field generated enough parasitoids in May to account for immigration into 25 late-sown fields. They suggested the use of early sowing, but recognised the potential problems of aphid and virus carry over, and parasitoid host preference effects. The effectiveness of parasitoids emerging early from overwintering cereal aphid populations has also been demonstrated by Powell (1983), Chambers and Adams (1986) and Vorley (1986), and grassland may also act as a reservoir for overwintering parasitoids (Vickerman, 1982; Vorley and Wratten, 1987).

In an attempt to experimentally reproduce early-season activity, *Metopolophium festucae* (Theobald), which had been exposed to *Aphidius rhopalosiphi* De Stefani Perez, was released into winter wheat plots (Wratten and Powell, 1991). Subsequent *Sitobion avenae* populations were significantly lower than in nearby control plots. From the evidence above, it can be concluded that parasitoids often hold the key to aphid population regulation, but that synchrony between parasitoid and aphid activity during the initial aphid population increase is vital.

1.1.4b Limitations on Aphid Parasitoids

Several factors may limit the impact of parasitoids on aphid populations. The important early-season synchrony between parasitoids and their hosts is frequently poor, due to factors such as crop sowing date, pesticide use and unfavourable climate. For instance, mild winter weather is known to favour early-season parasitoid activity (Bode, 1980; Vorley, 1986), while extended periods of

cold weather kill anholocyclic aphids in winter crops such as cereals, killing any parasitoids they contain in the process.

A number of studies have investigated the effects of insecticides on aphid parasitoids. Insecticide applied at field rates inhibited the emergence, and reduced the post-emergence survival of *Diaeretiella rapae* (Hsieh and Allen, 1986). Süss (1983) found that several compounds were toxic to larval *Aphidius ervi* Haliday, but that the mummy gave some degree of protection from pirimicarb. In laboratory trials (Gu and Waage, 1990), sublethal doses of pirimicarb disrupted the typical upward searching pattern of *D. rapae* on plants, as described by Ayal (1987). Similar results were reported by Umoru *et al.* (1996), who extended these studies to show that *D. rapae* spent less time searching on pirimicarb treated plants, even when aphids were present, and that the effect persisted for at least 24 hours after application. Longley (1994) found that deltamethrin prevented *Aphidius rhopalosiphi* from locating honeydew on filter paper disks. However, it was not clear whether this effect was due to repellency or to the masking of honeydew odours. In field trials, application of contact and systemic insecticides to winter wheat had no effect on percentage parasitism or parasitoid abundance (Giller *et al.*, 1995). However, it is possible that these applications were made prior to aphid and parasitoid immigration. Furthermore, effects may occur in subsequent parasitoid generations (P. Umoru, personal communication), but these were not addressed in this study.

Aphidiines are themselves attacked by secondary parasitoids (hyperparasitoids), belonging to the families Cynipidae, Encyrtidae, Eulophidae, Megaspilidae and Pteromalidae. Their biology and taxonomy are reviewed by Sullivan (1987, 1988). Hyperparasitoids may be either endoparasitic or ectoparasitic, depending upon whether they place an egg within the aphidiine larva in the live aphid prior to mummification, or onto the surface of the aphidiine larva inside the mummy itself. Hyperparasitism has been studied in cereal aphids (Höller *et al.*, 1993), pea aphids (van den Bosch *et al.*, 1982) and cabbage aphids (Horn, 1989).

Opinions differ regarding the severity of hyperparasitoid effects on aphid parasitoid effectiveness. During the latter part of the growing season, secondary parasitism can be spectacularly high, sometimes exceeding 90% (Sullivan and van den Bosch, 1971; Horn 1989; Völkl, 1992; Höller *et al*, 1993). However, Fougeroux *et al.* (1988) recorded levels of only 3% in French cereal crops at this time, and Vickerman and Wratten (1979) also considered that hyperparasitoids had little impact in their cereal study crop. Mackauer and Völkl (1993) proposed that aphidiines were limited by foraging efficiency and oviposition decisions, not by hyperparasitoids. It has even been argued that hyperparasitoids may prove beneficial, by damping population fluctuations and increasing stability in some ecosystems (Sullivan, 1987, 1988).

Höller *et al.* (1993) suggested that parasitoid females actively leave areas which have high hyperparasitoid densities. Höller *et al.* (1994) present evidence of leaving by parasitoids in the presence of female, but not male, hyperparasitoids, and suggest that this is mediated through detection of a hyperparasitoid-produced volatile semiochemical. The importance of hyperparasitoids is still unclear, especially since their major impact appears to occur late in the growing season, whereas aphid parasitoids have their greatest effect as control agents at the start the season.

1.2 Host Selection Process in Aphid Parasitoids

Female parasitoids must be able to locate suitable hosts in which to oviposit, ensuring the production of viable progeny. Although in many of their actions parasitoids resemble predators, parasitoids are distinguished by a high correlation between the number of hosts attacked and offspring produced. Since each host attacked usually results in a new parasitoid, host selection has a critical influence upon the final rate of parasitisation, and an understanding of the host selection process is vital when considering the use of parasitoids in biological control.

The ability to locate suitable hosts is particularly important for aphid parasitoids because female wasps may find themselves located some distance away from aphid populations. This may be the result of emerging in an environment which lacks suitable hosts, such as within a crop from which aphids have dispersed or on an aphid primary host plant (Starý, 1988b), or it may be the result of the parasitoids themselves needing to disperse from a habitat which is no longer suitable (Vinson, 1981).

Parasitoids locate and select hosts by gradually reducing the area to be searched, via a series of behavioural steps. In this review, the terminology used will be that of Vinson (1976, 1981, 1984), who has described 5 steps to successful parasitisation:

1. Host habitat location: The female parasitoid locates a habitat containing suitable host plants and hosts.

2. Host location (host finding): the parasitoid conducts a local search for hosts, on or near the host plant.

3. Host acceptance (host recognition): the parasitoid examines the host and determines its suitability for oviposition.

4. Host suitability: development of the parasitoid egg is determined by genetic and physiological factors of the host.

5. Host regulation: the developing parasitoid may influence the development, behaviour and physiology of the host.

Steps 1-3 concern the behaviour of the adult parasitoid and the use of olfactory, visual, tactile and other cues to physically locate and assess the host. Semiochemicals from the host, the host plant or the plant-host complex have been shown to be particularly important in this process. Only steps 1-3 will be discussed here, but Vinson (1976, 1984) reviews the entire host selection process for parasitoids in general, and Hågvar and Hofsvang (1991) provide an account relating specifically to the Aphidiinae. Habitat location is reviewed by Vinson

(1981) and host location by Weseloh (1981). Arthur (1991) reviews host acceptance and Vinson and Iwantsch (1980a,b) deal with host suitability and host regulation.

1.2.1 Habitat Location

This is the initial stage in the location of hosts by female parasitoids. Some authors have further divided it into three steps; habitat preference, habitat location and potential host-community location (Vinson, 1984; Hågvar and Hofsvang, 1991). Aphid parasitoids use visual and olfactory cues in habitat location, with odours emitted by host plants and/or the plant-host complex being important in attraction to, and arrestment within the host-community (Vet and Dicke, 1992).

Two studies have addressed the role of vision in habitat location. Vater (1971) demonstrated that *Diaeretiella rapae* was attracted to light from the green region of the electromagnetic spectrum, and that females were able to discriminate between several shades of green. *Aphidius ervi* was also attracted to green targets in a flight chamber (Goff and Nault, 1984).

A great deal of interest in habitat location by aphid parasitoids has focused on the use of olfactory cues, particularly by the crucifer-specialist parasitoid, *D. rapae*. In olfactometer tests, Read *et al.* (1970) demonstrated attraction of *D. rapae* females to collard (*Brassica oleraceae* L.) foliage and leaf juices, and to allyl isothiocyanate (although allyl isothiocyanate is found in only a few crucifer species, it is often used in experiments as a cheaper alternative to more widely occurring isothiocyanates). The same authors found that when female *D. rapae* were given a choice between *Myzus persicae* feeding on collard or beet, they preferentially attacked those feeding on collard. The application of an allyl isothiocyanate solution to the beet plants did not render them attractive to the parasitoids. Akinlosotu (1980) also found that *D. rapae* was attracted to allyl isothiocyanate in an olfactometer, and that Brussels sprouts leaves were more attractive than 50 cabbage aphids. In both cases, the authors concluded that

searching *D. rapae* use crucifer volatiles to reach the habitat, and then locate hosts via random search. Recently, allyl isothiocyanate was found to elicit an electrophysiological response from the antennae of female *D. rapae*, recorded by electroantennogram (EAG) (Vaughn *et al.*, 1996).

In wind tunnel tests, *D. rapae* showed an intrinsic preference for collard over a non-host plant (Sheehan and Shelton, 1989b). Wasps which were reared, or had post-emergence experience, on collards showed an increased flight response to these plants. However, the response to a non-host plant, potato, was not increased by post-emergence experience. The authors stressed the importance of habitat examining as well as habitat finding. Indeed, in further tests with *D. rapae* searching in patches of potted collards, larger patches were not more likely to be found than smaller ones, nor were patches containing hosts more likely to be found than host-free patches (Sheehan and Shelton, 1989a). In dispersal cages, the leaving rate of *D. rapae* was found to decrease with increasing patch size, although the presence of hosts again had no effect (Sheehan and Shelton, 1989a). It appears from these experiments that, in *D. rapae*, leaving rate and residence time in the habitat are at least equally important as habitat location.

Titayavan and Altieri (1990) studied the effects of applying an emulsion of allyl isothiocyanate to broccoli plants in the field. An application of 0.25 ml/plant resulted in increased parasitisation of *Brevicoryne brassicae* by *D. rapae*, compared with applications of water or wild mushroom extract. However, in field trapping experiments with water traps (Sheehan and Shelton, 1989a), releasing allyl isothiocyanate at ecologically relevant levels did not increase the capture of *D. rapae*. The authors suggested that these cues may operate over only a short distance, and that other chemicals may be active in long range attraction. Horn (1984) found that parasitisation of *Myzus persicae* was lower in collard plots in which weeds had been allowed to grow, compared to weedless plots. This suggests that a complex vegetational background may interfere with habitat finding by a specialist parasitoid such as *D. rapae*.

In olfactometer tests, Reed *et al.* (1995) found no attraction of *D. rapae* to cabbage leaves. Wasps were however attracted to cabbage leaves infested with *B. brassicae,* and the response to these was greater than to wheat leaves infested with Russian wheat aphid, *Diuraphis noxia* Mordwilko. This suggests an innate preference for the crucifer plant-aphid system, and the results indicate that the plant-host complex may be important in habitat location by *D. rapae.*

For other parasitoid species, there is inconsistent evidence as to the relative importance of volatiles from the plant alone, or from the plant-host complex. In a Y-tube olfactometer, *Lysiphlebus testaceipes* (Cresson) responded equally strongly to sorghum leaves and to its host aphid *Schizaphis graminum* (Rondani) (Schuster and Starks, 1974). The authors suggested that responses to the plant and to the aphid could operate either simultaneously or sequentially. In wind tunnel tests, naive *L. testaceipes* made significantly more upwind flights to wheat infested with *S. graminum* than to uninfested wheat (Grasswitz and Paine, 1993). This suggests an innate response, although the authors recognised that the response may have been influenced by a 'chemical legacy' from the larval meconium or cocoon (Corbet, 1985). It is possible that Y-tube olfactometer and wind tunnel bioassays test parasitoid responses at different stages of foraging. Depending upon whether the insect is walking or flying, volatile cues may assume different priorities.

The cereal aphid parasitoids, *Aphidius uzbekistanicus* Luzhetski and *Aphidius ervi*, responded to the uninfested leaves of their host plants in an olfactometer (Powell and Zhi-Li, 1983). Wickremasinghe and van Emden (1992) tested the responses of several aphid parasitoids, including *A. ervi*, *A. rhopalosiphi* and *Praon* species, to aphids and host plants in a Y-tube olfactometer. They found that the response to the plant on which the wasps were reared was greater than to their host aphids, but that the strongest response was to a plant-host complex. The same authors found that *A. rhopalosiphi* was attracted to three volatile chemicals, *cis*-3-hexenyl acetate, *cis*-3-hexen-1-ol and *trans*-2-hexenal, which occur in wheat

leaves (Buttery *et al.,* 1985), and that naive *A. rhopalosiphi* showed a preference for the particular variety of wheat on which they had developed. When tested in a 4-choice olfactometer, *Aphidius nigripes* Ashmead showed no response to potato or barley, the food plants of its hosts (Bouchard and Cloutier, 1985). Since *A. nigripes* is known to attack a range of polyphagous aphids, it may be disadvantageous for it to respond strongly to specific plant odours. In this case, the response to a plant-host complex was not tested.

Hågvar and Hofsvang (1987) released and observed *Ephedrus cerasicola* Starý in glasshouse compartments. When an array of plants with increasing densities of *M. persicae* were presented, wasps accumulated on the plants with the highest aphid densities. However, when all plants were uninfested, parasitoids were recovered in significantly fewer numbers from the plants. Timed observations revealed that this was due to parasitoids failing to locate uninfested plants rather than leaving from them in greater numbers. It was concluded that *E. cerasicola* uses aphid-induced plant volatiles in habitat location.

Aphidius ervi also uses herbivore-induced synomones in habitat location. In wind tunnel tests, wasps made more upwind flights in response to a plant-host complex than to either aphids or the plant alone (Guerrieri *et al.,* 1993). Wasps also responded to a host-damaged plant, from which the aphids had been removed. Du *et al.* (1996) also demonstrated the response of *A. ervi* to a plant-host complex (*Acyrthosiphon pisum* feeding on broad bean). This response was stronger than to undamaged or mechanically damaged plants. Furthermore, when presented with a choice between *A. pisum* and an inappropriate host, *Aphis fabae* feeding on bean, more flights were made to the *A. pisum* complex indicating the presence of aphid-specific, feeding-induced synomones.

1.2.2 Host Location

Once in a suitable habitat, the female parasitoid searches for hosts on, or near the host plant using physical, visual and olfactory stimuli. The olfactory cues used at

this stage are usually considered to operate over a relatively short range or on contact (Vinson, 1984), and may originate from the hosts themselves or from host products. They affect aspects of the wasp's behaviour such as walking speed, time spent searching and the angle and frequency of turning, and orientate the wasp to areas where hosts are, or have been, located.

Wickremasinghe and van Emden (1992) tested the responses of several parasitoid species in a Y-tube olfactometer. *Aphidius rhopalosiphi, Lysiphlebus fabarum* (Marsh.), a *Trioxys* species and a species of *Praon* were all attracted to their respective host aphids. *Aphidius ervi* was attracted to the nettle aphid *Microlophium carnosum* (Buckton). However, in another experiment *A. ervi* showed no response to nettle aphids (Powell and Zhi-Li, 1983), suggesting the existence of specialised races in the field. Genetic investigations (Nemec and Starý, 1983) and morphological and behavioural studies have resulted in the description of the nettle-system *A. ervi* as a separate species, *Aphidius microlophii* Pennacchio and Tremblay (Pennacchio and Tremblay, 1987). The *A. ervi* used in the experiments of Wickremasinghe and van Emden (1983) were collected from nettles, so their work supports this.

A. ervi has been shown to respond to the cereal aphid *Metopolophium dirhodum* (Powell and Zhi-Li, 1983) and the same authors demonstrated attraction of *Aphidius uzbekistanicus* to *Sitobion avenae* and *M. dirhodum. Lysiphlebus testaceipes* was attracted to its host aphid *Schizaphis graminum* in a Y-tube olfactometer (Schuster and Starks, 1974) and Bouchard and Cloutier (1985) used a 4-choice bioassay to show that *Aphidius nigripes* responded not only to its preferred hosts *Macrosiphum euphorbiae* (Thomas) and *Myzus persicae*, but also to the less favoured hosts *Aphis nasturtii* (Kaltenbach) and *Rhopalosiphum maidis* Fitch.

Plant architecture is an important factor in the host finding behaviour of *Aphidius rhopalosiphi* on wheat (Gardner and Dixon, 1985). Wasps prefer to search on the

leaves rather than on the ear, perhaps due to difficulty in walking on this structure. Since the ear is the preferred feeding site of *Sitobion avenae*, the parasitoids attack this aphid less once the ear has formed. Therefore, the host range of *A. rhopalosiphi* is a product of host finding behaviour as well as host preference.

Honeydew, an aphid waste product, has been the focus of considerable interest in parasitoid host finding studies. Gardner and Dixon (1985) investigated the searching behaviour of *A. rhopalosiphi* on wheat. The presence of honeydew significantly increased the time the wasp spent searching on the plant. *A. nigripes* spent twice as long searching on potatoes contaminated with the honeydew of its host *Macrosiphum euphorbiae*, than on clean plants (Cloutier and Bauduin, 1990). Plants washed with water no longer elicited a searching response in *A. nigripes* (Bouchard and Cloutier, 1984) indicating that the water soluble honeydew was the stimulant. Ayal (1987) determined a search pattern for *D. rapae* on Brussels sprouts. The wasps used honeydew as a contact kairomone and, on encountering it, initiated a series of behaviours which brought them into contact with aphids feeding on the plant.

Hågvar and Hofsvang (1989) released *Ephedrus cerasicola* into glasshouses containing paprika plants (*Capiscum annuum* L.). Counts revealed that parasitoids aggregated in greater numbers on plants which were contaminated with honeydew from *Myzus persicae*. These authors also concluded that honeydew was serving as a contact kairomone, stimulating wasps to increase their residence time in contaminated areas. In similar experiments, Budenberg *et al.* (1992) released *A. rhopalosiphi* into dispersal cages containing wheat plants. The residence time of wasps was higher on honeydew contaminated plants, but the application of artificial honeydew did not result in increased parasitisation of *Sitobion avenae*. In tests performed in a petri dish arena, *A. rhopalosiphi* increased its searching time and decreased its walking speed in response to honeydew (Budenberg, 1990). When wasps were separated from the honeydew by a gauze, they did not respond, suggesting that honeydew functions as a contact kairomone. It should be noted

though, that *A. nigripes* responded to honeydew in an airflow olfactometer, suggesting the presence of unidentified volatile components (Bouchard and Cloutier, 1985).

The use of aphid honeydew as a host finding kairomone appears to be widespread among the Aphidiinae, the following species also having shown behavioural responses; *Aphidius smithi* Sharma and Subba Rao (McGregor and Mackauer, 1989), *Praon pequodorum* Viereck (Hood-Henderson and Forbes, 1988), *Aphidius ervi* (Budenberg, 1990; Wickremasinghe and van Emden, 1992), *Lysiphlebus fabarum* and a *Trioxys* species (both Wickremasinghe and van Emden, 1992) and *Aphidius picipes* (Nees), *Praon volucre* (Haliday) and *Ephedrus plagiator* (Nees) (all Budenberg, 1990).

Honeydew is a food resource for adult parasitoids (Starý, 1988b) and it is possible that the parasitoid response is related purely to this (Budenberg, 1990). However, the wasps used in most of the tests described above were fed with honey prior to testing, and since honeydew has repeatedly been shown to increase searching in aphid parasitoids (e.g. Gardner and Dixon, 1985; McGregor and Mackauer, 1989; Budenberg, 1990), it is likely that it operates as a search stimulant. Bouchard and Cloutier (1994) found that the application of sucrose solution (a component of honeydew) to leaves did not induce the same response in *A. nigripes* as did honeydew.

Olfactory cues originating from the host are generally considered to act over relatively short distances or on contact (Vinson, 1984). However, in field experiments, parasitoids of the genus *Praon* were attracted to traps baited with synthetic aphid sex pheromones (Hardie *et al.,* 1991; Powell *et al.,* 1993). Sex pheromones are released in the autumn by the sexual females of holocyclic aphid species and act as a reliable signal of host presence. Female parasitoids of the genus *Praon* were captured in pheromone-baited traps placed in cereal fields in the autumn, a habitat with which they are not normally associated (Hardie *et al.*,

1994). This suggests that parasitoids may respond to these host-derived cues over relatively large distances. Aphid sex pheromones and their role in parasitoid host finding are reviewed in section 1.3 below.

1.2.3 Host Acceptance

After locating a host, the parasitoid must decide whether to accept or reject it for oviposition. Host acceptance may be influenced by factors such as the host species, abundance and quality, and the means by which aphidiines assess these factors include visual and tactile stimuli, and internal and external host kairomones.

Aphidiines often show a preference for a particular host species. Jackson *et al.* (1974) found that *Ephedrus plagiator* attacked four cereal-feeding aphids in preference to four other species. *E. plagiator* was never observed to ovipositor probe in non-host aphids, suggesting that wasps were employing external cues during host recognition. *Diaeretiella rapae* was found to have an innate order of preference for several hosts in terms of the number of mummies formed (Dhiman and Kumar, 1983). Host plants also appear to play a role in host acceptance. In tests conducted in Petri dish arenas, Braimah and van Emden (1994) found that *Aphidius rhopalosiphi* made significantly more oviposition stabs against *Sitobion avenae* when the aphid was on wheat rather than on filter paper. *A rhopalosiphi* also more frequently attacked *S. avenae* and *M. persicae* on wheat rather than on Brussels sprouts. Wasps showed no such preferences for aphids reared on an artificial diet, suggesting a role for plant-derived synomones in the interactions. Powell and Wright (1992) showed that *A. rhopalosiphi* increased its attack rate against a non-host aphid, *Acyrthosiphon pisum,* when wheat leaves were present, but that plant material had no effect on *Aphidius picipes* and *E. plagiator*, which attack aphids on a variety of plants. The authors suggested that cues from specific plants may not be used by polyphagous parasitoids during host acceptance.

Chow and Mackauer (1991) found that *Aphidius smithi* showed an innate preference for *Acyrthosiphon pisum* over *Macrosiphum creelii* Davis when both were available in equal numbers. When *M. creelii* was offered in greater numbers, the wasps did not switch to this host, indicating that differences in host quality influence host acceptance. Pungerl (1984) examined the host preferences of several *Aphidius* species collected from different populations. Wasps which were morphologically and electrophoretically similar were found to differ in their host preferences, indicating that prior conditioning has an effect on host choice. In host transfer trials, *Aphidius ervi* reared on *A. pisum* produced fewer mummies on *Microlophium carnosum*, and this was shown to be correlated with a reduced attack rate on the latter host (Powell and Wright, 1988). However, mummy production was greatly improved if the parasitoid's male parent had been raised on *M. carnosum*, indicating a genotypic influence on host preference. Cameron *et al.* (1984) showed that the reduced mummy production on transferring *A. ervi* to a new host can disappear after 4-5 generations. The change was associated with the loss of certain parasitoid esterase bands, shown by electrophoresis. Preference for a particular host or hosts seems to be the result of both genetic and conditioning factors. Chow and Mackauer (1992) considered that prior ovipositional experiences influence a parasitoid's expectations of the kind of hosts available, but do not alter its innate order of host preference.

The size, age and developmental stage of the host can affect host acceptance. Many species prefer to oviposit in aphids from the 2nd and 3rd larval instars, including *Diaeretiella rapae* (Takada, 1975), *Trioxys indicus* Subba Rao and Sharma (Singh and Sinha, 1982), *Aphidius rhopalosiphi* (Shirota *et al.,* 1983) and *Aphidius nigripes* (Cloutier *et al.,* 1984). *Aphidius matricariae* Haliday, however, showed no preference for a particular host stage ('t Hart *et al.,* 1978). This preference for younger larvae is believed in many cases to be the result of increased defensive behaviour by 4th larval instar and adult aphids. This can include striking with the legs, wings or antennae (Singh and Sinha, 1982; Shirota *et al.*, 1993; Liu *et al.,* 1984), falling from the plant (Chau and Mackauer, 1997)

or simply walking away (Hofsvang and Hågvar, 1986).

Parasitoids use visual and chemical cues in host assessment. Michaud and Mackauer (1994) investigated host acceptance by three *Aphidius* species. By elegant use of lighting and anaesthetised aphids, they showed that both colour and movement were important in host acceptance, although the final decision to lay an egg was taken after ovipositor probing. In host preference tests, *Aphidius microlophii*, probed non-host aphids with its ovipositor but did not deposit an egg (Pennacchio *et al.,* 1994), indicating that the wasp can detect internal cues via receptors on the ovipositor. Singh and Srivastava (1987) found that *Trioxys indicus* responded to haemolymph of its host *Aphis craccivora* Koch. painted onto filter paper squares, and that the parasitoid could distinguish between haemolymph from different larval instars. Whole body extracts and fresh cornicle secretion of *Rhopalosiphum padi* provoked antennal examination and attack behaviour in naive *Lysiphlebus testaceipes* (Grasswitz and Paine, 1992), and the application of *R. padi* cornicle wax onto a non-host aphid increased the frequency with which it was attacked. *Acyrthosiphon pisum* cornicle wax applied to glass beads elicited examination and attack behaviour in *Aphidius ervi* (Battaglia *et al.*, 1993). Again the response seemed to be innate. Since few aphids produce cornicle wax before parasitisation, it is possible that the same waxes may be present in the cuticle and act as contact cues in host acceptance (Grasswitz and Paine, 1992; D Battaglia, personal communication).

1.3 Aphid Sex Pheromones and their Role as Kairomones in Parasitoid Host Location

1.3.1 Discovery, Identification and Biology of Aphid Sex Pheromones

The first experimental evidence of sex pheromone production by aphids emerged in the early 1970's. Pettersson (1970) demonstrated that oviparae of *Schizaphis* species emitted chemical signals which attracted conspecific males in laboratory

bioassays and, soon after, the presence of a sex pheromone in the vetch aphid *Megoura viciae* Buckton was confirmed (Marsh, 1972, 1975).

The pheromone is released from glandular cells lying beneath porous 'scent plaques' on the tibiae of the hind legs of oviparous females (Pettersson, 1970, 1971; Eisenbach and Mittler, 1987; Pickett *et al.*, 1992). These legs are often raised and waved in the air during the typical calling behaviour of the sexual female aphid (Pettersson, 1971; Steffan, 1990). Male perception of the sex pheromone was thought to be via cells located in areas of the antennae known as the secondary rhinaria (Pettersson, 1971; Marsh, 1975; Eisenbach and Mittler, 1980) and this has been confirmed by the electrophysiological technique of single cell recording (SCR) (Dawson *et al.*, 1987, 1990).

Pettersson (1970) suggested that the sex pheromone was only released by the aphid during a specific phase of the oviparous stage, which he called the active copulatory period. This was characterised by the adoption of the calling stance i.e. with the hind legs raised. In fact, analysis of air entrainment samples has shown that only trace amounts of sex pheromone compounds are detectable when the aphid is not calling (Dawson *et al.*, 1990). Marsh (1972, 1975) demonstrated that pheromone release by *Megoura viciae* is under circadian control. In *Schizaphis graminum*, pheromone release is triggered by the onset of the light period in the L-D cycle (Eisenbach and Mittler, 1980), maximum pheromone production occurring 4-7 hours into the photophase, on days 6-8 of the adult stage. In *S. graminum* production and release of pheromone ends after mating (Eisenbach and Mittler, 1987) and, since a complete copulation was required, the authors implicated sperm or seminal substances in the cessation mechanism. Peak pheromone production has been shown to occur on day 6 of the adult stage in other aphid species (Marsh, 1972, 1975; Hardie *et al.*, 1990; Issacs, 1994).

The first chemical identification of an aphid sex pheromone was for *Megoura viciae* (Dawson *et al.*, 1987; the complete stereochemistry was established by

Dawson *et al.*, 1989).The active compounds were pinpointed by coupled gas chromatography of excised leg extracts and SCR on the male secondary rhinarium. Coupled gas chromatography-mass spectrometry was then used to identify the chemical structures. The sex pheromone of *M. viciae* was shown to consist of two monoterpenoid isomers: (+)-(4a*S*,7*S*,7a*R*)-nepetalactone I and (-)-(1*R*,4a*S*,7*S*,7a*R*)-nepetalactol II (figure 1.2). The nepetalactone I was active electrophysiologically but, in laboratory bioassays, was inactive compared to the leg extract. However, when presented in combination with the nepetalactol II, the activity was equivalent to that of the leg extract, indicating that the sex pheromone of *M. viciae* consists of a ratio of the two components (Dawson *et al.*, 1987).

The sex pheromones of many aphid species are now known, and have been shown to consist of either the nepetalactone I, the nepetalactol II or a specific ratio of the two. An exception is the damson-hop aphid, *Phorodon humuli* (Schrank), which produces only the stereoisomer (4a*R*,7*S*,7a*S*)-nepetalactol III (figure 1.2) (Campbell *et al.*, 1990). The stereochemistry at carbon-1 has not been fully characterised, although a mixture of 70% (1S) and 30% (1R) was active in laboratory bioassays.

Table 1.1 summarises the sex pheromone ratios identified for some economically important aphids. Species of *Cryptomyzus* produce both the nepetalactone I and nepetalactol II (Guldemond *et al.*, 1993). However, synthetic ratios did not have the same behavioural activity as ovipara leg extracts, and the authors suspected the presence of a third, as yet unidentified compound.

Dawson *et al.* (1990) have shown that male *Schizaphis graminum* have two types of olfactory cell in the secondary rhinarium, and that each is specialised to detect either the nepetalactone I or the nepetalactol II. The damson-hop aphid *P. humuli* produces only the nepetalactol III and has specialised receptors for this

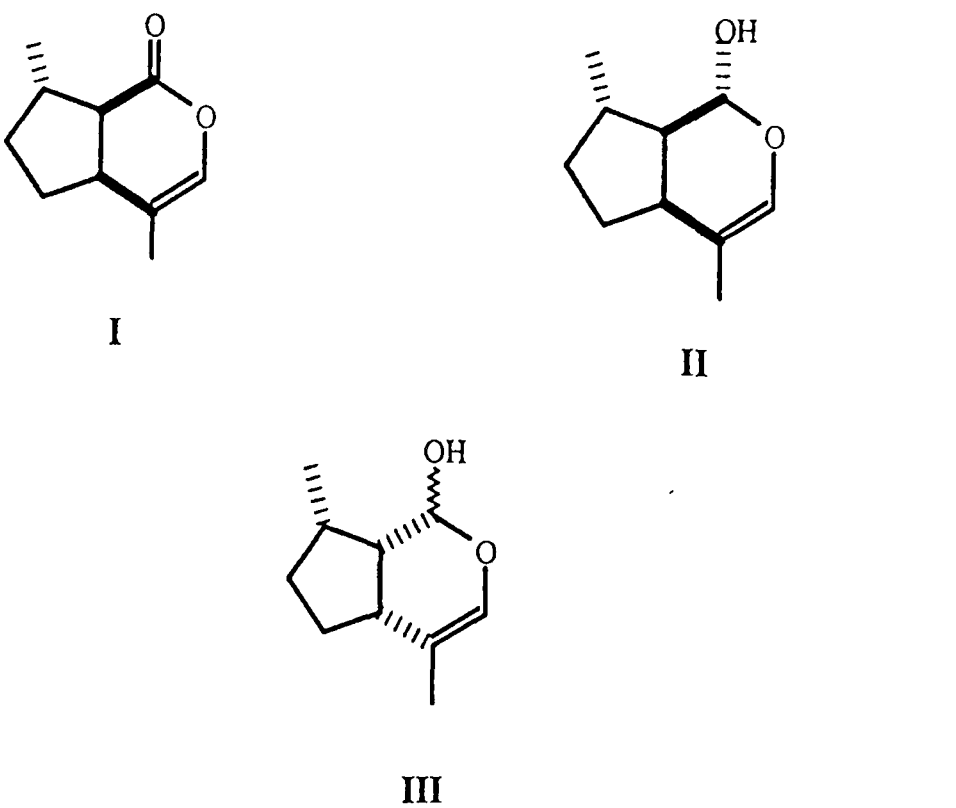

Figure 1.2 Chemical structures of aphid sex pheromone components.
(+)-(4a*S*,7*S*,7a*R*)-nepetalactone I; (-)-(1*R*,4a*S*,7S,7a*R*)-nepetalactol II; (4a*R*,7*S*,7a*S*)-nepetalactol III

compound in the secondary rhinaria (Campbell *et al.*, 1990). However this species also has separate receptor cells for the nepetalactone I and nepetalactol II. It has been suggested that production of two different sex pheromone components is one of the mechanisms by which aphids achieve species separation in the field (Hardie, 1991). The evidence concerning species specificity of sex pheromone blends is varied. Pettersson (1971) found that in four *Schizaphis* species, attraction of males to calling females in an olfactometer was not entirely species specific. In bioassays, males of *M. viciae* and *S. graminum* were preferentially attracted to calling conspecific females, but were also attracted to some degree by females of other aphid species (Dawson *et al.*, 1990). There appeared to be a correlation between the degree of attraction and the similarity of the heterospecific and conspecific pheromone ratios.

Table 1.1 The sex pheromones of some economically important aphid species. See figure 1.2 and text for explanation of chemicals.

Aphid species		Ratio I:II[1]	Reference
Acyrthosiphon pisum		1:1	Dawson *et al.* (1990)
Aphis fabae		29:1	Dawson *et al.* (1990)
Brevicoryne brassicae		1:0	Gabrys *et al.* (1997)
Megoura viciae	day 2-6	4:1-6:1	Hardie *et al.* (1990)
	day 7-8	12:1	
Myzus persicae		1:2	Pickett *et al.* (1992)
Rhopalosiphum padi		0:1	L. Wadhams (pers comm)
Phorodon humuli		0:1 (III)[2]	Campbell *et al.* (1990)
Schizaphis graminum		1:8	Dawson *et al.* (1988); Pickett *et al.* (1992)
Sitobion avenae		1:0	Pickett *et al.* (1992)
Sitobion fragariae (Walker)		1:0	Hardie *et al.* (1992)

[1]I= (+)-(4aS,7S,7aR)-nepetalactone I; II= (-)-(1R,4aS,7S,7aR)-nepetalactol II

[2]produces only (4aR,7S,7aS)-nepetalactol III

Hardie *et al.* (1990) were able to induce *M. viciae* males to attempt mating with live, conspecific virginoparae onto which 2ng of synthetic sex pheromone had been applied. Males attempted more matings when their species-specific sex pheromone ratio was applied, but also responded to ratios which mimicked the pheromone blends of other species. Sibling species of *Cryptomyzus* often occur on the same host plant and produce sexual females at the same time of year. However, Guldemond *et al.* (1994) reported that the circadian rhythm of pheromone production and male activity differed between the species and suggested that this contributed towards reproductive separation.

Evidence from field investigations more firmly supports a role for sex pheromones in species isolation. Males of the blackberry-cereal aphid *Sitobion fragariae* were attracted in significantly higher numbers to water traps baited with nepetalactone than to unbaited traps (Hardie *et al.*, 1992). Low numbers of males of other species were captured by the traps even though a nearby suction trap indicated that they were active in the area. Traps baited with the nepetalactol III specifically captured males of the damson-hop aphid *Phorodon humuli* (Campbell *et al.*, 1990), this species being the only one investigated so far which produces this compound. It is likely that the differences in the ratios of sex pheromones employed by aphids do contribute to species separation in the field. However, this is probably only one of several mechanisms at work. Hardie (1991) considered species separation to be mediated by; spatial and temporal separation (including primary host plant preference), visual and tactile stimuli, courtship behaviour and postmating mechanisms (e.g. production of infertile eggs and hybrid sterility).

The early laboratory experiments (Pettersson, 1970; Marsh, 1975) suggested that males were able to detect and respond to the sex pheromone over only relatively short distances. This led to the theory that, when searching for females, males would first need to locate the primary host plant by long range olfactory and visual cues and then initiate a local search for calling females (Steffan, 1987; Guldemond, 1990; Pickett *et al.*, 1992). However, field trials have now demonstrated that male aphids can detect and orient to sex pheromones over greater distances. Campbell *et al.* (1990) placed water-filled traps, baited with the *P. humuli* sex pheromone nepetalactol III, both inside and outside of a hop garden. Even though no primary host plants of these aphids grew in the area, the traps attracted large numbers of males, suggesting that long range attraction to the pheromone alone may have taken place. Males of *S. fragariae* were attracted in large numbers to clear plastic water traps baited with nepetalactone, while a nearby suction trap indicated that few males of this species were flying in the area (Hardie *et al.*, 1992), again suggesting a long range attraction. The characteristic raised hindleg posture adopted by calling females may be an adaptation for

pheromone dispersal over relatively large distances (Steffan, 1990).

1.3.2 Role of Aphid Sex Pheromones in Parasitoid Host Location

Selection should act upon phytophagous insects to make them as unapparent as possible to their enemies. However, they must still communicate with conspecifics, sometimes over large distances, and many do so with pheromones. There is evidence that several aphidiine species have developed the ability to use the sex pheromones of their aphid hosts as kairomones during host location.

The first evidence that parasitoids were attracted to aphid sex pheromones was seen during field experiments designed to observe the response of field-flying male aphids to synthetic sex pheromone (Hardie *et al.*, 1991). Clear plastic, water-filled traps were placed in semi-natural woodland during the autumn and were baited with lures releasing nepetalactone. Besides capturing male aphids, large numbers of parasitoids of the genus *Praon* were recovered from traps, the species being mainly *P. dorsale* (Haliday) and *P. abjectum* (Haliday). All the wasps were female, and 98.5% were found in pheromone baited-traps as opposed to unbaited controls. Since clear traps and synthetic pheromone were used, all other visual and olfactory cues were ruled out, clearly demonstrating a role for aphid sex pheromone in parasitoid host finding.

In the autumn, parasitoids of the genus *Praon* are thought to be associated with environments containing the woody host plants of holocyclic, host alternating aphids (Vorley, 1986). The results of the initial trapping experiments are consistent with this. However, when the experiments were repeated during the following autumn in winter cereal fields, attraction to nepetalactone was again demonstrated (Powell *et al.*, 1993; Hardie *et al.*, 1994). This time, 89% of captured wasps were *Praon volucre*, over 99% occurring in pheromone baited traps. The trials were replicated at sites in south western, central and northern England, and in northern Germany, and the response to sex pheromone was apparent at all sites which had parasitoid populations. In several of the trials, the

nepetalactol II component was also included but the response to this was significantly lower than to the nepetalactone. Trap height was important; parasitoids were captured almost exclusively in traps placed just above the crop canopy, compared to traps 1m higher. Representatives of six other aphidiine genera were found in traps, but there was no evidence that species other than *Praon* were attracted.

Why do parasitoids respond to aphid sex pheromones? Many aphid species in Britain are either permanently holocyclic, exist in holocyclic clones on a geographic basis or are facultatively holocyclic in response to climate, and therefore produce sexual females (oviparae) from September onwards (Dixon, 1973; Blackman, 1974). The oviparae do not survive the winter, but produce overwintering eggs which are unsuitable for attack by aphidiines. So for parasitoids attacking these species, the ovipara represents the final stage available for attack and in which to overwinter as diapausing prepupae within the mummy, ensuring co-location with aphid colonies in the spring. It would be advantageous for wasps to be able to efficiently locate this stage, and they do so by using the sex pheromone as a host finding cue.

Although parasitisation of sexual aphids has been recorded (Starý, 1970; Höller, 1990), most aphid parasitoids prefer to oviposit in the larval stages of their hosts (Starý, 1988b). However, oviparae do not produce sex pheromone until they reach the adult stage. If aphids exist in mixed instars colonies, calling females would indicate the presence of larvae nearby. In this case, parasitoids could use pheromone-producing adults to locate the less conspicuous larvae via an 'infochemical detour' (Vet and Dicke, 1992).

Praon volucre is a generalist parasitoid with a wide host range (Starý, 1976). Different aphid species have been shown to produce specific ratios of components in their sex pheromone blends, and *P. volucre* is known to attack several aphid species which employ different ratios (Starý, 1976). However, this parasitoid

seems to require only nepetalactone in order for full attraction to take place, and this may be an adaptation to a generalist lifestyle. Attraction to aphid sex pheromones has been demonstrated in aphid parasitoids other than *Praon*. In laboratory bioassays, *Aphidius matricariae* responded to nepetalactone (Issacs, 1994), *Diaeretiella rapae* was attracted to traps baited with nepetalactone in the field (Gabrys *et al.*, 1997) and *Lysiphlebia japonica* Ashmead showed electroantennogram responses to both pheromone components (Hou and Yan, 1995). Intriguingly, there is evidence that although parasitoids with a wide host range may respond fully to nepetalactone, more specialised parasitoids may require the exact ratio of components produced by their host aphid (Y-J Du, personal communication)

When the initial trapping experiments were repeated in the spring rather than the autumn, parasitoids were not captured in large numbers (Hardie *et al.*, 1994). At the time, it was concluded that the parasitoid response to aphid sex pheromones was either induced by environmental stimuli (i.e. the changes in temperature and photoperiod which occur in the autumn) or developed in response to parasitoids learning to associate suitable hosts with the presence of pheromone by attacking sexual aphids when they appeared in the autumn. However parasitoids have since been shown to respond to sex pheromones in laboratory bioassays under controlled conditions (Issacs, 1994; Lilley *et al.*, 1994; Y-J Du, personal communication). Wasps were reared and tested at temperatures and photoperiods which did not resemble those which exist in the field in autumn, thus ruling out the environmental induction theory. Furthermore, since test insects were prevented from having contact with either sexual aphids or sex pheromones, the response does not appear to be learned via association. Therefore, parasitoid response to aphid sex pheromones is likely to be innate.

Initial attempts have been made to attract parasitoids to aphid colonies in the field using sex pheromones. Potted barley plants were artificially infested with *Sitobion avenae* and exposed in the field, with or without nepetalactone lures (Lilley *et al.*,

1994). The presence of nepetalactone significantly increased parasitism of *S. avenae* by *P. volucre*, 57% of the aphids on plants with lures being parasitised compared to 35% on control plants. The sex pheromone released by oviparae of *Acyrthosiphon pisum* consists of a 1:1 ratio of nepetalactone:nepetalactol, and the presence of lures releasing this ratio on pea plants infested with *A. pisum* significantly increased parasitisation by *Aphidius ervi* and *Aphidius eadyi* Starý (Y-J Du, D Brooks, personal communications). Since the experiments described above used only virginoparae on the infested plants, it can be concluded that although when parasitoids are responding to the sex pheromone they are 'expecting' to locate sexual aphids, they will also attack other host stages if these are present around lures.

The distance over which parasitoids can detect and respond to aphid sex pheromones is unknown. Traps placed in cereal fields in the autumn attracted female *P. volucre* (Powell *et al.*, 1993; Hardie *et al.*, 1994). However, since sexual aphid morphs are uncommon in cereal fields in Britain at this time (Hand, 1989) and *P. volucre* is associated more with the aphids' primary host plants (Vorley, 1986), it is possible that parasitoids were attracted into the fields from other habitats. This suggests a long range attraction of parasitoids to the sex pheromone (Hardie *et al.*, 1994) but remains to be investigated experimentally.

1.4 Responses of Other Parasitoids and Predators To Host Pheromones

The best known interactions outside of the Aphidiinae are those between hymenopteran egg and larval parasitoids and their lepidopteran hosts. Lewis *et al.* (1982) showed that, in glasshouse bioassays, the presence of *Heliothis zea* (Boddie) abdominal tips or synthetic sex pheromone increased the rate of parasitisation by the egg parasitoid *Trichogramma pretiosum* Riley (Hymenoptera: Trichogrammatidae). Parasitisation rates were also increased in the field by baiting plots with synthetic *H. zea* sex pheromone, released from fibres attached to the vegetation. The authors suggested that the ability of the

wasps to detect host pheromone enabled them to achieve synchrony with the initial upsurge in moth populations, and avoid competition with other predators which build up later during a generation. Detailed studies on the orientation of *T. pretiosum* to calling female *H. zea* and to synthetic sex pheromone in a wind tunnel (Noldus, 1988; Noldus *et al.*, 1991a) have since suggested that the nature of the wasps' response is arrestment rather than attraction, and this could explain the increased parasitisation in field plots (Lewis *et al.*, 1982).

Trichogramma evanascens Westwood (Hym.: Trichogrammatidae) also responds to the sex pheromone of its host, *Mamestra brassicae* L. (Noldus and van Lenteren, 1985; Noldus *et al.*, 1991a,b). Again in wind tunnel tests, the pheromone seemed to stimulate arrestment rather than attraction (Noldus *et al.*, 1991a). Zaki (1985) showed that *T. evanascens* responds to 7 different lepidopteran sex pheromone components from *Pectinophora gossypiella* (Saunders), *Earias insulana* (Boisduval) and *Spodoptera littoralis* (Boisduval), and that the application of *P. gossypiella* sex pheromone to *S. littoralis* and *Heliothis armigera* Hubner eggs increased parasitisation by *T. evanascens* in the laboratory.

It appears that *Trichogramma* species use their hosts' sex pheromones as kairomones in host location. However, pheromone is produced by adult moths, whereas the wasps always attack the egg. Moreover, *H. zea* and *M. brassicae* are nocturnal, and release pheromone during the night (Noldus and Potting, 1990; Pope *et al.*, 1984), whereas *Trichogramma* search for eggs during daylight (Ashley *et al.*, 1973). However, if traces of sex pheromone were left by the adult at the egg laying site, parasitoids could still use this as a reliable indicator of the presence of eggs. Noldus *et al.* (1991b) used air which had been passed over calling female *M. brassicae* to contaminate Brussels sprouts leaves. The residence time of *T. evanascens* on treated leaves was significantly increased, and the effect persisted for over 24 hours. They suggested that contamination of the vegetation with sex pheromone provided a 'bridge in time' for the searching parasitoids.

It follows that parasitoids which attack the larval rather than the egg stage may be less likely to respond to host sex pheromones, since the persistence of the chemical may not be great enough to bridge the gap in time until the larvae are present. The sex pheromone of *Spodoptera frugiperda* (Smith) appeared to have no effect on the oviposition behaviour of its larval parasitoid *Cotesia marginiventris* (Cresson) (Hym.: Braconidae) (Tingle *et al.*, 1989). However, *Telenomus remus* Nixon (Hym.: Scelionidae), which attacks the eggs of *S. frugiperda*, did respond to host abdominal tips and synthetic sex pheromone components (Nordlund *et al.*, 1983). The oviposition-deterring pheromone of the apple maggot fly *Rhagoletis pomonella* (Walsh) retained the parasitoid *Opius lectus* Gahan (Hym.: Braconidae) on egg infested fruit (Prokopy and Webster, 1978). *O. lectus* parasitises early instar larvae, but the pheromone had no effect on another parasitoid, *Opius alloeus* Muesbeck (Hym.: Braconidae), which attacks only late instar larvae.

It may benefit a parasitoid to detect semiochemicals released by a host stage different to that under attack if the host occurs in mixed stages at the odour site. The larval parasitoid *Leptopilina heterotoma* (Thomson) (Hym.: Eucoilidae) is attracted to an aggregation pheromone produced by *Drosophila* species (Wiskerke *et al.,* 1993). The pheromone is produced by males, but transferred to the female during copulation. The female then applies the pheromone to the substrate, in colonies containing *Drosophila* of mixed ages. The parasitoids in the examples above, responding to pheromones produced by stages other than those which they attack are employing a so called 'infochemical detour' (Vet and Dicke, 1992). They use highly detectable stimuli from an unsuitable host stage to locate a less conspicuous but suitable stage.

Parasitoids of scale insects, which can be serious pests of fruit crops, respond to female sex pheromones of their hosts. The parasitoids *Aphytis melinus* De Bach (Hym.: Aphelinidae) and *Aphytis coheni* De Bach (Hym.: Aphelinidae) were attracted to traps baited with live, virgin females of the California red scale,

Aonidiella aurantii (Maskell) (Sternlicht, 1973), which are known to produce a sex pheromone (Tashiro and Chambers, 1967). The ectoparasitoid *Aphytis mytilaspidis* (Le Baron) (Hym.: Aphelinidae) was attracted to traps baited with chloroform extracts of virgin female red pear scale, *Epidiaspis leperii* Sign. (Abdel-Kareim and Kozár, 1988), and *Coccidoxenoides peregrinus* (Timberlake) (Hym.: Encyrtidae) was captured in traps baited with live, virgin females of *Planococcus citri* (Risso) and *Pseudococcus calceolariae* (Maskell) (Rotundo and Tremblay, 1975). Since these studies used live females or extracts of whole female, they do not rule out the presence of other host kairomones, besides the sex pheromone, which may have attracted parasitoids. However *Aphytis* species were attracted to traps baited with synthetic *A. aurantii* sex pheromone (Grout and Richards, 1991), and *Encarsia perniciosi* (Tower) (Hym.: Aphelinidae) was captured in traps baited with synthetic sex pheromone of San José scale, *Quadraspidiotus perniciosus* (Comstock) (Kyparissoudas, 1987).

Mitchell and Mau (1971) found that caged, male southern green stinkbugs, *Nezara viridula* L., attracted the parasitic tachinid fly *Trichopoda pennipes* (F.) to sticky traps in the field. The males of this pentatomid bug are known to produce a sex pheromone (Borges *et al.*, 1987; Aldrich *et al.*, 1989), and naturally occurring parasitisation in the field is significantly higher in males than in females (Mitchell and Mau, 1971). Males of another pentatomid, the soldier bug *Podisus maculiventris* (Say), also produce a pheromone. Caged males attracted two species of tachinid fly to traps, as well as egg-parasitoids of the genus *Telenomus* (Aldrich *et al.*, 1984). These are particularly interesting since the parasitoids are phoretic upon *Podisus* females, travelling attached to the body and hopping off when the female produces an egg mass. Attraction to calling male bugs would also bring them into close contact with mated females. The interactions between the true bugs and their parasites and predators are reviewed by Aldrich (1995).

There is one report of a parasitoid responding to a lepidopteran larval pheromone. Larvae of the flour moth, *Anagasta kuehniella* (Zeller), produce an epideictic

pheromone in the mandibular gland secretion which elicits ovipositor striking in the larval parasitoid *Venturia canescens* (Grav.) (Hym.: Ichneumonidae) (Corbet, 1971). Battisti (1989) demonstrated attraction of the egg parasitoid *Ooencyrtus pityocampae* Mercet (Hym.: Encyrtidae) to monitoring traps baited with sex pheromone of the pine processionary moth, *Thaumetopoea pityocampa* Denis and Schiffermüller. The parasitoids were captured in such large numbers that the author suggested the use of such traps may be detrimental to the control of the moth. Isidoro and Bin (1988) reported attraction of the egg-larval parasitoid *Platygaster dryomyiae* Silv. (Hym.: Platygasteridae) to traps baited with newly emerged virgin females of the gall midge *Dryomyia licthensteini* Fr. Lw.

The aggregation pheromones of bark beetles have been some of the longest studied semiochemicals, and Bedard (1965) provided one of the earliest experimental examples of possible parasitoid attraction to a host pheromone. The pteromalid *Tomicobia tibialis* Ashmead was attracted to traps containing *Ips paraconfusus* (Le Cônte) boring in pine, but no specific pheromones had been identified at that stage. *T. tibialis* was also attracted to traps baited with *Ips confusus* (Le Cônte) (Rice, 1969) and *Ips pini* (Say) (Lanier *et al.*, 1972). Kennedy (1979, 1984) reported the attraction of the parasitoid *Cheiropachus quadrum* (F.) (Hym.: Pteromalidae) to several synthetic bark beetle pheromone components, most strongly to the *Scolytus multistiatus* (Marsham) compounds multistriatin and cubebene. Four other parasitoid species were attracted, including one possible hyperparasitoid.

Bark beetle pheromones also evoke responses in several species of predator. Stephen and Dahlsten (1976) observed the arrival of various bark beetle predators, including the fly *Medetera aldrichii* Wheeler (Diptera: Dolichopodidae), during and shortly after the mass attack of pine by *Dendroctonus brevicomis* Le Cônte. The clerid beetle, *Temnochila chlorodia* (Mann), responded specifically to the *D. brevicomis* pheromone compound exo-brevicomin, when the substance was released from cotton wicks in field traps (Bedard *et al.*, 1980) and Vité and

Williamson (1970) demonstrated attraction of the clerid *Thanasimus dubius* F. to synthetic frontalin, an aggregation pheromone component of *Dendroctonus frontalis* Zimm. The authors suggested that the response by the predator allows it to both intercept prey and encounter conspecifics, providing enhanced spatial synchrony of foraging and mating. The clerid beetle *Enoclerus lecontei* (Wolc.) is attracted to pheromone components of both *Ips confusus* (Wood *et al.*, 1968) and *Ips pini* (Lanier *et al.*, 1972). Byers (1995) briefly reviews the responses of parasitoids and predators to bark beetle pheromones.

1.5 Use of Aphidiinae for Biological Control of Aphids in the Field

Biological control has been defined as 'the study and utilization of parasites, predators and pathogens for the regulation of host population densities' (DeBach, 1964). One of the earliest and most quoted examples is that of the citrus damaging coccoid *Icerya purchasi* Maskell in the United States which was controlled by the introduction from Australia of a coccinellid, *Rodolia cardinalis* (Mulsant) (Caltagirone and Doutt, 1989). Following the success of this program in 1888, there has been considerable interest in the use of predators and parasitoids against pest insects. Biological control can take the form of either classical biological control (the introduction of a novel enemy into a new country or region), augmentation (inundative release of large numbers of enemies at strategic times during a pest infestation) or enhancement (conservation and manipulation of enemies via habitat manipulation).

1.5.1 Introduction and Inundative Release of Aphidiinae

The majority of the literature concerning the use of Aphidiinae deals with the introduction of exotic species. Greathead (1989) surveyed the use of Aphidiinae in classical control and found that 23 aphidiine species had been introduced worldwide. In 32 out of 55 introductions, the parasitoids became established in the target location. Hågvar and Hofsvang (1991) briefly review the use of Aphidiinae in biological control, and Carver (1989) and Hughes (1989) both give

more thorough reviews of the biological control of aphids, with reference to aphidiine parasitoids.

Some of the earliest and most successful attempts to introduce aphid parasitoids were importations of *Trioxys* species into the United States. The most successful of these was the introduction from Iran of *Trioxys pallidus* (Haliday) to control the walnut aphid *Chromaphis juglandicola* (Kaltenbach) in California (Frazer and van den Bosch, 1973). Wasps were released in 1968 and, the following season, dramatic reductions in the expected peak aphid populations were recorded. The parasitoid is now credited with almost total commercial control of the walnut aphid in the area (van den Bosch *et al.*, 1982). A European biotype of *T. pallidus* has been introduced into Oregon, USA for control of the filbert aphid, *Myzocallis coryli* (Goeze), a pest of hazelnuts (Messing and Aliniazee, 1989). Thirty thousand wasps were released, and overwintering survival rates were high. The following season, peak aphid numbers in hazelnut orchards were reduced by 33-48%. The earliest reported success of aphid control by an imported *Trioxys* species involved the use of *T. complanatus* Quilis against the spotted alfalfa aphid *Therioaphis trifolii* (Monel) forma *maculata* (van den Bosch *et al.*, 1959, 1964) in the USA. *T. complanatus* was also introduced into southern Australia between 1977 and 1979 to control the same aphid, and is now established in most lucerne growing areas (Wilson *et al.*, 1982; Walters and Dominiak, 1984).

Trioxys curvicaudus Mackauer has been successful in controlling the linden aphid *Eucalipterus tiliae* (L.), as has *Trioxys tenuicaudus* Starý against the elm aphid *Tinocallis platani* (Kaltenbach), both in California (Olkowski *et al.*, 1982a,b). Recently, *Trioxys brevicornis* (Haliday) has been imported into California from Czechoslovakia to control the European asparagus aphid, *Brachycorynella asparagi* Mordwilko (Daane *et al.*, 1992). Between 1989 and 1990, 68 000 wasps were released and became established in the release area. Parasitisation rates of 10% were subsequently recorded, and the authors suggested that the introduced species may control aphid numbers in conjunction with the native enemy

complex.

In California, the pea aphid *Acyrthosiphon pisum* has been controlled by the introduction of *Aphidius smithi* from India (Hagan and Schlinger, 1960). The parasitoids were released in 1959 and, within one year, were successful in controlling pea aphids on alfalfa in coastal regions, while failing to affect aphid populations in the drier central valley area. The authors concluded that there was greater synchrony between pest and parasitoid populations under coastal climatic conditions. *A. pisum* has been controlled in New Zealand by the introduction of *Aphidius eadyi* (Cameron *et al.*, 1981). Within 3 years of release, the parasitoids were well dispersed and parasitisation rates of 30-40% were recorded in the field. In Australia, *Aphidius ervi* has been used in the control of *Acyrthosiphon kondoi* Shinji populations on lucerne (Milne, 1986). Wasps were imported from New Zealand in 1977 and by 1980, the usual spring increase in aphid populations was no longer recorded in the release areas, and parasitisation by *A. ervi* often exceeded 90%.

The cereal aphid parasitoid, *Aphidius rhopalosiphi*, was introduced from Europe into New Zealand and, within 3 years, parasitisation levels of 50-100% were recorded for cereal aphids (Farrell and Stufkens, 1990). A cost-benefit analysis of this introduction estimated that benefits to the nation from the biocontrol of *Metopolophium dirhodum* ranged from NZ$300 000 to NZ$5 million (Grundy, 1990). In 1977, *A. ervi* and *A. rhopalosiphi* were introduced into Chile, in order to improve control of the cereal aphids *M. dirhodum* and *Sitobion avenae* (Norambuena, 1981). In the season following the releases, the imported wasps parasitised 14.7% of aphids in spring wheat, and the authors concluded that the species may become important regulators of aphid populations in the region. The Russian wheat aphid *Diuraphis noxia* first colonised the southern USA in 1986, and by 1989 was causing serious grain losses (Pike and Harwood, 1989). Surveys in the Middle East identified over 20 species of aphidiine parasitoids and 5 species, including *Diaeretiella rapae* and *A. rhopalosiphi*, were subsequently

imported (González *et al.*, 1992). Between 1988 and 1992, 200 000 parasitoids were released at 53 sites, and initial indications were that some species had succeeded in becoming established (Tanigoshi *et al.*, 1995).

Attempts to mass release aphidiine species have been uncommon, and results have been variable. Halfhill and Featherstone (1973) released 100-300 million *Aphidius smithi* into alfalfa fields in an attempt to reduce populations of *Acyrthosiphon pisum* migrating to pea crops. They recorded significantly reduced aphid numbers in areas where parasitoids had been released, compared to areas where none had been released or insecticidal control had been used. However the authors considered this method of control to be impractical. Shands *et al.* (1975) attempted to control several potato feeding aphids, including *Macrosiphum euphorbiae*, by releasing laboratory reared *Diaeretiella rapae* and an unidentified *Praon* species. After releasing 192 000 *Praon* and 63 000 *D. rapae*, only 5 mummies were recovered in experimental plots. Parasitoids had been laboratory reared on *Myzus persicae* prior to release and it is likely that host preference effects accounted for their low efficiency in the field. Stinner (1966) reviews the strategy of mass release of control agents, with some reference to Aphidiinae.

There has been considerable use of aphidiine parasitoids in the control of aphids in glasshouses. This work is briefly reviewed by Hågvar and Hofsvang (1991).

1.5.2 Enhancement of Parasitoid Activity by Habitat Manipulation with Reference to Aphidiinae

Annual crop monocultures, and the cultural practices associated with them, are often counterproductive to biological control as they do not provide adequate resources for natural enemies (Rabb *et al.*, 1976). Furthermore, the resource concentration hypothesis (Root, 1973) predicts that monophagous and oligophagous herbivores are more likely to locate and build up in these cropping systems, where their host plants are concentrated.

The effects of vegetational diversification on natural enemies has been widely debated, with authors reaching different conclusions. Increased diversity has often been found to result in reduced pest populations (Perrin and Phillips, 1978; Risch *et al.*, 1983; Andow, 1985) but some authors have argued that increased vegetational diversity does not lead to increased ecosystem stability (Goodman, 1975; Murdoch, 1975). Risch *et al.* (1983) reviewed 150 published studies on the effects of agroecosystem diversification on pest abundance. In 53% of cases, the pest was shown to be less abundant, and in 18% of cases was more abundant.

The enemies hypothesis (Root, 1973; Russell, 1989) predicts that populations of natural enemies will be greater, and consequent herbivore populations lower, in diversified habitats due to increased availability of alternative prey/hosts, food sources and suitable microhabitats. Russell (1989), reviewing 18 studies which tested the enemies hypothesis, found that in 9 studies, pest mortality rates due to predation or parasitisation were higher, in 2 they were lower and in 2 there was no difference. Although Risch *et al.* (1983) concluded that the reduction of pest numbers in diverse ecosystems owed more to herbivore movement patterns and resource finding than to enemy activity, Sheehan (1986) argued that enemy activity was important, and that enemy foraging behaviour and population dynamics should be viewed in terms of the concentration of hosts and host containing vegetation. It is likely that both the enemies and resource concentration hypotheses act in a complementary fashion (Russell, 1989).

It is possible that the inconsistency in the results of habitat manipulation experiments is due to the adoption of a general approach, rather than targeting a specific tritrophic system. Powell (1986) writes,

'...it is not the establishment of diversity *per se* which is important but rather the addition of specific resources to the ecosystem via diversity.'

The same author sets out four mechanisms by which the resources necessary for

improving parasitoid effectiveness may be provided;

1. Provision of alternative hosts at times when the pest host is scarce.
2. Provision of food (pollen and nectar) for adult parasitoids.
3. Provision of refugia (e.g. for overwintering)
4. Maintenance of small populations of the pest host over extended periods to ensure the continued survival of the parasitoid population.

In the Aphidiinae, the adult food source is aphid honeydew (Starý, 1988b). However, provision of alternative hosts and use of refugia, weeds and intercropping have been attempted. Reviews of habitat manipulation for the enhancement of natural enemy activity (including references to Aphidiinae) are provided by Rabb *et al.*, 1976; Ables and Ridgway, 1981; Altieri and Letourneau, 1982; Powell, 1986; van Emden, 1988 and Altieri *et al.*, 1993.

Starý (1974) studied the aphid fauna of *Galium* species in central Europe. The aphids on this host plant were found to be mostly benign to agricultural crops, however several important parasitoid species, including *Praon volucre* and *Diaeretiella rapae*, were found to use the aphids as alternative hosts. The author suggested that *Galium* species may be useful reservoirs of aphid parasitoids alongside cultivated areas. In the USA, parasitism of *Schizaphis graminum* by *Lysiphlebus testaceipes* on sorghum was found to be higher when sunflowers were grown nearby (Eikenbary and Rogers, 1973). The sunflowers were infested with the sunflower aphid, *Aphis helianthi*, (Monel) and *L. testaceipes* was able to utilise these as alternative hosts to survive periods when *S. graminum* was scarce.

Aphidius ervi has been recorded as attacking both the pest aphids, *Acyrthosiphon pisum* and *Sitobion avenae*, as well as the innocuous species *Microlophium carnosum* which feeds on the perennial stinging nettle *Urtica dioica* L.. It has been suggested that nettles growing alongside crops could act as reservoirs of alternative hosts for *A. ervi* (Perrin, 1975; Starý, 1983). However, results of host

switching trials (Cameron *et al.*, 1984; Pungerl, 1984; Pennacchio *et al.*, 1994) and olfactometer bioassays (Powell and Zhi-Li, 1983) suggested that the *A. ervi* populations attacking *A. pisum* and *M. carnosum* were separate races or biotypes, and did not naturally transfer between the two hosts. On the basis of behavioural and morphological analysis, *A. ervi* attacking *M. carnosum* has now been described as a separate species, *Aphidius microlophii* (Pennacchio and Tremblay, 1987). This does question the potential value of using alternative hosts in the field, however it should be noted that the above experiments relied heavily on laboratory reared insects, and that laboratory populations are known to suffer from effects such as low founder numbers and genetic drift (Unruh *et al.*, 1983). This means that the results of laboratory trials may not always be applicable in the field.

Attempts have been made to enhance the effectiveness of Aphidiinae by manipulating the crop environment, and by the use of intercropping. Some of these attempts have been successful. Schlinger and Dietrick (1960) studied the impact of imported aphid parasitoids on the spotted alfalfa aphid *Therioaphis trifolii* f. *maculata* in California. The number of adult wasps per acre was four times greater in alfalfa fields which had been strip harvested than in conventionally harvested fields, because unharvested strips provided a refuge during cutting and pesticide spraying. Strip harvesting improved the ability of *Aphidius smithi* to control *Acyrthosiphon pisum* in Californian alfalfa fields (van den Bosch *et al.*, 1967). During the hot summer period when aphids were scarce, wasps were able to survive in uncut strips and were more closely synchronised with the subsequent increase in aphid populations during the autumn. Vegetational complexity, particularly the presence of the weed *Chenopodium album* L., has been credited with encouraging parasitism of *Aphis fabae* on beet crops, and with reducing levels of hyperparasitism (Barczak, 1992).

Some attempts have been made to improve control of aphid populations by provision of within-field refugia, headland management and the creation of field

margins (Thomas and Wratten, 1988; Nentwig, 1989; Hassall *et al.*, 1992; Lagerlöf and Wallin, 1993), although the specific impact on aphid parasitoids has not been investigated in all cases. Lagerlöf and Wallin (1993) studied the activity and abundance of various beneficial insect groups in artificially-created field margin strips. Plots containing a mixture of grasses were found to harbour more small parasitoids (<5mm) than ploughed plots or plots sown with clover or flowering plants.

Two studies have suggested that habitat manipulation via vegetational diversity may not necessarily aid specialist parasitoids. Smith (1976) found that parasitisation of *Brevicoryne brassicae* by *Diaeretiella rapae* in Brussels sprouts was four times greater in plots where weeds had been managed than in unmanaged plots, and Horn (1984) reported similar results with *D. rapae* attacking *Myzus persicae* in weedless compared to weedy collard plots. Sheehan (1986) speculates that the enemies hypothesis may not apply to specialised parasitoids, because the factors which attract and retain specialists such as large patch size, specific chemical and visual stimuli and low chemical and structural diversity of surrounding vegetation may be more prevalent in a simple environment. This emphasises the importance of identifying the target system and providing specific resources through diversity (Powell, 1986).

1.6 Introduction to The Current Work

Although there is strong evidence that early season parasitoid activity is a key factor in aphid population regulation (section 1.1.4), this activity is apparently rare in agroecosystems due to poor synchrony between aphid and parasitoid populations. If this synchrony could be enhanced, the frequency with which aphid populations are successfully regulated by the natural enemy complex may be increased, leading to a reduction in insecticide applications.

The discovery and initial characterisation of the parasitoid response to aphid sex pheromones (section 1.3.2) has provided the means with which to manipulate parasitoid behaviour to improve early season activity in the crop. This manipulation could form the basis of a strategy in which parasitoids are attracted into field margins alongside crops by the deployment of aphid sex pheromone lures. The field margin habitats would be managed to provide suitable vegetation and aphid hosts on which parasitoids could overwinter. If a reservoir of overwintering parasitoids could be established near the crop, emerging wasps would be more effectively located to tackle the early season build up of colonising aphids.

The aim of the work described here is to examine the effectiveness of the proposed strategy by further investigating the parasitoid response to aphid sex pheromones and testing the prospects for the manipulation of parasitoid populations in the field.

1.7 General Methods

1.7.1 Insects

1.71a Parasitoids

Parasitoid cultures were maintained in ventilated perspex cages (45x 66 x 45 cm) in a controlled environment. Environmental chambers operated at 15±1 °C, 70±5 % relative humidity with a 16hr L: 8 D photoperiod.

Cultures were established, and subsequently infused with field collected aphids and parasitoids. Details of the rearing of parasitoid species used in the experiments are provided in table 1.2.

Parasitoids were collected for experimental use by carefully removing mummies from the plant surface with a paintbrush (except *Praon* mummies which were

removed attached to a small piece of plant material). Mummies were placed in a Petri dish, and adult parasitoids emerged into small cone shaped plastic cages. Honey solution (1:1 honey:water) on a cotton wool wick was provided as a food source. Emergence cages were kept in a controlled environment room at 18 °C, 16hr L :8 D photoperiod. All parasitoids used in laboratory experiments were 2-3 day-old, mated individuals.

Table 1.2 Details of parasitoid cultures used in the experimental work

Parasitoid species	**Aphid species**	**Plant**	**Culture established**
Aphidius eadyi	*Acyrthosiphon pisum*	broad bean	1994
Aphidius ervi	*Acyrthosiphon pisum*	broad bean	1995
Aphidius rhopalosiphi	*Sitobion avenae*	winter barley	1996
Diaeretiella rapae	*Myzus persicae*	Chinese cabbage	1990
Ephedrus plagiator	*Sitobion avenae*	winter barley	1988
Praon myzophagum	*Acyrthosiphon pisum*	broad bean	1994
Praon myzophagum	*Myzus persicae*	Chinese cabbage	1992
Praon volucre	*Acyrthosiphon pisum*	broad bean	1996
Praon volucre	*Sitobion avenae*	winter barley	1995

1.7.1b Aphids

Aphid cultures were maintained in a similar way to the parasitoid cultures. Cages were kept in a separate room operating at 15±1 °C, 70±5 % RH and 16hr L :8 D photoperiod. *A. pisum* was reared on broad bean (culture established in 1990) and *S. avenae* on winter barley (established 1990).

1.7.2 Aphid Sex Pheromones

The aphid sex pheromone component (+)-(4aS,7S,7aR)-nepetalactone (98.5% pure by GC analysis) was obtained by steam distillation of catmint (*Nepeta cataria* L. (Laminaceae: Labiatae)) foliage, followed by column chromatography through silica. The (-)-(1R4aS,7S,7aR)-nepetalactol was produced by reduction of the lactone using diisobutylaluminium hydride (DIBAL) (Dawson *et al.*, 1989). A range of nepetalactone isomers were synthesised in the laboratory (Dawson *et. al* 1996).

1.7.2a Laboratory Bioassay

For laboratory bioassays, 1 mg/ml in HPLC hexane solutions of nepetalactone and nepetalactol were used.

1.7.2b Field Lures

Field lures were prepared by dispensing 50 µl of 200 mg/ml nepetalactone or nepetalactol solution (in diethyl ether AR) into amber coloured glass vials (08CPV Chromacol), giving a loading of 10 mg pheromone per vial. A hole was drilled in the plastic vial stopper to allow pheromone release. The release rate was approximately 250 µg/day (L Merrit, personal communication).

Chapter 2

Flight Responses of Aphid Parasitoids to Aphid Sex Pheromones

2.1 Introduction

Evidence that aphid parasitoids respond to aphid sex pheromones was obtained for the first time from field trapping experiments (Hardie *et al.*, 1991). Three parasitoid species, belonging to a single genus, *Praon*, were captured in pheromone-baited water traps (Hardie *et al.*, 1991; Powell *et al.*, 1993). Subsequent studies have indicated that other parasitoid species are attracted to aphid sex pheromones. *Diaeretiella rapae* was captured in pheromone-baited field traps (Gabrys *et al.*, 1997) and laboratory bioassays have been used to identify responses in *Aphidius ervi* (Poppy *et al.*, 1997) and *Aphidius matricariae* (Issacs, 1994).

It is possible that the response to aphid sex pheromones is a widespread phenomenon amongst aphid parasitoid species. If so, the potential would exist to manipulate parasitoids in a range of different crops, and test the aphid control strategy described in section 1.6.

In this chapter, the results of wind tunnel bioassays to examine the flight responses of parasitoids to aphid sex pheromones are presented. Wind tunnels have frequently been used to investigate the host location behaviour of parasitoids, particularly those which attack lepidopteran hosts. Studies have focused on the response to hosts and host products (Ma *et al.*, 1992; Agelopoulos *et al.*, 1995) and to plant host complexes (Eller *et al.*, 1988a; Turlings *et al.*, 1990; Kaiser and Cardé, 1992). Flight responses to host pheromones have also been investigated (Noldus *et al.*, 1991a; Wiskerke *et al.*, 1993).

Only three aphid parasitoid species have been studied using a wind tunnel bioassay; *Diaeretiella rapae* (Sheehan and Shelton, 1989b; Reed *et al.*, 1995; Vaughn *et al.*, 1996), *Lysiphlebus testaceipes* (Grasswitz and Paine, 1993) and *Aphidius ervi* (Guerrieri *et al.*, 1993; Du *et al.*, 1996). All of these studies investigated parasitoid responses to plants, aphids or plant-host complexes, however, recently *Aphidius ervi* was shown to respond to aphid sex pheromones in a wind tunnel (Poppy *et al.*, 1997).

The ability to study the flight behaviour of parasitoids provides the means to focus on a stage of the host location process which may involve the use of relatively long range cues (Eller *et al.*, 1988a; Du *et al.*, 1996). Therefore, if parasitoids respond to aphid sex pheromones in the wind tunnel, this would support previous evidence that the pheromone acts as a long range attractant (Hardie *et al.*, 1994).

The aims of the work presented in this chapter were to identify responses to aphid sex pheromones in previously unstudied parasitoid species, investigate the effects of the host aphid on parasitoid response and to test the activity of a synthetic sex pheromone component.

2.2 Methods

2.2.1 Insects

Parasitoids were obtained for experiments as described in section 1.7.1a.

2.2.2 Wind Tunnel Bioassay

The wind tunnel was specially designed for studying parasitoid flight behaviour (Du *et al.*, 1996). It consisted of a Plexiglas chamber (90 x 30 x 30 cm) through which an airflow was created using an extraction fan at one end. Air entered the tunnel from the opposite end via glass wool and charcoal filters, and passed through a mesh panel which helped to ensure a uniform airflow. This was tested

by visualising the odour plume using titanium chloride. Exhaust air was vented outside the room.

Wind speed was controlled by varying the speed of the fan, and lighting (provided by 3 U-shaped fluorescent tubes suspended above the tunnel) was adjusted by placing translucent paper between the lights and the roof of the tunnel. Both wind speed and light intensity were found to influence parasitoid flight performance, and different species required different conditions (partly related to the size of the parasitoid). Combinations of wind speed and lighting conditions used for different parasitoid species are presented in table 2.1.

Table 2.1 Wind speed and lighting conditions for aphid parasitoid species tested in wind tunnel bioassays.

Parasitoid Species	**Wind Speed (cms^{-1})**	**Light Intensity (Lux)**
Aphidius eadyi	22 ±2	3600
Aphidius ervi	22 ±2	3600
Aphidius rhopalosiphi	22 ±2	2500
Diaeretiella rapae	15 ±2	2500
Ephedrus plagiator	15 ±2	2500
Praon myzophagum	22 ±2	3600
Praon volucre	22 ±2	2500

Aphid sex pheromones (nepetalactone and nepetalactol) were released into the air stream from filter paper (Whatman No. 1) targets (1 x 2 cm) which were suspended from a vertical glass column. Nepetalactone and nepetalactol (1 mg/ml in hexane, see section 1.7.2) were applied to the filter paper using a microcapillary dispenser, and each application, whether a single component or a ratio of both,

amounted to 10 µl of solution. The control treatment consisted of 10 µl distilled hexane, applied in the same manner.

Parasitoids were released individually from an open ended glass tube (5 cm long x 1.5 cm diameter) positioned horizontally within the odour plume, 20 cm downwind from the odour source. Wasps were given 3 minutes in which to take-off from the release tube, and each wasp was tested only once. Parasitoids failing to take-off within 3 minutes were recorded as 'no take-off' and removed from the tunnel. A specific number of wasps were tested against each experimental treatment and all treatments were tested during a session, in a randomised order according to a quasi-complete Latin square, to account for variations in response both during and between sessions. The wind tunnel was cleaned with 95% ethanol between treatments.

For each parasitoid, the following information was recorded; time between release and take-off (time to take-off), whether the parasitoid took-off, made an oriented, upwind flight, or landed on the target. An oriented flight was defined as a straight, zig-zag or casting flight upwind, towards the target. Experiments were conducted in a controlled environment room (22 ±1 °C), between 2 and 5 hours into the parasitoid's scotophase, the time during which they were found to be most responsive.

2.2.3 Statistical Analysis

Means for each parameter measured were calculated for each test session, and session means were then averaged to give the overall response. Except where stated, session means were analysed by ANOVA using the SX Statistical Software (NH Analytical Systems) and compared using a Tukey test (Tukey's w procedure).The Tukey test was used in preference to other multiple comparison tests since it is more appropriate when the means of several samples are to be compared (Jones, 1984). Percentage responses were normalised using a logit transformation prior to ANOVA. The transformation was:

$$\text{Logit} = \ln \frac{P}{(1-P)}$$

where P is the proportion of wasps responding, determined by

$$P = \frac{x + 0.5}{n + 1}$$

and x is the number of wasps responding and n is the number tested.

For *D. rapae,* which was tested against only two treatments, the mean time to take-off was compared using a t-test, and the mean percentage taking-off was compared using a Chi-squared (χ^2) contingency test (Pearson method) in GENSTAT (Payne *et al.*, 1993). Mean time to take-off was calculated using the average times of those wasps which actually took-off from the release tube; non-responding wasps were excluded from this analysis.

2.3 Flight Responses of Four Previously Uninvestigated Aphid Parasitoid Species

2.3.1 Introduction

The growing number of parasitoid species which have been shown to respond to aphid sex pheromones suggests that the response may occur widely throughout the Aphidiinae. To test this further, four parasitoid species which had not previously been tested in the laboratory were chosen. If aphid sex pheromones are to be used to manipulate parasitoid populations in the field, it would be advantageous to be able to target parasitoids which attack the major aphid pests of different crops. The parasitoid species chosen for this study were; *Aphidius rhopalosiphi*, a parasitoid of cereal aphids, *Aphidius eadyi*, a specialist pea aphid

parasitoid, *Diaeretiella rapae*, which attacks aphids on brassicas, and *Ephedrus plagiator*, a generalist parasitoid with a wide host range.

Although different aphid species produce different ratios of the sex pheromone components nepetalactone and nepetalactol (table 1.1), previous studies have concentrated on the parasitoid response to nepetalactone (Issacs, 1994; Lilley *et al.*, 1994). An exception is the work of Du (unpublished data) who tested *Aphidius ervi* against nepetalactone, nepetalactol and a 1:1 ratio of the two components. The parasitoid gave a significantly stronger response to the 1:1 ratio, the exact ratio produced by its host aphid *Acyrthosiphon pisum* (Dawson *et al.*, 1990). In this study it was not possible to test a wide range of different ratios (due to limited availability of insects), so parasitoids were tested against nepetalactone, nepetalactol and a 1:1 ratio of the two. An exception was *Diaeretiella rapae* which was tested only against nepetalactone. The *D. rapae* experiment was unique in that male wasps were also tested (since field trapping experiments had reported attraction of males (Gabrys *et al.*, 1997; section 5.4).

2.3.2 Results

Details of the ANOVA results are given in the Appendix, in table A1.

2.3.2a Aphidius rhopalosiphi

The flight responses of female *A. rhopalosiphi* to aphid sex pheromones are shown in table 2.2. Parasitoids made significantly more oriented, upwind flights in response to nepetalactone, than to nepetalactol, a 1:1 ratio of nepetalactone :nepetalactol or to the control ($P< 0.05$).

Wasps only landed on the filter paper target in response to nepetalactone, but the proportion doing so was very low. There were no significant differences between treatments in the time to take-off ($P>0.05$) or in the percentage of wasps taking off ($P>0.05$)

Table 2.2 Responses of female *Aphidius rhopalosiphi* to nepetalactone and nepetalactol (10 µl of 1 mg/ml in hexane solution), a 1:1 ratio (5 µl:5 µl nepetalactone:nepetalactol 1 mg/ml in hexane) and control (10 µl hexane) in a wind tunnel bioassay

Treatment[1]	Time to take-off (s)	Mean % of wasps		
		Taking off	Making oriented flight	Landing on target
Nepetalactone	39.8 a	93.3 a	46.8 a	5.0 a
Nepetalactol	38.3 a	85.0 a	12.5 b	0.0 a
1:1 Ratio	43.6 a	80.8 a	9.3 b	0.0 a
Control	40.3 a	82.5 a	2.5 b	0.0 a

Column means followed by different letters are significantly different (ANOVA, Tukey test; $P< 0.05$).

[1]Forty parasitoids were tested to each treatment.

2.3.2b Aphidius eadyi

The flight responses of female *A. eadyi* to aphid sex pheromones are shown in table 2.3. Parasitoids made significantly more upwind flights to both nepetalactone and the 1:1 ratio than to the control ($P< 0.05$). Nepetalactol did not elicit a significant amount of oriented flights compared to the control ($P> 0.05$).

There were no significant differences between treatments in take-off times ($P>0.05$) or numbers of wasps taking-off ($P>0.05$), and parasitoids never landed on the filter paper target.

Table 2.3 Responses of female *Aphidius eadyi* to nepetalactone and nepetalactol (10 µl of 1 mg/ml in hexane solution), a 1:1 ratio (5 µl:5 µl nepetalactone:nepetalactol 1 mg/ml in hexane) and control (10 µl hexane) in a wind tunnel bioassay

Treatment[1]	Time to take-off (s)	Mean % of wasps		
		Taking off	Making oriented flight	Landing on target
Nepetalactone	40.4 a	92.8 a	27.6 a	0.0 a
Nepetalactol	39.6 a	71.3 a	8.1 ab	0.0 a
1:1 Ratio	24.2 a	97.0 a	31.8 a	0.0 a
Control	30.5 a	77.5 a	0.0 b	0.0 a

Column means followed by different letters are significantly different (ANOVA, Tukey test; $P< 0.05$).

[1]Thirty eight parasitoids were tested to each treatment.

2.3.2c Ephedrus plagiator

The flight responses of female *E. plagiator* to aphid sex pheromones are shown in table 2.4. Nepetalactol elicited significantly more oriented, upwind flights than did the control ($P< 0.05$), but not significantly more than a 1:1 ratio of nepetalactone:nepetalactol ($P> 0.05$). Nepetalactone did not elicit significantly more upwind flights than did the control ($P> 0.05$). There were no significant differences between treatments in either the time to take-off ($P>0.05$) or the number of wasps taking off ($P>0.05$), and wasps never landed on the filter paper target.

Table 2.4 Responses of female *Ephedrus plagiator* to nepetalactone and nepetalactol (10 µl of 1 mg/ml in hexane solution), a 1:1 ratio (5 µl:5 µl nepetalactone:nepetalactol 1 mg/ml in hexane) and control (10 µl hexane) in a wind tunnel bioassay

Treatment[1]	Time to take-off (s)	Mean % of wasps		
		Taking off	Making oriented flight	Landing on target
Nepetalactone	19.0 a	100 a	15.7 ab	0.0 a
Nepetalactol	24.8 a	98.3 a	37.8 a	0.0 a
1:1 Ratio	27.4 a	94.4 a	27.7 a	0.0 a
Control	25.0 a	100 a	0.0 b	0.0 a

Column means followed by different letters are significantly different (ANOVA, Tukey test; $P< 0.05$).

[1]Forty four parasitoids were tested to each treatment.

2.3.2d Diaeretiella rapae

The flight responses of *D. rapae* to nepetalactone are shown in table 2.5. Nepetalactone did not elicit significantly more oriented, upwind flights by female wasps than did the control. However nepetalactone did cause a significant increase in the number of female wasps taking off from the release tube (χ^2 contingency test; 1 d.f., $\chi^2=8.29$, $P< 0.001$), and a significant reduction in the time to take-off of these wasps (t-test, $P< 0.05$). There was no evidence of a behavioural response to nepetalactone by male *D. rapae*. Neither male nor female wasps landed on the filter paper target.

Table 2.5 Responses of *Diaeretiella rapae* to nepetalactone (10 µl of 1 mg/ml in hexane solution), and control (10 µl hexane) in a wind tunnel bioassay

Treatment[1]	Time to take-off (s)	Mean % of wasps		
		Taking off	Making oriented flight	Landing on target
Females				
Nepetalactone	55.8*	79.6***	8.1	0.0
Control	96.6	49.9	4.8	0.0
Males				
Nepetalactone	42.8	79.8	5.5	0.0
Control	45.3	88.4	10.9	0.0

*Column means significantly different at $P< 0.05$ (t-test)

***Column means significantly different at $P< 0.01$ (χ^2 contingency test)

[1]Fifty five parasitoids were tested to each treatment.

2.3.3 Discussion

2.3.3a Aphidius rhopalosiphi

Female *A. rhopalosiphi* showed a strong response to the aphid sex pheromone component nepetalactone, 46.8% of wasps making an oriented, upwind flight towards this odour source in the wind tunnel. It is likely that this species can use the aphid sex pheromone as a cue during the host location process. Furthermore, since nepetalactone elicits a flight response, the cue is likely to operate as a relatively long-range attractant. Although *A. rhopalosiphi* showed a strong response to nepetalactone, it showed only a weak response to nepetalactol. Another aphid parasitoid, *Praon volucre,* showed a weak, but significant response to nepetalactol-baited traps in the field (Hardie *et al.*, 1994). Both *A. rhopalosiphi* and *P. volucre* attack cereal aphids, but *P. volucre* also attacks a wide range of

other aphid species. *A. rhopalosiphi* is a relative specialist on cereal aphids, but does attack both *Sitobion avenae* which produces only nepetalactone, and *Rhopalosiphum padi* which produces only nepetalactol (table 1.1).

Another difference between *A. rhopalosiphi* and *P. volucre* is that whereas *P. volucre* is closely associated with environments containing the primary hosts of holocyclic, host-alternating aphids (Vorley, 1986), *A. rhopalosiphi* is more commonly found in cereals and grasses. Its host aphids often pass the winter anholocyclicly in Britain, depending on the weather (Hand, 1989), so *A. rhopalosiphi* may not need to locate sexual aphid colonies in most years. Therefore, this parasitoids' response to aphid sex pheromones may be an ancestral adaptation to a stage in its evolutionary history when its host aphids were host-alternating.

When presented with a 1:1 ratio of nepetalactone to nepetalactol in the wind tunnel, *A. rhopalosiphi* made significantly fewer upwind flights than to the nepetalactone alone. This may be due to the lower amounts of each component applied to the target (5 µl of each instead of 10 µl of a single component). However, other work has shown that parasitoids can distinguish between different pheromone blends presented as the same overall amount of material (section 2.4). Although *A. rhopalosiphi* would not normally encounter aphids which produce a 1:1 or similar pheromone ratio in its usual habitat, the reduced response may be an adaptation for avoiding non-host aphids.

2.3.3b Aphidius eadyi

A. eadyi responded strongly to both nepetalactone and a 1:1 ratio of nepetalactone to nepetalactol. *A. eadyi* is a specialist parasitoid of pea aphid *Acyrthosiphon pisum* (Cameron *et al.*, 1981), which produces a 1:1 ratio as its sex pheromone (table 1.1). The strong response to the 1:1 ratio would allow *A. eadyi* to efficiently locate colonies of holocyclic pea aphids. Du (unpublished data) found that another pea aphid parasitoid, *Aphidius ervi*, responded more strongly to a 1:1 ratio than to nepetalactone alone in a wind tunnel, and suggested that this species, being

relatively specialised, may have a preferential response to the exact pheromone ratio produced by its host (Y-J. Du, personal communication). Since *A. eadyi* has an even narrower host range than *A. ervi*, the strong response in this experiment to the nepetalactone alone is surprising. In the experiments of Du (unpublished data), the 1:1 ratio consisted of twice as much pheromone solution (10 µl lactone + 10 µl lactol) than the single nepetalactone treatment (10 µl). Indeed, in his experiments 10 µl nepetalactone elicited more upwind flights than did a 1:1 ratio consisting of 5 µl of each component (10 µl total). Therefore the strength of the attraction to nepetalactone may have been underestimated. The response to nepetalactone by *A. eadyi* may be ancestral; the parasitoid may have gradually become specialised on pea aphid, but it may also enable this species to locate other hosts if required.

2.3.3c Ephedrus plagiator

Female *E. plagiator* displayed responses to all pheromone treatments, although the response to nepetalactone was relatively weak. *E. plagiator* is a generalist parasitoid with a wide host range; Starý (1976) lists over 20 recorded host aphids. The parasitoid's broad response to aphid sex pheromone components may be an adaptation to this generalist lifestyle. It would find a wide range of aphids, producing a range of sex pheromone combinations, suitable for attack, and would benefit by being able to efficiently locate these colonies in the autumn.

2.3.3d Diaeretiella rapae

D. rapae exhibited a very low amount of upwind flight, and nepetalactone had no significant effect on this behaviour. However, female wasps did respond to nepetalactone with an increased tendency to take-off and a reduced time to take-off. There is one report of an odour source causing parasitoid flight initiation in a wind tunnel (Eller *et al.*, 1988a), and a reduction in the time to take-off in response to volatiles in a wind tunnel has been reported for *Cotesia rubecula* (Keller, 1990). The present study clearly demonstrates that *D. rapae* has a behavioural response to nepetalactone. Why this response was not manifested as upwind flight, as in other species, is not known. *D. rapae* was observed to be a

weak flier in the wind tunnel, having difficulty making upwind progress, and while the airflow of 15 cms^{-1} could have been lowered, wind speeds below this were shown by titanium chloride vapour not to support coherent odour plumes. Alternatively, the response of *D. rapae* may not involve a component of long range attraction, but the results of field trapping do not support this (Gabrys *et al.*, 1997; section 5.4).

Male *D. rapae* did not show any response to nepetalactone. Consistent responses by male parasitoids to aphid sex pheromones have not previously been reported (Issacs, 1994; Lilley *et al.*, 1994; section 2.5). Indeed it is difficult to propose a role for aphid sex pheromones in the ecology of male parasitoids. Males could perhaps use them to locate sites likely to contain host-searching females, but since many female parasitoids produce sex pheromones of their own (section 1.1.3a), this would appear unnecessary.

2.2.2e Flight responses and implications for parasitoid manipulation

The results of these experiments support the hypothesis that the parasitoid response to aphid sex pheromones may be a widespread phenomenon amongst the Aphidiinae. They also lend weight to the argument that the sex pheromones function as attractants, and over relatively large distances. The four species tested here differed in their responses, both in terms of the combinations of pheromone components to which they were attracted and the strength of that attraction (measured by percentage upwind flight). One species, *Diaeretiella rapae*, showed evidence of flight initiation in response to aphid sex pheromone.

It should be remembered that parasitoids within the same group (Aphidiinae) may show differences in response, and that this difference may be particularly apparent between specialist and generalist species. However, it is also clear that parasitoids with different host associations and habitats possess this response. The current experiment has demonstrated this for a specialist parasitoid, *Aphidius eadyi*, two which are specialised at the level of the plant, *Aphidius rhopalosiphi* and *Diaeretiella rapae* and a generalist, *Ephedrus plagiator*.

It is interesting therefore to speculate on how and why the parasitoid response to aphid sex pheromones evolved. It is possible that the response is an ancestral one which evolved before aphids became parthenogenetic, and therefore produced sexual females throughout the year. However, parthenogenesis is believed to have appeared very early in the evolution of aphids, perhaps 200 million years ago (Dixon, 1987). There are no aphidiines recorded in the fossil record until much later (Starý, 1970) and the main radiation of the Apocrita took place about 100 million years ago (Gauld and Bolton, 1988). So it is possible that the first aphidiines were associated with aphid species which had already developed their present day lifecycles. About 30-50 million years ago, host-alternation became a feature of aphid ecology (Moran, 1992). Therefore parasitoids may have begun to exploit aphid sex pheromones in order to follow aphid populations back to their winter host plants. Although only 10% of aphid species are host-alternating today, many more were in the past (Moran, 1992), so this may have been a selection factor in the evolution of the parasitoid response.

Although parasitoids made upwind flights directed towards the filter paper target, very few actually landed on the target. This may be due to the low level of visual information available to the wasp as it approached the target. Female *Aphidius ervi*, flying upwind to a plant host complex, did make a high number of landings on the plant, which obviously presents a powerful visual target (Du *et al.*, 1996). However, the lack of a visual component of orientation in these experiments supports the view that aphid sex pheromones may attract parasitoids over relatively large distances.

The parasitoids tested in these experiments attack a range of aphid species, which in turn attack some of the major crops grown in the UK (table 2.6). This enhances the potential to exploit the parasitoid response to aphid sex pheromones and attempt to manipulate parasitoid populations in agroecosystems.

Table 2.6 Parasitoid species responding to aphid sex pheromones in a wind tunnel bioassay, the aphids which they attack and the crops in which they are active

Parasitoid Species	Aphids Attacked	Active in Crops
Aphidius eadyi	*Acyrthosiphon pisum*	legumes
Aphidius rhopalosiphi	*Metopolophium dirhodum*	cereals
	Rhopalosiphum padi	
	Sitobion avenae	
Diaeretiella rapae	*Brevicoryne brassicae*	oilseed rape, brassica
	Macrosiphon euphorbiae	vegetables, potatoes
	Myzus persicae	
Ephedrus plagiator	*Acyrthosiphon pisum*	cereals, legumes
	Aphis fabae	
	cereal aphids	

2.4 Flight Responses of Parasitoid Species Reared on Two Different Host Aphids

2.4.1 Introduction

The aphid host in which a female parasitoid develops has an influence on the adult parasitoid's preferences and responses. Host preference effects are apparent within the Aphidiinae, with females often showing a preference for the host in which they developed (Pungerl, 1984; Powell and Wright, 1988). Host preference may be expressed through differential responses to chemicals originating from the plant host complex (Du *et al.*, 1996), the aphid cuticle (Grasswitz and Paine, 1992) or from inside the aphid's body (Pennacchio *et al.*, 1994). Parasitoids could

potentially use the particular sex pheromone blend released by different aphids to locate their preferred hosts.

To investigate whether the host aphid influences parasitoid response to aphid sex pheromones, two parasitoid species which had been laboratory reared on different host aphids were used. Since the parasitoid response to aphid sex pheromones is believed to be innate (Poppy *et al.*, 1997), one would not expect it to be modified by internal cues experienced by the parasitoid developing inside the host. However, differences in response between parasitoids from two hosts could arise from the parasitoid cultures being established from two genetically different populations or sub-populations, each associated with a particular aphid host.

2.4.2 Results

Details of the ANOVA results are given in the Appendix, in tables A2-A3.

2.4.2a Praon myzophagum reared on Acyrthosiphon pisum and Myzus persicae

The flight responses of *P. myzophagum* reared on *A. pisum* and *M. persicae* are shown in table 2.7. Parasitoids reared on *A. pisum* made significantly more upwind flights in response to a 1:1 ratio of nepetalactone to nepetalactol than to any other treatment ($P<0.05$). No other treatment elicited a significant response. Parasitoids reared on *A. pisum* landed on the 1:1 target significantly more often than on other targets ($P< 0.05$). There were no significant differences between treatments in the time to take-off, or the number of wasps taking off ($P>0.05$).

Parasitoids reared on *M. persicae* made significantly more upwind flights to both the 1:1 and 1:2 ratios ($P< 0.05$), both of which were equally attractive. The nepetalactone and nepetalactol treatments elicited a lower response which was not significantly different to the control ($P> 0.05$). There were no significant differences in the number of wasps landing on the target. There were no significant differences between treatments in the time to take-off, or the number of wasps taking off ($P>0.05$).

Table 2.7 Responses of female *Praon myzophagum* reared on two host species to nepetalactone and nepetalactol (10 µl of 1 mg/ml in hexane solution), a 1:1 ratio (5 µl:5 µl nepetalactone:nepetalactol 1 mg/ml in hexane), a 1:2 ratio (3.3 µl:6.6 µl nepetalactone:nepetalctol) and control (10 µl hexane) in a wind tunnel bioassay

Treatment	Time to take-off (s)	Mean % of wasps		
		Taking off	Making oriented flight	Landing on target
Reared on *Acyrthosiphon pisum*[1]				
Nepetalactone	49.4 a	96.6 a	27.0 a	0.0 a
Nepetalactol	60.8 a	95.0 a	35.4 a	8.3 ab
1:1 Ratio	49.6 a	100 a	68.8 b	29.8 b
1:2 Ratio	52.8 a	100 a	31.0 a	2.0 ab
Control	35.4 a	97.6 a	23.9 a	0.0 a
Reared on *Myzus persicae*[2]				
Nepetalactone	62.8 a	82.6 a	24.6 ab	0.0 a
Nepetalactol	60.8 a	96.6 a	22.0 ab	0.0 a
1:1 Ratio	52.6 a	98.6 a	41.4 b	7.4 a
1:2 Ratio	66.8 a	92.6 a	44.0 b	4.0 a
Control	77.6 a	78.0 a	20.6 a	0.0 a

Column means followed by different letters are significantly different (ANOVA, Tukey test; $P< 0.05$).

[1]Forty two parasitoids were tested to each treatment.

[2]Fifty five parasitoids were tested to each treatment.

2.4.2b Praon volucre reared on Acyrthosiphon pisum and Sitobion avenae

The flight responses of *P. volucre* reared on *A. pisum* and *S. avenae* are shown in table 2.8. Parasitoids from both hosts made significantly more upwind flights to both nepetalactone and a 1:1 ratio of nepetalactone to nepetalactol than to the control ($P< 0.05$), and both of these treatments were equally attractive. There were no significant differences between treatments in time to take-off, number of wasps taking off or landing on the target.

Table 2.8 Responses of female *Praon volucre* reared on two host species to nepetalactone (10 µl of 1 mg/ml in hexane solution), a 1:1 ratio (5 µl:5µl nepetalactone:nepetalactol 1 mg/ml in hexane) and control (10 µl hexane) in a wind tunnel bioassay

		Mean % of wasps		
Treatment	**Time to take-off (s)**	**Taking off**	**Making oriented flight**	**Landing on target**
Reared on *Acyrthosiphon pisum*[1]				
Nepetalactone	42.3 a	100 a	37.7 a	0.0 a
1:1 Ratio	37.0 a	100 a	43.0 a	6.7 a
Control	41.7 a	100 a	12.2 b	0.0 a
Reared on *Sitobion avenae*[2]				
Nepetalactone	40.7 a	97.7 a	45.7 a	2.3 a
1:1 Ratio	41.0 a	97.7 a	40.7 a	9.0 a
Control	52.3 a	93.3 a	9.0 b	0.0 a

Column means followed by different letters are significantly different (ANOVA, Tukey test; $P< 0.05$).

[1]Twenty five parasitoids were tested to each treatment.

[2]Thirty five parasitoids were tested to each treatment.

2.4.3 Discussion

Praon myzophagum showed a different pattern of responses to sex pheromones depending upon which host it was cultured on. Wasps reared on pea aphid *Acyrthosiphon pisum* responded only to a 1:1 ratio of pheromone components, which is the ratio produced by pea aphid oviparae (table 1.1), whilst wasps reared on *Myzus persicae* responded strongly to both a 1:1 ratio as well as a 1:2 ratio, which is the ratio produced by *M. persicae*. These results could be explained in terms of adaptation to a particular host aphid if the cultures had been established using parasitoids collected from two distinct field populations. However, this does not seem to have been the case. The recorded history of the culture suggests that the initial source of *P. myzophagum* was from *M. persicae*, and that a subculture was later established on *A. pisum*. It is thought that the subculture was initiated about three years prior to these experiments by transferring parasitoids from *M. persicae* onto *A. pisum*.

Therefore it appears that during this three year period, *P. myzophagum* on *A. pisum* has lost its response to the 1:2 ratio. The ability to discriminate between a 1:1 and a 1:2 ratio of two pheromone components implies a very subtle and sensitive response on the part of the parasitoid. However, there is evidence that aphid parasitoids can discriminate between aphid sex pheromone ratios in a behavioural bioassay (Y-J. Du, unpublished data). The loss of this response is puzzling since the female parasitoids should have experienced no selective pressure within the cultures to respond to a particular sex pheromone blend, because sexual aphids were not present in the cultures. Also it is difficult to propose an effect of 'chemical legacy' conditioning within the body of the host aphid (Corbet, 1985), since no sex pheromone material should be present and the parasitoid response is believed to be innate and genetically governed (Poppy *et al.*, 1997).

Praon volucre, reared on two different host aphids, showed no such differences in response to sex pheromones, but the *P. volucre* culture which was established on *S. avenae* was subcultured on *A. pisum* less than six months prior to the

experiment. The results of these two experiments considered together point to an effect of laboratory rearing on the change in response shown by *P. myzophagum*. Due to the small founder numbers and low population sizes associated with parasitoid cultures, genetic drift can easily occur (Mackauer, 1976), and this has been shown for the aphid parasitoid *Aphidius ervi* (Unruh *et al.*, 1983). It is possible that a chance effect in establishing the *pisum*-subculture using a very low number of individuals, or subsequent inbreeding or loss of alleles from the population has resulted in the loss of response to the 1:2 ratio. Unfortunately, not enough is known about the genetics of parasitoid responses to olfactory cues to be able to suggest a mechanism by which this could happen. It is not known whether this effect could be so subtle as to switch off a response to a 1:2 ratio of components whist leaving the response to a 1:1 ratio intact, although this could conceivably occur by a process of desensitisation.

The implications of this effect for manipulating parasitoids in the field are probably not serious, since field populations should not suffer the same genetic problems as laboratory cultures. Field populations of the same parasitoid species could exist as specialised subpopulations, associated with different host aphids, and this has been shown for *Aphidius ervi* (Cameron *et al.*, 1984). Differential responses to semiochemical cues could be one mechanism by which such a separation arises, but this has yet to be demonstrated. There would be more serious implications for a strategy which combined mass release of parasitoids with manipulation by pheromones. If parasitoid responses to pheromones can change over time under laboratory rearing, then care should be taken with the management of cultures. The responses of the individuals used to establish the desired culture would have to be checked. The culture would need to be regularly augmented with field collected wasps, and the responses of the laboratory reared wasps should be periodically assessed to ensure that changes such as those described in this experiment have not occurred.

2.5 Flight Responses of *Praon volucre* to Synthetic and Plant-Extracted Nepetalactone

2.5.1 Introduction

All previous studies on both aphids and aphid parasitoids have used sex pheromones which were obtained by extraction from the catmint plant, *Nepeta cataria* (section 1.7.2). Nepetalactone exists in *N. cataria* as a secondary plant metabolite and its function is unknown, although it may have a role in plant defence (Eisner, 1964). Although it is produced in volatile form by mechanically damaged plants, there are no reports of *Nepeta* plants attracting either male aphids or female parasitoids.

Chemists have recently been able to produce aphid sex pheromone components of high purity via fully synthetic pathways (Dawson *et al.*, 1996), and these compounds have been tested against male aphids in the field. In trapping experiments (Hardie *et al.*, 1997), traps baited with 99% pure, synthetic (4aS,7S,7aR)-nepetalactone captured 50% more male *Sitobion fragariae* than traps baited with plant derived (4aS,7S,7aR)-nepetalactone. In similar experiments (Hardie *et al.*, 1997), 99% pure, synthetic (1R,4aS,7S,7aR)-nepetalactol attracted three times as many male *Rhopalosiphum padi* than did the plant derived lactol. The authors suggested that the differences may be due to the presence of unknown plant volatiles in the *Nepeta* extracted pheromones which deter male aphids.

If aphid parasitoids also discriminate between synthetic and plant-extracted pheromones, this would have implications for their manipulation in the field using sex pheromone lures. *Praon volucre* is an important parasitoid species which attacks both *Sitobion fragariae* and *Rhopalosiphum padi*, and is known to respond to nepetalactone (Hardie *et al.*, 1991; Lilley *et al.*, 1994). Therefore, the responses of *P. volucre* to synthetic and plant-extracted nepetalactone were investigated in the wind tunnel.

2.5.2 Results

Flight responses of *P. volucre* to synthetic and plant-extracted nepetalactone are shown in table 2.9. Details of the ANOVA results are given in the Appendix, in table A4.

Table 2.9 Responses of *Praon volucre* to synthetic and plant-extracted nepetalactone (10 µl of 1 mg/ml in hexane solution), and control (10 µl hexane) in a wind tunnel bioassay

		Mean % of wasps		
Nepetalactone source[1]	**Time to take-off (s)**	**Taking off**	**Making oriented flight**	**Landing on target**
Females				
Synthetic	55.5 a	89.7 a	31.6 a	4.0 a
Plant-extracted	47.7 a	94.8 a	34.5 a	2.0 a
Control	47.8 a	92.2 a	3.3 b	0.0 a
Males				
Synthetic	70.6 a	91.6 a	0.0 a	0.0 a
Plant-extracted	73.8 a	94.2 a	2.0 a	0.0 a
Control	64.4 a	98.0 a	0.0 a	0.0 a

Column means for males and females followed by different letters are significantly different (ANOVA, Tukey test; $P < 0.05$).

[1]Fifty eight parasitoids were tested to each treatment.

Female parasitoids made significantly more oriented, upwind flights to both synthetic and plant-extracted nepetalactone than to the control ($P< 0.05$). Both pheromone treatments were equally attractive. There were no significant differences in the time to take-off, numbers of wasps taking off or landing on the target. Male *P. volucre* showed no behavioural responses to the sex pheromones.

2.5.3 Discussion

Praon volucre was attracted equally to both synthetic and plant-extracted aphid sex pheromones at the stimulus concentrations used, and these results are similar to those obtained with *Aphidius ervi* which also showed an equal response to both (Y-J. Du, personal communication). This suggests that, in the laboratory at least, parasitoids do not show the same degree of discrimination between synthetic and plant-extracted pheromones as do aphids. Hardie *et al.* (1997) suggest that, since the plant-extracted nepetalactone is quite pure in terms of enantiomeric composition, there could be undetected plant volatiles in the extract which either repel male aphids or mask the active compound. If this is so, then aphid parasitoids appear to be unaffected by such chemicals. This difference could arise because, whereas male aphids may use plant compounds to locate conspecific females (Campbell *et al.*, 1990) or avoid unsuitable hosts (Hardie *et al.*, 1997), parasitoids of the genus *Praon*, which attack aphids on a wide range of host plants (Starý, 1976), may rely more on the presence of aphid sex pheromones than on specific plant volatiles.

If parasitoids do not respond more strongly to synthetic pheromone, then the implications for parasitoid manipulation in the field are contrasting. On one hand it means that there is no immediate improved pheromone to use as a more powerful attractant. On the other it may mean that, although parasitoids are strongly attracted to sex pheromone lures, the attraction of male aphids may be sub-optimal, decreasing potential problems of contributing to pest aphid populations. It also means that sex pheromones can continue to be obtained by plant extraction, a considerably cheaper method than laboratory synthesis.

It should be noted that this experiment was carried out in a laboratory, using a single choice bioassay and laboratory-reared parasitoids. It would prove valuable to test parasitoid responses when given a choice of sex pheromones, and also to test the synthetic pheromone in a field situation, where the natural range of stimuli (e.g. the plant host complex) are present.

Chapter 3

Laboratory Investigations into the Nature of the Parasitoid Response to Aphid Sex Pheromones

3.1 Introduction

There is evidence that the parasitoid response to aphid sex pheromones consists, at least partially, of a relatively long range attraction (Hardie *et al.*, 1994; section 2.3). This is encouraging for the prospects of manipulating parasitoid populations in the field (section 1.6). However, aphid sex pheromones may have more subtle effects on parasitoid behaviour which could affect the approach taken towards a behavioural manipulation strategy. So far these have remained uninvestigated.

It is particularly important to ascertain whether learning or experience modifies the way in which parasitoids respond to aphid sex pheromones. It would also be valuable to know whether, as well as attracting parasitoids, sex pheromones affect other aspects of the wasps searching behaviour such as attack rate, or time spent in pheromone baited areas.

This chapter presents the results of investigations into the effects of experience on both parasitoid responses to aphid sex pheromones and attack rates, and on the effects of pheromones on parasitoid retention on plants. The results will be valuable when considering the use of aphid sex pheromones to manipulate parasitoid behaviour in the field.

3.2 Effect of Experience on Aphid Parasitoid Response to Aphid Sex Pheromones in a Four-Way Olfactometer

3.2.1 Introduction

Studies have shown that the responses of parasitoids to cues encountered during host location can be modified by experience (Vet and Groenewold, 1990; Turlings *et al.*, 1993), and this may occur via learning. Parasitoids appear to be particularly adept at learning host associated cues, with learning having been reported in over 20 species (Turlings *et al.*, 1993). Current theory proposes that parasitoids have developed the capacity for learning to enable them to locate hosts in a complex and changing environment, and that they should learn cues from the host's environment more readily than from the host itself (Vet and Dicke, 1992; Turlings *et al.*, 1993). This is because host-derived cues provide a reliable indication of host presence and often elicit strong innate responses, whereas environmental cues give less reliable information, and often elicit weaker innate responses (Vet and Dicke, 1992). In this way, parasitoids retain the flexibility to exploit the prevailing foraging conditions (Vet *et al.*, 1995). Learning is considered to be of greater significance for generalist parasitoids which can utilise a wide range of hosts, than for specialists which need only locate one or a few hosts, although this is not a hard and fast rule (Vet *et al.*, 1995).

Associative learning is said to have occurred when a response to a stimulus is newly acquired or enhanced by linking with a reinforcing stimulus (usually a host-derived product or the host itself) (Vet and Dicke, 1992). When an existing, weak response is enhanced by association, the process can be further classified as 'alpha conditioning' (Vet *et al.*, 1990a; Menzel *et al.*, 1993; Vet *et al.*, 1995), and this has been demonstrated in parasitoids (Eller *et al.*, 1988b; Cardé and Lee, 1989).

Associative learning has been shown in three aphid parasitoid species. *Lysiphlebus testaceipes* increased its response to a plant host complex (PHC) after oviposition on the PHC (Grasswitz and Paine, 1993) and *Diaeretiella rapae*

increased its response to a less preferred host after oviposition experience (Sheehan and Shelton, 1989b). Recently *Aphidius ervi* was shown to acquire a response to an undamaged plant via association with a host product (aphid honeydew) (Du *et al.*, 1997).

Aphid sex pheromone is a host-derived cue which is a highly reliable indicator of the presence of hosts, and the parasitoid response is believed to be innate (Poppy *et al.*, 1997). Therefore, aphid parasitoids do not need to acquire the response by associative learning. However, the strength of the response could be increased by association via the alpha conditioning process outlined above (Vet *et al.*, 1990a; Vet *et al.*, 1995). This would improve the parasitoids' ability to exploit sexual aphid colonies when they appear in the autumn.

In this experiment, the effect of associative learning on the response to aphid sex pheromone is examined in a relatively specialised parasitoid, *Aphidius ervi* and a more generalist species, *Ephedrus plagiator*. If parasitoid responses could be enhanced in this way then higher levels of attraction to field lures may be observed. It could also open the possibility of 'priming' parasitoids in the laboratory by contact with pheromone before mass releasing them into pheromone-baited crops.

3.2.2 Methods

3.2.2a Insects

Female *Aphidius ervi* and *Ephedrus plagiator* were obtained as described in section 1.7.1a.

3.2.2b Four-way Olfactometer Bioassay

The four-way olfactometer used to test parasitoid responses to aphid sex pheromones was based on a design used by Pettersson (1970) and Vet *et al.* (1983). It measured 11 cm in diameter by 1.5 cm in height and consisted of two perspex layers which, when sandwiched together, formed four arm regions and a

central area (figure 3.1). Air was removed from the centre of the olfactometer by a vacuum pump, buffered by a 1l jar and adjusted with a flowmeter to 400 ml/min. This airflow caused air to enter the olfactometer via holes at the ends of the arms, setting up four distinct regions of airflow (verified by visualisation with titanium chloride vapour).

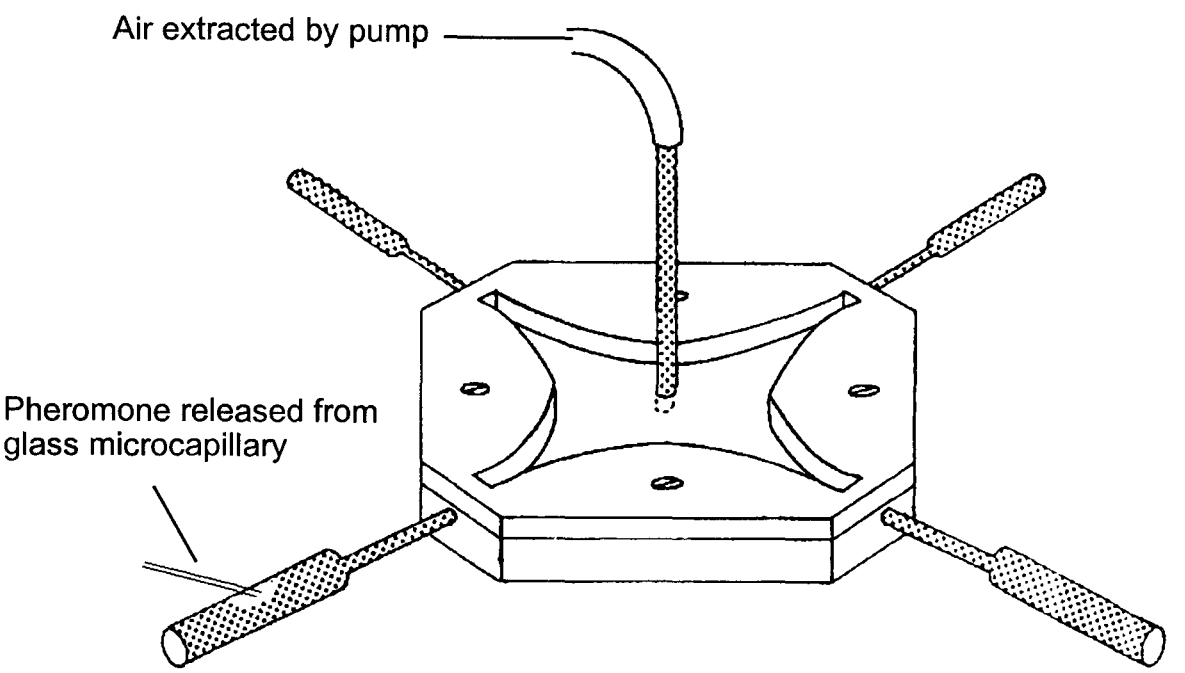

Figure 3.1 Four-way olfactometer showing the four arm regions. Aphid sex pheromone was released from a 10 µl microcapillary which was inserted through a hole in a Pasteur pipette.

Pheromones were introduced via microcapillary tubes (Drummond 10 µl) which were inserted at an angle of 45° into a hole blown in the tapered end of a Pasteur pipette. The pipette was then placed into the hole at the end of the test arm (figure 3.1). Pheromone (10 µl of 1 mg/ml in hexane solution) was introduced at 0.6 µl/min by evaporation. With *A. ervi*, nepetalactone and nepetalactol were released from separate microcaps to give a 1:1 ratio, since this species was shown to respond strongly to this combination (Poppy *et al.*, 1997). With *E. plagiator* a single microcap was used to release nepetalactol since *E. plagiator* responded to

this compound in a wind tunnel bioassay (section 2.3.2c). During experiments, pheromone was introduced into one arm, and into the other three arms hexane was introduced in an identical manner as a control. A small piece of terylene netting ensured that parasitoids did not leave the olfactometer and enter the odour release tubes.

The olfactometer was confined within a blackened cardboard box and illuminated from above by a small, fluorescent lamp, diffused by translucent paper. Exhaust air from the vacuum pump was vented outside the room.

A single parasitoid was introduced into the olfactometer by allowing it to enter via a hole in the floor of the chamber and, after entry, the hole was sealed with a Teflon stopper. The wasp was observed for 8 minutes, and the olfactometer was rotated through 90° each minute to eliminate directional bias. The total time spent by a wasp in each area, and the number of entries made into each area were recorded using The Observer behavioural software (Noldus Information Technology, The Netherlands). Between 17 and 19 parasitoids from each pre-experience treatment were tested.

The arm containing the pheromone source was changed systematically after every two parasitoids to control for equipment bias, and the olfactometer was cleaned with detergent and 90% ethanol each time the odour arm was changed. A small amount of anti-static fluid was applied to internal surfaces prior to use.

3.2.2c Experimental Treatments

Groups of five parasitoids were placed into a sealed 9 cm Petri dish and exposed to one of five experimental treatments:

I Aphid sex pheromone

II Host aphids + Hexane

III Host aphids + aphid sex pheromone at the same time

IV Host aphids followed separately by aphid sex pheromone

V None (hexane alone)

Aphids

Twenty aphids of the species on which the parasitoid was reared were placed in the Petri dish. Only larval instars were used as these are often preferred by parasitoids for oviposition. The sides of the Petri dish were coated with Fluon to confine aphids to the floor of the arena. With *A. ervi*, 20 *Acyrthosiphon pisum* were used and with *E. plagiator*, 20 *Sitobion avenae* were provided. All wasps were observed to attack at least one aphid during the treatment period.

Chemicals

Aphid sex pheromones and hexane control were introduced into the Petri dish by release from a microcapillary tube (Drummond 10 µl). The microcap was inserted into a short piece of Teflon tubing, which in turn was inserted through a small hole in the lid of the Petri dish. Another Teflon tube left the dish by a second hole in the lid and was attached to a vacuum pump, which was adjusted to 200 ml/min. Aphid sex pheromone (10 µl of 1 mg/ml in hexane solution) was released at 0.3 µl/min from the microcap by evaporation. Hexane control was released in an identical manner. For *A. ervi*, nepetalactone and nepetalactol were released from separate microcaps to give a 1:1 ratio, and for *E. plagiator* a single microcap released nepetalactol.

All treatments lasted 30 minutes, except treatment IV. This treatment was introduced as a control to break any temporal association between the sex

pheromone and oviposition. Wasps in this treatment were given aphids for 30 minutes, followed immediately by pheromone in a separate Petri dish for a further 30 minutes. After treatment, wasps were placed in stoppered glass tubes with access to 50% honey solution. They were kept at 18 °C for 2 hours before testing to allow their egg load to return to normal, since reduced egg loads may affect the responses of aphid parasitoids to foraging cues (Du *et al.*, 1997).

3.2.2d Statistical Analysis

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. Time and entries in the central area (where wasps could make a choice between the four arms) were excluded from the analysis. 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 (1 df). The null hypothesis was that, since there are four arms, parasitoids should make 25% of entries into and spend 25% of time in both the pheromone-treated arm and in each of the control arms (mean value).

3.2.3 Results

3.2.3a Aphidius ervi

The number of entries into, and the total time spent in pheromone and control arms by *A. ervi* subjected to different treatments are shown in table 3.1. Parasitoids from treatments II-IV (see 3.2.2c) all showed significant responses to aphid sex pheromone ($P< 0.01$-$P< 0.001$). The response was apparent as both an increased percentage of entries into the pheromone-baited arm (table 3.1a) and an increased percentage time spent in the pheromone-baited arm (table 3.1b). Wasps subjected to treatment I (exposed only to aphid sex pheromone) did not show a significant response to pheromone compared with the control ($P> 0.05$).

Table 3.1 Response of *Aphidius ervi*, subjected various pre-treatments, to aphid sex pheromone in a four-way olfactometer

a % time spent in control and pheromone-treated arms

Experience	n	mean % time spent in arm		χ^2 (1 df)
		Pheromone[1]	Control[2]	
I Pheromone	19	33.8	22.1	3.44^{NS}
II Host aphids	19	42.9	19.0	14.26^{**}
III I with II	19	43.6	18.8	15.38^{***}
IV I followed by II	17	37.1	20.1	6.81^{**}
V None	19	39.4	20.2	9.21^{**}

b % no. entries into control and pheromone-treated arms

Experience	n	mean % entries into arm		χ^2 (1 df)
		Pheromone[1]	Control[2]	
I Pheromone	19	33.1	22.3	2.91^{NS}
II Host aphids	19	37.5	20.1	7.21^{**}
III I with II	19	43.6	18.8	15.38^{***}
IV I followed by II	17	40.5	19.8	10.69^{**}
V None	19	45.9	17.9	19.49^{***}

,* $P< 0.01$, $P< 0.001$ respectively (Chi-squared test); NS= no significant difference between pheromone and control.

[1]1:1 ratio nepetalactone:nepetalactol (1 mg/ml in hexane)

[2]Mean of 3 control arms

3.2.3b Ephedrus plagiator

Table 3.2 Response of *Ephedrus plagiator*, subjected to various pre-treatments, to aphid sex pheromone in a four-way olfactometer

a % time spent in control and pheromone-treated arms

Experience	n	mean % time spent in arm		χ^2 (1 df)
		Pheromone[1]	Control[2]	
I Pheromone	19	19.4	26.8	1.38^{NS}
II Host aphids	17	23.9	25.3	0.052^{NS}
III I with II	18	46.0	18.0	19.56^{***}
IV I followed by II	18	19.6	26.8	1.30^{NS}
V None	19	30.1	23.0	1.20^{NS}

b % no. entries into control and pheromone-treated arms

Experience	n	mean % entries into arm		χ^2 (1 df)
		Pheromone[1]	Control[2]	
I Pheromone	19	24.9	25.0	0.001^{NS}
II Host aphids	17	22.5	25.8	0.28^{NS}
III I with II	18	38.4	20.5	7.99^{**}
IV I followed by II	18	17.7	27.4	2.36^{NS}
V None	19	30.6	23.1	1.39^{NS}

,* $P< 0.01$, $P< 0.001$ respectively (Chi-squared test); NS= no significant difference between pheromone and control.

[1]Nepetalactol (1 mg/ml in hexane)

[2]Mean of 3 control arms

The number of entries into, and the total time spent in pheromone and control arms by *E. plagiator* subjected to different treatments are shown in table 3.2. Parasitoids subjected to treatment III (exposed to aphid sex pheromone at the same time as host aphids) showed a highly significant response to pheromone in the olfactometer in terms of percentage time spent in the pheromone-treated arm ($P< 0.001$; table 3.2a) and a significant response in terms of percentage number of entries into the pheromone-treated arm ($P< 0.01$; table 3.2b). Wasps from all other treatments did not show a significant response to pheromone compared with the control ($P> 0.05$).

3.2.4 Discussion

3.2.4a Aphidius ervi

A. ervi which were naïve (i.e. received no experience prior to testing) responded positively to aphid sex pheromone in the olfactometer, providing further evidence that the parasitoid response to aphid sex pheromone is innate. Wasps which were allowed to oviposit in host aphids while contacting aphid sex pheromone also responded positively, but the response was not high enough to suggest that it had been enhanced by this treatment. Therefore, the response of *A. ervi* to aphid sex pheromone was not enhanced by associative learning (alpha conditioning).

Vet *et al.* (1990a, 1995) propose a variable-response model of parasitoid foraging in which the potential of a response to be modified by learning rises with decreasing strength, and increasing variability of the response. This means that although strong responses to reliable, host-derived cues should be less open to associative learning, they may still be enhanced in this manner, via alpha conditioning (Vet *et al.*, 1990a; Menzel *et al.*, 1993; Vet *et al.*, 1995). Aphid sex pheromones are host-derived cues and the parasitoid response to them is innate. However, in nature they are only reliable indicators of host presence for a short time in autumn when sexual aphids are present. Furthermore, although the parasitoid response is innate, it could be argued that it is not strong or is variable since, in wind tunnel bioassays, often only 30-40% of wasps respond (Chapter 2).

In the model of Vet *et al.* (1990a, 1995) this would allow the response to be enhanced by alpha conditioning. However, the results of the current experiment suggest that the response is outside of the range of responses which can be enhanced in this way (i.e. it is too strong). *A. ervi* is a relative specialist, and since specialists do not require a high level of behavioural flexibility, they may be less inclined to modify their responses by learning (Vet *et al.*, 1995).

If the response of *A. ervi* to aphid sex pheromones cannot be enhanced artificially, then this removes the possibility of 'priming' this species prior to release into pheromone-baited crops. It would be interesting to test whether the sex pheromone could act as the key stimulus (reward) in conditioning a weaker response via associative learning (Vet *et al.*, 1990a).

The effect of exposing wasps to aphid sex pheromone alone (without the reward of oviposition) was to diminish the response to the pheromone to the extent that it was no longer significantly different to the control. This suggests that *A. ervi* habituated to the pheromone. It is unlikely that the loss of the response was due simply to prolonged exposure resulting in sensory fatigue since wasps exposed to both pheromone and aphids did show a significant response. It is possible, therefore, that exposure to sex pheromone without the reward of host contact, caused a repression in parasitoid response which lasted for at least the two hours between treatment and testing.

This phenomenon has been reported previously. Males of the lepidopteran *Epiphyas postvittana* (Walk.), exposed to conspecific female sex pheromone, showed a reduced response for up to five hours after exposure (Bartell and Lawrence, 1973). Among parasitoids, *Trichogramma* wasps have been shown to habituate to kairomones by a gradual waning of response during searching (Gardner and van Lenteren, 1986; Zaborski *et al.*, 1987). Thomson and Stinner (1990) showed that, when exposed to both scales and eggs of its host, *Trichogramma* increased its response to scales, but when exposed only to scales, with no reward, the response was reduced by habituation. A similar effect was

observed when the parasitoid *Campoletis sonorensis* (Cameron) was exposed to host frass without oviposition; the wasp had a reduced response to a plant host complex (McAuslane *et al.*, 1991). Papaj *et al.* (1994) found that negative (unrewarded experience) in one microhabitat caused the parasitoid *Leptopilina heterotoma* to decrease its response to that microhabitat in favour of a second. The authors proposed either habituation or negative associative learning to explain the reduction. The aphid parasitoid *Aphidius rhopalosiphi* was shown to habituate to honeydew sprayed onto plants after a period spent searching on the plants (Budenberg *et al.*, 1992).

It is interesting that parasitoids which experienced sex pheromone immediately after host aphids retained their response. The wasps were exposed to pheromone in a separate Petri dish, so no aphid substances were carried over. However, aphid substances may have adhered to the parasitoids themselves. The treatments were administered one immediately after the other, and it is possible that the parasitoids' experience of hosts was sufficiently recent to preclude any habituation during subsequent exposure to pheromone, or that it increased the time taken for the parasitoid to habituate.

Because, when it occurs naturally, aphid sex pheromone is very closely linked with the presence of host aphids (c.f. host scales), it is difficult to see an advantage in a habituation mechanism; parasitoids should only encounter aphid sex pheromone in the presence of aphids. There is, however, a situation in which it may become advantageous. Parasitoids prefer to oviposit in larval aphids, whereas only adult, sexual females produce sex pheromone. It is possible that parasitoids use the pheromone to locate mixed instar colonies via an 'infochemical detour' (Vet and Dicke, 1992). However, if a parasitoid locates a colony containing only adult females, it may benefit the parasitoid to stop responding to the sex pheromone for a time and disperse to a different area. This could easily be tested in the laboratory by providing the parasitoid with less preferred hosts or host stages, along with aphid sex pheromone.

A habituation response may have implications for the use of sex pheromone lures in the field. If lures are deployed in areas with no or few hosts, then attracted parasitoids may acquire reduced responses to lures or may even disperse. This effect has been implicated in the failure of foliar kairomone applications to increase parasitisation of Lepidoptera by *Chelonus* sp. parasitoids in the field (Chiri and Legner, 1983). However, as Bartell and Lawrence (1973) point out, exposure to pheromone in the laboratory is artificial and is unlikely to mimic conditions in pheromone-baited areas in the field. In the current experiment, the pheromone release rate during treatment was 0.3 µg/min (during testing it was 0.6 µg/min). The release rate from field lures is approximately 250 µg/day (L Merritt, personal communication), which is of the same order of magnitude (nominally 0.2 µg/min). However, although there was a constant throughput of pheromone-laden air through the Petri dish during treatment, it is likely that higher concentrations of pheromone built up in the dish. Therefore further experiments are required to establish the ecological importance of the habituation effect found in this experiment.

The four-way olfactometer was chosen to test parasitoid responses as it allows examination of parasitoid walking behaviour, and short range contact with pheromone-laden air. Consequently it is more useful than a wind tunnel in examining parasitoid foraging in pheromone-baited patches. The fact that *A. ervi* responded positively in the olfactometer suggests that, as well as longer range attraction, the nature of the parasitoid response may include short range attraction or even arrestment. *A. ervi* spent significantly longer in the pheromone-treated arm of the olfactometer than in any control arm. Parasitoids also made significantly more entries into the pheromone-treated arm. Observations suggested that this was due to the parasitoid crossing the edge of the pheromone odour field (as visualised with TiCl vapour), and immediately turning and re-entering the odour field. There was no evidence that wasps were either more or less active in the pheromone-treated arm compared to the control, or that they engaged in any unique behaviour such as wing fanning or oviposition stance. The results suggest that searching *A. ervi* may be retained in pheromone-treated areas.

3.2.4b Ephedrus plagiator

Naïve *E. plagiator* did not respond to the aphid sex pheromone component nepetalactol in the olfactometer. This was both surprising and disappointing since this species had previously responded to nepetalactol in a wind tunnel bioassay (section 2.3.2c). There are two possible explanations for this failure to respond. Firstly the olfactometer may test parasitoid behaviour at a stage of host location during which aphid sex pheromones are a less important cue for this species. Alternatively, the pheromone concentration in the olfactometer may have been either above or below the behavioural threshold for *E. plagiator* response.

Parasitoids which were allowed to oviposit in host aphids while contacting nepetalactol did show a strong, positive response in the olfactometer, and were the only treatment group to do so. The response was not induced simply by exposure to pheromone or host aphids alone, or by exposure to pheromone immediately after contact with aphids. Therefore, the response appears to have been induced by associative learning.

The fact that naïve *E. plagiator* did not respond to nepetalactol in this experiment, but did respond previously in the wind tunnel, makes interpretation of this result difficult. Because naïve wasps did not respond in the olfactometer, it could be argued that a novel response was induced by the pairing of a neutral stimulus (nepetalactol) with oviposition. Parasitoids have been shown to learn novel odours including perfume (De Jong and Kaiser, 1991) and vanilla essence (Lewis and Tumlinson, 1988) by association. However nepetalactol has an ecological significance for aphid parasitoids, and the attraction already demonstrated in the wind tunnel suggests that the effect does not fall into this category.

If the pheromone concentration was below the behavioural threshold for *E. plagiator* response, associative learning may have increased the parasitoid's sensitivity, allowing the response to become apparent. There is evidence that the sensitivity of parasitoid olfactory receptors can be affected by associative learning (Vet *et al.*, 1990b). Alternatively, the pheromone concentration may have been

higher than the optimal for parasitoid response. In this case, a learning experience may have changed the dose-response curve, so that the higher concentration elicited a response. This effect has been demonstrated in learning experiments with honey bees (L Wadhams, personal communication). It is worth noting that *E. plagiator* is a generalist parasitoid, and as such may be expected to be more receptive to learning than the relatively more specialised *Aphidius ervi* (Vet *et al.*, 1985).

There are many questions arising from these data, but they can easily be answered by further experiments. Firstly the experiment should be repeated with higher pheromone concentrations to establish whether failure of naïve parasitoids to respond was due to a behavioural threshold. This would help to determine whether the associative learning observed can be classified as alpha conditioning. The nature of the learning could be investigated by using host products such as exuviae or honeydew as the conditioning stimulus, rather than oviposition. Thirdly, it would be interesting to use nepetalactol as the conditioning stimulus to affect a weaker response (e.g. to an undamaged aphid food plant). This would provide more evidence that nepetalactol is innately recognised by *E. plagiator*. A response to an undamaged plant has been induced in *A. ervi* via contact with aphid honeydew (Poppy *et al.*, 1997).

If responses to aphid sex pheromones can be acquired or enhanced by associative learning in some aphidiine species, then the possibility of 'priming' parasitoids prior to release could be exploited. Since wasps are reared in cages where they will oviposit in hosts and contact host products, it would be straightforward to introduce the kairomone at this stage. Wasps primed in this way might be more likely to locate or remain in pheromone-baited areas in crops or glasshouses. Parasitoid learning has been used to improve microhabitat finding (Papaj and Vet, 1990), and retention and parasitisation (Lewis and Martin, 1990) in the field.

3.3 Effect of Pheromone Pre-Exposure on *Aphidius ervi* Attack Rate Against Two Aphids

3.3.1 Introduction

Parasitoid foraging behaviour can be modified by prior experience (Turlings *et al.*, 1993; Vet *et al.*, 1995). The previous experiment investigated how the parasitoid response to aphid sex pheromone might be affected by associative learning, but there is a second mechanism by which parasitoid behaviour may be influenced by experience, and this is known as priming. Priming is the process by which an insect comes into contact with an innately recognised stimulus and subsequently becomes more receptive to other cues (not necessarily present during the experience) to which it already has a response (Turlings *et al.*, 1993). This may be manifested as an increase in responsiveness to all foraging cues as opposed to an increased preference for the specific cues encountered (preference learning) (Turlings *et al.*, 1993). Priming has been demonstrated in parasitoids, commonly involving contact with the host insect (reviewed by Turlings *et al.*, 1993). However, Hare (1996) found that *Aphytis melinus* reared on one host, increased its attack rate against another host after exposure to a synthetic kairomone associated with the second host.

Two experiments were designed to investigate the effect of pre-exposure to aphid sex pheromone on the behaviour of *Aphidius ervi*. The aim of Experiment I was to determine whether exposure to the innately recognised aphid sex pheromone component nepetalactone would affect parasitoid acceptance of either the aphid in which the parasitoid developed, or a second, less preferred species. This could happen if the exposed parasitoid became more receptive to host-related foraging cues via priming. It would be particularly interesting if the attack rate against a less preferred host could be increased in this way. Experiment II extended this to assess the effect of the presence of sex pheromone during host contact itself, in order to see whether the pheromone would stimulate host attack.

In experiment I, *A. ervi* (from *Acyrthosiphon pisum)* were tested against *A. pisum* and the less preferred *Sitobion avenae*, after having been pre-exposed to sex pheromone or a control. In experiment II, *A. ervi* (from *A. pisum*) were tested against *S. avenae* after having been pre-exposed to sex pheromone or a control, but with a further treatment of either pheromone or control administered during the attack rate bioassay itself. Nepetalactone was used as the sex pheromone stimulus in both experiments since it is the pheromone produced by *S. avenae* and elicits a behavioural response in *A. ervi* (Poppy *et al.*, 1997).

3.3.2 Methods

3.3.2a Insects

Female *A. ervi* and the aphids *A. pisum* and *S. avenae* were obtained as described in section 1.7.1.

3.3.2b Attack Rate Bioassay

Bioassays were performed in plastic Petri dish arenas (9 cm diameter). The floor of the dish was lined with filter paper, and the sides were coated with Fluon, to confine aphids to the base of the arena. Twenty aphids of either *A. pisum* or *S. avenae* (larval instars only) were introduced into the dish, followed by a single female *A. ervi*. The behaviour of the wasp was observed for 10 minutes, and the following information was recorded; time to first oviposition stab, number of aphids contacted with the antennae (antennation), number of aphids approached in the oviposition stance (without an oviposition stab) and the number of oviposition stabs. An oviposition stab was recorded whenever a wasp made physical contact with an aphid with the ovipositor, while in an oviposition stance (ovipositor curled forward under the abdomen). The behaviours were regarded as a series of events and only the final event was recorded (e.g. if a wasp antennated an aphid then stabbed it, this was recorded as a stab, not an antennation). In this way, each contact between a wasp and an aphid was classified as only one of the above categories. A fresh group of aphids in a clean dish were used to test each parasitoid. All treatment-test combinations were performed at least once per

session, and were performed in alternation within the session. In experiment II, either nepetalactone or hexane control was administered to the Petri dish during the bioassay. The chemicals were introduced in the same way as for the pre-exposure, described in 3.2.2c below. Between 17 and 24 parasitoids were tested against each treatment.

3.3.2c Experimental Treatments

Prior to bioassay, wasps were subjected in groups of 5-10 individuals to one of two experimental treatments. Pheromone treated wasps were exposed in a Petri dish to nepetalactone (1 mg/ml in hexane; release rate: 0.3 µg/min) for 30 min, as previously described in section 3.2.2c. Control wasps were similarly exposed to hexane. After treatment, wasps were held in stoppered, glass tubes at 18 °C for 1 hour prior to testing, to allow them to settle after handling.

3.3.2d Statistical Analysis

For each behavioural parameter recorded, means were calculated. In experiment I, means of pheromone and control treated groups attacking each species were compared with a t-test to check for effects of pheromone exposure. Means of all groups were compared with ANOVA, to check for effects of the aphid species offered. In experiment II, means of all treatments were compared by ANOVA.

3.3.3 Results

3.3.3a Experiment I

The effects of pre-exposure to aphid sex pheromone on *A. ervi* response to *A. pisum* and *S. avenae* in the attack rate bioassay are shown in table 3.3. Pre-exposure to nepetalactone had no significant effect on the behaviour of *A. ervi* against either *A. pisum* or *S. avenae* (t-test, $P > 0.05$). There were significant differences, irrespective of pre-exposure, between *A. ervi* attacking *A. pisum* and *S. avenae* in terms of time to first oviposition stab (ANOVA; 3 d.f., sed=81.0, $F=7.76$, $P<0.001$), mean number of oviposition stances (ANOVA; 3 d.f., sed=1.75, $F=8.49$, $P<0.0001$) and mean number of oviposition stabs (ANOVA; 3

d.f., sed=2.33, F=17.3, P< 0.0001). There was no significant difference in the number of antennations that *A. ervi* made against *A. pisum* or *S. avenae* (ANOVA; 3 d.f., sed=0.80, F=0.12, P> 0.05).

Table 3.3 The effect of pre-exposure to aphid sex pheromone on various aspects of the host acceptance behaviour of *Acyrthosiphon pisum*-reared *Aphidius ervi* against *A. pisum* and *Sitobion avenae* (mean ± standard error).

Pre-exposure[1]	**n**	**Time to 1st Ovip. Stab (s)**	**No. Antenn.**	**No. Ovip. Stance**	**No. Ovip. Stabs**
vs *A. pisum*					
Pheromone	24	64.4 ±13.9	10.8 ±1.7	3.5 ±0.5	16.2 ±1.7
Control	22	60.8 ±12.4	10.6 ±2.2	2.3 ±0.5	12.9 ±2.4
vs *S. avenae*					
Pheromone	23	128.7 ±22.0	10.1 ±1.7	1.2 ±0.3	2.9 ±0.9
Control	22	177.9 ±33.6	9.5 ±1.5	0.8 ±0.2	3.1 ±1.1

[1]Pheromone: Nepetalactone (1 mg/ml in hexane; release rate 0.3 µg/min); Control: Hexane (0.3 µg/min)

3.3.3b Experiment II

The effects of pre-exposure to aphid sex pheromone on *A. ervi* response to *S. avenae*, with and without pheromone present during the bioassay are shown in table 3.4 There were no significant differences between any of the treatments in terms of time to first stab (ANOVA; 3 d.f., sed=51.9, F=0.59, P> 0.05), antennations (ANOVA; 3 d.f., sed=1.43, F=0.29, P>0.05), stances (ANOVA;

3d.f., sed=0.70, F=1.13, P>0.05) or stabs (ANOVA; 3 d.f., sed=0.62, F=0.06, P>0.05), indicating that the sex pheromone did not affect the behaviour of *A. ervi.*

Table 3.4 The effect of pre-exposure to aphid sex pheromone on various aspects of the host acceptance behaviour of *Acyrthosiphon pisum*-reared *Aphidius ervi* against *Sitobion avenae*, with and without pheromone present during the bioassay (mean ± standard error).

Pre-exposure[1]	n	Time to 1st Ovip. Stab (s)	No. Antenn.	No. Ovip. Stance	No. Ovip. Stabs
Pheromone present[2]					
Pheromone	17	238.8 ±44.6	9.1 ±1.7	0.5 ±0.3	5.6 ±1.5
Control	17	277.8 ±41.2	9.3 ±1.9	0.7 ±0.6	5.2 ±1.3
Pheromone absent[3]					
Pheromone	17	307.7 ±50.4	7.0 ±2.0	1.5 ±0.3	4.5 ±1.7
Control	17	249.2 ±54.1	8.7 ±1.7	1.5 ±0.7	5.4 ±2.3

[1]Pheromone: Nepetalactone (1 mg/ml in hexane; release rate 0.3 µg/min); Control: Hexane (0.3 µg/min)

[2]Nepetalactone (1 mg/ml in hexane; release rate 0.3 µg/min)

[3]Hexane (0.3 µg/min)

3.3.4 Discussion

A. ervi could not be induced to increase its attack rate against either its host aphid or a less preferred aphid by pre-exposure to aphid sex pheromone (Experiment I). The presence of aphid sex pheromone during the bioassay did not stimulate *A. ervi* to increase its attack behaviour, irrespective of whether it had been pre-exposed to pheromone or not (Experiment II). Although aphid sex pheromones

almost certainly attract *A. ervi* (Poppy *et al.*, 1997), and may even cause it to remain in treated areas (section 3.2), they do not seem to increase this parasitoids' attack rate by either priming prior to host contact or stimulation during host contact. The implications for this in the field are that parasitoids coming into contact with pheromone lures will not exhibit heightened responses to other host location cues, and parasitoids searching in pheromone-baited areas will not show an increased attack rate against aphid colonies. In particular, pheromone will have no effect on a parasitoids' response to a less preferred aphid species. On the other hand, the presence of pheromone did not interfere with or disrupt the parasitoids' host attack behaviour, which is an advantage. Therefore, it seems likely that aphid sex pheromones can be used to attract parasitoids into baited areas, but not to directly influence their host acceptance behaviour once there.

In Experiment I, although there were no significant differences between pheromone and control treated parasitoids on either host aphid, there were differences between parasitoids attacking *A. pisum* (on which they were reared) and *S. avenae*, a less preferred host. Parasitoids made significantly fewer oviposition stabs against *S. avenae* than against *A. pisum*. This was expected since the host aphid in which a parasitoid develops can have an influence upon its preferred choice of host as an adult, and *A. ervi*, reared on *A. pisum*, has been shown to prefer this host aphid species over *S. avenae* (Powell and Zhi-Li, 1983; Powell and Wright, 1988, 1992). It was interesting that wasps made significantly fewer oviposition stabs against *S. avenae* since the parasitoid may assess a non host aphid internally via the ovipositor before deciding to deposit an egg (Mackauer, 1992). However, there is evidence that parasitoids can also use external host cues during host acceptance (Grasswitz and Paine, 1992; Battaglia *et al.*, 1993) and it is interesting that parasitoids made a similar number of antennations (without an oviposition stab) against each aphid species.

3.4 Effect of Experiencing Aphid Sex Pheromone at Parasitoid Emergence on Response of *Praon myzophagum.*

3.4.1 Introduction

A previous experiment suggested that a generalist aphid parasitoid, *Ephedrus plagiator*, may change its response to aphid sex pheromone after associative learning (section 3.2). However, it was not clear whether the response was enhanced or newly acquired. A further experiment was designed using another generalist parasitoid, *Praon myzophagum*, to examine the effect on the parasitoid of emerging from the mummy into a pheromone-baited area without live aphids. This could happen in the field when aphid populations have declined in a particular area, and it would be advantageous to attract parasitoids to a new pheromone-baited area.

In this experiment, parasitoid responses were tested in a wind tunnel bioassay, in order to investigate effects on longer-range orientation. Experiencing pheromone during and after emergence from the mummy could affect subsequent response in two ways. Firstly the parasitoid could enhance its response to pheromone by alpha conditioning (section 3.2.4), induced by associative learning with cues from the aphid mummy as the conditional stimulus. Secondly, there could be a priming effect which occurs during emergence. Aphid parasitoids have been shown to acquire responses to cues detected from the aphid mummy at emergence (van Emden *et al.*, 1996), and they may be particularly receptive to other cues at this time.

3.4.2 Methods

3.4.2a Insects

Female *Praon myzophagum* reared on *Acyrthosiphon pisum* were obtained as described in section 1.7.1a.

3.4.2b Wind Tunnel Bioassay

The wind tunnel bioassay method was the same as that described in section 2.2.2, except that the control treatment in this case consisted of 10 µl diethyl ether, not hexane, since the pre-exposure to pheromone used aphid sex pheromone field lures which are formulated in diethyl ether (section 1.7.2b).

3.4.2c Experimental Treatments

Pheromone-experienced wasps were obtained by allowing adults to emerge into a small, conical plastic cage as described in section 1.7.1a. Two aphid sex pheromone field lures (section 1.7.2b) one releasing nepetalactone, the other nepetalactol were placed inside the cage. A 1:1 ratio of sex pheromone components was chosen since *P. myzophagum* (reared on *A. pisum*) responded to this combination in a wind tunnel bioassay (section 2.4). Control wasps were obtained by allowing adults to emerge into a separate cage which contained control field lures releasing diethyl ether. Mummies were held in glass Petri dishes inside the cages, and these were verified to be clear of any aphids or other aphid products prior to placement in the emergence cages.

3.4.2c Statistical Analysis

Data were transformed where necessary and analysed by ANOVA as described in section 2.2.3. Details of the ANOVA results are given in the Appendix, in table A5.

3.4.3 Results

The flight responses of pheromone and control experienced *P. myzophagum* to a 1:1 ratio of nepetalactone and nepetalactol in a wind tunnel bioassay are shown in table 3.5. Wasps from both pheromone experienced and control groups made significantly more upwind flights to the pheromone compared to the control ($P< 0.05$), but there was no significant difference between treatment groups in the proportion of wasps making upwind flights to either the pheromone or the control ($P> 0.05$). A significantly higher proportion of pheromone experienced wasps landed on the filter paper target compared to wasps from all other treatments ($P<$

0.05). There were no significant differences between treatments in either the time to take-off, or the proportion of wasps taking off (P> 0.05).

Table 3.5 Responses of female *Praon myzophagum* which experienced aphid sex pheromone during emergence, to aphid sex pheromone (5 µl:5µl nepetalactone:nepetalactol 1 mg/ml in hexane) and control (10 µl diethyl ether) in a wind tunnel bioassay

		Mean % of wasps		
Treatment[1]	**Time to take-off (s)**	**Taking off**	**Making oriented flight**	**Landing on target**
Pheromone experienced				
Pheromone	62.6 a	95.0 a	46.8 a	20.5 a
Control	59.5 a	95.0 a	10.3 b	0.0 b
Control experienced				
Pheromone	49.8 a	100 a	41.5 a	5.5 b
Control	60.3 a	97.5 a	12.5 b	0.0 b

Column means followed by different letters are significantly different (ANOVA, Tukey test; P< 0.05).

[1]Thirty nine parasitoids were tested to each treatment.

3.4.4 Discussion

Exposure to aphid sex pheromone during and after emergence did not affect the response of *P. myzophagum* in terms of upwind flight towards the pheromone target. However, exposure to pheromone did increase the parasitoids' propensity to land on the target. The response to aphid sex pheromone may have been enhanced via associative learning in the emergence cage. In this case, contact with aphid mummies would have provided the conditional stimulus. During the

experiments presented in this thesis, newly emerged female parasitoids were often seen to adopt an oviposition stance towards aphid mummies in the emergence cages. Van Emden *et al.* (1996) proved that emerging parasitoids can acquire responses to cues present in the mummy. It is possible that this is brought about by associative learning.

If associative learning did occur in the current experiment, it is unclear why the landing response was enhanced, whereas the general attraction response was not. It is possible that, since the parasitoid associated pheromone with the presence of hosts, the response was manifested as landing, which would bring parasitoids into contact with hosts. Alternatively, learning may have increased the parasitoids olfactory sensitivity to pheromone (Vet *et al.*, 1990b), allowing it to navigate more successfully along the odour plume, or may have reduced the variability in response amongst the treated group. Naïve *P. myzophagum* were previously shown to land significantly more often on a pheromone treated target than a control target (section 2.4.2a), but naïve parasitoids did not do so in this experiment. Therefore it could be argued that rather than an enhancement in the landing response of the pheromone-treated group, landing in the control group was depressed. However, it is difficult to see a mechanism by which this could occur, and the upwind flight response of the control group was unaffected. It is not unusual for the responses of parasitoids in the wind tunnel to vary between occasions. Barometric flux in particular has been shown to affect the flight responses of parasitoids to semiochemical cues (Steinberg *et al.*, 1992).

Associative learning in aphid parasitoids via contact with the mummy is an area which could be explored further. Since each parasitoid must emerge from a mummy, there may be potential for it to acquire or enhance responses to cues associated with the aphid, the aphid's diet or the surrounding environment. This may affect the parasitoid's foraging choices in adult life. An increase in landing response after associative learning of aphid sex pheromone would be an advantage when attempting to concentrate parasitoids in pheromone-baited areas in the field.

3.5 Effect of Aphid Sex Pheromone on Retention of Parasitoids on Cereal Plants in the Laboratory

3.5.1 Introduction

The positive responses of parasitoids towards aphid sex pheromone in the olfactometer bioassay (section 3.2) suggest that, as well as attracting parasitoids, the pheromone may also cause them to spend more time in a particular area, perhaps by arrestment. If this effect could be demonstrated on an aphid-infested plant, then it would have advantages for the use of sex pheromone lures in the field. Pheromone might cause parasitoids to spend more time on baited plants by decreasing their leaving rate from the plant, or by affecting their search behaviour on the plant, leading to increased rates of parasitisation. Aphid honeydew acts as a contact kairomone for aphid parasitoids, and Budenberg *et al.* (1992) found that the leaving rate of parasitoids was significantly lower from honeydew-treated plants. However, the retention of wasps on treated plants did not lead to increased parasitisation in this study.

In the field, an arrestment effect could help retain parasitoids which are attracted to sex pheromone lures. It could also have a similar effect on parasitoids which emerge into pheromone-baited areas. Information on whether this occurs may also aid in the interpretation of the results of field experiments (Chapters 5 and 6).

In this experiment, the effect of aphid sex pheromone on the retention of parasitoids is examined by measuring the leaving rate of parasitoids from aphid-infested cereal plants inside laboratory cages. The parasitoids *Praon volucre* and *Aphidius rhopalosiphi* were used, since these are important enemies of cereal aphids, and were the species recorded in the field experiments (Chapters 5 and 6). The effect of sex pheromone on *P. volucre* was measured at high host density (200 aphids/plant) and at low host density (20 aphids/plant). Host density has an effect on the amount of time a parasitoid spends on a plant (Sheehan and Shelton, 1989a; Budenberg *et al.*, 1992), and the sex pheromone may be particularly useful

in retaining parasitoids in areas of low host density, which often occur during the early part of the season in the field.

3.5.2 Methods

3.5.2a Insects

Female *Praon volucre* and *Aphidius rhopalosiphi* (both from *Sitobion avenae*) were obtained as described in section 1.1.7a.

3.5.2b Leaving Rate Experiment

Experiments were conducted inside two wood-framed laboratory rearing cages, measuring 45 x 45 x 60 cm. The cages were painted white, and had glass roofs and doors and terylene sides to allow ventilation. Two pots of 10 day old winter barley plants (var. Puffin) (50 seedlings/pot) were infested with the host aphid *Sitobion avenae* 24 hours prior to the experiment to allow aphids to settle on the plant. At the start of the experiment, an aphid-infested potted plant was placed into each cage, and a nepetalactone field lure (release rate 250 µg/day, section 1.7.2b) was added to one of the plant pots, attached to a small cane. Ten female parasitoids were introduced onto each plant by allowing them to walk out of glass vials and onto the surface of the plant. Occasionally a wasp would leave the plant before all ten wasps were introduced and, in this case, it was recaptured and released onto the plant for a second time. When all ten wasps had been successfully released onto the plant, the door of the cage was closed and the experiment started.

The cages were examined every hour to determine the number of parasitoids which had left the plant. Those which had done so were easily visible against the sides of the cages, and were counted and then removed from the cages. Observations were continued for 8 hours after the start of the experiment and initial observations indicated that the final numbers remaining on the plant altered little after this time, even up to 24 hours later. At the end of the experiment, the plants were removed from the cages and the wasps which remained on them were

carefully removed, until all had been accounted for. The plants were placed in terylene sleeves and kept at 18 °C, 16:8 hours L:D for 14 days, after which time the number of mummies on the plants was recorded.

The experiments were performed in a controlled environment chamber at 22 ±1 °C, between 2 and 10 hours into the parasitoids' photophase. Pheromone and control cages were placed at opposite ends of the room, which was well ventilated. The position of pheromone and control cages was alternated between replicates.

Three separate experiments were performed. *P. volucre* was tested at a high host density (200 *S. avenae*/plant) and a low host density (20 *S. avenae*/plant), and *A. rhopalosiphi* was tested at 200 *S. avenae*/plant. Seven replicates were completed for each experiment.

3.5.2c Statistical Analysis

The mean number of wasps remaining on pheromone and control plants at each time interval was calculated. The means were then compared with a t-test. The mean percentage parasitisation on the plants was calculated from the number of mummies developing. The means for pheromone and control plants were very similar and were not analysed statistically.

3.5.3 Results

The effect of aphid sex pheromone on the retention of *P. volucre* on aphid-infested plants is shown in figure 3.2. At 200 aphids/plant (figure 3.2a), the mean number of *P. volucre* remaining on the pheromone-baited plant was significantly higher than on the control plant at a single time interval, 7 hours ($P < 0.05$, t-test). There were no significant differences between pheromone-baited and control plants at any other time interval. At 20 aphids/plant (figure 3.2b), there were no significant differences between treatments in the number of *P. volucre* remaining in the plant at any time interval ($P > 0.05$, t-test).

Figure 3.3 shows the percentage parasitisation recorded on the plants for *P. volucre*. There were no significant differences between parasitisation rates on pheromone-baited and control plants at either 200 aphids/plant (figure 3.3a) or 20 aphids/plant (figure 3.3b).

The retention of *A. rhopalosiphi* at 20 aphids/plant is shown in figure 3.4. The mean number of *A. rhopalosiphi* remaining on pheromone-baited plants was significantly higher than on control plants at three time intervals, 2, 3 and 4 hours ($P < 0.05$, t-test). There was no significant difference in parasitisation rates recorded on pheromone-baited and control plants (figure 3.5).

3.5.4 Discussion

The effect of aphid sex pheromone on retaining *Praon volucre* on the plant should be considered marginal. Only at one time interval were significantly more parasitoids observed on the pheromone-baited plant, and this was 7 hours after the start of the 200 aphids/plant experiment. As expected, *P. volucre* left the plant with 20 aphids more quickly than they left the plant with 200 aphids, and this agrees with previous observations that aphid parasitoids spend more time in areas of high host density (Sheehan and Shelton, 1989a; Budenberg *et al.*, 1992). However, after an initially more rapid decrease, parasitoid numbers on the 20 aphid plant remained steady. Aphid sex pheromone had no effect at the lower host density. This was surprising because, with hosts more difficult to find, the pheromone may have been expected to have a greater influence on parasitoid retention.

It is difficult to argue that the significant effect on *P. volucre* at 7 hours in the 200 aphid experiment will be of great impact in the field. There does seem to be a general decline in parasitoid numbers on the control plant between 5 and 7 hours, during which time numbers on the pheromone-baited plant remain steady. It is possible that the pheromone retained wasps after they had exhausted their egg supply. In fact, levels of parasitisation were very similar on control and pheromone-baited plants.

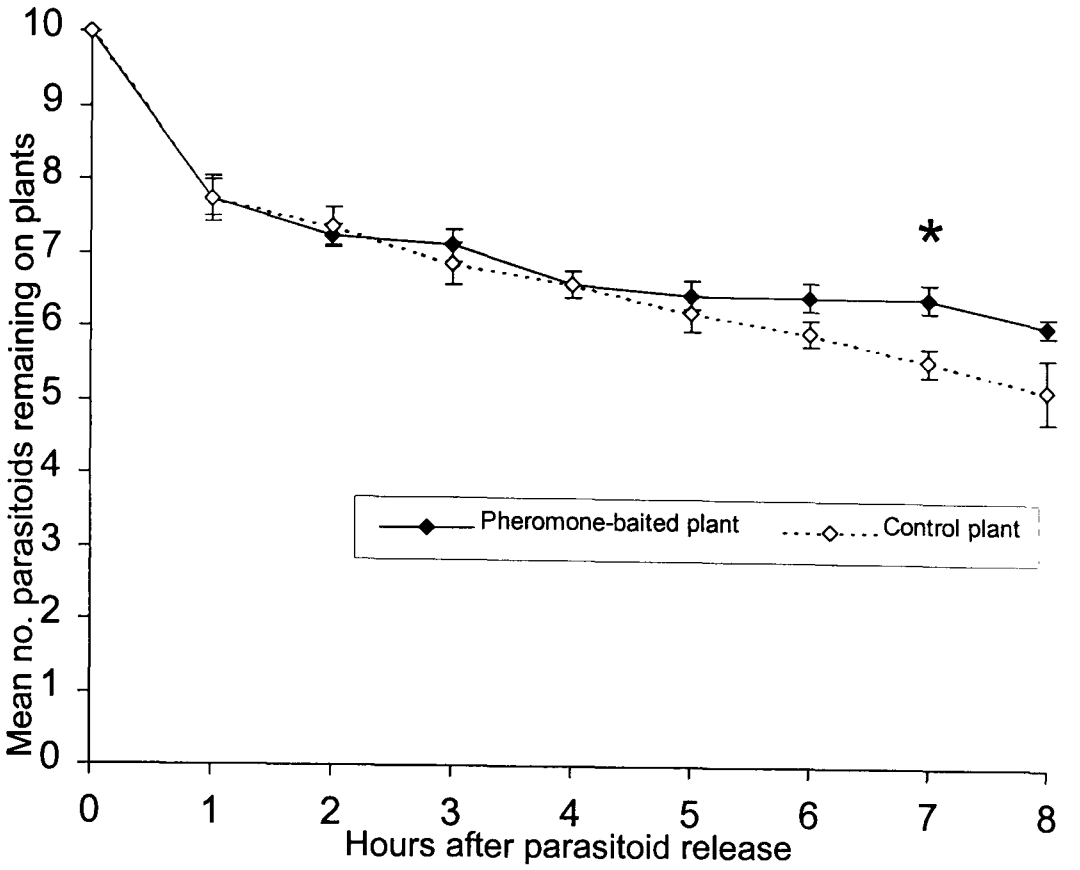

Figure 3.2a Effect of aphid sex pheromone on retention of female *Praon volucre* on caged barley plants infested with 200 *Sitobion avenae*.
*Significant difference between pheromone and control, P< 0.05 (t-test). (7 replicates, 10 parasitoids/plant/replicate). Error bars= +/- standard error.

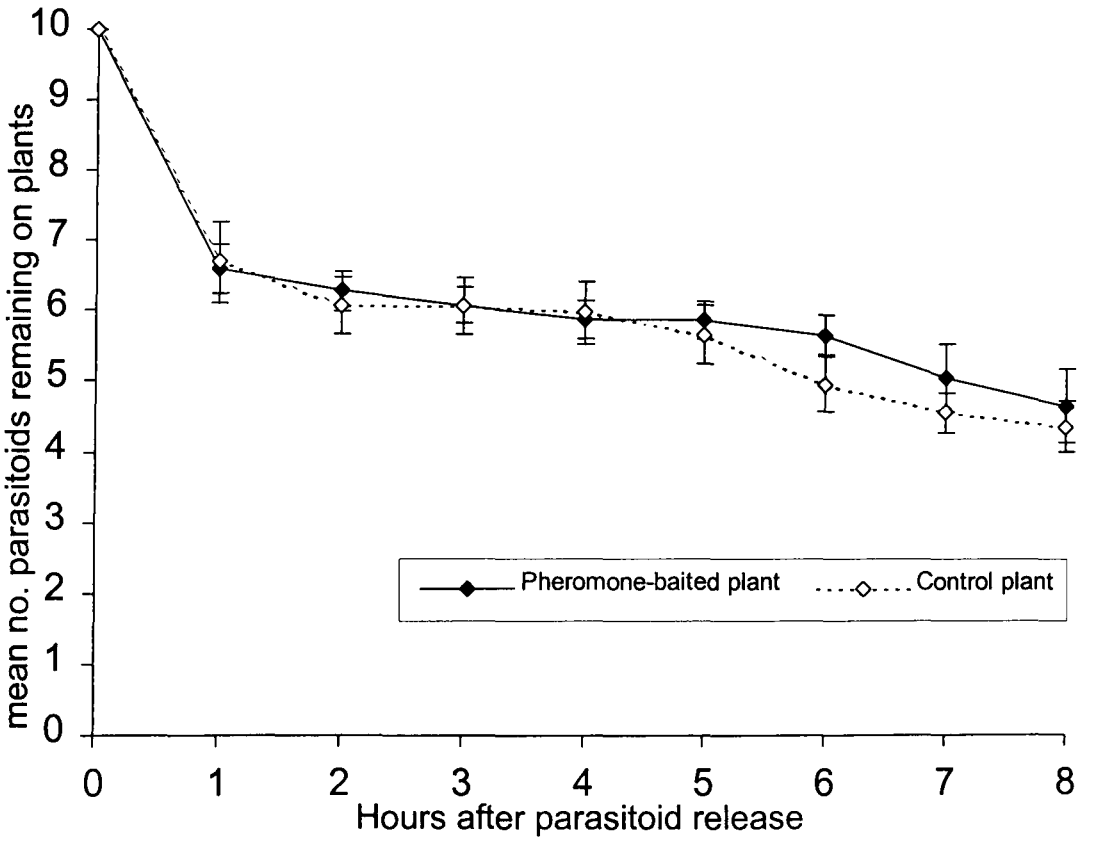

Figure 3.2b Effect of aphid sex pheromone on retention of female *Praon volucre* on caged barley plants infested with 20 *Sitobion avenae*.
(7 replicates, 10 parasitoids/plant/replicate). Error bars= +/- standard error.

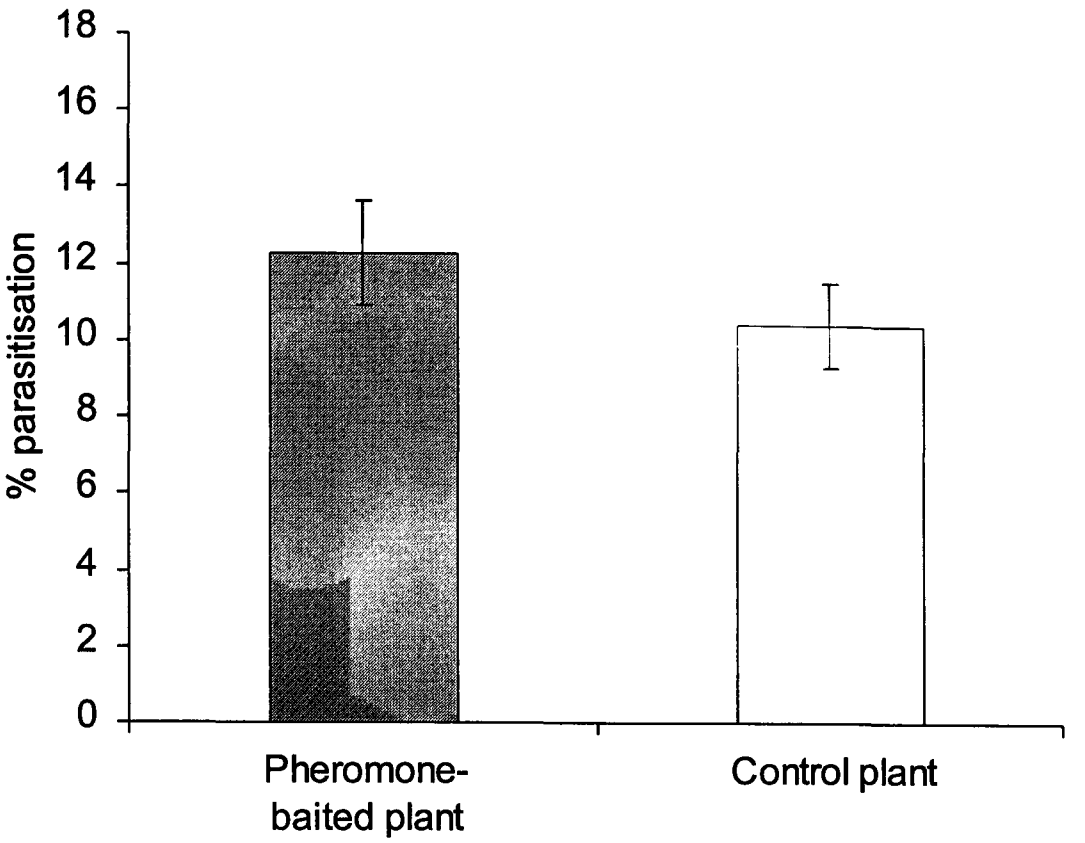

Figure 3.3a Mean % parasitisation (+/- standard error) by *Praon volucre* on caged barley plants infested with 200 *Sitobion avenae* . (7 replicates, 10 parasitoids/plant/replicate)

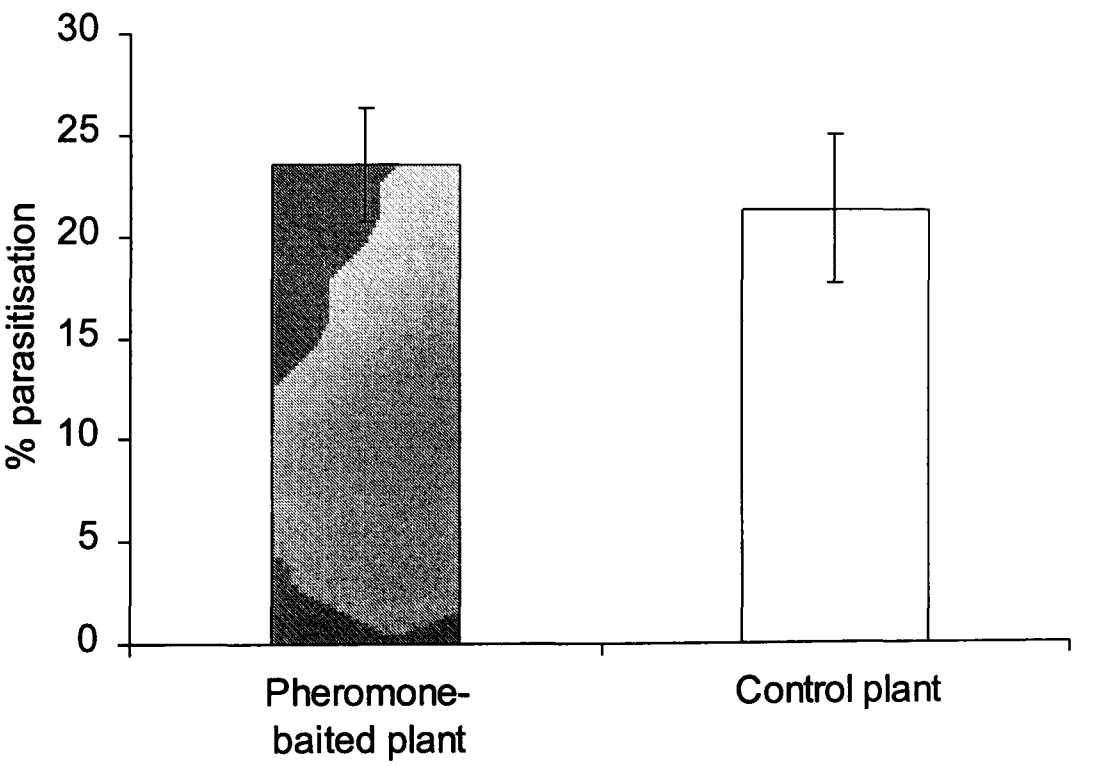

Figure 3.3b Mean % parasitisation (+/- standard error) by *Praon volucre* on caged barley plants infested with 20 *Sitobion avenae* . (7 replicates, 10 parasitoids/plant/replicate).

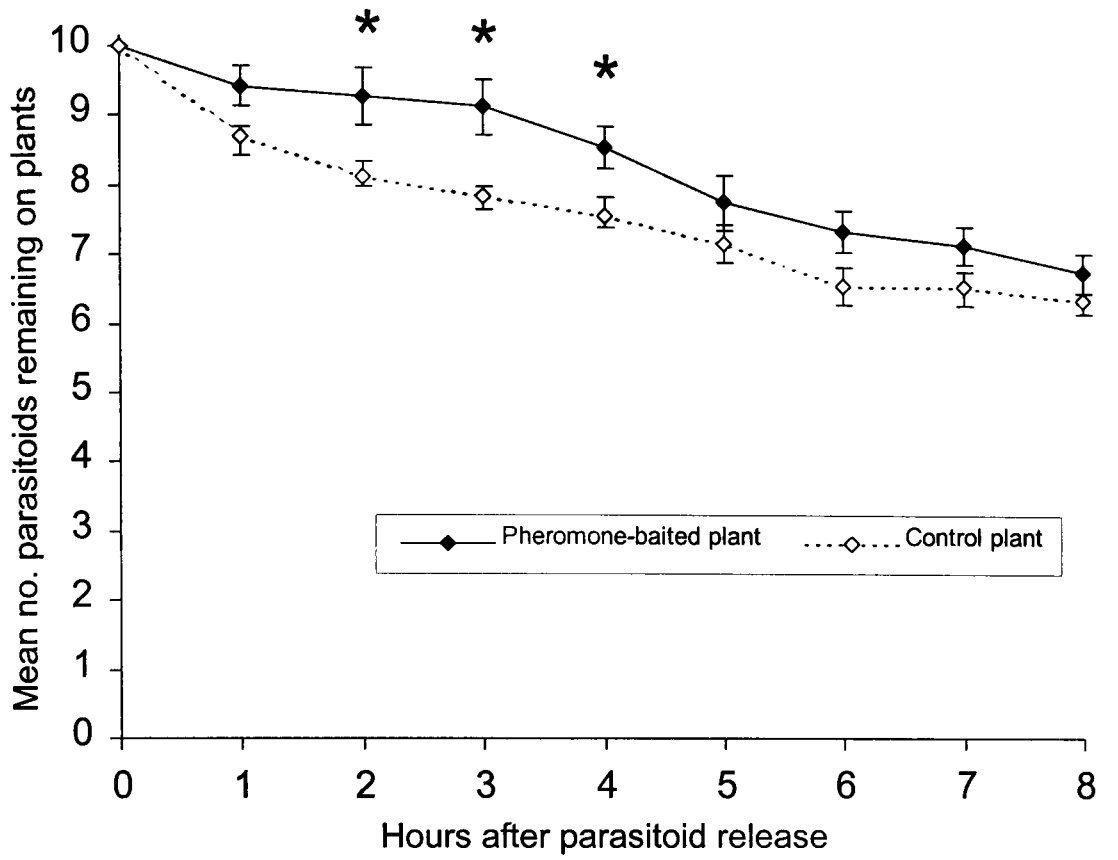

Figure 3.4 Effect of aphid sex pheromone on retention of female *Aphidius rhopalosiphi* on caged barley plants infested with 200 *Sitobion avenae*. *Significant difference between pheromone and control, P< 0.05 (t-test). (7 replicates, 10 parasitoids/plant/replicate). Error bars= +/- standard error.

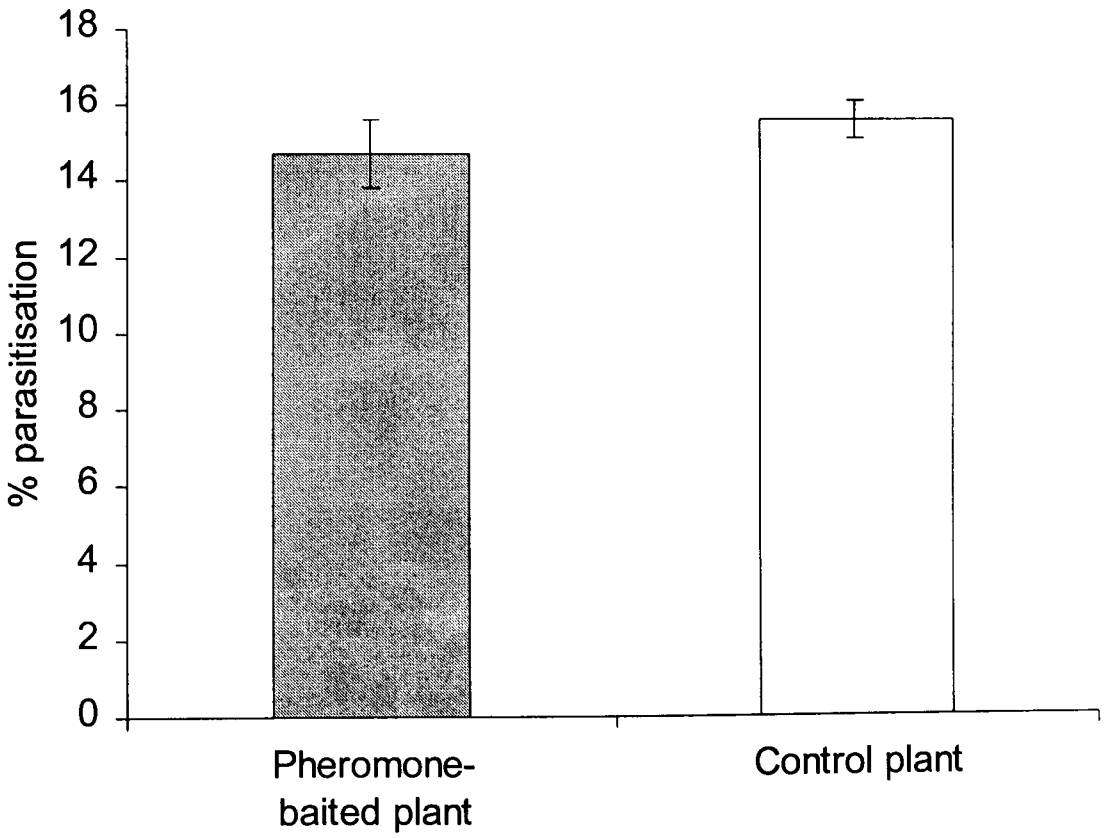

Figure 3.5 Mean % parasitisation (+/- standard error) by *Aphidius rhopalosiphi* on caged barley plants infested with 200 *Sitobion avenae*. (7 replicates, 10 parasitoids/plant/replicate).

For the sex pheromone to have a useful effect on *P. volucre* it would need to increase parasitisation, especially at low host density, and this was not demonstrated here. However, the aphid density of 20 aphids/plant may still be too high to see this effect, since parasitoids may be able to locate sufficient hosts.

Aphid sex pheromone seemed to have a greater effect on the retention of *Aphidius rhopalosiphi*. This time parasitoid numbers remained higher on the pheromone-baited plant in the early part of the experiment, between 2 and 4 hours after the start. This may have been due to an increase in the time allocated to searching on the plant. *A. rhopalosiphi* spent more time searching and resting on plants which were contaminated with aphid honeydew (Gardner and Dixon, 1985). An effect on searching or arrestment may more usually be associated with host contact kairomones such as body scales (Lewis *et al.*, 1979) or secretions (Strand and Vinson, 1982) rather than with pheromones. However, *Trichogramma pretiosum* was arrested by host sex pheromone in the laboratory (Noldus *et al.*, 1991a), and sex pheromone lures increased parasitisation on plants and in field plots (Lewis *et al.*, 1982). If the time spent searching by *A. rhopalosiphi* had been increased, then one might expect a commensurate increase in parasitisation levels, and this did not occur in the experiment. However, at 200 aphids/plant, searching wasps may be able to locate enough hosts irrespective of the pheromone. The *A. rhopalosiphi* experiment was not repeated at the lower host density due to time constraints, but this may provide clarification of these results.

Even though *A. rhopalosiphi* was retained to some extent on pheromone-baited plants, there was no increased parasitisation on these plants. Therefore the value of this response in the field may be low. Lewis *et al* (1979) found that *Trichogramma pretiosum* was retained in areas which were blanket treated with a kairomone, but that parasitisation rates were reduced because the wasp was prevented from concentrating its search in areas were hosts were locally abundant. If aphid sex pheromone was found to retain *A. rhopalosiphi* in areas which lack aphids entirely (which could be addressed with a further experiment using uninfested plants), then this would be a disadvantage in the field. However, after 4

hours in the current experiment, the numbers on the control and pheromone-baited plants were not significantly different, suggesting that, after this time, wasps began to habituate to the pheromone. A similar effect was observed for *A. rhopalosiphi* on honeydew-treated plants (Budenberg *et al.*, 1992).

There appears to be a difference in the way that *P. volucre* and *A. rhopalosiphi* responded to aphid sex pheromone in this experiment. A difference was also observed in field experiments designed to measure parasitisation rates on potted plants (section 5.2). It is possible that the two species use the pheromone in a different way, and this is discussed further in section 5.2.3. If the retention effect on *A. rhopalosiphi* led to increased parasitisation in pheromone-baited areas in the field, this would be an advantage for the use of pheromone in manipulating parasitoids in the field. However, if parasitoids are retained without parasitising more aphids, then the deployment of sex pheromone lures will have to be carefully managed, especially when aphid density is low.

Chapter 4

Interactions Between Aphid Sex Pheromone And The Plant-Host Complex In *Aphidius ervi*

4.1 Introduction

Parasitoids successfully find and parasitise hosts via the series of steps which comprise the host location process (Vinson, 1976). Volatile cues originating from the host plant, or from the interaction of the host insect with the plant, are exploited by foraging parasitoids during this process (Vinson, 1981, 1984). Of particular interest is the use by the parasitoid of chemicals which are produced by the plant in response to insect feeding damage, the so called plant-host complex (PHC) (e.g. Turlings *et al.*, 1990; Steinberg *et al.*, 1993).

Aphid parasitoids have been shown to respond to chemicals from the plants on which their host aphids feed (Schuster and Starks, 1974; Powell and Zhi-Li, 1983; Sheehan and Shelton, 1989b; Wickremasinghe and van Emden, 1992), and also to the PHC (Read *et al.*, 1970; Grasswitz and Paine, 1993; Reed *et al.*, 1995). Recent work involving the tritrophic system Broad bean (*Vicia faba*)-*Acyrthosiphon pisum-Aphidius ervi* has revealed in more depth the role of the PHC in aphid parasitoid foraging. In wind tunnel tests (Guerrieri *et al.*, 1993; Du *et al.*, 1996), *A. ervi* was not strongly attracted to the odour of either its host aphid, *Acyrthosiphon pisum* or an uninfested plant, but the PHC was highly attractive. When aphids were removed from the plant, the host damaged plant continued to be attractive to *A. ervi* (Guerrieri *et al.*, 1993; Du *et al.*, 1996) suggesting that the parasitoid was using volatile chemicals produced by the plant in response to aphid feeding. Foraging parasitoids face a reliability-detectabilty problem (Vet and Dicke, 1992), in that cues from the host are very reliable but may be difficult to detect, whereas cues from the host plant are more detectable but may not be reliable indicators of host presence. It has been suggested that *A. ervi* solves the

reliability-detectability problem by using herbivore-induced cues from the PHC (Guerrieri *et al.*, 1993; Du *et al.*, 1996).

Volatiles from the PHC may play an important role in the foraging of aphid parasitoids, many of which also respond to aphid sex pheromones, and if sex pheromone lures are deployed in the field they are expected to operate in conjunction with cues from the host plant and the PHC. Therefore it would be valuable to investigate the possible interaction of sex pheromone and PHC cues in the foraging behaviour of aphid parasitoids, and *A. ervi* is an ideal species to work with since its responses to both PHC (Guerrieri *et al.*, 1993; Du *et al.*, 1996) and aphid sex pheromone (Poppy *et al.*, 1997) have been well studied.

The flight responses of *A. ervi* to aphid sex pheromones in combination with the PHC *Vicia faba-Acyrthosiphon pisum* were investigated in a wind tunnel bioassay. The interaction between the PHC and the sex pheromone, and the relative importance of these cues were studied in two experiments.

Experiment I

In this experiment, the responses of *A. ervi* to combinations of plant and aphid sex pheromone cues were investigated. Parasitoids were tested against a clean, uninfested plant (P) and a plant-host complex (PHC) consisting of 100 *Acyrthosiphon pisum* feeding on *Vicia faba*. Previous work has shown that *A. ervi* has only a weak response to uninfested plants, but a strong response to the PHC (Guerrieri *et al.*, 1993; Du *et al.*, 1996). To study the interaction between the aphid sex pheromone and the plant cues, parasitoids were also tested against the uninfested plant plus pheromone (P + Ph) and the plant-host complex plus pheromone (PHC + Ph). The effect of the sex pheromone may be to enhance the parasitoid response to the plant cues. However, if the parasitoid has a hierarchy of cues to which it responds, then the sex pheromone may assume a lesser importance when reliable and detectable herbivore-induced cues are available. In this case, the pheromone would not greatly affect parasitoid response to the PHC.

Experiment II

The relative importance to the parasitoid of sex pheromone and PHC were investigated by testing the effect of pheromone on responses to a stronger and a weaker PHC. Work has shown that *A. ervi* response to the PHC is influenced by the length of time the aphids have been feeding on the plant (Guerrieri *et al.*, 1996). *A. ervi* showed a higher response to 40 aphids feeding for 72 hours than to 40 aphids feeding for 24 hours. This may be because the longer aphid infestation is necessary for the plant to produce the attractive damage-related cues in their fullest quantity. Thus, if *A. ervi* has a hierarchy of cues to which it responds, then the sex pheromone may be expected to have less of an effect on responses to the stronger PHC than to the weaker PHC.

4.2 Methods

4.2.1 Insects

Female *Aphidius ervi* (from *Acyrthosiphon pisum*) were obtained as described in section 7.1.1a.

4.2.2 Wind Tunnel Bioassay

The wind tunnel method was as described in section 2.2.2. The following targets were tested in the wind tunnel:

Experiment I

P: An uninfested broad bean plant. A filter paper target was attached to a thin metal wire which was supported in the soil of the plant pot so that the target was aligned in the odour plume of the plant. Hexane (10 µl) was applied to the filter paper.

P + Ph: The uninfested bean plant plus pheromone. Nepetalactone and nepetalactol (5 µl:5 µl of 1 mg/ml in hexane solution) were applied to the filter paper.

PHC: A plant-host complex. The plant was infested with 100 mixed instar *A. pisum*, 24 hours before testing. Hexane (10 µl) was applied to the filter paper.

PHC + Ph: The plant host complex plus pheromone. Nepetalactone and nepetalactol were applied to the filter paper as above.

Experiment II

PHC24: The plant host complex plus hexane as in Experiment I. The plant was infested with 100 *A. pisum* 24 hours before testing

PHC24 + Ph: The above plus pheromone instead of hexane as in Experiment I.

PHC48: A plant-host complex plus hexane. The plant was infested with 100 *A. pisum* 48 hours before testing.

PHC48 + Ph: The above plus pheromone instead of hexane.

All plants used in the experiments were 10 cm tall broad bean (*Vicia faba* cv. Sutton) seedlings with 2 or 3 pairs of open leaves. Targets were placed 20 cm upwind of the parasitoid release tube. Each target was tested during a session, and the order in which they were tested was randomised according to a quasi-complete Latin square. The PHCs were chosen on the basis that the PHC (100 aphids feeding for 24 hours) previously elicited a response in *A. ervi* (Du *et al.*, 1996), and the PHC48 would presumably elicit a stronger response (Guerrieri *et al.*, 1996).

4.2.3 Statistical Analysis

Data were transformed where necessary and analysed by ANOVA as described in section 2.2.3. Details of the ANOVA results are given in the Appendix, in tables A6-A7.

4.3 Results

4.3.1 Experiment I

The flight responses of *A. ervi* to uninfested plants and plant-host complex, with and without aphid sex pheromone are shown in table 4.1.

Table 4.1 Responses of female *Aphidius ervi* to uninfested plants and plant-host complex (PHC) in combination with aphid sex pheromone (Ph) (5 µl nepetalactone:5 µl nepetalactol, 1 mg/ml in hexane solution).

Treatment[1]	Time to take-off (s)	Mean % of wasps		
		Taking off	Making oriented flight	Landing on target
Plant	45.4 a	73.8 a	15.6 a	0.0 a
Plant + Ph	33.5 a	85.0 ab	36.9 b	17.5 b
PHC	43.2 a	95.0 b	41.9 b	10.0 ab
PHC + Ph	51.8 a	97.5 b	63.8 c	29.4 b

Column means followed by different letters are significantly different (ANOVA, Tukey test; $P < 0.05$).

[1]Thirty eight parasitoids were tested to each treatment

The addition of aphid sex pheromone to the uninfested plant (P) and the plant-host complex (PHC) significantly increased the proportion of wasps making upwind flights to these targets ($P < 0.05$). The strongest response was to PHC + Ph, and this was significantly greater than to P + Ph ($P < 0.05$). Pheromone increased the proportion of parasitoids which landed on the PHC and significantly increased the proportion landing on the P ($P < 0.05$). The proportion of wasps taking-off in response to the PHC was significantly higher than in response to the

P (P< 0.05). There was no significant difference between treatments in the time to take-off (P> 0.05).

4.3.2 Experiment II

The flight responses of *A. ervi* to PHC (24 and 48 aphid feeding hours), with and without aphid sex pheromone are shown in table 4.2.

Table 4.2 Responses of female *Aphidius ervi* to plant-host complex with 24 and 48 hours of aphid feeding (PHC24, PHC48) in combination with aphid sex pheromone (Ph) (5 µl nepetalactone:5 µl nepetalactol, 1 mg/ml in hexane solution).

		Mean % of wasps		
Treatment[1]	**Time to take-off (s)**	**Taking off**	**Making oriented flight**	**Landing on target**
PHC24	46.6 a	100 a	19.5 a	6.8 a
PHC24 + Ph	56.5 a	91.8 a	41.3 bc	22.0 a
PHC48	50.8 a	93.8 a	37.0 b	8.8 a
PHC48 + Ph	43.9 a	97.0 a	53.1 c	18.6 a

Column means followed by different letters are significantly different (ANOVA, Tukey test; P< 0.05).

[1]Thirty eight parasitoids were tested to each treatment

Parasitoids made a significantly higher proportion of oriented flights to the PHC48 than to the PHC24 (P< 0.05). The addition of aphid sex pheromone to PHC24 and PHC48 significantly increased the proportion of oriented flights to these treatments (P< 0.05), however there was no significant difference in the number of wasps flying to the PHC24 + Ph and the PHC48 + Ph (P> 0.05). The

addition of pheromone increased the proportion of parasitoids landing on the PHC24 and PHC48, but the difference was not significant (P= 0.06). There were no significant differences between treatments in the time to take-off or the number of wasps taking-off (P> 0.05).

4.4 Discussion

In Experiment I, *A. ervi* showed a relatively weak response to the uninfested plant and a strong response to the PHC. This agrees with the results of previous studies (Guerrieri *et al.*, 1993; Du *et al.*, 1996). The addition of aphid sex pheromone to the plant targets significantly increased the parasitoids' response to the targets. In both cases, the proportion of wasps making oriented flights to the plant targets was increased by about 20% by the addition of pheromone, suggesting an additive rather than synergistic effect. Aphid sex pheromone alone was not included as a treatment in this experiment as it would have presented an inconsistent visual target.

The fact that aphid sex pheromone increased the response to the PHC in Experiment I suggests that, even when herbivore-induced plant cues are available for use by the parasitoid, the pheromone still affects the insect's behaviour. Therefore, if *A. ervi* has a hierarchy of cues to which it responds, the aphid sex pheromone is likely to retain equal status with the level of herbivore-induced cues used in this experiment.

However, evidence that the relative importance of volatile cues may change was obtained in Experiment II. Again the addition of aphid sex pheromone increased the response of *A. ervi* to the plant targets, but the increase in response to the longer aphid infestation (PHC48) was less pronounced than to the shorter infestation (PHC24), and the magnitude of the response to PHC48 and PHC24 was not significantly different. Previous work has shown that when *A. pisum* feed on broad bean, high levels of herbivore-induced volatiles may not be released by the plant until 48-72 hours after infestation (G Poppy, personal communication), presumably due to a delay in production of the chemicals by the plant. Therefore

the PHC48 should represent a more powerful stimulus to *A. ervi* than does the PHC24, being a more detectable and more reliable indicator of host presence.

It appears that when *A. ervi* is responding to this stronger stimulus, the aphid sex pheromone has a lesser influence on its behaviour. This would make sense in a field situation, where the aphid sex pheromone lures may prove valuable in attracting parasitoids into crops early in the season when aphid densities are low. When aphid populations increase and large amounts of herbivore-induced volatiles are present, it may be more advantageous for the parasitoid to respond to these rather than being 'distracted' by aphid sex pheromone lures which may not reliably indicate the presence of hosts.

The results of both experiments suggest that the aphid sex pheromone may have an influence on parasitoid landing. In both experiments the presence of pheromone increased the proportion of wasps landing on the plant targets, although the difference was not always statistically significant. In Experiment I, pheromone increased landing on an uninfested plant, suggesting that orienting wasps did not require the presence of host or PHC cues in order to decide to land. Increased landing by parasitoids in response to aphid sex pheromones would prove valuable in the field, where it may help to increase parasitisation levels in pheromone-baited areas. It may also help to explain the results of field experiments (Chapters 5 and 6).

The interactions between aphid sex pheromones and the PHC could be explored further. To gain more information on the relative importance of the two cues, choice tests could be performed, particularly between the PHC and PHC + Ph. Choice tests have been used successfully in the wind tunnel (Du *et al.*, 1996, 1997), and may prove more valuable in revealing any hierarchy in parasitoid responses. It would also be interesting to test the effect of aphid sex pheromone on parasitoid response to a PHC containing an inappropriate host. Du *et al.* (1996) showed that *A. ervi* did not respond to plants infested with 100 *Aphis fabae*, which is an unsuitable host for this parasitoid. It would be interesting to see if aphid sex

pheromone overcomes this lack of attraction, or whether cues from the unsuitable PHC override attraction to the pheromone.

Chapter 5

Field Responses of Aphid Parasitoids to Pheromone-Baited Plants and Traps

5.1 Introduction

Substantial information now exists concerning the responses of aphid parasitoids to aphid sex pheromones under laboratory conditions. However, if the idea of manipulating parasitoids to improve aphid control is to be pursued, then information about responses in the field is required. It is necessary to demonstrate that, as well as attracting parasitoids, aphid sex pheromone lures will increase rates of parasitisation. Previous work in the field has demonstrated the attraction of *Praon* species to water traps (Hardie *et al.*, 1991; Powell *et al.*, 1993) and to aphid-infested cereal plants (Lilley *et al.*, 1994). The aim of the work in this chapter was to confirm that sex pheromone lures can increase parasitisation rates on plants, and to assess the distance over which lures may be active. Further experiments investigated the responses of the brassica aphid specialist *Diaeretiella rapae* to aphid sex pheromones, and the responses of the generalist parasitoid *Praon volucre* to a range of synthetic pheromone components.

5.2 Active Distance of Aphid Sex Pheromone Lures, Measured by Parasitisation Rates on Aphid-Infested Plants

5.2.1 Introduction

The distance over which aphid sex pheromone lures can influence parasitisation is an important consideration when devising a parasitoid manipulation strategy. If such a strategy were to be successfully implemented, it would be desirable to achieve the maximum efficiency of lure deployment. Information on pheromone active distance is also interesting in the context of the behaviour of parasitoids, and how they use the pheromone as a foraging cue. So far, the distance over

which parasitoids can detect and respond to aphid sex pheromones is not known, although there is indirect evidence that they may be attracted over relatively large distances. *Praon* species were captured in pheromone-baited traps placed in cereal fields in the autumn, a habitat with which they are not normally associated at that time of year, suggesting they were attracted into the field from other habitats (Hardie *et al.*, 1994). The results of wind tunnel experiments (Chapter 2) also support the existence of a relatively long-range flight response.

In this series of experiments, the range of influence of aphid sex pheromone lures was assessed by measuring parasitisation rates on aphid-infested potted plants. Unbaited potted plants have been used successfully to record aphid parasitoid activity in the field (Carter *et al.*, 1982; Vorley, 1986; Dedryver *et al.*, 1991; Milne, 1995), and aphid sex pheromone lures have been shown to increase parasitisation of aphids on plants by *Praon* species (Lilley *et al.*, 1994), and by *Aphidius ervi* and *Aphidius eadyi* (D Brooks, personal communication). Therefore this method was used to measure parasitisation rates at various distances from pheromone lures, mimicking the situation which may occur in a crop or field margin habitat. The influence of lures was tested over distances of 20 cm, 1m and 3m.

5.2.2 Methods

5.2.2a Potted Plant Method

Pots containing 10-day old winter barley (var. Puffin) (50 seedlings/pot) were used in the experiments. Plants were infested with 200 mixed-instar *Sitobion avenae* virginoparae and left overnight for the aphids to settle. The aphid-infested plants were exposed in the field for 3 days, after which time they were returned to the laboratory and kept at 18 °C, 16:8 L:D for 2 weeks to allow development of aphid mummies. While in the field, pots stood in plastic dishes (23 cm diameter), which contained water to maintain plant quality and to act as a barrier against walking predators. Another plastic dish (23 cm diameter) was supported by wire

above each plant to act as a rain shelter. Plants were checked daily and any aphid predators, excluding parasitoids, were removed.

Pheromone-baited plants received an aphid sex pheromone field lure (section 1.7.2b) which was attached to a cane and supported in the soil of the plant pot. Control pots received control lures, and lures were replaced every four weeks. On retrieving plants from the field, the number of aphids still present was recorded, and the plant was thoroughly searched for aphid predators, including parasitoids, which were removed. After two weeks in storage, the plants were examined and the number of mummies that had formed was recorded. Mummies were identified as either *Praon* or *Aphidius* species, according to the shape of the mummy.

5.2.2b Experimental Design

The distance of influence of aphid sex pheromone lures was measured by deploying an aphid-infested plant, baited with a lure, then placing unbaited, aphid-infested plants at varying distances from the baited plant. In the 20 cm experiment, a single unbaited plant was placed 20 cm away from the baited plant, and two unbaited plants were placed 15m away as a control (figure 5.1a). In the 1m and 3m experiments, an array of four unbaited plants was placed around the baited plant, to control for effects of wind direction (figure 5.1b). A similar array of unbaited plants, 15m away, was used as a control. Between four and six replicates were completed at each distance, and the position of pheromone-baited and control plant arrays was alternated between replicates.

The following experiments were performed:

20 cm distance	29/8/96 - 12/9/96	6 replicates
1m distance	26/9/96 - 25/10/96	4 replicates
	12/7/97 - 6/9/97	6 replicates
3m distance	15/8/97 - 26/9/97	5 replicates

The 20 cm, 1m (1996) and 3m experiments were sited on harvested cereal fields. Early replicates of the 1m (1997) experiment were sited on a grass field, with later replicates on a harvested cereal field.

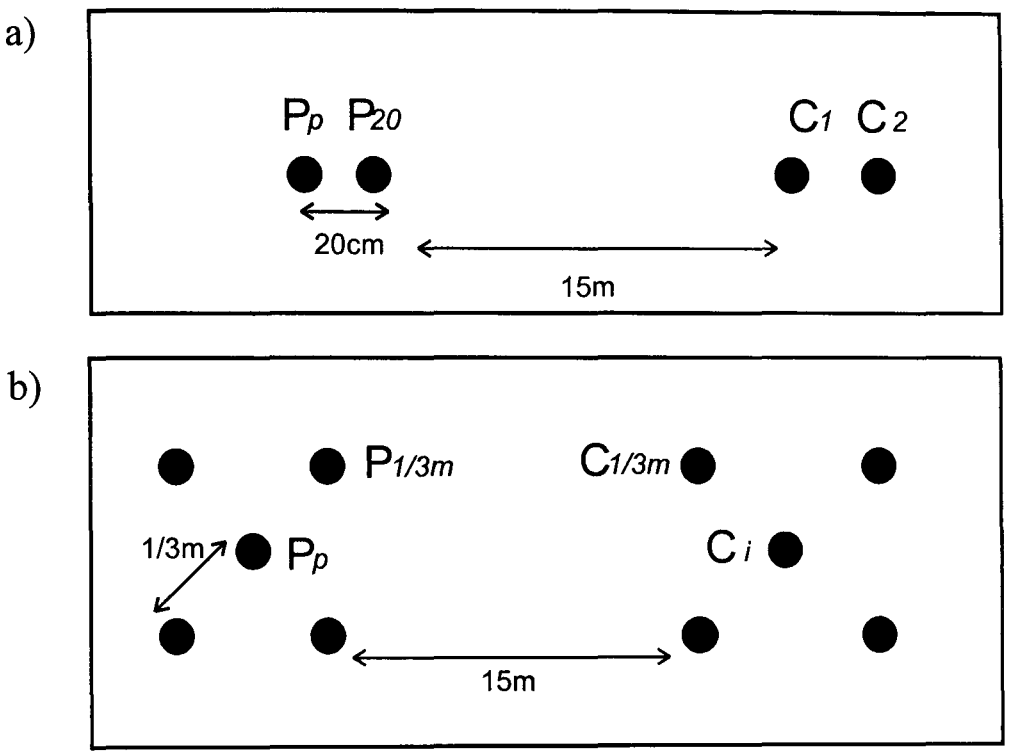

Figure 5.1 Experimental layout of potted plant experiments in the field.

● represents an aphid-infested plant. a) 20 cm experiment; P_p= pheromone-baited plant, P_{20} , C_1 and C_2= unbaited plants. b) 1m and 3m experiments; P_p= pheromone-baited plant, $P_{1/3m}$, C_i and $C_{1/3m}$= unbaited plants.

5.2.2c Statistical Analysis

The mean numbers of mummies which developed on each of the plants was calculated. Initial analyses showed that there were no significant differences between the numbers of mummies which developed on the four outer plants in each array of five in the 1m and 3m experiments. Therefore the mean number of mummies for the four outer plants was used for further comparisons. Means were

analysed by ANOVA and compared with a Tukey test, using the SX Statistical Software (NH Analytical Systems).

5.2.3 Results

For clarity, only the means for the number of mummies recorded on plants are presented in the results. However, the full data relating to aphid survival and number of mummies recorded from the experiments are presented in the Appendix, in tables A11-A20. In no experiment were there significant differences in the numbers of aphids surviving on the plants at the end of the exposure period. Details of the ANOVA results for experiments in this section are given in the Appendix, in table A8.

5.2.3a 20 cm Experiment

The mean number of *Praon* mummies recorded on pheromone-baited plants, and on unbaited plants placed 20 cm or 15m away from the baited plant are shown in figure 5.2.

Very low numbers of *Aphidius* mummies were recorded and these were excluded from the analysis. The number of mummies on the pheromone-baited plant (P_p) was significantly higher ($P< 0.05$) than on the unbaited control plants (C_1 and C_2), 15m away from P_p. However, there was no significant difference between numbers of mummies on P_p and on the unbaited plant placed 20 cm away from P_p (P_{20}).

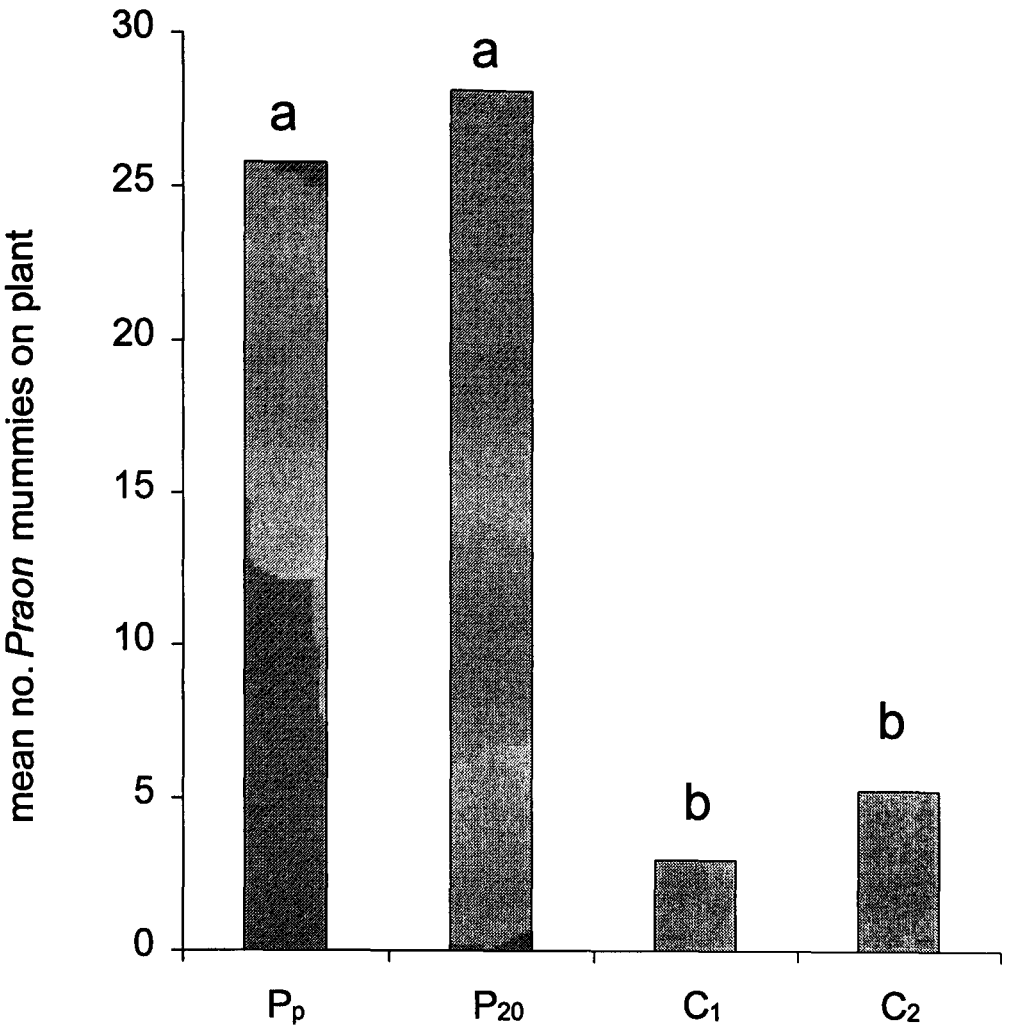

Figure 5.2 Mean number of *Praon* mummies recorded on aphid-infested plants. P_p= pheromone-baited plant; P_{20}= unbaited plant 20 cm away from baited plant; C_1 and C_2= unbaited control plants (see figure 5.1a). Columns with different letters are significantly different (ANOVA, Tukey test; $P< 0.05$).

5.2.3b 1m Experiment 1996

The mean number of mummies recorded from aphid-infested plants placed in 1m arrays (figure 5.1b) is shown in figure 5.3. Mummies of both *Praon* and *Aphidius* species were recorded, and are shown separately. Only four replicates were completed in 1996 and the data were not analysed statistically. However, the results suggest that aphid sex pheromone did increase parasitisation by both *Praon* and *Aphidius* on the baited plant (P_p), and that P_p had a greater influence over parasitisation on unbaited plants 1m away (P_{1m}) for *Aphidius* than it did for *Praon*.

5.2.3c 1m Experiment 1997

The mean number of mummies recorded from aphid-infested plants placed in 1m arrays (figure 5.1b) is shown in figure 5.4. Mummies of both *Praon* and *Aphidius* species were recorded, and are shown separately. For *Praon*, the number of mummies on the pheromone-baited plant (P_p) was significantly higher ($P< 0.05$) than on unbaited plants (P_{1m}, C_i and C_{1m}). The pheromone-baited plant did not influence parasitisation on unbaited plants placed 1m away (P_{1m}). For *Aphidius*, the number of mummies on the pheromone-baited plant (P_p) was significantly higher ($P< 0.05$) than on the unbaited control plants placed 15m away (C_i and C_{1m}), but not significantly higher than on the unbaited plant placed 1m away (P_{1m}), indicating that the pheromone influenced parasitisation rates over a distance of 1m.

5.2.3d 3m Experiment

The mean number of *Praon* mummies recorded from aphid-infested plants placed in 3m arrays (figure 5.1b) is shown in figure 5.5. Very low numbers of *Aphidius* mummies were recorded and were excluded from the analysis. The number of mummies on the pheromone-baited plant (P_p) was significantly higher ($P< 0.05$) than on the unbaited plants (P_{3m}, C_i and C_{3m}).

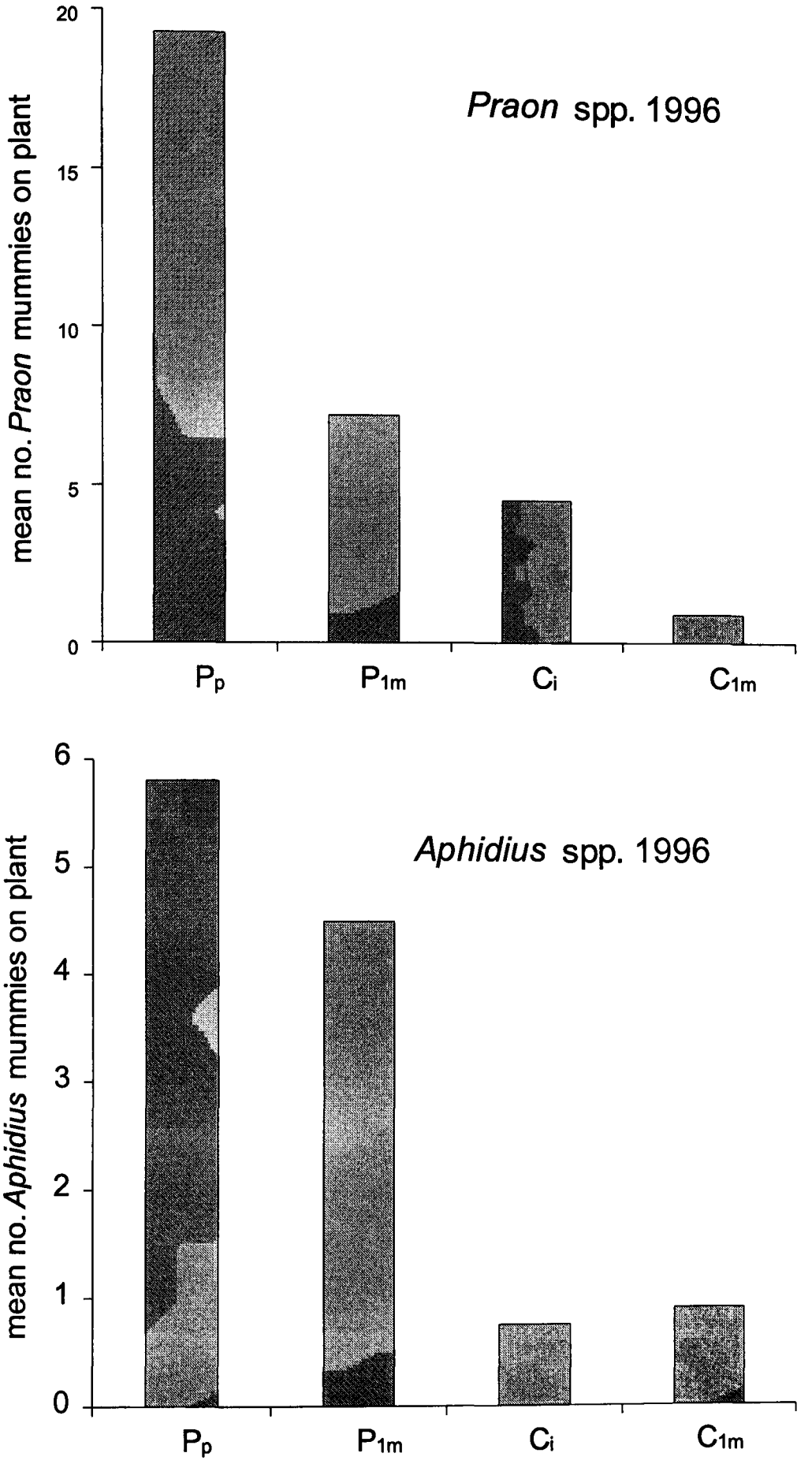

Figure 5.3 Mean number of *Praon* and *Aphidius* mummies recorded on aphid-infested plants in 1996. P_p= pheromone-baited plant; P_{1m}= unbaited plant 1m away from baited plant; C_i and C_{1m}= unbaited control plants (see figure 5.1a).

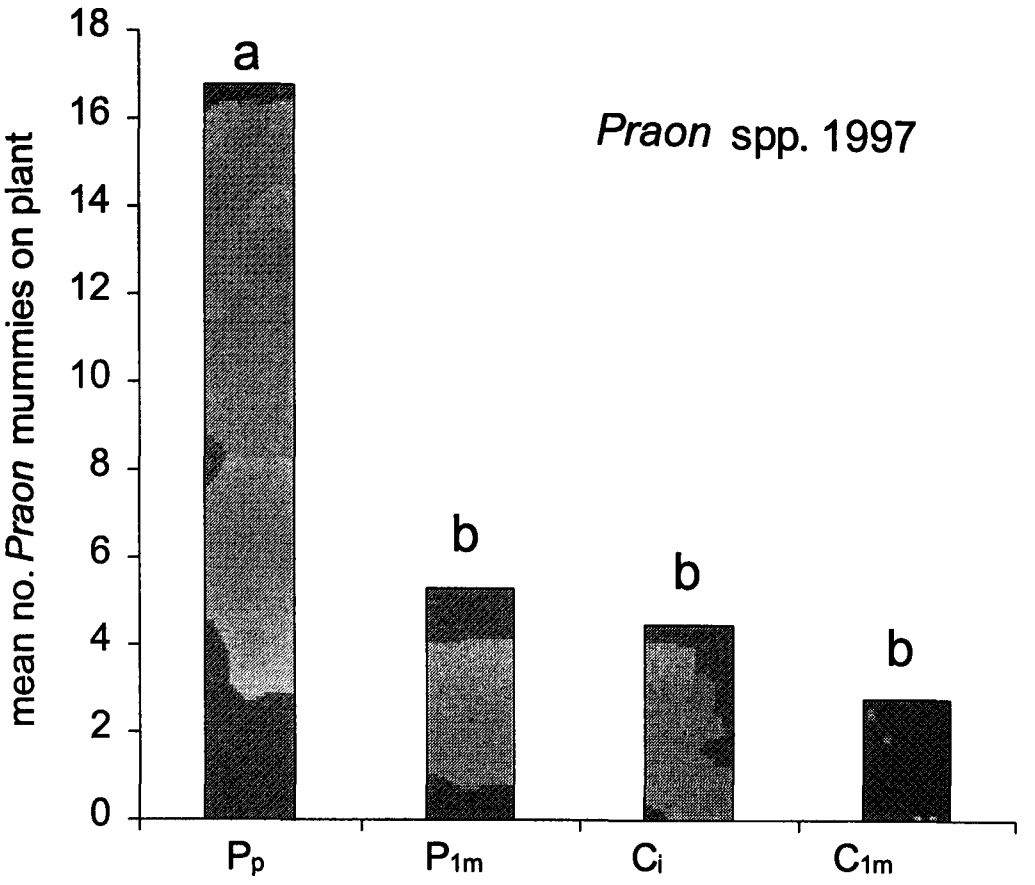

Figure 5.4 Mean number of *Praon* and *Aphidius* mummies recorded on aphid-infested plants in 1997. P_p= pheromone-baited plant; P_{1m}= unbaited plant 1m away from baited plant; C_i and C_{1m}= unbaited control plants (see figure 5.1a). Columns with different letters are significantly different (ANOVA, Tukey test; $P< 0.05$).

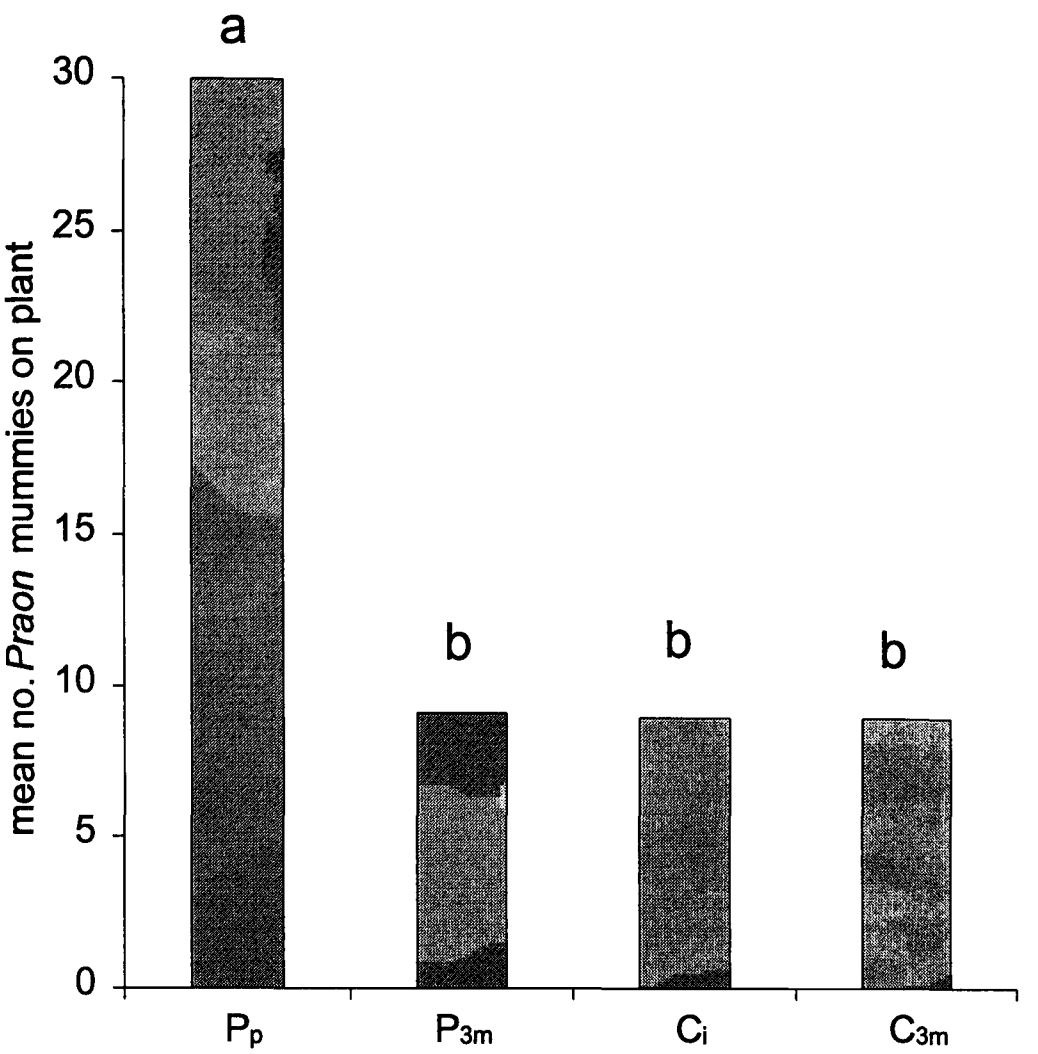

Figure 5.5 Mean number of *Praon* mummies recorded on aphid-infested plants. P_p= pheromone-baited plant; P_{3m}= unbaited plant 3m away from baited plant; C_i and C_{3m}= unbaited control plants (see figure 5.1a). Columns with different letters are significantly different (ANOVA, Tukey test; $P< 0.05$).

5.2.4 Discussion

The presence of aphid sex pheromone lures increased parasitisation rates on aphid-infested plants in all of the experiments. This shows that lures not only attract parasitoids, but that attracted parasitoids will attack aphids they encounter around the lures. Since only virginoparae were present on the plants, the results also show that parasitoids responding to the pheromone will attack host stages other than that which produces the pheromone in the field (the ovipara). In these two respects the present results agree with previous studies (Lilley *et al.*, 1994; D Brooks, personal communication). The demonstration of increased parasitisation

is an important step towards the use of aphid sex pheromone lures to improve aphid population control.

The investigation into the distance over which lures may be effective provided useful and interesting information. In the 20 cm experiment, only *Praon* species were recorded. The pheromone lure on the baited plant clearly influenced the rate of parasitisation on the unbaited plant placed 20 cm away. Whether this was due to parasitoids being attracted equally to both plants, or being attracted to the baited plant initially before dispersing to the unbaited plant is not possible to determine here. However, it is encouraging that the increased parasitisation was not confined to the point-source of pheromone release. There was no evidence of increased parasitisation on unbaited plants placed 15m away along the same field edge, suggesting that the pheromone had no effect over this relatively large distance, although the experiment did not allow for the effect of wind direction.

It is possible that the increased parasitisation on pheromone-baited plants was not due to the attraction of large numbers of parasitoids, since a single female can oviposit in a large number of aphids. However, field observations often revealed large numbers of parasitoids searching on baited plants, with fewer on unbaited plants. Another possibility is that the pheromone increased searching or host attack by a low number of parasitoids after they had located the plant. However, the results of experiments in Petri dish arenas (section 3.3) and on caged plants (section 3.5) do not support this. Therefore, it is likely that the increased parasitisation on pheromone baited plants was due to the attraction of higher numbers of parasitoids.

In the 1m experiments, both *Praon* and *Aphidius* were found parasitising aphids on the plants. Identification of emerging wasps using Powell (1982) showed that the *Aphidius* parasitoids were *Aphidius rhopalosiphi*. There appeared to be a difference between the two parasitoids in the distribution of mummies on the plants. In 1996 this was apparent as a trend since overall numbers were considered too low for analysis, but in 1997 the differences were supported by statistical

analysis. Parasitisation by both parasitoids was higher on pheromone-baited than on unbaited plants. However, for *A. rhopalosiphi*, parasitisation was also increased on unbaited plants 1m away from the baited plant, whereas for *Praon*, parasitisation on plants 1m away was unaffected. This suggests that there is a difference between the foraging strategies of the two parasitoids. The difference could be explained if *Praon* flew more directly to the source of the pheromone, whereas *Aphidius rhopalosiphi* ended its flight and began to forage on suitable plants at least 1m away from the source.

This behaviour could arise in three ways. Firstly, there may be a difference in the way in which *Praon* and *A. rhopalosiphi* respond to the pheromone itself. *Praon* may use the pheromone purely as an attractant, whereas with *A. rhopalosiphi* it may also have an arrestment effect, which would cause parasitoids to land once inside a pheromone-laden area. In pheromone trap experiments (Hardie *et al.*, 1994), there was evidence that *Praon* was attracted over a relatively large distance, but *A. rhopalosiphi* has not previously been recorded in pheromone water traps (W Powell, personal communication). *Praon* is associated with habitats containing the winter hosts of host-alternating aphids (Vorley, 1986) and may use aphid sex pheromones to locate sparsely distributed aphid colonies in the autumn. *A. rhopalosiphi*, on the other hand, is more usually found attacking aphids in grasses and cereals, where hosts may be more easily located, and the role of aphid sex pheromone less important. Lewis *et al.* (1982) found that parasitisation by *Trichogramma* on plants was enhanced by the presence of host sex pheromone lures, and laboratory experiments (Noldus *et al.*, 1991a) confirmed that the effect was due to arrestment of searching parasitoids by the pheromone.

A second explanation for the difference may lie in the way in which *Praon* and *A. rhopalosiphi* use cues from the plant host complex (PHC). If the PHC is relatively more important to *A. rhopalosiphi* , then this parasitoid may have ceased flying to the pheromone source when the PHC cues from the outer plants were detected. *Praon* species are generalists and attack aphids on a wide range of plants, and it

may be disadvantageous for a generalist to respond to a particular PHC. Bouchard and Cloutier (1985) found that another generalist aphid parasitoid, *Aphidius nigripes*, did not respond to host food-plant odours in an olfactometer and proposed the same explanation. *A. rhopalosiphi*, on the other hand, is a specialist on grasses and cereals, and has been shown to respond strongly to a cereal PHC in an olfactometer (Micha and Wyss, 1995). A final explanation may simply be that the two parasitoids have different behavioural thresholds of response to the pheromone. If *A. rhopalosiphi* was more sensitive, then its threshold would be reached at a further distance away from the pheromone source. The difference between *Praon* and *A. rhopalosiphi* is supported by cage experiments, in which *A. rhopalosiphi* but not *P. volucre* was retained on pheromone-baited plants (section 3.5), although there was no increased parasitisation in this case.

Unfortunately, *A. rhopalosiphi* was not recorded in the 3m experiment. However, the result supports the findings of the 1m experiment, suggesting that *Praon* flies directly to the pheromone source.

The fact that, for *A. rhopalosiphi*, pheromone lures seem to influence parasitisation over at least 1m is encouraging for the use of lures in the crop. *A. rhopalosiphi* is usually the dominant species in cereal crops (Powell, 1982; Vickerman, 1982; Wratten and Powell, 1991), and in a field trial (section 6.3.1), increased parasitisation by *Aphidius* was evident up to 2-3m away from pheromone lures. In both crops and field margins, where the distribution of aphids cannot be controlled, lures may be more effective in establishing populations of *A. rhopalosiphi* rather than *Praon* species parasitoids.

5.3 The Response of Aphid Parasitoids to Synthetic and Plant-Extracted Nepetalactone in the Field

5.3.1 Introduction

The nepetalactone used in laboratory and field experiments with both aphids and parasitoids is usually obtained by extraction from the catmint plant *Nepeta cataria* (Laminaceae: Labiatae), in which it occurs as a secondary metabolite. No function has yet been determined for it in the plant, although a defensive role has been suggested (Eisner, 1964). Chemists have recently developed pathways to produce synthetic nepetalactones (and nepetalactols) of known purity and enantiomeric structure (Dawson *et al.*, 1996), and it has been suggested that plant-extracted nepetalactone may contain enantiomeric or plant-derived impurities which reduce its attractivity to male aphids (Hardie *et al.*, 1997). In a wind tunnel experiment (section 2.5), *Praon volucre* was attracted equally to plant-extracted and 99% pure synthetic nepetalactone. However, it was suggested that testing these compounds in the field would be valuable, and chemists have recently produced a wider range of compounds which can now be field tested against parasitoids.

The plant-extracted nepetalactone has a particular stereochemical structure (figure 1.2), and is termed (4aS,7S,7aR)-nepetalactone (referred to hereafter as (7S)-lactone). By changing the stereochemistry at C-7, the enantiomer (4aR,7R,7aS) (referred to as (7R)-lactone), can be produced (Dawson *et al.*, 1996; Hardie *et al.*, 1997). In field trapping experiments (Hardie *et al.*, 1997), the 99% pure (7S)-lactone and 99% (7S)-nepetalactol attracted significantly more males of *Sitobion fragariae* and *Rhopalosiphum padi* respectively than did the plant-extracted equivalents.

Since previous attempts to attract parasitoids to water traps had proved unsuccessful during the current project, it was decided to compare the plant-extracted and synthetic nepetalactones using the potted plant method which was used successfully in the previous experiment (section 5.2).

5.3.2 Methods

5.3.2a Experimental Design

The potted plant method was as described in section 5.2.2a. Five experimental treatments were tested:

A 98% (7R)-nepetalactone

B 99% (7S)-nepetalactone

C 50% (7S)-nepetalactone:50% (7R)-nepetalactone

D Plant-extracted nepetalactone

E Control

Synthetic nepetalactones A and B were synthesised from the equivalent citronellol, and the mixture C from the racemic citronellol (Dawson *et al.*, 1996). Plant-extracted nepetalactone was obtained from *Nepeta cataria* as described in section 1.7.2. All compounds were released from glass-vial field lures (section 1.7.2b). Pheromone compounds (10 mg) were carried in diethyl ether, and control vials contained only diethyl ether. Lures were attached to canes which were placed in the soil of the plant pot.

The trial ran from 20 August to 26 September 1997 on a field from which cereals had recently been harvested. Potted plants were placed 15m apart along a field edge. A single pot was used for each treatment in each replicate, and pots were arranged according to a quasi-complete Latin square design. Ten replicates were completed, and the positions of treatments were re-randomised between replicates.

5.3.2b Statistical Analysis

The mean number of parasitoid mummies which developed on plants of each treatment was calculated. Means were analysed by ANOVA and compared with a Tukey test, using the SX Statistical Software (NH Analytical Systems). Details of the ANOVA results are given in the Appendix, in tables A9-A10.

5.3.3 Results

For clarity, only the means for the number of mummies recorded on plants are presented in the results. However, the full data relating to aphid survival and number of mummies recorded from the experiments are presented in the Appendix, in tables A21-A22. There were no significant differences between plants in the percentage of aphids recovered at the end of the exposure period (ANOVA, $P > 0.05$). Parasitisation of aphids on the plants was mainly due to *Praon* species parasitoids. Very low numbers of *Aphidius* mummies were recorded and were excluded from the analysis.

The mean number of *Praon* mummies which developed on plants from each of the treatments is shown in figure 5.6. The highest number of mummies was recorded on the plants baited with the synthetic 99% (7S)-lactone (B), although it was not significantly higher than that recorded on the plant-extracted nepetalactone plant (D). The number of mummies on plants baited with the synthetic 98% (7R)-lactone and the 50% (7R):50% (7S)-lactone (A and C) were not significantly higher than on the control plant (E).

5.3.4 Discussion

The synthetic (7S)-lactone was responsible for higher rates of parasitisation than the plant-extracted lactone, although the difference was not significant. Since the plant-extracted lactone is relatively pure in terms of its enantiomeric composition (Hardie *et al.*, 1997), the reduced response to it could be caused by plant-related contaminants, although generalist parasitoids such as *Praon*, which attack aphids on a wide range of plants, may not be sensitive to specific plant cues (Bouchard and Cloutier, 1985).

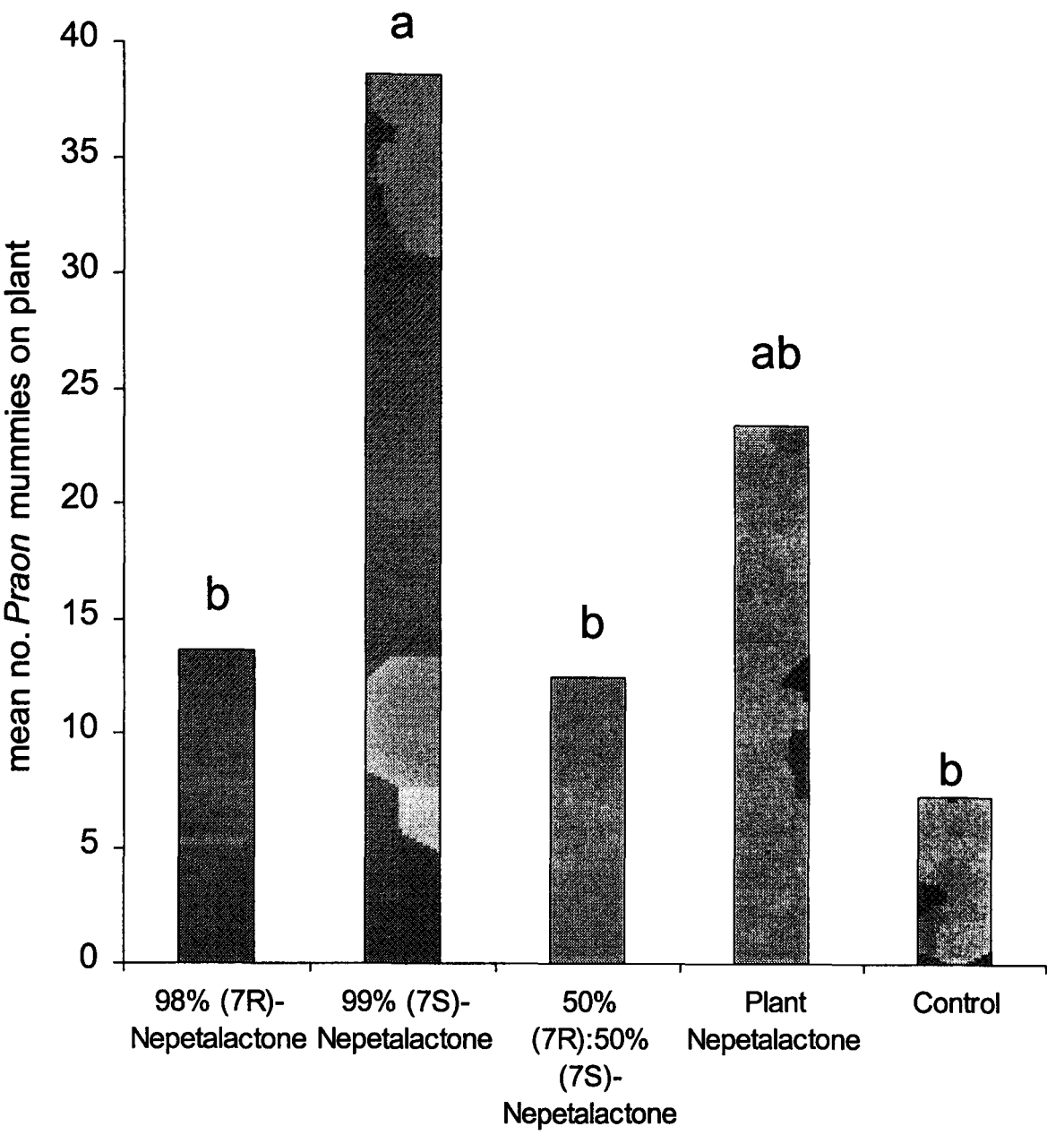

Figure 5.6 The mean number of *Praon* species mummies recorded from aphid-infested plants baited with synthetic or plant-extracted nepetalactone, after exposure in the field for 3 days. Columns followed by different letters are significantly different (ANOVA, Tukey test; $P < 0.05$).

The major effect on *Praon* parasitisation was due to the (7R)-lactone enantiomer. Parasitisation rates on plants baited with (7R)-lactone were not significantly higher than on the unbaited control plant, and the presence of (7R)-lactone reduced the attractiveness of the (7S)-lactone to a non-significant level. This suggests that *Praon* species parasitoids can detect the different enantiomeric configurations of nepetalactone and that they have a differential behavioural response to the two enantiomers. It is likely that, for *Praon* parasitoids, the

enantiomeric purity of the pheromone is more critical than the presence of possible contaminants of plant origin. In field trapping experiments (Hardie *et al.*, 1997), the presence of (7R)-nepetalactol in the racemic mixture reduced the previously high capture of *Rhopalosiphum padi* by the (7S)-nepetalactol. However, the racemic mixture and the (7R)-lactol alone were still significantly more attractive then the unpurified plant-extracted lactol. Therefore, for aphids, there seems to be an effect of both enantiomeric purity and plant contaminants.

The fact that the presence of (7R)-lactone in the racemic mixture inhibited attraction to the (7S)-lactone indicates that *Praon* can detect the (7R) enantiomer, and that the behavioural response to the (7S) enantiomer is modified as a consequence. To elucidate the mechanism by which this occurs will require electrophysiological studies on the parasitoid antenna. It is possible that *Praon* has separate receptor cells for the two pheromone enantiomers, as is the case in the gypsy moth *Lymantria dispar* L. (Hansen, 1984), or that the same cell can separately detect both enantiomers, as has been shown for *Dendroctonus* species beetles (Payne *et al.*, 1982); Dickens *et al.*, 1985).

Behavioural and electrophysiological responses to pheromone enantiomers have received attention in other insect species. The bark beetle predator *Thanasimus dubius* was found to specifically use one enantiomer of its hosts' aggregation pheromone as a kairomone (Payne *et al.*, 1984). Males of the braconid parasitoid *Macrocentrus grandii* responded preferentially to a particular enantiomer of the female sex pheromone, both in the laboratory and the field (Swedenborg *et al.*, 1994). For several insect species there are reports of one pheromone enantiomer eliciting a reduced response (Dickens and Mori, 1989; Levinson *et al.*, 1990; Phillips *et al.*, 1990), or no response at all (Pierce *et al.*, 1988; Schaner *et al.*, 1989) compared to a second enantiomer.

This experiment should be repeated in order to clarify the comparative attractiveness of the plant-extracted and the synthetic (7S) lactones. If the (7S)-lactone proves to be more effective in increasing parasitisation rates, this would

lead to an improved field lure with which to manipulate parasitoid populations. The results suggest that, although the (7R)-lactone does not actually repel or deter parasitoids, it may reduce or eliminate the response to the (7S)-lactone. This would provide a useful tool for controlling the movements of parasitoids in the field.

5.4 Responses of *Diaeretiella rapae* to Aphid Sex Pheromone-Baited Water Traps in the Field

5.4.1 Introduction

A previous experiment in a laboratory wind tunnel (section 3.3.2d) showed that female *D. rapae* respond to the aphid sex pheromone nepetalactone. However, the response was manifested as a reduction in time to take-off, and an increased proportion of wasps taking-off, rather than as oriented, upwind flight towards the pheromone target as noted for other parasitoid species (Poppy *et al.*, 1997); Chapter 2). The experiment therefore yielded ambiguous information about the nature of the parasitoid's response to the sex pheromone. A reason for the lack of upwind flight may have been that *D. rapae* was reluctant to fly under wind tunnel conditions. Therefore, the sex pheromone was tested against a field population of *D. rapae*.

The responses of *D. rapae* in the field were investigated using monitoring traps. Gabrys *et al.* (1997) reported that *D. rapae* was attracted to pheromone-baited traps in oilseed rape and cabbage crops in Poland, and traps have previously been used successfully to capture *Praon* species parasitoids in the United Kingdom and Germany (Hardie *et al.*, 1991, 1994; Powell *et al.*, 1993). As well as providing a means of testing the responses of a particular parasitoid, monitoring traps, if effective, could also be used to survey the aphid parasitoid fauna in a particular area or habitat.

5.4.2 Methods

5.4.2a Monitoring Traps

Parasitoid monitoring traps were similar to those used by Hardie *et al.* (1991), Issacs (1994) and Gabrys *et al.* (1997). Traps consisted of a clear plastic Petri dish (14 cm diameter) which was attached to a short length of plastic pipe. The plastic pipe fitted over the end of a bamboo cane which could be pushed into the ground to support the trap at a height which was level with the canopy of the crop. Aphid sex pheromone lures were suspended from a wire support, which was attached to the centre of the Petri dish and also supported a plastic 'rain hat' to protect the lure. Once deployed in the field, traps were filled with water, containing approximately 2% detergent in order to capture and retain insects. A small section of the wall of the Petri dish was cut away and replaced with plastic mesh in order to allow excess water to drain away while retaining trapped insects. Traps were emptied by pouring the contents through a piece of muslin, which was then placed into a vial and returned to the laboratory for sorting.

5.4.2b Experimental Design

Experiments were performed in two years, and on different sites. All the sites were fields which contained spring-sown oilseed rape, and which supported populations of the cabbage aphid, *Brevicoryne brassicae*, and the peach-potato aphid, *Myzus persicae*. In 1995, traps were deployed between 29 June and 16 July on three sites, and in 1996 traps were deployed between 8 August and 18 August on two sites. Four pheromone treatments were tested in each experiment:

A Nepetalactone

B Nepetalactol

C 1:1 ratio lactone: lactol (1995)
  1:2 ratio lactone: lactol (1996)

D Control

In 1995 a 1:1 ratio of nepetalactone to nepetalactol was tested, but this was modified to a 1:2 ratio in 1996 since this is the ratio released by *Myzus persicae* (Pickett *et al.*, 1992), which is a host for *D. rapae*. The other major host for *D. rapae* is *Brevicoryne brassicae*, which produces only the nepetalactone (Gabrys *et al.*, 1997). At each site, four traps (one of each treatment) were placed 10m apart along the edge of the oilseed rape crop. Traps were arranged according to a quasi-complete Latin square design (each row representing a line of traps), and were re-randomised and emptied every day. Captured *D. rapae* were identified according to Powell (1982), and the numbers of males and females were recorded.

5.4.2c Statistical Analysis

The numbers of male and female *D. rapae* captured were analysed by ANOVA using GENSTAT. Means were compared using a Tukey test. Details of the ANOVA results are given in the Appendix, in table A8.

5.4.3 Results

For clarity, only the mean numbers of *D. rapae* captured are presented in the results. However, the numbers of *D. rapae* captured during each replicate are presented for each site in the Appendix in tables A23-A27.

The mean numbers of male and female *D. rapae* captured in traps baited with different aphid sex pheromone components in 1995 and 1996 are shown in figure 5.7. In 1995, traps baited with all pheromone treatments captured significantly more female *D. rapae* than did the control trap ($P < 0.05$). In the same experiment, the number of males captured in the nepetalactone-baited trap was significantly higher than in the control trap ($P < 0.05$), although fewer males than females were captured overall. In 1996, total numbers of *D. rapae* were much lower than in 1995. Traps baited with nepetalactone captured significantly more female *D. rapae* than did the control trap ($P < 0.05$), but not significantly more than the traps baited with nepetalactone or a 1:2 ratio. There were no significant differences between the numbers of males captured in the traps in 1996.

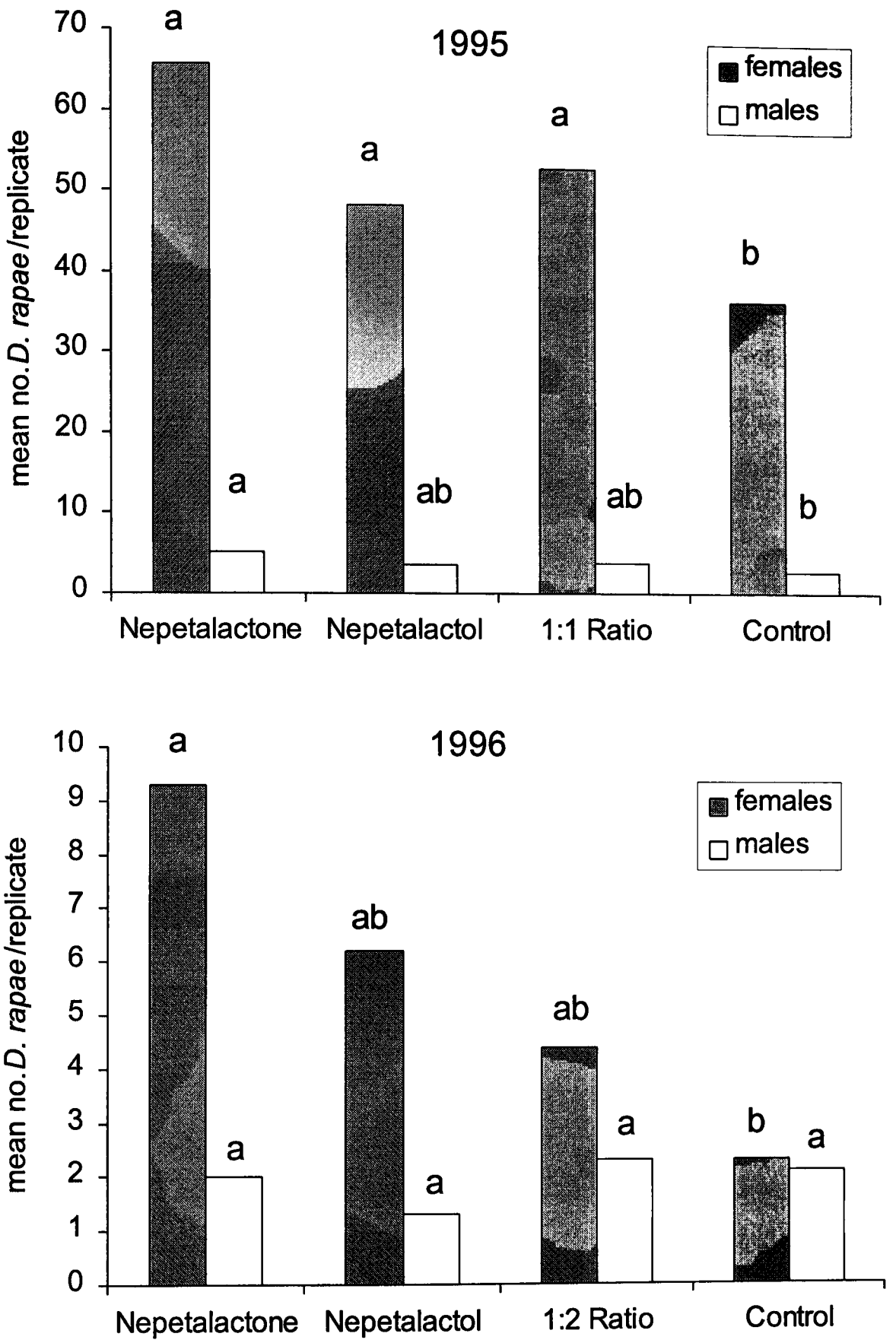

Figure 5.7 Capture of male and female *Diaeretiella rapae* in pheromone-baited water traps placed in oilseed rape fields in the summers of 1995 and 1996. Columns followed by a different letter (for males and females separately) are significantly different (ANOVA, Tukey test; $P< 0.05$).

5.4.4 Discussion

The results support previous evidence from wind tunnel bioassays that female *D. rapae* respond to aphid sex pheromones (section 3.3.2d). In the wind tunnel, parasitoids did not show a flight response towards the pheromone target, so the field trapping experiment more clearly indicates that they are attracted to the pheromone source. In 1995, both nepetalactone and nepetalactol, as well as a 1:1 ratio of the two compounds were equally attractive whereas, in 1996, nepetalactone appeared to be more attractive than nepetalactol or a 1:2 ratio. The difference may be due to the presence of very high numbers of *D. rapae* in 1995, which were the result of a *Brevicoryne brassicae* population explosion on oilseed rape in response to hot, dry weather.

The current results are in agreement with those of Gabrys *et al.* (1997), who demonstrated attraction of *D. rapae* to nepetalactone-baited traps in oilseed rape and cabbage fields in Poland. The major aphid hosts of *D. rapae* are the cabbage aphid, *Brevicoryne brassicae*, and the peach-potato aphid, *Myzus persicae*. Since *B. brassicae* releases nepetalactone as its sex pheromone (Gabrys *et al.*, 1997) and *M. persicae* releases a 1:2 ratio of lactone to lactol (Pickett *et al.*, 1992), a response to nepetalactone would allow *D. rapae* to locate sexual colonies of both these species in the field.

In 1995, significantly more male *D. rapae* were captured in the nepetalactone-baited trap than in the control trap. However, it is unlikely that this is an indication of a male parasitoid response to the aphid sex pheromone. Female *D. rapae* have been shown to produce a sex pheromone which is attractive to males (Read *et al.*, 1970; Askari and Alishah, 1979), and males were probably attracted by the large numbers of females either swarming around, or on the water surface, of the nepetalactone-baited trap. Capture of male *D. rapae* in nepetalactone-baited traps has previously been reported, probably for the same reason (Gabrys *et al.*, 1997; B Gabrys, personal communication). In wind tunnel experiments (section 3.3.2d), there was no evidence for a male behavioural response to nepetalactone.

These results provide further evidence that Petri dish monitoring traps are effective at capturing some aphid parasitoids. As well as providing a means of testing the field responses of particular parasitoids to aphid sex pheromones, monitoring traps may provide a useful tool for assessing the aphid parasitoid fauna in a particular habitat or crop system. This information will be of use in devising strategies for manipulating parasitoid populations in order to improve the control of aphids.

Chapter 6

Field Trial: Effect of Aphid Sex Pheromone on Parasitisation Rates in a Winter Wheat Crop

6.1 Introduction

One possible exploitation of the parasitoid response to aphid sex pheromones would be to use pheromone lures to manipulate parasitoids in the field, in order to enhance the control they already exert on aphid populations. As discussed in section 1.1.4, for parasitoids to be effective in the control of aphids they need to arrive in the crop early in the season and achieve synchrony with colonising aphid populations (Carter *et al.*, 1980; Wratten and Powell, 1991). If parasitoids achieve this early season synchrony, they can slow the initial upsurge in aphid populations, allowing other natural enemies to keep aphid numbers below economic thresholds later in the season. Synchrony between aphids and their parasitoids is often poor in agroecosystems, but might be improved by using aphid sex pheromone lures to establish overwintering parasitoid populations in field margin habitats (section 1.6). The following spring, parasitoids would be able to enter the crop immediately, rather than having to arrive from more dispersed locations.

Since the initial discovery of the parasitoid response to aphid sex pheromone (Hardie *et al.*, 1991), laboratory studies have yielded valuable information on the nature of the response (Issacs, 1994; Lilley *et al.*, 1994; Poppy *et al.*, 1997; Chapters 2-4, this thesis), and small scale field experiments have shown that parasitoids which are attracted to sex pheromone lures will attack aphids present around the lures (Lilley *et al.*, 1994; Chapter 5). The research has now reached the stage at which the parasitoid response to sex pheromone needs to be investigated at a larger scale, i.e. in a crop ecosystem, to test whether parasitoids can be manipulated in order to improve aphid control.

The use of semiochemicals to manipulate insects in the field is well established. However, the target organism has usually been the pest itself, with strategies such as mating disruption (Neumann, 1992; Cardé and Minks, 1995) and 'push-pull' (Miller and Cowles, 1990; Smart *et al.*, 1994) being employed to reduce feeding damage. The use of kairomones to enhance the effectiveness of parasitoids is less widely reported. Kairomones extracted from the host or from host products, and applied directly to foliage in the field, increased parasitisation of pyralid larvae by *Apanteles cypris* Nixon (Hu and Chen, 1987), and of the aleyrodid *Aleurocanthus spiniferus* Quaint. by *Amitus longicornis* Foster (Li *et al.*, 1993.) In a glasshouse study, a synthetic kairomone of *Pseudaletia separata* Walker (Noctuidae) doubled the percentage parasitisation by *Apanteles kariyai* (Watanabe) when applied to plants (Takabayashi and Takahashi, 1988). Kairomones extracted from the larvae of *Agrotis ipsilon* (Hufnagel) and *Spodoptera littoralis* increased parasitisation by their respective larval parasitoids *Apanteles ruficrus* Haliday and *Microplitis demolitor* Wilkinson when applied to field plots (Zaki, 1996).

Host pheromones have also been used in the field. Lewis *et al.* (1985) used a formulation containing the sex pheromone and other volatiles of *Heliothis zea* to increase parasitisation of eggs by *Trichogramma pretiosum* in a cotton crop. Lewis *et al.* (1982) achieved a similar effect using synthetic *H. zea* sex pheromone released from lures (Conrel fibres). In pear orchards, parasitisation of eggs of the moth *Grapholita molesta* (Busck) was 80% higher in areas baited with its synthetic sex pheromone than in unbaited areas (Meng *et al.*, 1985).

If parasitoids can be attracted to field margin habitats in the autumn, and overwinter successfully, adults emerging the following spring may disperse from the field edges rather than entering the crop itself. Aphid sex pheromone lures within the crop might provide a stimulus for overwintered parasitoids to enter the crop, and may also lead directly to increased parasitisation of aphids. Although aphids only produce the sex pheromone in the autumn, parasitoids respond throughout the year.

To investigate the effectiveness of manipulating parasitoids within the crop in the spring, and to ascertain whether parasitoid behaviour can be influenced on a wider scale, field trials were performed in a crop of winter wheat. In 1996 the effect of baiting plots with different numbers of aphid sex pheromone lures was investigated. In 1997 the effect of aphid sex pheromone was again tested, both on its own and in combination with a plant volatile, methyl salicylate, which was previously shown to reduce colonisation of the crop by cereal aphids (Pettersson *et al.*, 1994).

6.2 Methods

6.2.1 Experimental Sites

In both 1996 and 1997, experiments were performed in a crop of winter wheat at Rothamsted Farm. Full details of the experimental crops are shown in table 6.1.

Table 6.1 Details of experimental winter wheat crops in which aphid sex pheromone field trials were performed

Experiment	Winter wheat variety	Seed rate	Sowing date
1996	Hereward	380/m^2	20 October 1995
1997	Mercia	380/m^2	8 October 1996

Herbicides, fungicides and fertilisers were applied according to standard farm practice, but no insecticide sprays or insecticidal seed dressings were used.

6.2.2 Experimental Treatments

The semiochemical treatments which were assigned to replicated plots in the 1996 and 1997 field trials are listed in table 6.2.

Table 6.2 Semiochemical treatments with which experimental plots were baited in aphid sex pheromone field trials.

Treatment code	Experimental treatment/plot	Lure type	Release Rate
1996			
C	None	----	----
P1	1 nepetalactone lure	Glass vial	250 µg/day/vial
P2	2 nepetalactone lures	Glass vial	250 µg/day/vial
P5	5 nepetalactone lures	Glass vial	250 µg/day/vial
1997			
C	None	----	----
P	1 nepetalactone lure	Glass vial	250 µg/day/vial
P/MS	1 nepetalactone lure +	Glass vial	250 µg/day/vial
	6 methyl salicylate lures	Polythene bag	30-35 mg/day/bag

Nepetalactone lures were supported on bamboo canes in the centre of the plot (figure 6.3). Lures were attached to bulldog clips, allowing their position to be regularly adjusted so they were always level with the height of the crop, and were protected from rainfall by a plastic dish (9 cm diameter). Lures consisted of the glass vial dispensers described in section 1.7.2b. Methyl salicylate lures were sealed bags made of polythene (1000 gauge) which contained a square of household cleaning sponge impregnated with methyl salicylate (1 ml). Six polythene bags were placed in the plot, in a rectangular arrangement about half

way into the plot (figure 6.4). Bags were attached to bamboo canes and were adjusted to just below the height of the crop. Nepetalactone lures were replaced every 4 weeks, and methyl salicylate lures every 6 weeks.

In the 1997 trial, five experimental treatments were actually assessed. However, for relevancy to this thesis, and for clarity, only those involving the aphid sex pheromone are considered here. The other treatments were methyl salicylate alone, and an antifeedant (polygodial).

6.2.3 Experimental Design

In both 1996 and 1997, a replicated plot design based on a neighbour balanced Latin square was used. This design controls for the possible effects of treatments on neighbouring plots, and for the colonisation of insects from a particular direction. In 1996, a 4x4 Latin square was used (figure 6.1), and in 1997 a 5x5 Latin square was used (figure 6.2). In both trials, plots measured 6x6m, and were separated by 6m paths.

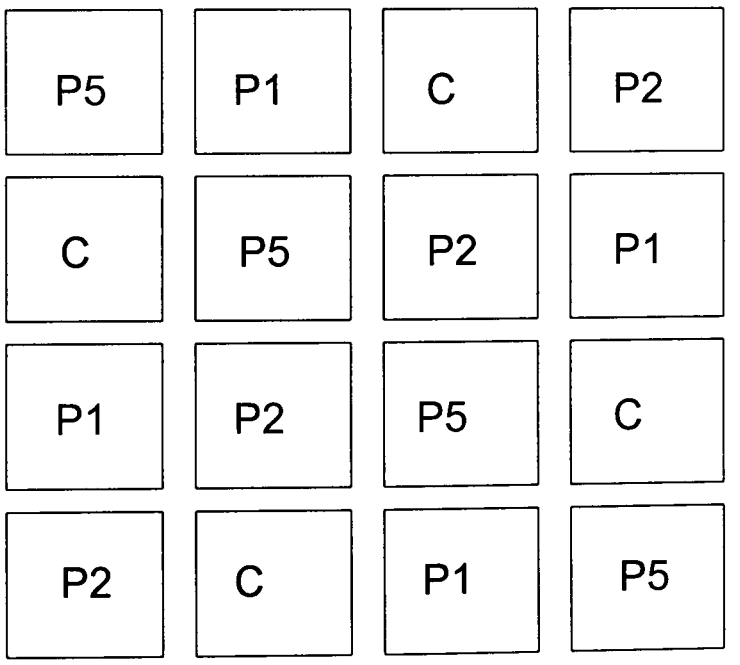

Figure 6.1 4x4 neighbour balanced Latin square design used in 1996. C= control, P1, P2, P5= 1, 2 or 5 nepetalactone vials/plot (table 6.2).

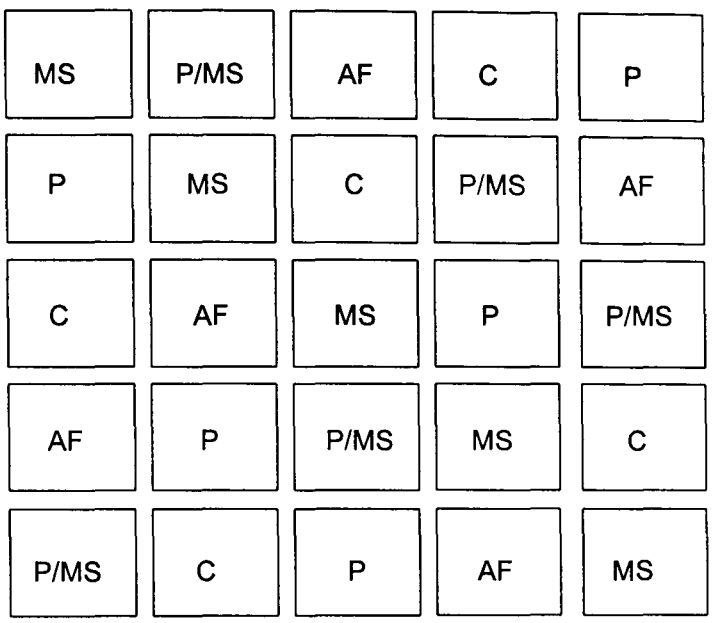

Figure 6.2 5x5 neighbour balanced Latin square design used in 1997.

C= control, P= nepetalactone, P/MS= nepetalactone + methyl salicylate, MS= methyl salicylate, AF= antifeedant (polygodial).

6.2.4 Assessment of Aphid Numbers and Parasitisation Levels

The numbers of live and parasitised aphids present in experimental plots was sampled at weekly intervals during the course of the field trials. Weekly counts were made between 1 June and 30 July in 1996, and between 15 May and 22 July in 1997. Aphids were assessed by counting adult and larval aphids, and noting the species. Parasitisation was measured by counting aphid mummies, and noting whether the mummy was of *Praon* or *Aphidius* according to the shape of the mummy. No aphids or mummies were removed from the plots during sampling.

Experimental plots were sampled according to a predetermined protocol which differed slightly between the two trials. In both 1996 and 1997, plots were divided into two diagonal transects which bisected the centre of the plot. Sampling was carried out at regular points along each transect. In 1996, there were 8 sampling

points per transect (figure 6.3), 4 on each side of the centre, giving a total of 16 sampling points per plot. At each sampling point, 6 tillers of wheat were chosen at random, and the numbers of aphids and aphid mummies present on each tiller recorded. In 1997, there were 6 sampling points per transect (figure 6.4), 3 on either side of the centre, giving a total of 12 sampling points per plot. At each sampling point, 5 tillers of wheat were chosen at random. The sampling protocol allowed for the assessment of aphid and mummy numbers at varying distances from the nepetalactone lure in the centre of the plot (and in 1997 around the methyl salicylate lures).

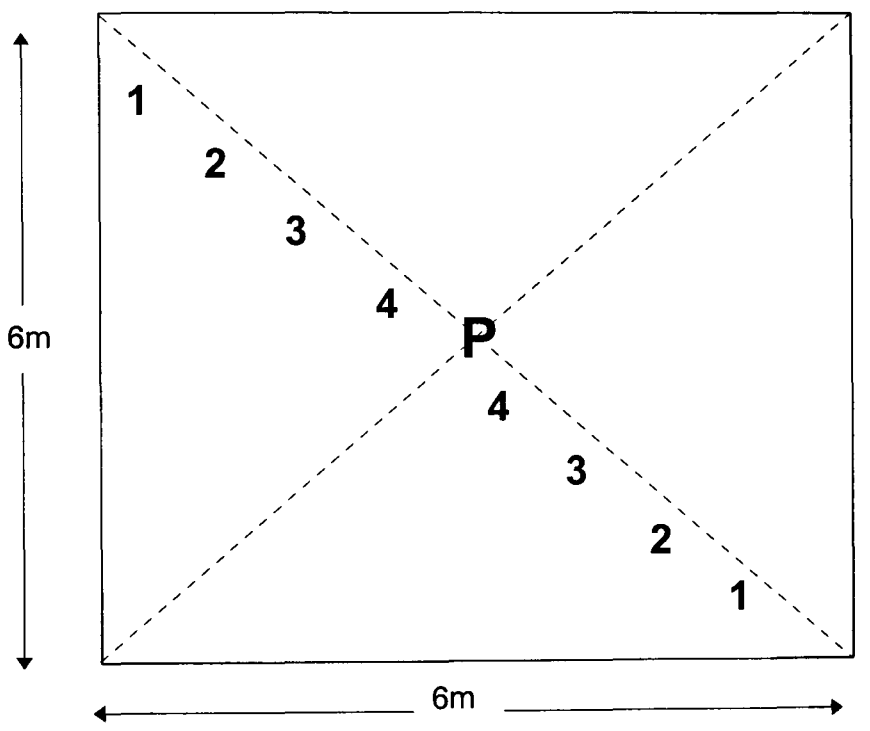

Figure 6.3 Layout of experimental plot and sampling protocol in 1996 trial.
Six wheat tillers were sampled at each of points 1-4 along both diagonal transects.
P= position of nepetalactone lures.

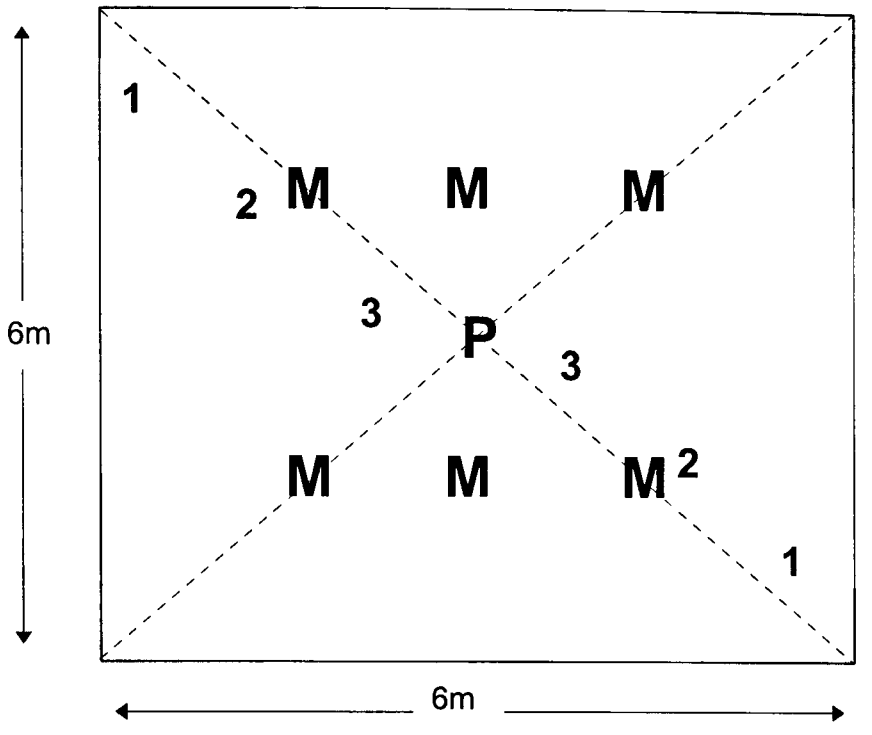

Figure 6.4 Layout of experimental plot and sampling protocol in 1997 trial.
Five wheat tillers were sampled at each of points 1-3 along both diagonal transects. P= position of nepetalactone lures, M= position of methyl salicylate lures.

6.2.5 Statistical Analysis

Numbers of aphids/tiller and aphid mummies/plot from both trials were analysed by ANOVA in GENSTAT. Prior to analysis, data were subjected to the transformation $x = \log_{(y + 0.5)}$, where x is the transformed data value and y is the untransformed data value.

The raw data for total numbers of aphids and mummies recorded from the plots in the 1996 and 1997 field trials are shown in the Appendix in tables A28-A31.

6.3 Results

6.3.1 1996 Field Trial

6.3.1a Results of ANOVA

Numbers of aphids and mummies recorded in the experimental plots were sufficient for statistical analysis on four sampling occasions between 10 July and 30 July. Tables 6.3 and 6.4 show the results of ANOVA on aphid and mummy numbers for the comparisons between the three levels of pheromone treatment, P1 v P2 v P5, and between the mean value for the pheromone treatments, P_{mean}, and the control treatment, C.

Table 6.3 Results of ANOVA on the number of aphids/tiller for comparisons between three levels of pheromone treatment (P1 v P2 v P5) (table 6.2), and between the mean for the pheromone treatments and the control, (P_{mean} v C) in the 1996 field trial.

Sample Date	Comparison	S.E.D.	F	P
10 July	P1 v P2 v P5	0.058	0.11	0.894
	P_{mean} v C	0.047	0.02	0.888
16 July	P1 v P2 v P5	0.017	0.03	0.968
	P_{mean} v C	0.014	16.3	0.007**
24 July	P1 v P2 v P5	0.017	0.54	0.607
	P_{mean} v C	0.014	0.17	0.694
30 July	P1 v P2 v P5	0.089	0.30	0.752
	P_{mean} v C	0.073	0.00	0.948

Table 6.4 Results of ANOVA on the number of mummies/plot for comparisons between three levels of pheromone treatment (P1 v P2 v P5) (table 6.2), and between the mean for the pheromone treatments and the control, (P_{mean} v C) in the 1996 field trial.

Sample Date	Comparison	S.E.D	F	P
10 July	P1 v P2 v P5	1.691	1.61	0.275
	P_{mean} v C	1.389	3.96	0.094
16 July	P1 v P2 v P5	1.204	1.23	0.357
	P_{mean} v C	0.997	8.26	0.028*
24 July	P1 v P2 v P5	1.873	0.05	0.954
	P_{mean} v C	1.539	7.17	0.037*
30 July	P1 v P2 v P5	2.019	0.13	0.877
	P_{mean} v C	1.642	2.17	0.191

There were no significant differences between the three pheromone treatments P1, P2 and P5 in terms of the number of aphids or mummies at any sample date. Therefore, all further comparisons between pheromone-treated and control plots are based on the means for the pheromone-treated plots, P_{mean}. The number of aphids/tiller was significantly higher in control plots than in pheromone plots on one occasion, 16 July ($P< 0.01$). The number of mummies/plot was significantly higher in pheromone plots than in control plots on two occasions, 16 July and 24 July ($P< 0.05$).

6.3.1b Percentage Parasitisation

Levels of parasitisation in pheromone and control plots in terms of percentage parasitisation is shown in figure 6.5. Percentage parasitisation overall was relatively low, but was generally higher in pheromone plots than in control plots.

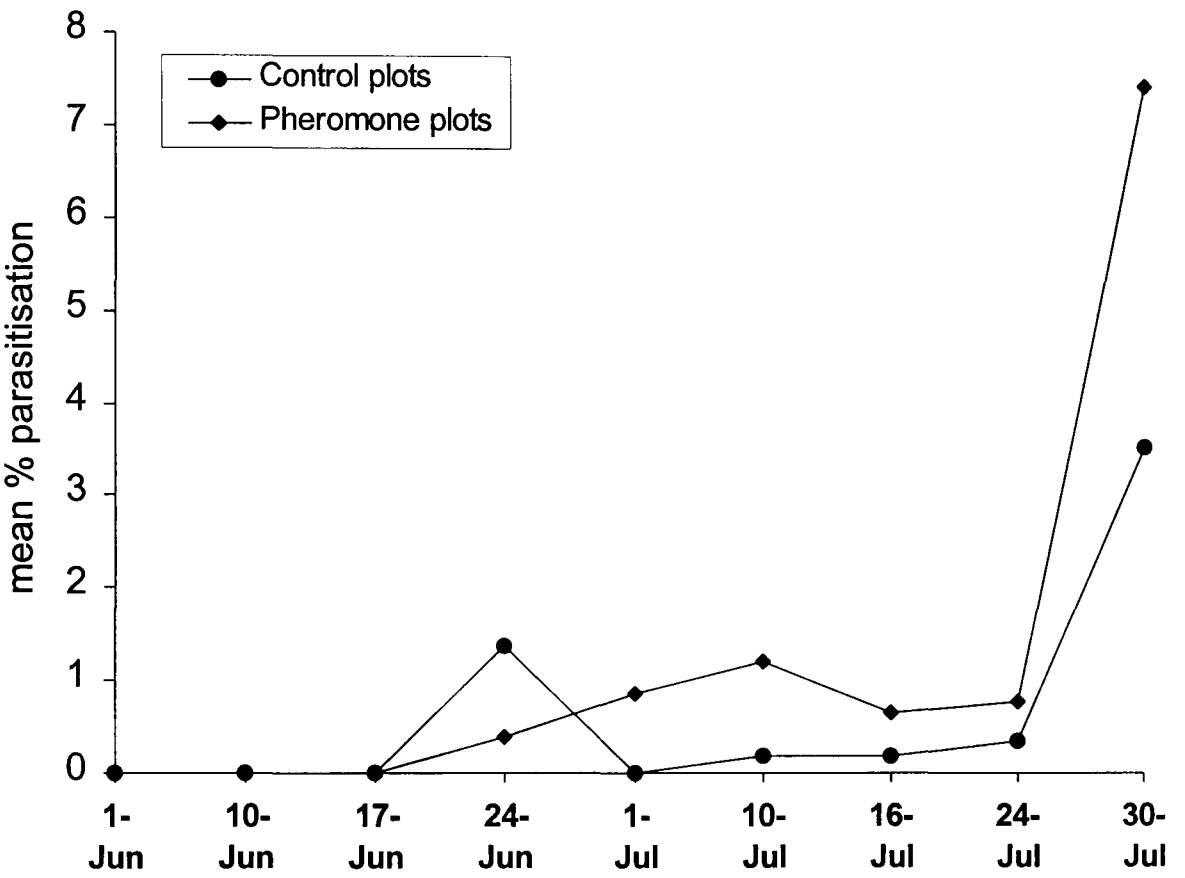

Figure 6.5 Percentage parasitisation of aphids in pheromone-treated and control winter wheat plots in 1996 field trial.

6.3.1c Aphid Populations

The development of aphid populations during the field trial is shown in figure 6.6. Aphid populations (mean no. aphids/tiller) were similar in pheromone-baited and control plots, except for 16 July when they were significantly higher in control plots (table 6.3). Aphids recorded during the trial were almost exclusively *Sitobion avenae*, with very low numbers of *Metopolophium dirhodum*.

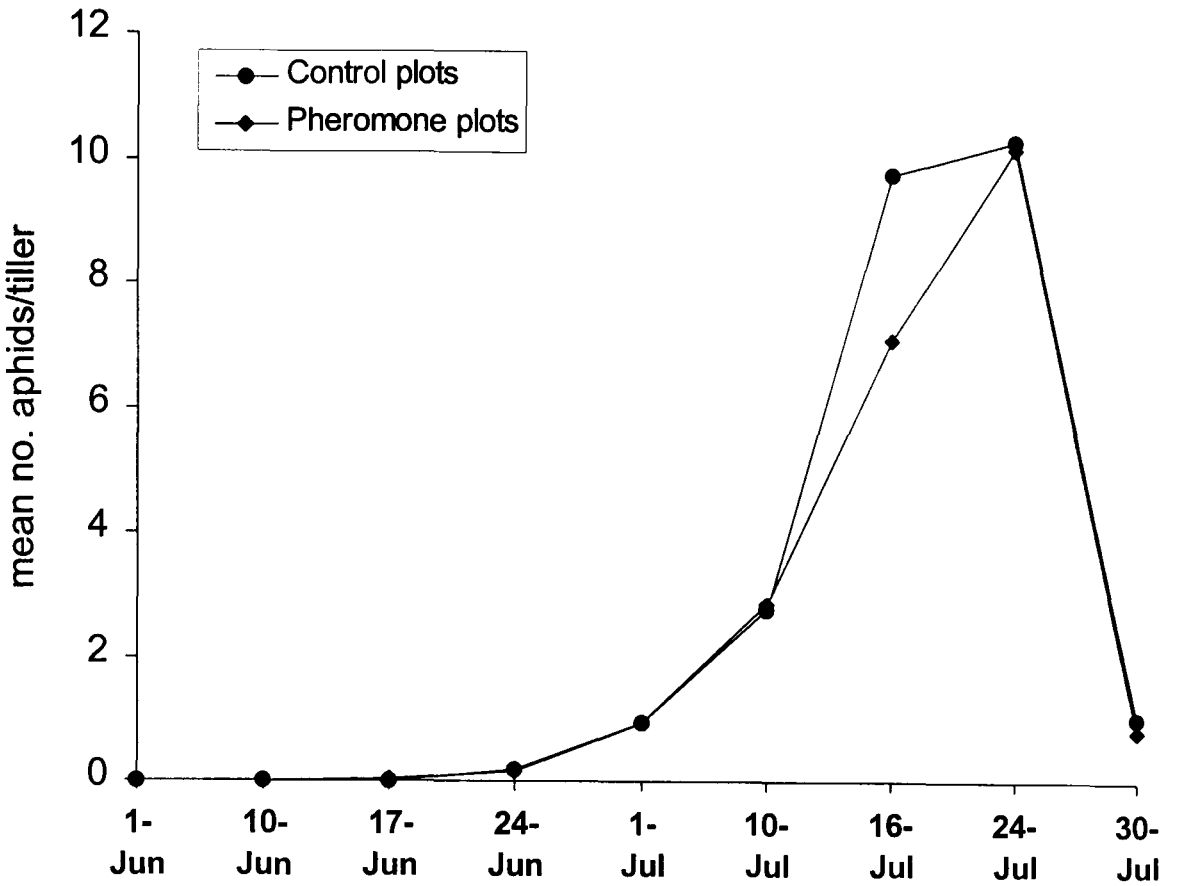

Figure 6.6 Aphid populations (mean no. aphids/tiller) in pheromone-baited and control winter wheat plots in 1996 field trial.

6.3.1d Synchrony of Aphids and Parasitoids

Since the critical period for aphid parasitoid activity is early in the season, during the initial aphid population increase, it is valuable to compare parasitoid activity with changes in the aphid population. Figure 6.7 shows the number of mummies recorded in pheromone and control plots, along with the aphid population (recorded from control plots). The number of mummies in pheromone-treated plots was generally higher than in control plots, and was significantly higher on 16 July and 24 July (table 6.4). The number of mummies increased earlier in pheromone-baited plots than in control plots, and was more closely synchronised with the increase in aphid populations.

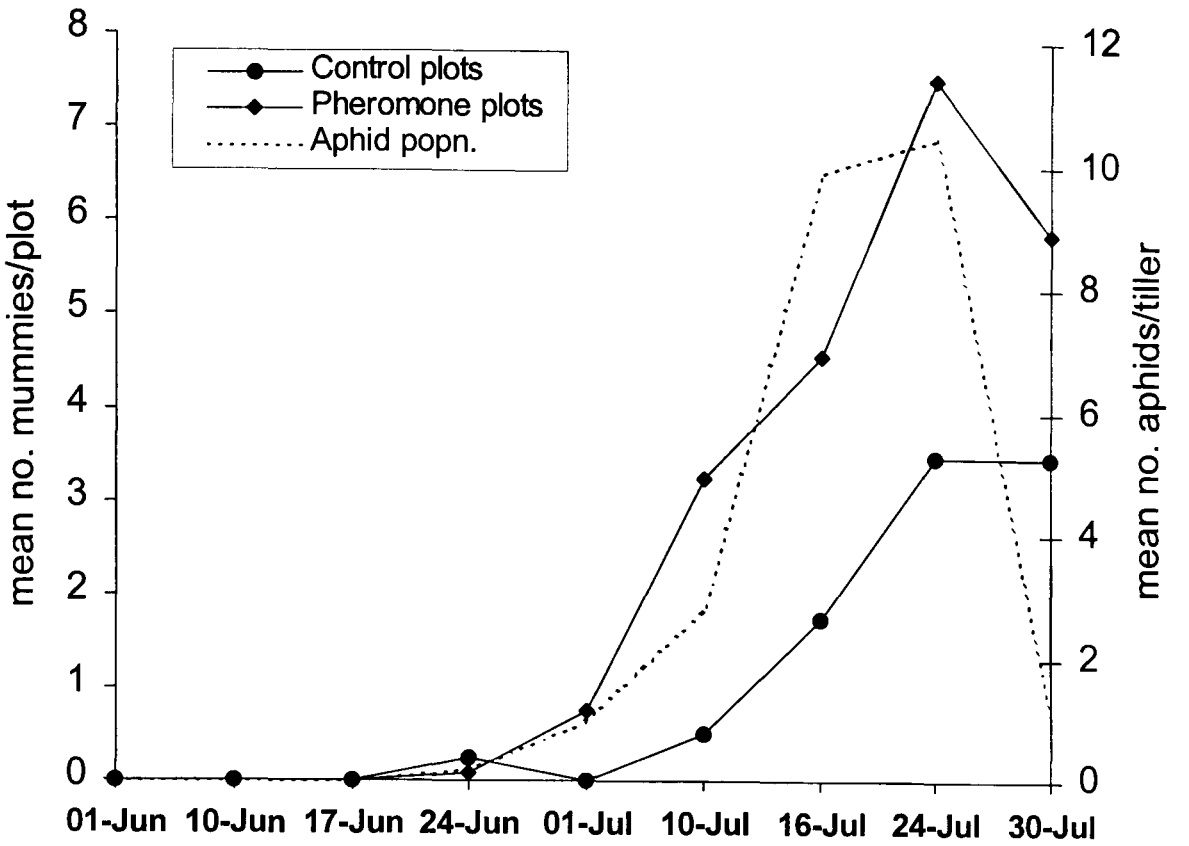

Figure 6.7 Numbers of mummies in pheromone-baited and control winter wheat plots, and the aphid population in 1996 field trial.

6.3.1e Position of Mummies Within Plots

Mummies were recorded at all sampling positions within both pheromone and control plots. Logistic regression analysis showed that there were no significant differences in the distribution of mummies between the sampling positions in either pheromone or control plots ($P > 0.05$). The mean number of mummies recorded at each sampling position per sampling occasion is shown in figure 6.8. In pheromone plots, the number of mummies recorded at the inner positions (3 and 4) was higher than at the outer positions (1 and 2) (see figure 6.3), although the difference was not significant. Numbers of mummies at the outer positions, a distance of 2-3m from the pheromone lure, were higher than in the equivalent positions in control plots, but the difference between pheromone and control plots grew larger towards the centre positions, nearer the lure.

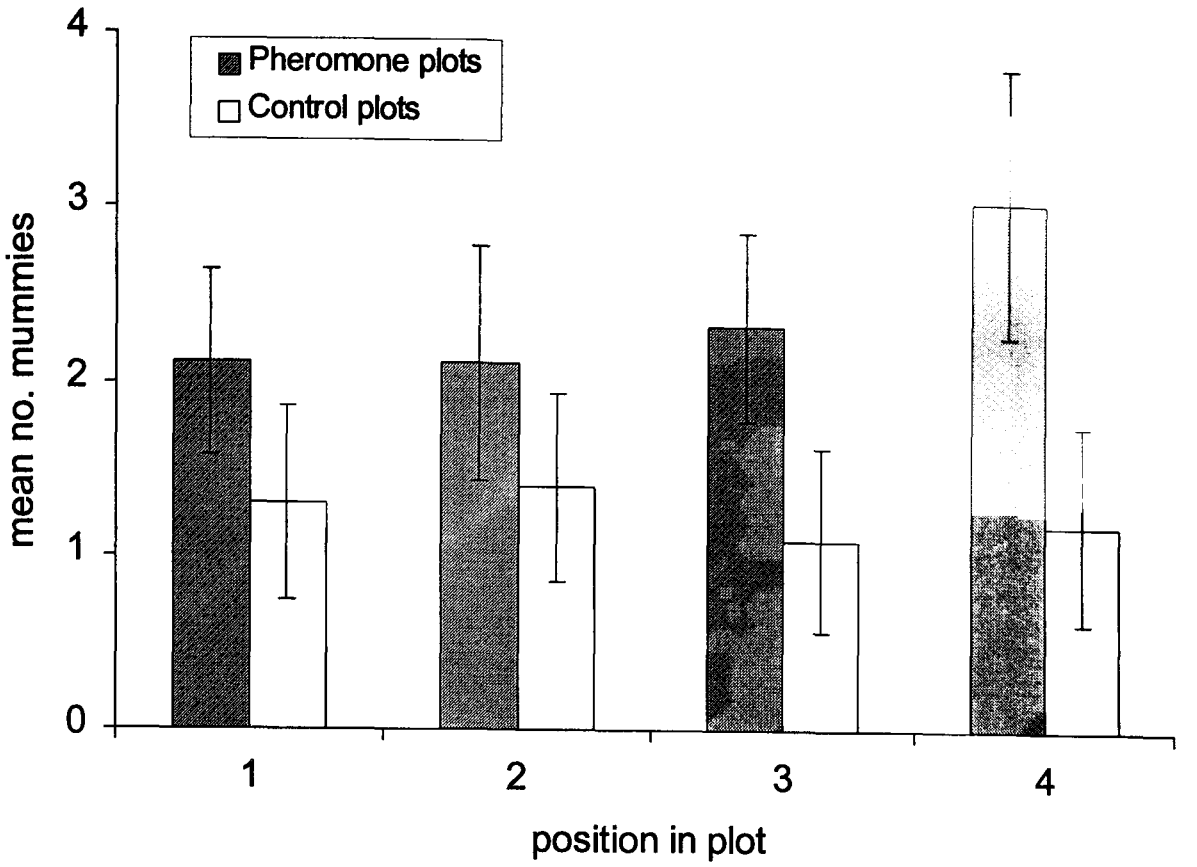

Figure 6.8 Mean number of mummies/sample occasion (± standard error) at different positions within pheromone and control winter wheat plots in 1996 field trial. Positions are in order 1-4 from the outer edge to the centre of the plot (next to the lure) (See figure 6.3).

6.3.1f Species of Mummies

Both *Praon* and *Aphidius* mummies were recorded in the experimental plots. Mummies were not removed from the plots and hence emerging wasps were not identified to species. However, a collection of mummies made from another area of the same field revealed that *Praon* mummies were mostly *P. volucre* and *Aphidius* mummies were exclusively *A. rhopalosiphi*. The total number of mummies of each genus recorded from pheromone and control plots is shown in figure 6.9. Parasitisation in the experimental plots was mainly due to *Aphidius*, with low numbers of *Praon* mummies recorded.

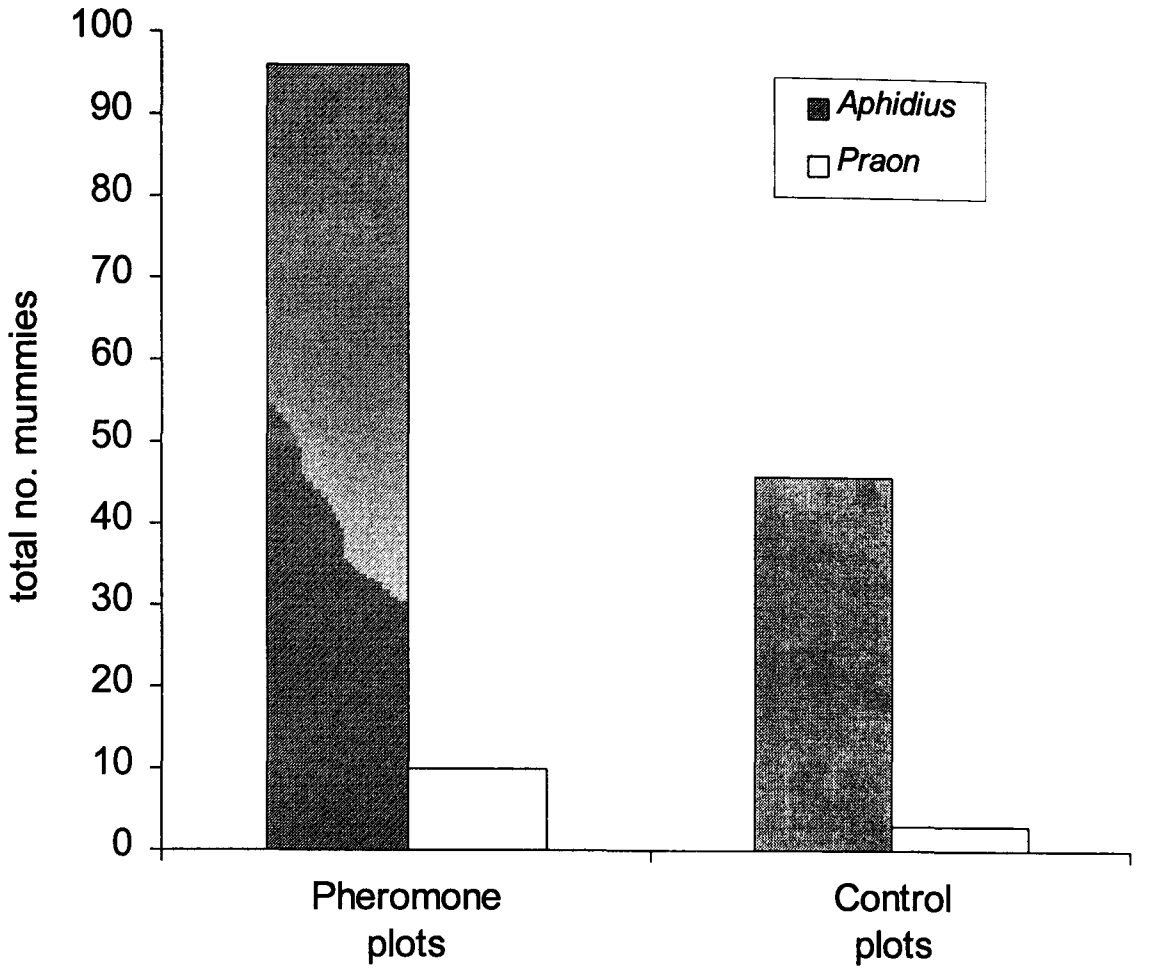

Figure 6.9 Total numbers of *Praon* and *Aphidius* mummies recorded from pheromone and control winter wheat plots in 1996 field trial.

6.3.1g Crop Yield

The grain yields in tonnes/hectare from the wheat plots are shown in table 6.5. There was no significant difference between the yields from the plots (ANOVA; $F= 0.59$, $P= 0.644$)

Table 6.5 Grain yields from winter wheat plots treated with 1, 2 or 5 pheromone vials and from control plots in 1996 field trial.

Treatment	Yield (tonnes/hectare)
P1	8.17
P2	8.02
P5	8.04
C	8.34

6.3.2 1997 Field Trial

6.3.2a Results of ANOVA

Numbers of aphids were sufficient for statistical analysis on six occasions between 17 June and 22 July. Numbers of mummies were analysed on five occasions between 1 July and 22 July. Tables 6.6 and 6.7 show the results of ANOVA for the comparison between control plots, C, and plots baited with pheromone, P, and pheromone + methyl salicylate, P/MS.

There were no significant differences between any of the treatments at any sample date in terms of number of aphids/tiller or number of mummies/plot.

Table 6.6 Results of ANOVA on the number of aphids/tiller for comparisons between treatments P, P/MS and C (table 6.2) in the 1997 field trial.

Sample Date	Comparison	S.E.D.	F	P
17 June	P v P/MS v C	0.329	0.23	0.917
24 June	P v P/MS v C	0.199	1.86	0.182
1 July	P v P/MS v C	0.337	0.64	0.642
8 July	P v P/MS v C	0.396	1.44	0.279
15 July	P v P/MS v C	0.245	0.63	0.652
22 July	P v P/MS v C	0.207	0.90	0.492

Table 6.7 Results of ANOVA on the number of mummies/plot for comparisons between treatments P, P/MS and C (table 6.2) in the 1997 field trial.

Sample Date	Comparison	S.E.D.	F	P
1 July	P v P/MS v C	0.069	1.53	0.256
8 July	P v P/MS v C	0.108	0.59	0.680
15 July	P v P/MS v C	0.091	0.81	0.542
22 July	P v P/MS v C	0.114	0.11	0.977

6.3.2b Percentage Parasitisation

The percentage parasitisation recorded in the plots is shown in figure 6.10. The overall level of parasitisation was low, and there were no discernible trends in parasitisation levels between the different treatments.

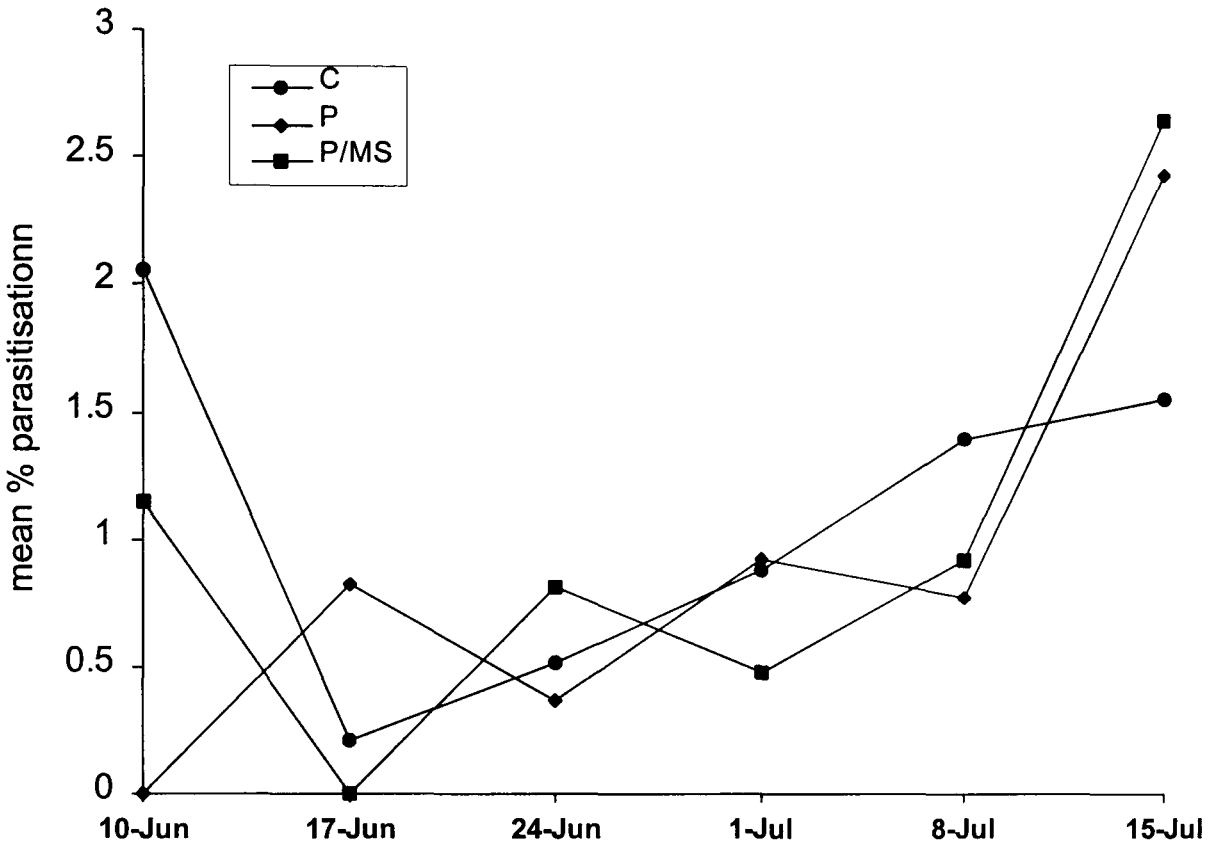

Figure 6.10 Percentage parasitisation in winter wheat plots treated with semiochemicals in 1997 field trial. C= control plots, P= pheromone baited plots, P/MS= pheromone + methyl salicylate baited plots.

6.2.3c Aphid Populations

The development of aphid populations in plots during the field trial is shown in figure 6.11. There were no significant differences between plots in aphid populations (mean no. aphids/tiller) (table 6.6). Aphids were mainly *Sitobion avenae* and *Metopolophium dirhodum,* although low numbers of *Rhopalosiphum padi* were also recorded.

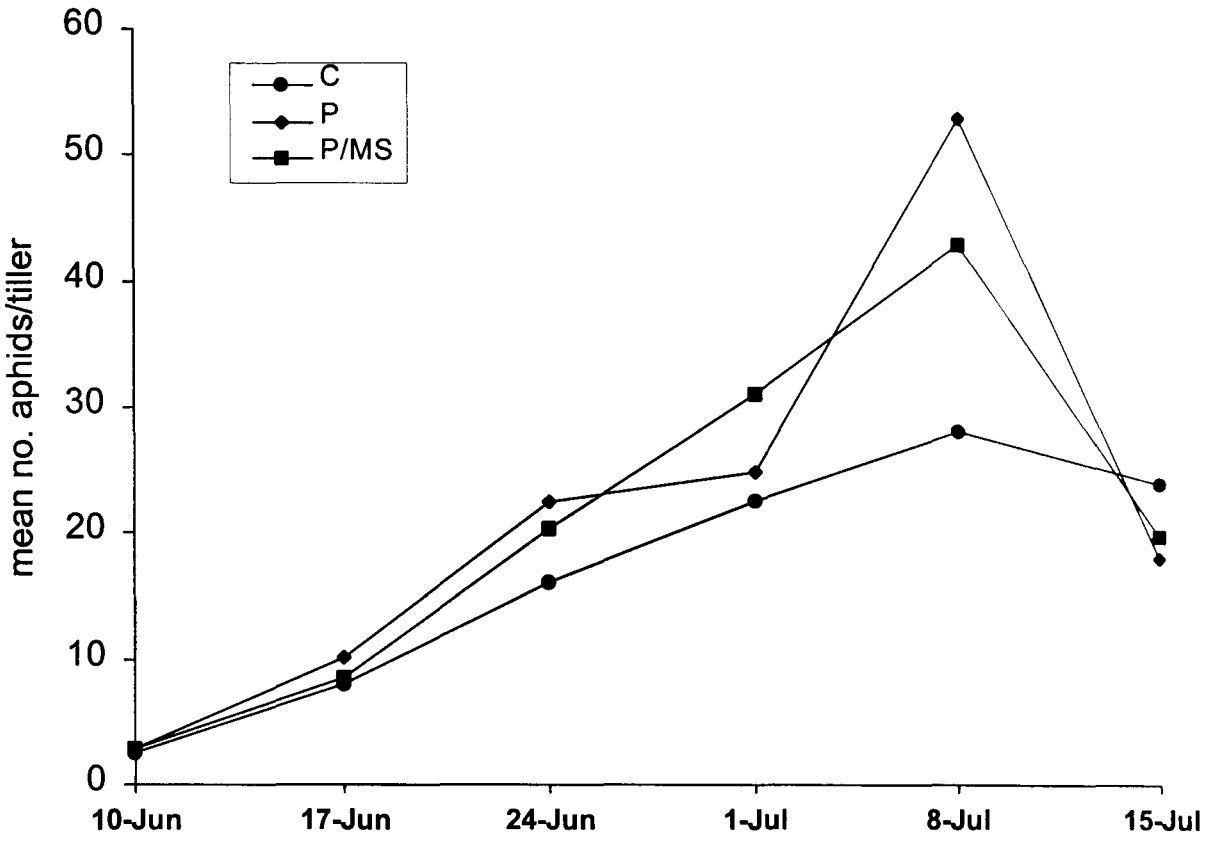

Figure 6.11 Aphid populations (mean no. aphids/tiller) in winter wheat plots treated with semiochemicals in 1997 field trial. C= control plots, P= pheromone-baited plots, P/MS= pheromone + methyl salicylate baited plots.

6.2.3d Position of Mummies in Plots

The number of mummies recorded from each sampling position per sampling occasion is shown in figure 6.12. Numbers of mummies were variable and were not analysed statistically. In the control and pheromone + methyl salicylate baited plots, the number of mummies appeared to decrease from the outer edge of the plot (position 1) towards the centre (position 3), whereas in pheromone-baited plots, the number of mummies was as high in the centre as at the edge of the plots.

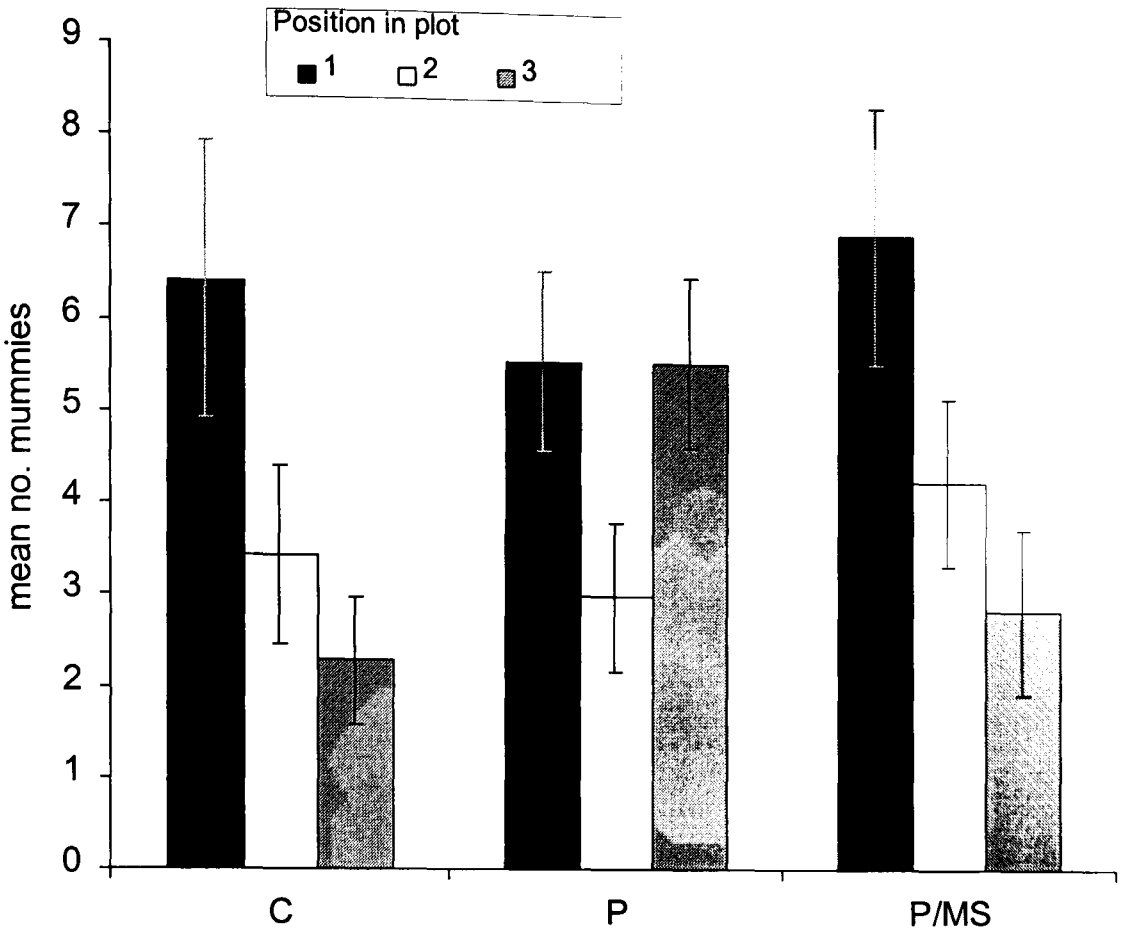

Figure 6.12 Mean number of mummies/sampling occasion (± standard error) at different positions within winter wheat plots in 1997 field trial. Positions are in order 1-3 from the outer edge of the plot to the centre (see figure 6.4). C= control plots, P= pheromone-baited plots, P/MS= pheromone + methyl salicylate baited plots.

6.2.3e Species of Mummies

Both *Praon* and *Aphidius* mummies were recorded in the experimental plots. A collection of mummies made from another area of the same field revealed that *Praon* mummies were mostly *P. volucre* and *Aphidius* mummies were exclusively *A. rhopalosiphi*. The total number of mummies of each genus recorded from pheromone and control plots is shown in figure 6.13. Parasitisation in the experimental plots was mainly due to *Aphidius*, with low numbers of *Praon* mummies recorded

6.2.3f Crop Yield

The grain yields in tonnes/hectare from the wheat plots are shown in table 6.8. There was no significant difference between the yields from the plots (ANOVA; F= 1.09, P= 0.403).

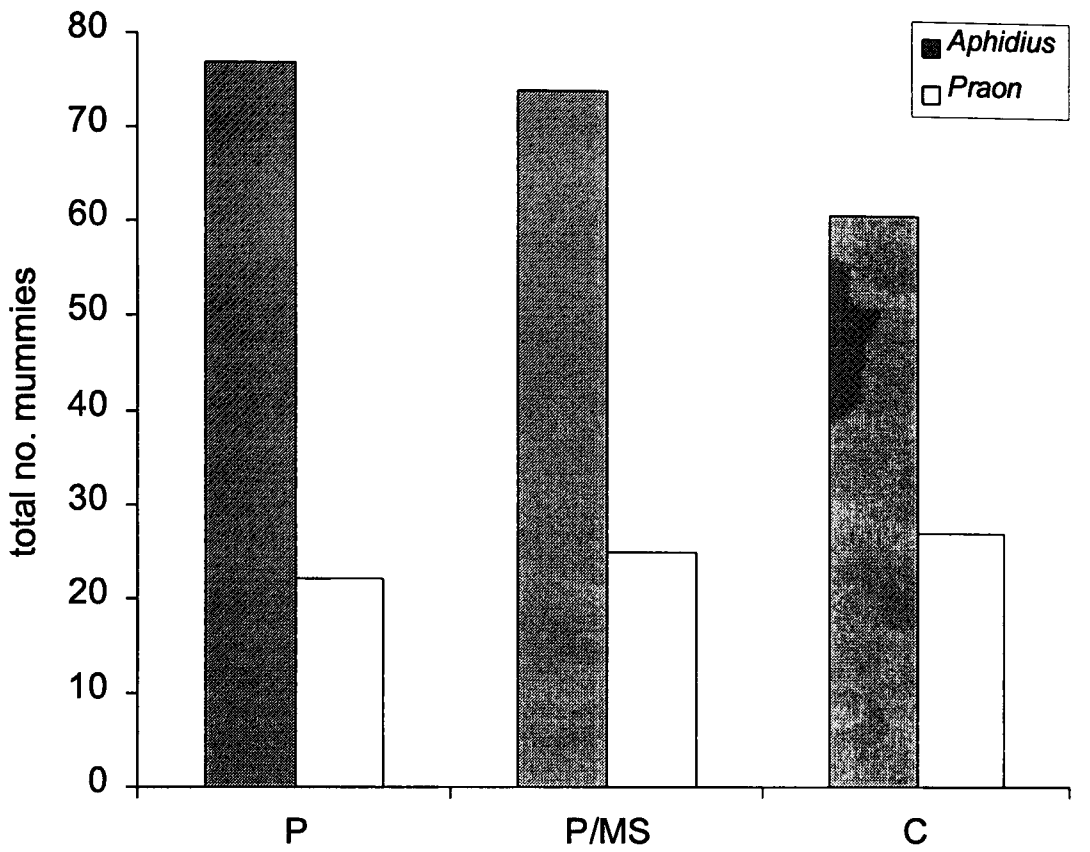

Figure 6.13 Total numbers of *Praon* and *Aphidius* mummies recorded from winter wheat plots treated with semiochemicals in 1997 field trial. P= pheromone-baited plots, P/MS= pheromone + methyl salicylate baited plots, C= control plots.

Table 6.8 Grain yields from winter wheat plots treated with pheromone, P, pheromone + methyl salicylate, P/MS and from control plots, C, in 1997 field trial

Treatment	Yield (tonnes/hectare)
P	8.35
P/MS	8.37
C	8.23

6.4 Discussion

6.4.1 1996 Field Trial

Aphid sex pheromone lures appeared to increase parasitisation levels in the wheat plots, although the number of mummies recorded was significantly higher statistically on only two sampling occasions. More encouraging was the closer synchrony between the increase in aphid and mummy numbers in the pheromone plots compared to the control plots. Parasitoid activity during the early phase of aphid population build-up is critical for parasitoids to fulfil their potential as aphid control agents (Carter *et al.*, 1980; Powell, 1983; Fougeroux *et al.*, 1988; Wratten and Powell, 1991). Of the organisms that comprise the aphid natural enemy complex, parasitoids have the greatest ability to achieve this early activity (Dean, 1974, Chambers *et al.*, 1986, Langer *et al.*, 1987), but frequently fail to do so under field conditions. Aphid sex pheromone therefore appeared to promote synchrony, especially during the important initial period of aphid population increase.

Despite the positive effect of pheromone on parasitisation levels, there were no major differences between aphid populations in control and pheromone plots. The aphid population was significantly higher in control plots on one sample occasion, but subsequently returned to a level equivalent to that in the pheromone plots. Although significant increases in parasitisation were demonstrated, overall levels were low, peaking at only 8% in pheromone plots. Fougeroux *et al.* (1988) found that fields with 20% parasitisation early in the season had subsequent aphid populations which remained below economic thresholds. It is likely that comparable levels of parasitisation would be necessary to significantly affect aphid populations. In 1996, cold, wet weather in June resulted in the late arrival of aphids in the crop. Parasitoids may have reached the crop earlier, before lures were deployed, and then dispersed in response to the absence of hosts. By the time aphid populations began to build up in the crop, ear development on the wheat was at an advanced stage and *Sitobion avenae*, which prefers to feed on the ear, was mainly confined to this part of the plant. Laboratory experiments have

shown that *S. avenae* suffers significantly less parasitisation by *A. rhopalosiphi* when on the ear (Gardner and Dixon, 1985), possibly gaining protection there. Therefore, the low levels of parasitisation may have resulted from a combination of climatic conditions and parasitoid behaviour.

Although parasitisation in pheromone plots remained higher than in control plots throughout the trial, the difference did not increase greatly after the initial early divergence. This is consistent with the theory that aphid parasitoids may have a hierarchy of cues to which they respond. At low host density, parasitoids may respond strongly to the aphid sex pheromone, whereas once aphid populations have increased, the aphid-induced volatiles from the crop may become more important as foraging cues since they are highly reliable indicators of host presence.

The distribution of mummies within the plot, according to the distance from the lure, shows that, although there is a trend towards increased parasitisation near the lure in pheromone plots, parasitisation is by no means concentrated around the lure. Parasitoids orienting to the pheromone may have landed in the plot in response to volatiles from the plant-host complex, rather than completing their flight to the lure. The results of potted plant experiments (section 5.2) suggest that lures may influence parasitisation by *Aphidius rhopalosiphi* over a distance of at least 1m. Laboratory cage experiments (section 3.5) have shown that *A. rhopalosiphi* may be retained for longer periods in pheromone-baited areas, which would add to the effects of increased attraction. It is also possible that the pheromone retained parasitoids emerging from mummies inside baited plots, although this remains to be investigated.

The relatively low number of *Praon* mummies recorded in the trial is consistent with the fact that *Praon* is a generalist parasitoid associated with a range of habitats, not only with cereal crops (Vorley, 1986), whereas *A. rhopalosiphi* is usually the dominant parasitoid in cereals (Powell, 1982; Vickerman, 1982; Wratten and Powell, 1991). However, it was disappointing that the sex

pheromone did not attract *Praon* from outside of the crop, as had previously been achieved with pheromone traps deployed in winter wheat in the autumn (Hardie *et al.*, 1994). The relative level of *Praon* parasitisation may be underestimated by counting mummies since parasitised aphids often leave the plant (Höller, 1991), and those parasitised by *Praon* seem relatively more inclined to do so (personal observation; a large proportion of *Praon* mummies in laboratory cultures form on the sides of cages and plant pots, and many of the *Praon* mummies recovered from the potted plant experiments in Chapter 5 were found on the sides of the plant pots.).

6.4.2 1997 Field Trial

The results of the 1997 trial were not as encouraging as those obtained in 1996. The aphid sex pheromone had no effect on parasitisation levels or aphid populations. The aphid population in the pheromone and pheromone + methyl salicylate plots was higher than in control plots on one or two sampling occasions, but the difference was not statistically significant and may have resulted from a localised aphid population explosion. There is no published evidence that female aphids are attracted to aphid sex pheromones. Parasitisation levels in 1997 were even lower than in 1996, so even if the pheromone had significantly increased parasitisation, it is unlikely to have greatly affected aphid populations.

Methyl salicylate has previously been effective in reducing colonisation and subsequent populations of cereal aphids in field plots (Pettersson *et al.*, 1994). It is thought to be effective against *Rhopalosiphum padi* since it is a component of the aphid's winter host plant, *Prunus padus,* and against *Sitobion avenae* since it may also play a general role in plant defence. It was included alongside the sex pheromone in the hope that parasitoids may have had a more dramatic effect on the consequently lower initial aphid populations. However, analysis of initial aphid populations, and of aphid numbers around methyl salicylate lures (not presented) showed that methyl salicylate was ineffective in this trial. Despite this failure, it was encouraging to note that parasitisation levels were not lower either in methyl salicylate-baited plots or around methyl salicylate lures, indicating that

methyl salicylate may be used in the future with no deleterious effects on aphid parasitoids.

6.4.3 Conclusions and Future Directions

Although the 1996 field trial suggested that aphid sex pheromone may be effective in manipulating parasitoid behaviour over a large scale, the overall conclusion from both trials must be that the effect was probably marginal and that further work must be done in situations with greater insect numbers in order to advance this approach. Both trials suffered from low parasitoid numbers, and although significantly higher parasitisation was demonstrated, the level of parasitisation would have to be considerably higher before impacts on aphid populations are seen. However, the use of pheromone in the crop itself is not the major emphasis of the parasitoid manipulation strategy. The initial goal is to establish overwintering populations of parasitoids in field margins. If large numbers of parasitoids can be concentrated alongside the crop in the autumn, there may be a greater abundance the following spring which can then be manipulated within the crop. Further basic information about the distances over which lures are effective, and how parasitoids use the sex pheromone will be valuable when designing future experiments.

Previous experiments of this type have not always proved successful. Jennings and Jones (1986) found that the application of moth scale extracts to trees failed to increase parasitisation by *Trichogramma* species because the pattern of kairomone application interfered with the parasitoid's host searching behaviour. A similar problem was encountered by Chiri and Legner (1983) who found that foliar coverage of *Spodoptera* scale extract failed to increase parasitisation by *Chelonus* species, possibly due to habituation, distraction and dispersal by the parasitoid. Lewis *et al.* (1979) found that blanket application of *Heliothis zea* scale extract actually reduced parasitisation by *Trichogramma pretiosum*, because the parasitoid was prevented from concentrating its search in host-containing areas. Therefore, a degree of care in the application of pheromones in the field should be exercised.

The field-plot experiment discussed in this chapter could be tested in other crop types, since responses to aphid sex pheromones have been identified in a range of parasitoids (Chapter 2). It may emerge that this strategy is better suited to small scale, high value horticultural crops, rather than vast agroecosystems where distances are greater and aphids may not always pose a significant threat.

Before embarking on the development of this kind of strategy, the wider implications of manipulating parasitoid populations should be considered. If lures are deployed before aphids arrive in the crop, then attracted parasitoids may disperse following habituation to the stimulus. Laboratory experiments have already suggested that habituation to aphid sex pheromone may occur in *Aphidius ervi* (section 3.2). It is likely that, since it is innate, the parasitoid response is influenced by genotype (Poppy *et al.*, 1997). Consequently, attracting large numbers of parasitoids to the pheromone may inadvertently select for parasitoids which have a strong response, and this could lead to a change in the genetic variability of the population. This would have more profound implications if the alleles governing pheromone response were found to be linked to those governing other characteristics, and could be particularly serious if attracted parasitoids find no hosts present, and suffer a selective disadvantage as a result. The distance over which lures attract parasitoids has not been firmly established, so the possibility remains that parasitoid populations may be depleted in certain areas, and concentrating large numbers of parasitoids in certain areas carries the risk that they may be exterminated by a chance event. Lastly parasitoids may be induced to alter their host preferences by being moved between different habitats. However, with further behavioural and field experiments, and with current advances in genetic techniques, all of these potential problems can be addressed.

Chapter 7

General Discussion

McNeil (1992), discussing the use of semiochemicals in insect management programmes, writes

'... a solid understanding of the behavioural ecology underlying the pheromone-mediated communication systems we wish to modify could greatly improve the degree of success'

The aim of this project was to study the behavioural ecology of the parasitoid response to aphid sex pheromones in order to advance the prospects of developing a sustainable aphid control strategy. Basic information was sought from laboratory experiments, and some aspects of the strategy were tested in the field. In a field trial, the first attempt to manipulate aphid parasitoids using sex pheromone lures was made.

The exploitation of natural enemies within integrated pest management (IPM) programmes is becoming an increasingly desirable prospect because intensive pesticide use is leading to the widespread development of resistance, especially in aphids (Devonshire, 1989). With the acceptance that insecticides can damage natural enemy populations (Jepson, 1989) and growing public concern over the real and perceived dangers of pesticides, the demand for alternative control methods may now be increasing. Manipulating the resident parasitoid fauna using semiochemicals may have advantages over traditional approaches to biological control. Mass release of aphid parasitoids would be expensive and would need to overcome the problems of parasitoid host preference and long-term laboratory rearing, and the classical introduction of novel parasitoid species is inappropriate in European agricultural systems. Also, when parasitoids are artificially released into crop habitats, they often disperse, rather than attacking the pest population.

The emphasis of biological control may now be focusing more closely on the potential of conserving and manipulating parasitoids. In a recent article, Lewis (in press) writes

'...we need to understand, promote and maximise the effectiveness of indigenous natural enemies.'

and the same author advises that '...these approaches must be based on a thorough knowledge of the ecosystem.'.

The work in Chapter 2 demonstrated that a number of aphid parasitoids, which have different host exploitation strategies, all possess an innate behavioural response to aphid sex pheromones. This suggests that the response may be a general phenomenon among the Aphidiinae. Responses were identified in both generalist and specialist parasitoids, but the hypothesis that specialists respond optimally to the exact pheromone blend produced by their host aphid has yet to be substantiated. There are over 400 species of Aphidiinae worldwide (Starý, 1988b), so there is considerable scope for investigating the extent of the response. It will be particularly interesting to relate the response of different parasitoid species to their ecology, especially in ecosystems in warmer regions, where aphids do not undergo a sexual generation. In an experiment in which parasitoids were reared on two different hosts (Chapter 2), there was evidence that long-term laboratory rearing may affect the specificity of the response to aphid sex pheromone components. Since the response is innate (Poppy *et al.*, 1997), it is likely to be influenced by genotype, and this is another area of research which merits investigation. The elucidation of the genetics that underpin the behavioural response would be valuable for work with aphid parasitoids, and may have general implications for the rearing of parasitoids for biological control.

Thus there is potential to use aphid sex pheromones in a variety of crop systems. However, one intriguing question has yet to be answered. There is no published evidence that parasitoids actually use aphid sex pheromones to locate sexual aphid

colonies in the field. Although this seems the most obvious reason why parasitoids now show the response, it may have originally evolved under conditions that were different from the current ones. Knowledge of the evolutionary development and significance of the response can only enhance our understanding of how to exploit it.

The overriding conclusion from the work in Chapter 3 is that the parasitoid response to aphid sex pheromones is innate, and this confirms previous findings. This means that the response should not be easily modified by learning (Vet and Dicke, 1992). The relative specialist *Aphidius ervi* showed no evidence of learning, and although there was an indication of associative learning with the generalist *Ephedrus plagiator*, it was ambiguous and will require further investigation. Experiments also showed that the presence of pheromone does not affect the host-attack behaviour of *A. ervi*. There are both advantages and disadvantages from these findings. On one hand there would seem to be little prospect of manipulating reared parasitoids artificially, to enhance or 'tune' their response prior to release. However, although this would be a bonus, it is not the premise on which the proposed aphid control strategy is based. In fact, the resistance to modification shown by the parasitoid response may be an advantage, as it means that we can be more confident in predicting the behaviour of parasitoids in the field, and that parasitoids may be less often side-tracked from the task we wish them to perform.

One particularly important finding from this section of work was the suggestion that *A. ervi* may habituate to the aphid sex pheromone, if it experiences it without a reward. Although the experiment was performed under artificial laboratory conditions, if the same response occurred in the field it would have serious consequences for the use of sex pheromone lures. In particular the timing of lure deployment relative to aphid arrival would become critical, which may reduce the ease of use of any strategy which is developed.

A particularly pleasing aspect of the project was that information was gathered from experiments in the field as well as the laboratory. The potted plant experiments in Chapter 5 confirmed that sex pheromone lures can increase parasitisation of nearby aphids, as previous work had suggested (Lilley *et al.*, 1994, D Brooks, personal communication). This is an important step from demonstrating responses in the laboratory towards manipulating parasitoids in the crop. The potted plant method was successfully employed to investigate the distance over which pheromone lures may be effective. The results were interesting, while by no means conclusive. However they did highlight that there can be differences in behaviour between aphid parasitoids, in this case *Praon volucre* and *Aphidius rhopalosiphi*. It is important to bear this in mind, since it is easy to assume that insects we consider to be closely related will always act in the same manner.

The difference between *P. volucre* and *A. rhopalosiphi* may stem from their differential use of the aphid sex pheromone and/or volatiles from the plant-host complex (PHC). At a more fundamental level, this may be due to their different host exploitation strategies, *A. rhopalosiphi* being a specialist on cereal aphids, and *P. volucre* a generalist. The differences between the foraging strategies of generalists and specialists, and how this affects their relative effectiveness as control agents, is an area that would reward further investigation, and may determine the approach taken toward their manipulation. The effectiveness of the potted plant method was re-affirmed by these experiments, and this technique could be used to survey the parasitoid fauna of different habitats (since monitoring traps have not always been effective). It could also be used to extend the work on pheromone-PHC interactions (Chapter 4) into the field.

The field trial (Chapter 6) was the first attempt to use aphid sex pheromone lures to manipulate parasitoids on a wider scale and, as such, the design and methodology may serve as a guide for future experiments of this type. Results from the 1996 trial were encouraging, particularly the indication that the pheromone enhanced the early season synchrony between aphid and parasitoid

populations. However, the failure to reproduce this effect in 1997 is a demonstration of the unpredictability of the ecosystems with which we must work, and of the importance of replicating such experiments in different years and conditions. This area will undoubtedly require further work before the prospects for exploiting the strategy can be fully assessed. The advantages, and potential concerns, regarding this approach are discussed in Chapter 6. However, it is worth emphasising that, if the adoption of IPM strategies are to be encouraged, the economic implications must be recognised. At present, it is relatively inexpensive for a farmer to treat a crop with a standard insecticide whereas, for the foreseeable future, there is likely to be an increased financial cost of implementing an IPM programme. However, with the strategy proposed here, although the initial costs (such as setting up field margins) may be high, once the strategy is established it may not carry a high financial premium. The strategy would probably be cheaper than traditional approaches to biological control, and the use of field margins has additional environmental benefits. The proposed strategy could be integrated with schemes involving field margins, buffer zones or conservation headlands which are already in existence. These considerations will have to be addressed, and the most appropriate target system for the strategy identified at a relatively early stage of its development.

The next stage, in terms of field work, should be the establishment of field margin habitats alongside the crop. In the autumn, the attraction of parasitoids to pheromone lures deployed in the margins should be measured, and the overwintering success of attracted parasitoids assessed. The success of this aspect of the strategy depends upon the provision of suitable host aphids and plants for attracted parasitoids. The ideal approach would be to encourage non-pest aphids in the margin habitats, providing alternative hosts for parasitoids and avoiding problems of pest or plant virus carry-over. However, because adult parasitoids may show a preference for the aphid species in which they developed (Pungerl, 1984; Powell and Wright, 1988), emerging parasitoids may not be efficient in attacking pest aphid populations in the adjacent crop. The presence of pest aphid species in the margins carries the risk of harbouring pests and plant viruses. In

terms of harbouring pests, the advantages of producing large numbers of parasitoids which will attack pest aphids may outweigh the disadvantage. The virus problem may be more serious, and this strategy may prove unsuitable for some crops if pest aphids are used in field margins.

The choice of pest or non pest aphids will depend in part on whether specialist or generalist aphid parasitoids are to be exploited. A generalist, such as *Praon*, may be more flexible in its host preferences but, in the field trials (Chapter 6), relatively low levels of *Praon* parasitisation was recorded compared to that by the specialist *Aphidius rhopalosiphi*.

The aphids which are supported in the field margins depends largely upon which plant species are present. This is another question which needs to be addressed. During this project, a field trial was set up to investigate feasibility of different field margin designs. The aim was to seed the plots with different aphid species and measure parasitoid attraction and overwintering, but due to limited availability of aphids, this was not possible. However, some useful information about plant suitability was generated. Plots which were seeded with the grasses *Dactylis glomerata* and *Poa annua* established successfully. *P. annua* is a short, tough grass which prevented the invasion of weeds. This design would support cereal aphids and their parasitoids. Plots seeded with red clover, *Trifolium repens*, and *P. annua* also established successfully with little weed invasion, and could be used alongside leguminous crops. The next step will be to establish field margins alongside the crop, and attempt to link the use of aphid sex pheromone in the autumn and spring.

Integrated pest management involves, by definition, a range of measures, not necessarily excluding the use of conventional pesticides. Since it is unlikely that a single semiochemical can alone bring about a significant reduction in pest numbers or plant damage, their future value lies in combined and integrated use (Pickett *et al.*, 1997). A great deal is known about the chemical ecology of aphids (Pickett *et al.*, 1992, 1997) so this approach has considerable potential. The use of

repellent, non-host plant volatiles (Issacs *et al.*, 1993; Pettersson *et al.*, 1994) and antifeedants (Griffiths *et al.*, 1989) has already been attempted with encouraging results. If numbers of colonising aphids can be reduced, then attracted parasitoids may be more successful in slowing the subsequent population build-up. The work on synthetic aphid sex pheromones merits further investigation. A synthetic compound which is more attractive than the plant extract would prove an obvious advantage. However, the use of synthetic enantiomers to 'switch-off' the parasitoid response (as suggested in the field experiment in Chapter 5) may also be a useful tool in the management of parasitoid populations.

Due to the level of understanding of the ecosystem which is required, this area of research is still at an early stage of development. However, information of the type presented in this thesis is vital to that development. When working with natural enemies such as parasitoids, it is important to understand their host location behaviour at different stages and in different situations, particularly in the field as well as the laboratory. An example from this thesis is the work with *Aphidius rhopalosiphi*. This parasitoid was shown to respond behaviourally to aphid sex pheromones in a laboratory bioassay (Chapter 2). Further laboratory experiments (Chapter 3) showed that aphid sex pheromone may increase retention of the parasitoid on aphid infested plants. In the field, pheromone lures were used to increase parasitisation by *A. rhopalosiphi* on potted plants (Chapter 5). Finally the behaviour of *A. rhopalosiphi* was manipulated in the crop itself (Chapter 6), indicating that this species may be an important component of future cereal aphid control strategies.

In conclusion, this project has provided valuable information on the parasitoid response to aphid sex pheromones, both in the laboratory and field, and has suggested that the response may form the basis of a novel aphid control strategy. However, as McNeil (1992) recognised, an even greater understanding of the behavioural ecology of the aphid-parasitoid interaction will be required in order to improve the degree of success with which we may exploit it.

REFERENCES

Abdel-Kareim, A.I. and Kozár, F. (1988) Extraction and bioassay of the sex pheromone of the red pear scale, *Epidiapsis leperii*. *Entomol. Exp. Appl.* **46**, 79-84.

Ables, J.R. and Ridgway, R.L. (1981) Augmentation of entomophagous arthropods to control pest insects and mites. In: *Biological Control in Crop Production.* Ed: C.G. Papavizas. pp. 273-303. Allanheld, Osmun.

Agelopoulos, N.G, Dicke, M. and Posthumus, M.A. (1995) Role of volatile infochemicals emitted by feces of larvae in host-searching behaviour of parasitoid *Cotesia rubecula* (Hymenoptera: Braconidae): a behavioural and chemical study. *J. Chem. Ecol.* **21**, 1789-1811.

Akinlosotu, T.A. (1980) Some aspects of the host finding behaviour of the female *Diaeretiella rapae* McIntosh (Hymenoptera, Aphidiidae). *Nigerian J. Entomol.* **1**, 11-18.

Aldrich, J.R. (1985) Chemical communication in the true bugs and parasitoid exploitation. In: *Chemical Ecology of Insects 2.* Eds: R.T. Cardé and W.J. Bell. pp. 318-363. Chapman and Hall, New York.

Aldrich, J.R., Kochansky, J.P. and Abrams, C.B. (1984) Attractant for a beneficial insect and its parasitoids: pheromone of the spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae). *Environ. Entomol.* **13**, 1031-1036.

Aldrich, J.R., Lusby, W.R, Marron, B.E., Nicolaou, K.C., Hoffmann, M.P. and Wilson, L.T. (1989) Pheromone blends of green stink bugs and possible parasitoid selection. *Naturwissenschaften* **76**, 173-175.

Alford, D.V., Cooper, D.A. and Williams, I.R. (1991) Insect pests of oilseed rape. *HGCA Oilseeds Research Review. No. OS1.*

Altieri, M.A., Cure, J.R. and Garcia, M.A. (1993) The role and enhancement of parasitic hymenoptera biodiversity in agroecosystems. In: *Hymenoptera and Biodiversity.* Eds: J. LaSalle and I.D. Gauld. pp. 257-275. CAB International, Wallingford.

Altieri, M.A. and Letourneau, D.K. (1982) Vegetation management and biological control in agroecosystems. *Crop Prot.* **1**, 405-430.

Andow, D. (1985) Plant diversification and insect population control in agroecosystems. In: *Some Aspects of Integrated Pest Management.* Ed: D. Pimental. pp. Dept. Entomol., Cornell Univ., Ithaca, New York.

Anon., (1983) *Lettuce Aphids. ADAS Leaflet No. 392.* MAFF Publications, London.

Anon., (1984) *Cabbage Aphid. ADAS Leaflet No. 269.* MAFF Publications, London.

Anon., (1994) *Pesticide Usage Survey Report 127: Arable Farm Crops in Great Britain 1992. Ministry of Agriculture, Fisheries and Food.* pp. 97. MAFF Publications, London.

Anon., (1995) *The Grower* **29**, 5.

Arthur, A.P. (1981) Host acceptance by parasitoids. In: *Semiochemicals: Their Role in Pest Control.* Eds: D.A. Nordlund, R.L. Jones and W.J. Lewis. pp. 97-120. John Wiley and Sons, New York.

Ashley, T.R., González, D. and Leigh, T.F. (1973) Reduction in effectiveness of laboratory-reared *Trichogramma*. *Environ. Entomol.* **2**, 1069-1073.

Askari, A. and Alishah, A. (1979) Courtship behaviour and evidence for a sex pheromone in *Diaeretiella rapae* (Hymenoptera: Braconidae), the cabbage aphid primary parasitoid. *Ann. Entomol. Soc. Amer.* **72**, 749-750.

Ayal, Y. (1987) The foraging strategy of *Diaeretiella rapae* I. The concept of the elementary unit of foraging. *J. Anim. Ecol.* **56**, 1057-1068.

Barczak, T. (1992) Functional response of the tritrophic system: *Aphis fabae* Scop.- primary parasitoids- hyperparasitoids to different types of habitat. Preliminary results. *Polskie Pismo Entomologiczne* **61**, 119-124.

Bartell, R.J. and Lawrence, L.A. (1973) Reduction in responsiveness of males of *Epiphyas postvittana* (Lepidoptera) to sex pheromone following previous pheromonal experience. *J. Ins. Physiol.* **19**, 845-855.

Battaglia, D., Pennacchio, F., Marincola, G. and Tranfaglia, A. (1993) Cornicle secretion of *Acyrthosiphon pisum* (Homoptera: Aphididae) as a contact kairomone for the parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). *Eur. J. Entomol.* **90**, 423-428.

Battisti, A. (1989) Field studies on the behaviour of two egg parasitoids of the pine processionary moth *Thaumetopoea pityocampa*. *Entomophaga* **34**, 29-38.

Bedard, W.D. (1965) The biology of *Tomicobia tibialis* parasitizing *Ips confusus* in California. *Contrib. Boyce Thomson Inst.* **23**, 77-81.

Bedard, W.D., Wood, D.L., Tilden, P.E., Lindahl Jr, K.Q., Silverstein, R.M. and Rodin, J.O. (1980) Field response of the western pine beetle and one of its predators to host- and beetle-produced compounds. *J. Chem. Ecol.* **6**, 625-641.

Blackman, R. (1974) *Invertebrate Types: Aphids.* Ginn and Company, London.

Bode, E. (1980) Aphids in winter wheat: abundance and limiting factors from 1976 to 1979. *Bull. SROP/WPRS* **III (4)**, 49-57.

Borges, M., Jepson, P.C. and Howse, P.E. (1987) Long-range mate location and close-range courtship behaviour of the green stink bug, *Nezara viridula* and its mediation by sex pheromones. *Entomol. Exp. Appl.* **44**, 205-212.

van den Bosch, R., Lagrace, C.F. and Stern, V.M. (1967) The interrelationship of the aphid, *Acyrthosiphon pisum*, and its parasite, *Aphidius smithi*, in a stable environment. *Ecology* **48**, 993-1000.

van den Bosch, R., Messenger, P.S. and Gutierrez, A.P. (1982) *An Introduction to Biological Control.* Plenum Press, New York, London.

van den Bosch, R., Schlinger, E.I., Dietrick, E.J. and Hall, I.M. (1959) The role of imported parasitoids in the biological control of the spotted alfalfa aphid in southern California. *J. Econ. Entomol.* **52**, 142-147.

van den Bosch, R., Schlinger, E.I., Hall, J.C. and Puttler, B. (1964) Studies on succession, distribution and phenology of imported parasites of *Therioaphis trifolii* (Monell) in southern California. *Ecology* **45**, 602-621.

Bouchard, Y. and Cloutier, C. (1984) Honeydew as a source of host-searching kairomones for the aphid parasitoid *Aphidius nigripes* (Hymenoptera: Aphidiidae). *Can. J. Zool.* **62**, 1513-1520.

Bouchard, Y. and Cloutier, C. (1985) Role of olfaction in host finding by aphid parasitoid *Aphidius nigripes* (Hymenoptera: Aphidiidae). *J. Chem. Ecol.* **11**, 801-808.

Braimah, H. and van Emden, H.F. (1994) The role of the plant in host acceptance by the parasitoid *Aphidius rhopalosiphi* (Hymenoptera: Braconidae). *Bull. Entomol. Res.* **84**, 303-306.

Budenberg, W.J. (1990) Honeydew as a contact kairomone for aphid parasitoids. *Entomol. Exp. Appl.* **55**, 139-148.

Budenberg, W.J., Powell, W. and Clark, S.J. (1992) The influence of aphids and honeydew on the leaving rate of searching aphid parasitoids from wheat plants. *Entomol. Exp. Appl.* **63**, 259-264.

Buttery, R.G., Xu, C. and Ling, L.C. (1985) Volatile components of wheat leaves (and stems): possible insect attractants. *J. Agric. Food Chem.* **33**, 115-117.

Byers, J.A. (1995) Host-tree chemistry affecting colonization in bark beetles. In: *Chemical Ecology of Insects 2.* Eds: R.T Cardé and W.J. Bell. pp. 154-213. Chapman and Hall, New York.

Caltagirone, L.E. and Doutt, R.L. (1989) The history of the vedalia beetle importation to California and its impact on the development of biological control. *Ann. Rev. Entomol.* **34**, 1-16.

Cameron, P.J., Powell, W. and Loxdale, H.D. (1984) Reservoirs for *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae), a polyphagous parasitoid of cereal aphids (Hemiptera: Aphididae). *Bull. Entomol. Res.* **74**, 647-656.

Cameron, P.J., Walker, G.P. and Allan, D.J. (1981) Establishment of the introduced parasite *Aphidius eadyi* (Hymenoptera: Aphidiidae) in the north island of New Zealand, and its initial effect on pea aphid. *N.Z. J. Zool.* **8**, 105-112.

Campbell, C.A.M., Dawson, G.W., Griffiths, D.C., Pettersson, J., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. (1990) Sex attractant pheromone of the damson-hop aphid *Phorodon humuli* (Homoptera: Aphididae). *J. Chem. Ecol.* **16**, 3455-3465.

Cardé, R.T. and Lee, H.-P. (1989) Effect of experience on the responses of the parasitoid *Brachymeria intermedia* (Hymenoptera: Chalcididae) to its host, *Lymantria dispar* (Lepidoptera, Lymantriidae), and to kairomone. *Ann. Entomol. Soc. Amer.* **82**, 652-657.

Cardé, R.T. and Minks, A.K. (1995) Control of moth pests by mating disruption: successes and constraints. *Ann. Rev. Entomol.* **40**, 559-585.

Carter, N., McLean, I.F.G., Watt, A.D. and Dixon, A.F.G. (1980) Cereal aphids- a case study and review. *Appl. Biol.* **5**, 271-348.

Carver, M. (1989) Biological control of aphids. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2C.* Eds: A.K. Minks and P. Harrewijn. pp. 141-165. Elsevier, Amsterdam.

Chambers, R.J. and Adams, T.H.L. (1986) Quantification of the impact of hoverflies (Diptera: Syrphidae) on cereal aphids in wheat: an analysis of field populations. *J. Appl. Ecol.* **23**, 895-904.

Chau, A. and Mackauer, M. (1997) Dropping of pea aphids from feeding site: a consequence of parasitism by the wasp *Monoctonus paulensis*. *Entomol. Exp. Appl.* **83**, 247-252.

Chiri, A.A. and Legner, E.P. (1983) Field applications of host-searching kairomones to enhance parasitization of the pink bollworm (Lepidoptera: Gelechiidae). *J. Econ. Entomol.* **76**, 254-255.

Chow, A. and Mackauer, M. (1991) Patterns of host selection by four species of aphidiid (Hymenoptera) parasitoids: influence of host switching. *Ecol. Entomol.* **16**, 403-410.

Chow, A. and Mackauer, M. (1992) The influence of prior ovipositional experience on host selection in four species of Aphidiid wasps (Hymenoptera: Aphidiidae). *J. Insect Behav.* **5**, 99-108.

Chua, T.H. (1977) Population studies of *Brevicoryne brassicae* (L.), its parasites and hyperparasites in England. *Res. Popul. Ecol.* **19**, 125-139.

Cloutier, C. and Bauduin, F. (1990) Searching behaviour of the aphid parasitoid *Aphidius nigripes* (Hymenoptera: Aphidiidae) foraging on potato plants. *Environ. Entomol.* **19**, 222-228.

Cloutier, C., Dohse, L.A. and Bauduin, F. (1984) Host discrimination in the aphid parasitoid *Aphidius nigripes*. *Can. J. Zool.* **62**, 1367-1372.

Corbet, S.A. (1971) Mandibular gland secretion of larvae of the flour moth, *Anagasta kuehniella*, contains an epideictic pheromone, and elicits ovipositional movements in a hymenopteran parasite. *Nature* **232**, 481-484.

Corbet, S.A. (1985) Insect chemosensory responses: a chemical legacy theory. *Ecol. Entomol.* **10**, 143-153.

Daane, K.M., Yokota, G.Y., Gill, R.F., Caltagirone, L.E, Hagen, K.S., González, D., Starý, P. and Chaney, W.E. (1992) Imported parasite may help control European asparagus aphid. *Calif. Agric.* **46**, 12-14.

Dawson, G.W., Griffiths, D.C., Janes, N.F., Mudd, A., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. (1987) Identification of an aphid sex pheromone. *Nature* **325**, 614:616.

Dawson, G.W., Griffiths, D.C., Merritt, L.A., Mudd, A., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. (1988) The sex pheromone of the greenbug, *Schizaphis graminum*. *Entomol. Exp. Appl.* **48**, 91-93.

Dawson, G.W., Griffiths, D.C., Merritt, L.A., Mudd, A., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. (1990) Aphid semiochemicals- a review, and recent advances on the sex pheromone. *J. Chem. Ecol.* **16**, 3019-3030.

Dawson, G.W., Janes, N.F., Mudd, A., Pickett, J.A., Shavin, A.M.Z., Wadhams, L.J. and Williams, D.J. (1989) The aphid sex pheromone. *Pure Appl. Chem.* **61**, 555-558.

Dawson, G.W., Pickett, J.A. and Smiley, D.W. (1996) The aphid sex pheromone cyclopentanoids: synthesis and the elucidation of structure and biosynthetic pathways. *Bioorganic and Medicinal Chemistry* **4**, 351-361.

Dean, G.J. (1974) Effects of parasites and predators on the cereal aphids *Metopolophium dirhodum* (Wlk.) and *Macrosiphum avenae* (F.) (Hem., Aphididae). *Bull. Entomol. Res.* **63**, 411-422.

Dean, G., Dewar, A.M., Powell, W. and Wilding, N. (1980) Integrated control of aphids. *Bull. SROP/WPRS* **III (4)**, 49-57.

Dean, G.J.W. and Wilding, N. (1973) Infection of cereal aphids by the fungus *Entomopthora*. *Ann. Appl. Biol.* **74**, 133-138.

DeBach, P. (1964) The scope of biological control. In: *Biological Control of Insect pests and weeds*. Ed: P. DeBach. pp. 3-20. Chapman and Hall, London.

Decker, U.M., Powell, W. and Clark, S.J. (1993) Sex pheromones in the cereal aphid parasitoids *Praon volucre* and *Aphidius rhopalosiphi*. *Entomol. Exp. Appl.* **69**, 33-39.

Dedryver, C.A., Creach, V. Rabasse, J.M. and Nenon, J.P. (1991) Spring activity assessment of parasitoids in western France by experimental exposure of cereal aphids on trap plants in a wheat field. *Bull. IOBC/WPRS* **14**, 82-93.

De Jong, R. and Kaiser, L. (1991) Odour learning by *Leptoplilina boulardi*, a specialist parasitoid (Hymenoptera: Eucoilidae). *J. Ins. Behav.* **4**, 743-750.

Devonshire, A.L. (1989) Resistance of aphids to insecticides. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2C.* Eds: A.K. Minks and P. Harrewijn. pp. 123-139. Elsevier, Amsterdam.

Dhiman, S.C. and Kumar, V. (1983) Host preference of *Aphidius rapae* (Curtis) (Hymenoptera: Braconidae). In: *Proc. Symp. Insect Ecology and Resource Management.* Ed: S.C. Groel. pp. 138-141. Sanatan Dharm Coll., Muzaffarnagar, India.

Dickens, J.C. and Mori, K. (1989) Receptor chirality and behavioural specificity of the boll weevil, *Anthonomus grandis* Boh. (Coleoptera: Curculionidae), for its pheromone (+)-grandisol. *J. Chem. Ecol.* **15**, 517-528.

Dickens, J.C., Payne, T.L., Ryker, L.C. and Rudinsky, J.A. (1985) Multiple receptors for pheromonal enantiomers on single olfactory cells in the Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopk. (Coleoptera: Scolytidae). *J. Chem. Ecol.* **11**, 1359-1370.

Dixon, A.F.G. (1973) *Biology of Aphids.* Edward Arnold, London.

Dixon, A.F.G. (1987) Evolution and adaptive significance of cyclical parthenogenesis in aphids. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2A.* Eds: A.K. Minks and P. Harrewijn. pp. 289-297. Elsevier, Amsterdam.

Doodson, J.K. and Saunders, P.J.W. (1970) Some effects of barley yellow dwarf virus on spring and winter cereals in field trials. *Ann. Appl. Biol.* **66**, 361-374.

Du, Y.J., Poppy, G.M. and Powell, W. (1996) The relative importance of semiochemicals from the first and second trophic level in the host foraging behaviour of *Aphidius ervi*. *J. Chem. Ecol.* **22**, 1591-1605

Du, Y.J., Poppy, G.M., Powell, W. and Wadhams, L.J. (1997) Chemically-mediated associative learning in the host foraging behaviour of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae). *J. Insect. Behav.* **10**, 509-522

Edwards, C.A., Sunderland, K.G. and George, K.S. (1979) Studies on the polyphagous predators of cereal aphids. *J. Appl. Ecol.* **16**, 811-823.

Eikenbary, R.D. and Rogers, C.E. (1973) Importance of alternate hosts in establishment of introduced parasites. In: *Proc. Tall Timbers Conf. on Ecological Animal Control by Habitat Manipulation No. 5.* pp. 119-133. Tallahassee, Florida. March, 1973.

Eisenbach, J. and Mittler, T.E. (1980) An aphid circadian rhythm: factors affecting the release of sex pheromone by oviparae of the greenbug, *Schizaphis graminum*. *J. Ins. Physiol.* **26**, 511-515.

Eisenbach, J. and Mittler, T.E. (1987) Effects of photoperiod and mating on sex pheromone release by oviparae of the aphid *Schizaphis graminum*. *Physiol. Entomol.* **12**, 293-296.

Eisner, T. (1964) Catnip: its raison d' être. *Science* **146**, 1318-1320.

Eller, F.J., Tumlinson, J.H. and Lewis, W.J. (1988a) Beneficial arthropod behaviour mediated by airborne semiochemicals: source of volatiles mediating the host-location flight behaviour of *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid of *Heliothis zea* (Boddie) (Lepidoptera: Noctuidae). *Environ. Entomol* **17**, 745-753.

Eller, F.J., Tumlinson, J.H. and Lewis, W.J. (1988b) Beneficial arthropod behaviour mediated by airborne semiochemicals II. Olfactometric studies of host location by the parasitoid *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae). *J. Chem. Ecol.* **14**, 425-434.

Elliot, N.C, French, B.W., Reed, D.K., Burd, J.D. and Kinder, S.D. (1994) Host species effects on parasitization by a Syrian population of *Diaeretiella rapae* M'Intosh (Hymenoptera: Aphidiidae). *Can. Entomologist* **126**, 1515-1517.

van Emden, H.F. (1988) The potential for managing indigenous natural enemies of aphids on field crops. *Phil. Trans. R. Soc. Lond. B.* **318**, 183-201.

van Emden, H.F., Sponagl, B., Wagner, E., Baker, T., Ganguly, S. and Douloumpaka, S. (1996) Hopkins' ' host selection principle', another nail in its coffin. *Physiol. Entomol.* **21**, 325-328.

Farrell, J.A. and Stufkens, M.W. (1990) The impact of *Aphidius rhopalosiphi* (Hymenoptera: Aphidiidae) on populations of the rose grain aphid (*Metopolophium dirhodum*) (Hemiptera: Aphididae) on cereals in Canterbury, New Zealand. *Bull. Entomol. Res.* **80**, 377-383.

Fougeroux, A., Bouchet, C., Reboulet, J.N. and Tisseur, M. (1988) Microhymenoptera for aphid population regulation in French cereal crops. In: *Integrated Crop Protection in Cereals.* Eds: R. Cavalloro and K.D. Sunderland. pp. 61-68. *Proc. Meeting EC Experts Group, Littlehampton, England, 1986.* A.A. Balkema, Rotterdam.

Frazer, B.D. (1988) Coccinellidae. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2B.* Eds: A.K. Minks and P. Harrewijn. pp. 231-247. Elsevier, Amsterdam.

Frazer, B.D. and van den Bosch, R. (1973) Biological control of the walnut aphid in California: the interrelationship of the aphid and its parasite. *Environ. Entomol.* **2**, 561-568.

Gabrys, B.J., Gadomski, H.J., Klukowski, Z., Pickett, J.A., Sobota, G.T., Wadhams, L.J. and Woodcock, C.M. (1997) Sex pheromone of cabbage aphid *Brevicoryne brassicae*: identification and field trapping of male aphids and parasitoids. *J. Chem. Ecol.* **23**, 1881-1890.

Gardner, S.M. and Dixon, A.F.G. (1985) Plant structure and the foraging success of *Aphidius rhopalosiphi* (Hymenoptera: Aphidiidae). *Ecol. Entomol.* **10**, 171-179.

Gardner, S.M. and van Lenteren, J.C. (1986) Characterization of the arrestment responses of *Trichogramma evanescens*. *Oecologia* **68**, 265-270.

Gauld, I. And Bolton, B. (1988) *The Hymenoptera*. Oxford University Press. pp. 332.

George, K.S. (1974) Damage assessment aspects of cereal aphid attack in autumn- and spring-sown cereals. *Ann. Appl. Biol.* **77**, 67-74.

Gilbert, N. and Guitierrez, A.P. (1973) A plant-aphid parasite relationship. *J. Anim. Ecol.* **42**, 323-340.

Giller, P.S., Ryan, B., Kennedy, T. and Connery, J. (1995) Aphid-parasitoid interactions in a winter cereal crop: field trials involving insecticide application. *J. Appl. Entomol.* **119**, 233-239.

Goff, A.M. and Nault, L.R. (1984) Response of the pea aphid parasite *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) to transmitted light. *Environ. Entomol.* **13**, 595-598.

González, D., Summers, C.G. and Qualset, C.O. (1992) Russian wheat aphid: natural enemies, resistant wheat offer potential control. *Calif. Agric.* **46**, 32-34.

Goodman, D. (1975) The theory of diversity-stability relationships in ecology. *Q. Rev. Biol.* **50**, 237-266.

Grasswitz, T.R. and Paine, T.D. (1992) Kairomonal effect of an aphid cornicle secretion on *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae). *J. Ins. Behav.* **5**, 447-457.

Grasswitz, T.R. and Paine, T.D. (1993) Effect of experience on in-flight orientation to host-associated cues in the generalist parasitoid *Lysiphlebus testaceipes*. *Entomol. Exp. Appl.* **68**, 219-229.

Greathead, D.J. (1989) Biological control as an introduction phenomenon: a preliminary examination of programmes against Homoptera. *The Entomologist* **108**, 28-37.

Griffiths, D.C., Pickett, J.A., Smart, L.E. and Woodcock, C.M. (1989) Use of insect antifeedants against aphid vectors of plant virus disease. *Pesticide Sci.* **27**, 269-276.

Grout, T.G. and Richards, G.I. (1991) Value of pheromone traps for predicting infestations of red scale, *Aonidiella aurantii* (Maskell) (Hom., Diaspididae), limited by natural enemy activity and insecticides used to control citrus thrips *Scirtothrips aurantii* Faure (Thys., Thripidae). *J. Appl. Entomol.* **111**, 20-27.

Grundy, T.P. (1990) A cost-benefit analysis of biological control of rose-grain aphid in New Zealand. *Proc. Forty Third N.Z. Weed and Pest Control Conf.* pp. 163-165.

Gu, D.J. and Waage, J.K. (1990) The effects of insecticides on the distribution of foraging parasitoids. *Diaeretiella rapae* (Hym., Braconidae) on plants. *Entomophaga* **35**, 49-56.

Guerrieri, E., Du, Y.J., Poppy, G.M., Powell, W., Pennacchio, F. and Tremblay, E. (1996) The role of host-induced plant synomones on in-flight orientation of the aphid parasitoid *Aphidius ervi* (Haliday) (Hymenoptera: Braconidae: Aphidiinae). *Proc. XX International Congr. Entomol. August 1996, Firenze, Italy.*

Guerrieri, E., Pennacchio, F. and Tremblay, E. (1993) Flight behaviour of the aphid parasitoid *Aphidius ervi* (Hymenoptera: Braconidae) in response to plant and host volatiles. *Eur. J. Entomol.* **90**, 415-421.

Guldemond, J.A. (1990) Choice of host plant as a factor in reproductive isolation of the aphid genus *Cryptomyzus* (Homoptera: Aphididae). *Ecol. Entomol.* **15**, 43-51.

Guldemond, J.A., Dixon, A.F.G., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. (1993) Specificity of sex pheromones, the role of host plant odour in the olfactory attraction of males, and mate recognition in the aphid *Cryptomyzus*. *Physiol. Entomol.* **18**, 137-143.

Guldemond, J.A., Tigges, W.T. and De Vrijer, P.W.F. (1994) Circadian rhythm of sex pheromone production and male activity of coexisting sibling species of *Cryptomyzus* aphids (Homoptera: Aphididae). *Eur. J. Entomol.* **91**, 85-89.

Hagan, K.S. and Schlinger, E.I. (1960) Imported Indian parasite of pea aphid established in California. *Calif. Agric.* **14**, 5-6.

Hågvar, E.B. and Hofsvang, T. (1987) Foraging by the aphid parasitoid *Ephedrus cerasicola* for patchily distributed hosts. *Entomol. Exp. Appl.* **44**, 81-88.

Hågvar, E.B. and Hofsvang, T. (1989) The effect of honeydew on plant colonization by the aphid parasitoid *Ephedrus cerasicola*. *Entomophaga* **34**, 495-501.

Hågvar, E.B. and Hofsvang, T. (1991) Aphid parasitoids (Hymenoptera: Aphidiidae): biology, host selection and use in biological control. *Biocontrol News and Inf.* **12**, 13-41.

Halfhill, J.E. and Featherstone, P.E. (1973) Inundative releases of *Aphidius smithi* against *Acyrthosiphon pisum*. *Environ. Entomol.* **2**, 469-472.

Hand, S.C. (1989) The overwintering of cereal aphids on Gramineae in southern England. *Ann. Appl. Biol.* **115**, 17-29.

Hansen, K. (1984) Discrimination and production of disparlure enantiomers by the gypsy moth and the nun moth. *Physiol. Entomol.* **9**, 9-18.

Hardie, J. (1991) Contribution of sex pheromone to mate location and reproductive isolation in aphid species (Homoptera: Aphidinea). *Entomol. Gener.* **16**, 249-256.

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

Hardie, J., Holyoak, M., Nicholas, J., Nottingham, S.F., Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. (1990) Aphid sex pheromone components: age dependent release by females and species-specific response. *Chemoecol.* **1**, 63-68.

Hardie, J., Nottingham, S.F., Dawson, G.W., Harrington, R., Pickett, J.A. and Wadhams, L.J. (1992) Attraction of field-flying aphid males to synthetic sex pheromone. *Chemoecol.* **3**, 133-117.

Hardie, J., Nottingham, S.F., Powell, W. and Wadhams, L.J. (1991) Synthetic aphid sex pheromone lures female aphid parasitoids. *Entomol. Exp. Appl.* **61**, 97-99.

Hardie, J., Peace, L., Pickett, J.A., Smiley, D.W.M., Storer, R.J. and Wadhams, L.J. (1997) Sex pheromone stereochemistry and purity affect field catches of male aphids. *J. Chem. Ecol.* **23**, 2547-2554.

Hare, J.D. (1996) Priming *Aphytis*: behavioural modification of host selection by exposure to a synthetic contact kairomone. *Entomol. Exp. Appl.* **78**, 263-269.

t' Hart, J., de Jonge, J., Collé, C., Dicke, M., van Lenteren, J.C. and Ramakers, P. (1975) Host selection, host discrimination and functional response of *Aphidius matricariae* Haliday (Hymenoptera: Braconidae), a parasite of the green peach aphid, *Myzus persicae* (Sulz.). *Meded. van de Fac. Landbouw. Rijkuniv. Gent.* **43**, 441-453.

Hassall, M., Hawthorne, A., Maudsley, M., White, P. and Cardwell, C. (1992) Effects of headland management on invertebrate communities in cereal fields. *Agric. Ecosyst. Environ.* **40**, 155-178.

Hodgson, C. and Aveling, C. (1988) Anthocoridae. In: *World Crop Pests. Aphids. Their biology, Natural Enemies and Control. Vol. 2B.* Eds: A.K. Minks and P. Harrewijn. pp. 279-292. Elsevier, Amsterdam.

Hofsvang, T. and Hågvar, E.B. (1986) Oviposition behaviour of *Ephedrus cerasicola* (Hym.: Aphidiidae) parasitizing different instars of its aphid host. *Entomophaga* **31**, 261-267.

Höller, C. (1990) Overwintering hymenopterous parasitism in autumn of the cereal aphid *Sitobion avenae* (F.) in northern Germany. *J. Appl. Entomol.* **109**, 21-28.

Höller, C. (1991) Movement away from the feeding site in parasitised aphids: host suicide or an attempt by the parasitoid to escape hyperparasitism? In: *Ecology of Aphidophaga IV*. Ed: I. Hodek. pp. 45-49. SPB Academic Publishing, The Hague.

Höller, C., Borgemeister, C., Haardt, T. and Powell, W. (1993) The relationship between primary parasitoids and hyperparasitoids of cereal aphids: an analysis of field data. *J. Anim. Ecol.* **62**, 12-21.

Höller, C., Micha, S.G., Schulz, S., Francke, W. and Pickett, J.A. (1994) Enemy-induced dispersal in a parasitic wasp. *Experientia* **50**, 182-185.

Hood-Henderson, D.E. and Forbes, A.R. (1988) Behavioural responses of a primary parasitoid *Praon pequodorum* Viereck (Hymenoptera: Aphidiidae) and a secondary parasitoid *Dendrocerus carpenteri* Kiefler (Hymenoptera: Megaspilidae) to aphid honeydew. *Proc., XVIII Int. Congr. Entomol. Vancouver, 3-9 July, 1988.* pp. 373.

Horn, D.J. (1984) Vegetational complexity and parasitism of green peach aphids (*Myzus persicae* (Sulzer) (Homoptera: Aphididae)) on collards. *J. N.Y. Entomol. Soc.* **92**, 19-26.

Horn, D.J. (1989) Secondary parasitism and population dynamics of aphid parasitoids (Hymenoptera: Aphidiidae). *J. Kansas Entomol. Soc.* **62**, 203-210.

Hou, Z. and Yan, F. (1995) Electroantennogram responses of *Lysiphlebia japonica* Ashmead (Hymenoptera: Aphidiidae) to some cotton plant volatiles and cotton aphid pheromones. *Entomologia Sinica* **2**, 253-264.

Hsieh, C.Y. and Allen, W.W. (1986) Effects of insecticides on emergence, survival, longevity and fecundity of the parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) from mummified *Myzus persicae* (Homoptera: Aphididae). *J. Econ. Entomol.* **79**, 1599-1602.

Hu, J.S. and Chen, C.M. (1987) A study of the host-searching kairomone of *Apanteles cypris* Nixon. *Acta Entomol. Sinica* **30**, 31-40.

Hughes, R.D. (1989) Biological control in the open field. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2C.* Eds: A.K. Minks and P. Harrewijn. pp. 167-198. Elsevier, Amsterdam.

Isidoro, N. and Bin, F. (1988) Kairomone activity of *Dryomia lichensteini* sexual pheromone on the egg larval parasitoid, *Platygaster dryomyiae. Proc. 15th Ital. Natn. Entomol. Congr. L'Aquila, 13-17 June, 1988.* pp. 939-944.

Issacs, I. (1994) *Studies on the Chemical Ecology of the Black Bean Aphid, Aphis fabae.* PhD. Thesis, University of London.

Issacs, R., Hardie, J., Hick, A.J., Pye, B.J., Smart, L.E., Wadhams, L.J. and Woodcock, C.M. (1993) Behavioural responses of *Aphis fabae* to isothiocyanates in the laboratory and field. *Pesticide Sci.* **39**, 349-355.

Jackson, H.B., Rogers, C.E., Eikenbary, R.D., Starks, K.J. and McNew, R.W. (1974) Biology of *Ephedrus plagiator* on different aphid hosts and at various temperatures. *Environ. Entomol.* **3**, 618-620.

Jennings, D.T. and Jones, R.L. (1986) Field tests of kairomones to increase parasitism of spruce budworm (Lepidoptera: Tortricidae) eggs by *Trichogramma* spp. (Hymenoptera: Trichogrammatidae). *Great Lakes Entomologist* **19**, 185-189.

Jepson, P.C. (1989) The temporal and spatial dynamics of pesticide side-effects on non-target invertebrates. In: *Pesticides and Non-Target Invertebrates.* Ed: P.C. Jepson. pp. 95-127. Intercept, Wimbourne, Dorset.

Jones, D. (1984) Use, and role of multiple-comparison procedures in ecological and agricultural entomology. *Environ. Entomol.* **13**, 635-649.

Kaiser, L. and Cardé, R.T. (1992) In-flight orientation to volatiles from the plant-host complex in *Cotesia rubecula* (Hym: Braconidae): increased sensitivity through olfactory experience. *Physiol. Entomol.* **17**, 62-67.

Keller, M.A. (1990) Responses of the parasitoid *Cotesia rubecula* to its host *Pieris rapae* in a flight tunnel. *Entomol. Exp. Appl.* **57**, 243-249.

Kelm, M. (1988) The effectiveness of *Diaeretiella rapae* (McIntosh) in the control of the cabbage aphid *Brevicoryne brassicae* (L.) on oilseed rape in Great Britain. In: *Ecology and Effectiveness of Aphidophaga*. Eds: E. Niemczyk and A.F.G. Dixon. pp. 249-252. SPB Academic Publishing, The Hague.

Kennedy, B.H. (1979) The effect of multilure on parasites of the European elm bark beetle *Scolytus multistriatus*. *Bull. Entomol. Soc. Amer.* **29**, 178-179.

Kennedy, B.H. (1984) Effect of multilure and its components on parasites of *Scolytus multistriatus* (Coleoptera: Scolytidae). *J. Chem. Ecol.* **10**, 373-385.

Kieckhefer, R.W. and Kantack, B.H. (1986) Yield losses in spring barley caused by cereal aphids (Homoptera: Aphididae) in South Dakota. *J. Econ. Entomol.* **79**, 749-752.

Kyparissoudas, D.S. (1987) The occurrence of *Encarsia perniciosa* in areas of northern Greece as assessed by sex pheromone traps of its host *Quadraspidiotus perniciosus*. *Entomol. Hellenica* **5**, 7-12.

Lagerlöf, J. and Wallin, H. (1993) The abundance of arthropods along two field margins with different types of vegetation composition: an experimental study. *Agric. Ecosyst. Environ.* **43**, 141-154.

Langer, A., Stilmant, D., Verbois, D. and Hance, Th. (1997) Seasonal activity and distribution of cereal aphid parasitoids in Belgium. *Entomophaga* **42**, 185-191.

Lanier, G.N., Birch, M.C., Schmitz, R.F. and Furniss, M.M. (1972) Pheromones of *Ips pini* (Coleoptera: Scolytidae): variation in response among three populations. *Can. Entomol.* **104**, 1917-1923.

Láska, P. (1984) A method of comparing the role of aphid parasitoids and predators exemplified by the cabbage aphid, *Brevicoryne brassicae. Acta Entomol. Bohemoslov.* **81,** 81-89.

Latgé, J.P. and Papierok, B. (1988) Aphid pathogens. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2B.* Eds: A.K. Minks and P. Harrewijn. pp. 323-335. Elsevier, Amsterdam.

Leather, S.R. (1993) Overwintering in six arable aphid pests: a review with particular relevance to pest management. *J. Appl. Entomol.* **116**, 217-233.

Levinson, H.Z., Levinson, A., Ren, Z. and Mori, K. (1990) Comparative olfactory perception of the aggregation pheromones of *Sitophilus oryzae* (L.), *S. zeamais* (Motsch.) and *S. granarius* (L.), as well as the stereoisomers of these pheromones. *J. Appl. Entomol.* **110**, 203-213.

Lewis, W.J. (in press) Biological from a sustainable agriculture perspective.

Lewis, W.J., Beevers, M., Nordlund, D.A., Gross Jr., H.R. and Hagen, K.S. (1979) Kairomones and their use for management of entomophagous insects IX. Investigations of various kairomone-treatment patterns for *Trichogramma* spp. *J. Chem. Ecol.* **5**, 673-680.

Lewis, W.J., Gross Jr, H.R. and Nordlund, D.A. (1985) Behavioural manipulation of *Trichogramma* (Hymenoptera: Trichogrammatidae). *Southwestern Entomologist* **suppl. 8**, 49-55.

Lewis, W.J. and Martin, J.R. (1990) Semiochemicals for use with parasitoids: status and future. *J. Chem. Ecol.* **16**, 3067-3089.

Lewis, W.J., Nordlund, D.A., Gueldner, R.C., Teal, P.E.A. and Tumlinson, J.H. (1982) Kairomones and their use for management of entomophagous insects. XIII. Kairomonal activity for *Trichogramma* sp. of abdominal tips, excretion, and a synthetic sex pheromone blend of *Heliothis zea* (Boddie) moths. *J. Chem. Ecol.* **8**, 1323-1332.

Lewis, W.J. and Tumlinson, J.H. (1988) Host detection by chemically mediated associative learning in a parasitic wasp. *Nature* **331**, 257-259.

Li, Z.X., Chen, C.M. and Tang, M.Y. (1993) Host searching kairomones of *Amitus longicornis* Foster. *Acta Entomol. Sinica* **36**, 45-50.

Lilley, R., Hardie, J. and Wadhams, L.J. (1994) Field manipulation of *Praon* populations using semiochemicals. *Norw. J. Agric. Sci. Suppl.* **16**, 221-226.

Liu, Shu-Sheng, Morton, R. and Hughes, R.D. (1984) Oviposition preferences of a hymenopterous parasite for certain instars of its aphid host. *Entomol. Exp. Appl.* **35**, 249-254.

Longley, M. (1994) The effect of deltamethrin on the response of *Aphidius rhopalosiphi* (Hym.: Aphidiidae) to honeydew in a filter paper bioassay. *Bull. OILB/SROP* **17**, 48-56.

Lopez, E.R. and van Driesche, R.G. (1989) Direct measurement of host and parasitoid recruitment for assessment of total losses due to parasitism in a continually breeding species, the cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). *Bull. Entomol. Res.* **79**, 47-59.

Ma, R.Z., Swedenborg, P.D. and Jones, R.L. (1992) Host-seeking behaviour of *Eriborus terebrans* (Hymenoptera: Ichneumonidae) toward the European corn borer and the role of chemical stimuli. *Ann. Entomol. Soc. Amer.* **85**, 72-79.

Mackauer, M. (1976) Genetic problems in the production of biological control agents. *Ann. Rev. Entomol.* **21**, 369-385.

Mackauer, M. and Chow, F.J. (1986) Parasites and parasite impact on aphid populations. In: *Plant Virus Epidemics. Monitoring, Modelling and Predicting Outbreaks.* Eds: G.D. McLean, R.G. Garret and W.G. Ruesink. pp. 95-118. Academic Press, Sydney.

Mackauer, M. and Völkl, W. (1993) Regulation of aphid populations by aphidiid wasps: does parasitoid foraging behaviour or hyperparasitism limit impact? *Oecologia* **94**, 339-350.

Maiteki, G.A. and Lamb, R.J. (1985) Spray timing and economic threshold for the pea aphid, *Acyrthosiphon pisum* (Homoptera: Aphididae), on field peas in Manitoba. *J. Econ. Entomol.* **78**, 1449-1454.

Marsh, D. (1972) Sex pheromone in the aphid *Megoura viciae*. *Nature* **238**, 31-32.

Marsh, D. (1975) Responses of male aphids to the female sex pheromone in *Megoura viciae* Buckton. *J. Entomol.* **50**, 43-64.

McAuslane, H.J., Vinson, S.B. and Williams, H.J. (1991) Stimuli influencing host microhabitat location in the parasitoid *Campoletis sonorensis*. *Entomol. Exp. Appl.* **58**, 267-277.

McGregor, R. and Mackauer, M. (1989) Toxicity of carbaryl to the pea-aphid parasite *Aphidius smithi*: influence of behaviour on pesticide uptake. *Crop. Prot.* **8**, 193-196.

McNeil, J.N. (1992) Evolutionary perspectives and insect pest control: an attractive blend for the deployment of semiochemicals in management programs. In: *Insect Chemical Ecology. An Evolutionary Approach*. Eds: B.D. Roitberg and M.B. Isman. pp. 334-353. Chapman and Hall, New York.

McNeil, J.N. and Brodeur, J. (1995) Pheromone-mediated mating in the aphid parasitoid, *Aphidius nigripes* (Hymenoptera: Aphidiidae). *J. Chem. Ecol.* **21**, 959-972.

Meng, X.Z., Wang, Y.H. and Ye, M.X. (1985) Field trials on mass trapping for the control of the oriental fruit moth with synthetic sex pheromone. *Acta Entomol. Sinica* **28**, 142-147.

Menzel, R., Greggers, U. and Hammer, M. (1993) Functional organization of appetitive learning and memory in a generalist pollinator, the honey bee. In: *Insect Learning. Ecological and Evolutionary Perspectives*. Eds: D.R. Papaj and A.C. Lewis. pp. 79-125. Chapman and Hall, New York.

Messing, R.H. and Aliniazee, M.T. (1989) Introduction and establishment of *Trioxys pallidus* (Hym.: Aphidiidae) in Oregon, U.S.A. for control of filbert aphid *Myzocallis coryli* (Hom.: Aphididae). *Entomophaga* **34**, 153-163.

Micha, S.G. and Wyss, U. (1995) The importance of plant odours for host searching of *Aphidius uzbekistanicus* (Hymenoptera, Aphidiidae), a parasitoid of the grain aphid (*Sitobion avenae*). *Gesunde-pflanzen* **47**, 300-307.

Michaud, J.P. and Mackauer, M. (1994) The use of visual cues in host evaluation by aphidiid wasps I. Comparison between three *Aphidius* parasites of the pea aphid. *Entomol. Exp. Appl.* **70**, 273-283.

Miles, P.W. (1989) Specific responses and damage caused by aphidoidea. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2C.* Eds: A.K. Minks and P. Harrewijn. pp. 23-47. Elsevier, Amsterdam.

Miller, J.R. and Cowles, R.S. (1990) Stimulo-deterrent diversion: a concept and its possible application to onion maggot control. *J. Chem. Ecol.* **16**, 3197-3212.

Milne, W.M. (1986) The establishment and dispersal of *Aphidius ervi* in blue-green aphid populations of lucerne in New South Wales, Australia. In: *Ecology of Aphidophaga.* Ed: I. Hodek. pp: 459-464. Academia Prague and Dr. W. Junk, Dortrecht.

Milne, W.M. (1995) Use of trap plants as a means of measuring the activity of cereal aphid parasitoids in the field. *Agric. Ecosyst. Environ.* **52**, 31-34.

Mitchell, W.C. and Mau, R.F.L. (1971) Response of the female southern green stink bug and its parasite *Trichopoda pennipes*, to male stink bug pheromones. *J. Econ. Entomol.* **64**, 856-859.

Moran, N.A. (1992) The evolution of aphid life cycles. *Ann. Rev. Entomol.* **37**, 321-348.

Murdoch, W.W. (1975) Diversity, stability and pest control. *J. Appl. Ecol.* **12**, 795-807.

Nazzi, F., Powell, W., Wadhams, L.J. and Woodcock, C.M. (1996) Sex pheromone of aphid parasitoid *Praon volucre* (Hymenoptera, Braconidae). *J. Chem. Ecol.* **22**, 1169-1175.

Nemec, V. and Starý, P. (1983) Elpho-morph differentiation in *Aphidius ervi* Hal. biotype on *Microlophium carnosum* (Bckt.) related to parasitization on *Acyrthosiphon pisum* (Harr.) (Hym., Aphidiidae). *Z. Angew. Entomol.* **95**, 524-530.

Nemec, V. and Starý, P. (1984) Population diversity of *Diaeretiella rapae* (M'Int.) (Hym., Aphidiidae), an aphid parasitoid in agroecosystems. *Z. Angew. Entomol.* **97**, 223-233.

Nentwig, W. (1989) Augmentation of beneficial arthropods by strip-management II. Successional strips in a winter wheat field. *J. Plant Diseases and Protection* **96**, 89-99.

Neumann, U. (1992) Avoiding pitfalls in confusion: a review on mating disruption. *Bull. OILB-SROP* **15**, 10-17.

New, T.R. (1988) Neuroptera. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2B.* Eds: A.K. Minks and P. Harrewijn. pp. 249-258. Elsevier, Amsterdam.

Nijveldt, W. (1988) Cecidomyiidae. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2B.* Eds: A.K. Minks and P. Harrewijn. pp. 271-277. Elsevier, Amsterdam.

Noldus, L.P.J.J. (1988) Response of the egg parasitoid *Trichogramma pretiosum* to the sex pheromone of its host *Heliothis zea. Entomol. Exp. Appl.* **48**, 293-300.

Noldus, L.P.J.J. and van Lenteren, J.C. (1985) Kairomones for the egg parasite *Trichogramma evanescens* Westwood. I. Effect of volatile substances released by two of its hosts, *Pieris brassicae* L. and *Mamestra brassicae* L. *J. Chem. Ecol.* **11**, 781-792.

Noldus, L.P.J.J., van Lenteren, J.C. and Lewis, W.J. (1991a) How *Trichogramma* parasitoids use moth sex pheromones as kairomones: orientation behaviour in a wind tunnel. *Physiol. Entomol.* **16**, 313-327.

Noldus, L.P.J.J. and Potting, R.P.J. (1990) Calling behaviour of *Mamestra brassicae* (Lepidoptera: Noctuidae): effect of age and photoperiod. *Entomol. Exp. Appl.* **56**, 23-30.

Noldus, L.P.J.J., Potting, R.P.J. and Barendregt, H.E. (1991b) Moth sex pheromone adsorption to leaf surface: bridge in time for chemical spies. *Physiol. Entomol.* **16**, 329-344.

Norambuena, M.H. (1981) Introduction and establishment of parasitoids (Hymenoptera: Aphidiidae) for biological control of *Metopolophium dirhodum* (Walker) and *Sitobion avenae* (Fabricius) (Homoptera: Aphididae) in southern Chile. *Agricultura Tecnica (Chile)* **41**, 95-102.

Nordlund, D.A., Lewis, W.J. and Gueldner, R.C. (1983) Kairomones and their use for management of entomophagous insects XIV. Response of *Telenomus remus* to abdominal tips of *Spodoptera frugiperda*, (z)-9-tetradecene-1-ol acetate and (z)-9-dodecene-1-ol acetate. *J. Chem. Ecol.* **9**, 695-701.

Olkowski, W., Olkowski, H. and van den Bosch, R. (1982a) Linden aphid parasite establishment. *Environ. Entomol.* **11**, 1023-1025.

Olkowski, W., Olkowski, H., van den Bosch, R., Hom, R., Zuparko, R. and Klitz, W. (1982b) The parasitoid *Trioxys tenuicuaudus* Starý established on the elm aphid *Tinocallis platani* Kaltenbach in Berkeley, California. *Pan-Pacific Entomol.* **58**, 59-63.

Papaj, D.R., Snellen, H, Swaans, K. and Vet, L.E.M. (1994) Unrewarding experiences and their effect on foraging in the parasitic wasp *Leptopilina heterotoma* (Hymenoptera: Eucoilidae). *J. Ins. Behav.* **7**, 465-481.

Papaj, D.R. and Vet, L.E. M. (1990) Odour learning and foraging success in the parasitoid, *Leptopilina heterotoma*. *J. Chem. Ecol.* **16**, 3137-3150.

Payne, R.W., Lane, P.W. Todd, A.D., Digby, P.G.N., Tompson, R., Harding, S.A., Tunnicliffe-Wilson, G., Leech, P.K., Welham, S.J., Morgan, G.W. and White, R.P. (1983) *GENSTAT 5 Release 3 Reference Manual.* Clarenden Press, Oxford.

Payne, T.L., Dickens, J.C. and Richerson, J.V. (1984) Insect predator-prey coevolution via enantiomeric specificity in a kairomone-pheromone system. *J. Chem. Ecol.* **10**, 487-492.

Payne, T.L., Richerson, J.V., Dickens, J.C., West, J.R., Mori, K., Berisford, C.W., Heddon, R.L., Vité, J.P. and Blum, M.S. (1982) Southern pine beetle: olfactory receptor and behaviour discrimination of enantiomers of the attractant pheromone frontalin. *J. Chem. Ecol.* **8**, 873-882.

Pennacchio, F., Digilio, M.C., Tremblay, E. and Tranfaglia, A. (1994) Host recognition and acceptance behaviour in two aphid parasitoid species: *Aphidius ervi* and *Aphidius microlophii* (Hymenoptera: Braconidae). *Bull. Entomol. Res.* **84**, 57-64.

Pennacchio, F. and Tremblay, E. (1987) Biosystematic and morphological study of two *Aphidius ervi* Haliday (Hymenoptera, Braconidae) 'biotypes' with the description of a new species. *Boll. Lab. Ent. Agr. Filippo Silvestri* **43**, 105-117.

Perrin, R.M. (1975) The role of the perennial stinging nettle, *Urtica dioica*, as a reservoir of beneficial natural enemies. *Ann. Appl. Biol.* **81**, 289-297.

Perrin, R.M. and Phillips, M.L. (1978) Some effects of mixed cropping on the population dynamics of insect pests. *Entomol. Exp. Appl.* **24**, 585-593.

Pettersson, J. (1970) An aphid sex attractant. I. Biological studies. *Entomol. Scand.* **1**, 63-73.

Pettersson, J. (1971) An aphid sex attractant. II. Histological, ethological and comparative studies. *Entomol. Scand.* **2**, 81-93.

Pettersson, J., Pickett, J.A., Pye, B.J., Quiroz, A., Smart, L.E., Wadhams, L.J. and Woodcock, C.M. (1994) Winter host component reduces colonization by bird-cherry-oat aphid *Rhopalosiphum padi* (L.) (Homoptera, Aphididae), and other aphids in cereal fields. *J. Chem. Ecol.* **20**, 2565-2574.

Phillips, T.W., Nation, J.P., Wilkinson, R.C., Foltz, J.L., Pierce Jr, H.D. and Oehlschlager, A.C. (1990) Response specificity of *Dendroctonus terebrans* (Coleoptera: Scolytidae) to enantiomers of its sex pheromone. *Ann. Entomol. Soc. Amer.* **83**, 251-257.

Pickett, J.A., Wadhams, L.J. and Woodcock, C.M. (1997) Developing sustainable pest control from chemical ecology. *Agric. Ecosyst. Environ.* **64**, 149-156.

Pickett, J.A., Wadhams, L.J., Woodcock, C.M. and Hardie, J. (1992) The chemical ecology of aphids. *Ann. Rev. Entomol.* **37**, 67-90.

Pierce Jr, H.D., Pierce, A.M., Johnston, B.D., Oehlschlager, A.C. and Borden, J.H. (1988) Aggregation pheromone of square-necked grain beetle, *Cathartus quadricollis* (Guer.). *J. Chem. Ecol.* **14**, 2169, 2184.

Pike, K.S. and Harwood, R. (1989) Russian wheat aphid biological control program, state of Washington. *Proc. 3rd Russian Wheat Aphid Conf., 25-27 October, 1989, Albuquerque, N.M.* pp. 129-139.

Plumb, R.T. (1976) Barley yellow dwarf virus in aphids caught in suction traps 1969-1973. *Ann. Appl. Biol.* **83**, 53-59.

Plumb, R.T. (1983) Barley yellow dwarf virus- a global pattern. In: *Plant Virus Epidemiology* Eds: R.T. Plumb and J.M. Thresh. pp. 185-198. Blackwell Scientific Publications, Oxford.

Polaszek, A. (1986) The effects of two species of hymenopterous parasitoid on the reproductive system of the pea aphid, *Acyrthosiphon pisum*. *Entomol. Exp. Appl.* **40**, 285-292.

Polgár, L.A., Darvas, B. and Völkl, W. (1995) Induction of dormancy in aphid parasitoids: implications for enhancing their field effectiveness. *Agric. Ecosyst. Environ.* **52**, 19-23.

Pope, M.M., Gaston, L.K. and Baker, T.C. (1984) Composition, quantification and periodicity of sex pheromone volatiles from individual *Heliothis zea* females. *J. Insect Physiol.* **12**, 943-945.

Poppy, G.M., Powell, W. and Pennacchio, F. (1997) Aphid parasitoid responses to semiochemicals- genetic, conditioned or learnt? *Entomophaga* **42**, 193-199.

Powell, W. (1982) The identification of hymenopterous parasitoids attacking cereal aphids in Britain. *Syst. Entomol.* **7**, 465-473.

Powell, W. (1983) The role of parasitoids in limiting cereal aphid populations. In: *Aphid Antagonists.* Ed: R. Cavalloro. pp. 50-56. A.A. Balkema, Rotterdam.

Powell, W. (1986) Enhancing parasitoid activity in crops. In: *Insect Parasitoids. 13th Symp. R. Entomol. Soc. Lond.* Ed: J. Waage and D. Greathead. pp. 319-340. Academic Press, London.

Powell. W., Hardie, J., Hick, A.J., Höller, C., Mann, J., Merritt, L., Nottingham, S.F., Wadhams, L.J., Witthinrich, J. and Wright, A.F. (1993) Responses of the parasitoid *Praon volucre* (Hymenoptera: Braconidae) to aphid sex pheromone lures in cereal fields in autumn: implications for parasitoid manipulation. *Eur. J. Entomol.* **90**, 435-438.

Powell, W. and Wright, A.F. (1988) The abilities of the aphid parasitoids *Aphidius ervi* Haliday and *Aphidius rhopalosiphi* Perez (Hymenoptera: Braconidae) to transfer between different known host species and the implications for the use of alternative hosts in pest control strategies. *Bull. Entomol. Res.* **78**, 683-693.

Powell, W. and Wright, A.F. (1992) The influence of host food plants on host recognition by four aphidiine parasitoids (Hymenoptera: Braconidae). *Bull. Entomol. Res.* **81**, 449-453.

Powell, W. and Zhi-Li, Z. (1983) The reactions of two cereal aphid parasitoids, *Aphidius uzbekistanicus* and *A. ervi* to host aphids and their food-plants. *Physiol. Entomol.* **8**, 439-443.

Prokopy, R.J. and Webster, R.P. (1978) Oviposition-deterring pheromone of *Rhagoletis pomonella*. A kairomone for its parasitoid *Opius lectus*. *J. Chem. Ecol.* **4**, 481-494.

Pungerl, N.B. (1984) Host preferences of *Aphidius* (Hymenoptera: Aphidiidae) populations parasitising pea and cereal aphids (Hemiptera: Aphididae). *Bull. Entomol. Res.* **74**, 153-161.

Rabb, R.L., Stinner, R.E. and van den Bosch, R. (1976) Conservation and augmentation of natural enemies. In: *Theory and Practice of Biological Control* Eds: C.B. Huffaker and P.S. Messenger. pp. 233-254. Academic Press, New York.

Read, D.P., Feeny, P.P. and Root, R.B. (1970) Habitat selection by the aphid parasite *Diaeretiella rapae* (Hymenoptera: Braconidae) and hyperparasite *Charips brassicae* (Hymenoptera: Cynipidae). *Can. Entomol.* **102**, 1567-1578.

Reed, H.C., Tan, S.H., Haapanen, K., Killmon, M., Reed, D.K. and Elliot, N.C. (1995) Olfactory responses of the parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae) to odour of plants, aphids and plant-aphid complexes. *J. Chem. Ecol.* **21**, 407-418.

Rice, R.E. (1969) Response of some predators and parasites of *Ips paraconfusus* (Lec.) (Coleoptera: Scolytidae) to olfactory attractants. *Contrib. Boyce Thompson Inst.* **24**, 189-194.

Risch, S.J., Andow, D. and Altieri, M.A. (1983) Agroecosystem diversity and pest control: data, tentative conclusions, and new research directions. *Environ. Entomol.* **12**, 625-629.

Root, R.B. (1973) Organization of plant-arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). *Ecol. Monogr.* **43**, 94-125.

Rotundo, G. and Tremblay, E. (1975) Sull' attrattivitá delle femine vergini de due specie di Pseudococcidi per un imenottero parasita. *Boll. Lab. Entomol. Agr. Portici* **32**, 172-179.

Russell, E.P. (1989) Enemies hypothesis: a review of the effect of vegetational diversity on predatory insects and parasitoids. *Environ. Entomol.* **18**, 590-599.

Schaner, A.M., Tanico-Hogan, L.D. and Jackson, L.L. (1989) (s)-2-pentadecyl acetate and 2-pentadecone, components of aggregation pheromone of *Drosophila busckii*. *J. Chem. Ecol.* **15**, 2577-2588.

Schlinger, E.I. and Dietrick, E.J. (1960) Biological control of insect pests aided by strip-farming alfalfa in experimental program. *Calif. Agric.* **14**, 15.

Schuster, D.J. and Starks, K.J. (1974) Response of *Lysiphlebus testaceipes* in an olfactometer to a host and a non-host insect and to plants. *Environ. Entomol.* **3**, 1034-1035.

Shands, W.A., Simpson, G.W. and Simpson, B.A. (1975) Evaluation of field introductions of two insect parasites (Hymenoptera: Braconidae) for controlling potato-infesting aphids. *Environ. Entomol.* **4**, 499-503.

Shaposhnikov, G. Ch. (1987) Evolution of aphids in relation to evolution of plants. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2A.* Eds: A.K. Minks and P. Harrewijn. pp. 409-414. Elsevier, Amsterdam.

Sheehan, W. (1986) Response by specialist and generalist natural enemies to agroecosystem diversification: a selective review. *Environ. Entomol.* **15**, 456-461.

Sheehan, W. and Shelton, A.M. (1989a) Parasitoid response to concentration of herbivore food plants: finding and leaving plants. *Ecology* **70**, 993-998.

Sheehan, W. and Shelton, A.M. (1989b) The role of experience in plant foraging by the aphid parasitoid *Diaeretiella rapae* (Hymenoptera: Aphidiidae). *J. Insect. Behav.* **2**, 743-759.

Shirota, Y., Carter, N., Rabbinge, R. and Ankersmit, G.W. (1983) Biology of *Aphidius rhopalosiphi*, a parasitoid of cereal aphids. *Entomol. Exp. Appl.* **34**, 27-34.

Singh, R. and Sinha, T.B. (1982) Bionomics of *Trioxys* (*Binodoxys*) *indicus* Subba Rao and Sharma, an aphidiid parasitoid of *Aphis craccivora* Koch. XIII. Host selection by the parasitoid. *Z. Angew. Entomol.* **93**, 64-75.

Singh, R. and Srivastava, M. (1987) Bionomics of *Trioxys indicus* Subba Rao and Sharma, an aphidiid parasitoid of *Aphis craccivora* Koch. 25. The role of host haemolymph in the host stage preference by the parasitoid. *G. Ital. Entomol.* **3**, 409-412.

Smart, L.E., Blight, M.M., Pickett, J.A. and Pye, B.J. (1994) Development of field strategies incorporating semiochemicals for the control of the pea and bean weevil, *Sitona lineatus* L. *Crop Protection* **13**, 127-135.

Smith, J.C. (1976) Influence of crop background on natural enemies of aphids on Brussels sprouts. *Ann. Appl. Biol.* **83**, 15-29.

Stinner, R.E. (1966) Efficacy of inundative releases. *Ann. Rev. Entomol.* **22**, 515-531.

Starý, P. (1970) *Biology of Aphid Parasites (Hymenoptera: Aphidiidae) with Respect to Integrated Control.* Dr. W. Junk, The Hague

Starý, P. (1974) Parasite spectrum (Hym., Aphidiidae) of aphids associated with *Galium*. *Entomol. Scand.* **5**, 73-80.

Starý, P. (1976) Aphid parasitoids (Hymenoptera, Aphidiidae) of the Mediterranean area. *Trans. Czechosl. Acad. Sci., Ser. Math. Nat. Sci.* **86**. 95pp. Dr. W. Junk, The Hague.

Starý, P. (1983) The perennial stinging nettle (*Urtica dioica*) as a reservoir of aphid parasitoids (Hymenoptera, Aphidiidae). *Acta. Entomol. Bohemoslov.* **80**, 81-86.

Starý, P. (1987) Subject bibliography of aphid parasitoids (Hymenoptera: Aphidiidae) of the world 1758-1982. *Monogr. Appl. Entomol.* **25**, 101pp.

Starý, P. (1988a) Aphelinidae. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2B.* Eds: A.K. Minks and P. Harrewijn. pp. 185-188. Elsevier. Amsterdam.

Starý, P. (1988b) Aphidiidae. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2B.* Eds: A.K. Minks and P. Harrewijn. pp. 171-184. Elsevier. Amsterdam.

Steffan, A.W. (1987) Fern-und nahorientierung geflugelter gynoparae und sexualis-mannchen bie blatthousen (Homoptera: Aphidinea: Aphididae). *Entomol. Gener.* **15**, 33-49.

Steffan, A.W. (1990) Courtship behaviour and possible pheromone spread by hindleg raising in sexual females of aphids (Homoptera: Aphidinea). *Entomol. Gener.* **15**, 33-49.

Steinberg, S. Dicke, M. and Vet, L.E.M. (1993) Relative importance of infochemicals from first and second trophic level in long-range host location by the larval parasitoid *Cotesia glomerata*. *J. Chem. Ecol.* **19**, 47-59.

Steinberg, S., Dicke, M., Vet, L.E.M. and Wanningen, R. (1992) Response of the braconid parasitoid *Cotesia* (= *Apanteles*) *glomerata* to volatile infochemicals: effects of bioassay set-up, parasitoid age and experience and barometric flux. *Entomol. Exp. Appl.* **63**, 163-175.

Stephen, F.M. and Dahlsten, D.L. (1976) The arrival sequence of the arthropod complex following attack of *Dendroctonus brevicomis* in ponderosa pine. *Can. Entomol.* **108**, 282-304.

Sternlicht, M. (1973) Parasitic wasps attracted by the sex pheromone of their coccid host. *Entomophaga* **18**, 339-342.

Strand, M.R. and Vinson, S.B. (1982) Behavioural response of the parasitoid *Cardiochiles nigriceps* to a kairomone. *Entomol. Exp. Appl.* **31**, 308-315.

Sullivan, D.J. (1987) Insect hyperparasitism. *Ann. Rev. Entomol.* **32**, 49-70.

Sullivan, D.J. (1988) Hyperparasites. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2B.* Eds: A.K. Minks and P. Harrewijn. pp. 189-203. Elsevier, Amsterdam.

Sullivan, D.J. and van den Bosch, R. (1971) Field ecology of the primary parasites and hyperparasites of the potato aphid, *Macrosiphum euphorbiae*, in the east San Francisco Bay area. *Ann. Entomol. Soc. Amer.* **64**, 389-394.

Sunderland, K.D. (1988) Carabidae and other invertebrates. In: *World Crop Pests. Aphids. Their Biology, Natural Enemies and Control. Vol. 2B.* Eds: A.K. Minks and P. Harewijn. pp. 293-310. Elsevier, Amsterdam.

Süss, L. (1983) Survival of pupal stage of *Aphidius ervi* Hal. in mummified *Sitobion avenae* F. to pesticide treatment. In: *Aphid Antagonists*. Ed: R. Cavalloro. pp. 129-134. A.A. Balkema, Rotterdam.

Swedenborg, P.D., Jones, R.L., Zou, H-Q, Shin, I. and Liu, H-W. (1994) Biological activity of (3R,5S,6R)- and (3S,5R,6S)-3-5-dimethyl-6(methylethyl)-3,4,5,6-tetrahydropyran-2-one, a pheromone of *Macrocentrus grandii* (Goidanich) (Hymenoptera: Braconidae). *J. Chem. Ecol.* **20**, 3373-3380.

Sylvester, E.S. (1989) Viruses transmitted by aphids. In: *World Crop Pests. Aphids. Their Biology, natural Enemies and Control. Vol. 2C.* Eds: A.K. Minks. and P. Harrewijn. pp. 65-87. Elsevier, Amsterdam.

Takabayashi, J. and Takahashi, S. (1988) Effect of kairomone on the parasitization rates of *Apanteles kariyai* to *Pseudaletia separata. J. Pesticide. Sci.* **13**, 283-286.

Takada, H. (1975) Differential preference for *Myzus persicae* (Sulzer) of two parasites, *Diaeretiella rapae* (M'Intosh) and *Aphidius gifuensis* Ashmead. *Jap. J. Appl. Entomol. Zool.* **19**, 260-266.

Tanigoshi, L.K., Pike, K.S., Miller, R.H., Miller, T.D. and Allison, D. (1995) Search for, and release of, parasitoids for the biological control of Russian wheat aphid in Washington state (USA). *Agric. Ecosyst. Environ.* **52**, 25-30.

Tashiro, H. and Chambers, D.L. (1967) Reproduction in California red scale *Aonidiella aurantii* (Homoptera: Diaspididae). I. Discovery and extraction of a female sex pheromone. *Ann. Entomol. Soc. Amer.* **60**, 1166-1170.

Tatchell, G.M. (1989) An estimate of the potential economic losses to some crops due to aphids in Britain. *Crop Prot.* **8**, 25-29.

Thomas, M.B. and Wratten, S.D. (1988) Manipulating the arable crop environment to enhance the activity of predatory insects. *Aspects. Appl. Biol.* **17**, 57-66.

Thomson, M.S. and Stinner, R.E. (1990) The scale response of *Trichogramma* (Hymenoptera: Trichogrammatidae): variation among species in host specificity, and the effect of conditioning. *Entomophaga* **35**, 7-21.

Tingle, F.C., Mitchell, E.R. and Heath, R.R. (1989) Mating and oviposition by *Cotesia* (=*Apanteles*) *marginiventris* (Hymenoptera: Braconidae) in presence of synthetic sex pheromone of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *J. Chem. Ecol.* **15**, 2045-2050.

Titayavan, M. and Altieri, M.A. (1990) Synomone-mediated interactions between the parasitoid *Diaeretiella rapae* and *Brevicoryne brassicae* under field conditions. *Entomophaga* **35**, 499-507.

Turlings, T.C.J., Tumlinson, J.H., Eller, F.J. and Lewis, W.J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* **250**, 1251-1253.

Turlings, T.C.J., Wäckers, F.L., Vet, L.E.M., Lewis, W.J. and Tumlinson, J.H. (1993) Learning of host-finding cues by hymenopterous parasitoids. In: *Insect Learning. Ecological and Evolutionary Perspectives*. Eds: D.R. Papaj and A.C. Lewis. pp. 51-78. Chapman and Hall, New York.

Umoru, P.A., Powell, W. and Clark, S.J. (1996) Effect of pirimicarb on the foraging behaviour of *Diaeretiella rapae* (Hymenoptera: Braconidae) on host-free and infested oilseed rape plants. *Bull. Entomol. Res.* **86**, 193-201

Unruh, T.R., White, W., González, D, Gordh, G. and Luck, R.F. (1983) Heterozygosity and effective size in laboratory populations of *Aphidius ervi* (Hym.: Aphidiidae). *Entomophaga* **28**, 245-258.

Vater, G. (1971) Über ausbreitung und orientierung von *Diaeretiella rapae* (Hymenoptera, Aphidiidae) unter berücksichtigung der hyperparasiten von *Brevicoryne brassicae* (Homoptera, Aphididae). *Z. Angew. Entomol.* **68**, 187-225.

Vaughn, T.T., Antolin, M.F. and Bjostad, L.B. (1996) Behavioural and physiological responses of *Diaeretiella rapae* to semiochemicals. *Entomol. Exp. Appl.* **78**, 187-196.

Vet, L.E.M. and Dicke, M. (1992) Ecology of infochemical use by natural enemies in a tritrophic context. *Ann. Rev. Entomol.* **37**, 141-172.

Vet, L.E.M. and Groenewold, A.W. (1990) Semiochemicals and learning in parasitoids. *J. Chem. Ecol.* **16**, 3119-3135.

Vet, L.E.M., van Lenteren, J.C., Heymans, M. and Meelis, E. (1983) An olfactometer for measuring olfactory responses of hymenopterous parasitoids and other small insects. *Physiol. Entomol.* **8**, 97-106.

Vet, L.E.M., Lewis, W.J. and Cardé, R.T. (1995) Parasitoid foraging and learning. In: *Chemical Ecology of Insects 2*. Eds: R.T. Cardé and W.J. Bell. pp. 65-101. Chapman and Hall, New York.

Vet, L.E.M., Lewis, W.J., Papaj, D.R. and van Lenteren, J.C. (1990a) A variable-response model for parasitoid foraging behaviour. *J. Ins. Behav.* **3**, 471-490.

Vet, L.E.M., De Jong, R., van Giessen, W.A. and Visser, J.H. (1990b) A learning related variation in electroantennogram responses of a parasitic wasp. *Physiol. Entomol.* **15**, 243-247.

Vickerman, G.P. (1982) Distribution and abundance of cereal aphid parasitoids (*Aphidius* spp.) on grassland and winter wheat. *Ann. Appl. Biol.* **101**, 185-190.

Vickerman, G.P. and Wratten, S.D. (1979) The biology and pest status of cereal aphids (Hemiptera: Aphididae) in Europe: a review. *Bull. Entomol. Res.* **69**, 1-32.

Vinson, S.B. (1976) Host selection by insect parasitoids. *Ann. Rev. Entomol.* **21**, 109-133.

Vinson, S.B. (1981) Host location. In: *Semiochemicals: Their Role in Pest Control.* Eds: D.A. Nordlund, R.L. Jones and W.J. Lewis. pp. 51-77. John Wiley and Sons, New York.

Vinson, S.B. (1984) Parasitoid-host relationship. In: *Chemical Ecology of Insects.* Eds: W.J. Bell and R.T. Cardé. pp. 205-233. Chapman and Hall, New York.

Vinson, S.B. and Iwantsch, G.F. (1980a) Host regulation by insect parasitoids. *Q. Rev. Biol.* **55**, 143-165.

Vinson, S.B. and Iwantsch, G.F. (1980b) Host suitability for insect parasitoids. *Ann. Rev. Entomol.* **25**, 397-419.

Vité, J.P. and Williamson, D.L. (1970) *Thanasimus dubius*: prey selection. *J. Insect Physiol.* **16**, 233-237.

Völkl, W. (1992) Aphids or their parasitoids: who actually benefits from ant-attendance? *J. Anim. Ecol.* **61**, 273-281.

Vorley, W.T. (1986) The activity of parasitoids (Hymenoptera: Braconidae) of cereal aphids (Hemiptera: Aphididae) in winter and spring in southern England. *Bull. Entomol. Res.* **76**, 491-504.

Vorley, W.T. and Wratten, S.D. (1987) Migration of parasitoids (Hymenoptera: Braconidae) of cereal aphids (Hemiptera: Aphididae) between grassland, early-sown cereals and late-sown cereals in southern England. *Bull. Entomol. Res.* **77**, 555-568.

Walters, P.J. and Dominiak, B.C. (1984) The establishment in New South Wales of *Trioxys complanatus* Quilis (Hymenoptera: Aphidiidae), an imported parasite of *Therioaphis trifolii* (Monell) f. *maculata*, spotted alfalfa aphid. *Gen. Appl. Entomol.* **16**, 65-67.

Walton, M.P., Powell, W., Loxdale, H.D. and Allen-Williams, L. (1990) Electrophoresis as a tool for estimating levels of hymenopterous parasitism in field populations of the grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae). *Entomol. Exp. Appl.* **54**, 271-279.

Weseloh, R.M. (1981) Host location by parasitoids. In: *Semiochemicals: Their Role in Pest Control.* Eds: D.A. Nordlund, R.L. Jones and W.J. Lewis. pp. 79-95. John Wiley and Sons, New York.

Wickremasinghe, M.G.V. and van Emden, H.F. (1992) Reactions of adult female parasitoids, particularly *Aphidius rhopalosiphi*, to volatile chemical cues from the host plants of their aphid prey. *Physiol. Entomol.* **17**, 297-304.

Wilson, C.G., Swincer, D.E. and Walden, K.J. (1982) The introduction of *Trioxys complanatus* Quilis (Hymenoptera: Aphidiidae), an internal parasite of the spotted alfalfa aphid, into South Australia. *J. Aust. Entomol. Soc.* **21**, 13-27.

Winfield, A.L. (1992) Management of oilseed rape pests in Europe. *Agric. Zool. Rev.* **5**, 51-95.

Wiskerke, J.S.C., Dicke, M. and Vet, L.E.M. (1993) Larval parasitoids use aggregation pheromone of adult hosts in foraging behaviour: a solution to the reliability-detectability problem. *Oecologia* **93**, 145-148.

Wood, D.L., Browne, L.E., Bedard, W.D., Tilden, P.E., Silverstein, R.M. and Rodin, J.O. (1968) Response of *Ips confusus* to synthetic sex pheromones in nature. *Science* **159**, 1373.

Wratten, S.D. and Powell, W. (1991) Cereal aphids and their natural enemies. In: *The Ecology of Temperate Cereal Fields. 32nd Symp. Brit. Ecol. Soc.* Eds: L.G. Fairbank, N. Carter, J.F. Darbyshire and G.R. Potts. pp. 233-257. Blackwell, Oxford.

Zaborski, E. Teal, P.E.A. and Laing, J.E. (1987) Kairomone-mediated host-finding by spruce budworm egg parasite, *Trichogramma minutum*. *J. Chem. Ecol.* **13**, 113-122.

Zaki, F.N. (1985) Reactions of the egg parasitoid *Trichogramma evanescens* Westw. to certain insect pheromones. *Z. Angew. Entomol.* **99**, 448-453.

Zaki, F.N. (1996) Effect of some kairomones and pheromones on two hymenopterous parasitoids *Apanteles ruficrus* and *Microplitis demolitor* (Hym., Braconidae). *J. Appl. Entomol* **120**, 555-557.

Appendix

ANOVA	d.f	s.e.d.	F	P
Aphidius rhopalosiphi				
Time to take-off	6	13.76	0.05	>0.05
% taking off	6	0.433	1.70	>0.05
% oriented flight	6	0.351	7.68	<0.01
Aphidius eadyi				
Time to take-off	6	13.79	0.61	>0.05
% taking off	6	0.433	1.70	>0.05
% oriented flight	6	0.351	7.68	<0.01
Ephedrus plagiator				
Time to take-off	6	6.30	0.64	>0.05
% taking off	6	0.411	0.27	>0.05
% oriented flight	6	0.519	7.43	<0.05

Table A1 ANOVA results for wind tunnel experiments (section 2.3). d.f.= residual degrees of freedom, s.e.d.= standard error of the difference of the means, F= value of F, P= probability.

ANOVA	d.f	s.e.d.	F	P
Praon myzophagum (*A. pisum*)				
Time to take-off	12	8.910	1.81	>0.05
% taking off	12	0.323	0.77	>0.05
% oriented flight	12	0.308	8.85	<0.001
% landing on target	12	0.426	4.02	<0.05
Praon myzophagum (*M. persicae*)				
Time to take-off	12	8.880	1.57	>0.05
% taking off	12	0.403	1.30	>0.05
% oriented flight	12	0.350	3.53	<0.05
% landing on target	12	0.390	1.21	>0.05

Table A2 ANOVA results for flight response of *Praon myzophagum* reared on two different hosts (section 2.4). d.f.= residual degrees of freedom, s.e.d.= standard error of the difference of the means, F= value of F, P= probability.

ANOVA	d.f	s.e.d.	F	P
Praon volucre (*A. pisum*)				
Time to take-off	2	3.740	1.21	>0.05
% oriented flight	2	0.214	7.22	<0.05
% landing on target	2	0.473	1.0	>0.05
Praon volucre (*S. avenae*)				
Time to take-off	2	5.310	3.13	>0.05
% taking off	2	0.630	0.09	>0.05
% oriented flight	2	0.195	95.17	<0.001
% landing on target	2	0.327	7.0	>0.05

Table A3 ANOVA results for flight response of *Praon volucre* reared on two different hosts (section 2.4). d.f.= residual degrees of freedom, s.e.d.= standard error of the difference of the means, F= value of F, P= probability.

ANOVA	d.f	s.e.d.	F	P
Praon volucre -females				
Time to take-off	2	8.370	0.47	>0.05
% taking off	2	0.082	1.0	>0.05
% oriented flight	2	0.310	23.22	<0.05
Praon volucre -males				
Time to take-off	2	9.380	0.54	>0.05
% taking off	2	0.098	1.0	>0.05
% oriented flight	2	0.163	1.0	>0.05

Table A4 ANOVA results for response of *Praon volucre* to synthetic and plant-extracted nepetalactone (section 2.5). d.f.= residual degrees of freedom, s.e.d.= standard error of the difference of the means, F= value of F, P= probability.

ANOVA	d.f	s.e.d.	F	P
Response of *Praon myzophagum*				
Time to take-off	6	8.81	0.82	>0.05
% taking off	6	0.389	1.67	>0.05
% oriented flight	6	0.549	2.11	>0.05
% landing on target	6	0.326	14.2	<0.01

Table A5 ANOVA results for response of *Praon myzophagum* exposed to sex pheromone at emergence (section 3.4). d.f.= residual degrees of freedom, s.e.d.= standard error of the difference of the means, F= value of F, P= probability.

ANOVA	d.f	s.e.d.	F	P
Response of *Aphidius ervi*				
Time to take-off	6	16.05	1.29	>0.05
% taking off	6	0.518	5.66	<0.05
% oriented flight	6	0.124	84.57	<0.001
% landing on target	6	0.436	8.04	<0.05

Table A6 ANOVA results for flight response of *Aphidius ervi* to sex pheromone and host-plant complex- experiment I (section 4.3). d.f.= residual degrees of freedom, s.e.d.= standard error of the difference of the means, F= value of F, P= probability.

ANOVA	d.f	s.e.d.	F	P
Response of *Aphidius ervi*				
Time to take-off	6	11.75	0.43	>0.05
% taking off	6	0.376	0.93	>0.05
% oriented flight	6	0.233	14.55	<0.001
% landing on target	6	0.615	2.45	>0.05

Table A7 ANOVA results for flight response of *Aphidius ervi* to sex pheromone and host-plant complex- experiment II (section 4.3). d.f.= residual degrees of freedom, s.e.d.= standard error of the difference of the means, F= value of F, P= probability.

ANOVA	d.f	s.e.d.	F	P
20cm Experiment				
% aphid recovery	3	17.8	0.35	>0.05
No. *Praon* mummies	3	18.8	15.73	<0.001
1m Experiment 1997				
% aphid recovery	3	7.90	0.15	>0.05
No. *Praon* mummies	3	9.01	10.45	<0.001
No. *Aphidius* mummies	3	9.39	7.51	<0.01
3m Experiment				
% aphid recovery	3	6.16	0.71	>0.05
No. *Praon* mummies	3	13.1	4.28	<0.05
Synthetic nepetalactone experiment				
% aphid recovery	4	2.52	0.06	>0.05
No. *Praon* mummies	4	17.5	4.84	<0.01

Table A8 ANOVA results for pheromone-baited potted plant experiments (sections 5.2 and 5.3). d.f.= residual degrees of freedom, s.e.d.= standard error of the difference of the means, F= value of F, P= probability.

ANOVA	d.f	s.e.d.	F	P
Capture of *D. rapae* 1995				
Females	18	0.066	8.76	<0.001
Males	18	0.069	3.46	<0.05

Table A9 ANOVA results for capture of *Diaeretiella rapae* in pheromone-baited traps in 1995 (section 5.4). d.f.= residual degrees of freedom, s.e.d.= standard error of the difference of the means, F= value of F, P= probability.

ANOVA	d.f	s.e.d.	F	P
Capture of *D. rapae* 1996				
Females	12	0.106	7.63	<0.01
Males	12	0.093	1.02	>0.05

Table A10 ANOVA results for capture of *Diaeretiella rapae* in pheromone-baited traps in 1996 (section 5.4). d.f.= residual degrees of freedom, s.e.d.= standard error of the difference of the means, F= value of F, P= probability.

	% Aphid recovery at each replicate date						
Potted plant	29.8.96	6.9.96	12.9.96	29.8.96	6.9.96	12.9.96	Mean
P_p	28.0	42.5	30.5	28.0	32.5	21.5	30.5
P_{20}	23.0	61.0	26.5	73.5	63.5	19.0	44.4
C_1	15.0	50.5	0.0	46.0	83.5	10.5	34.3
C_2	2.0	90.0	39.5	26.0	70.0	18.0	40.9

Table A11 Aphid recovery from 20 cm potted plant experiment (section 5.2). See figure 5.1 for explanation of potted plant treatments and positions.

	Total no. mummies at each replicate date						
Potted plant	29.8.96	6.9.96	12.9.96	29.8.96	6.9.96	12.9.96	Mean
P_p	25	42	18	28	11	31	25.8
P_{20}	28	24	26	42	23	26	28.2
C_1	0	0	0	12	2	3	2.8
C_2	0	21	0	11	0	0	5.3

Table A12 Number of mummies recorded from plants from 20 cm experiment (section 5.2). See figure 5.1 for explanation of potted plant treatments and positions

Potted plant	% Aphid recovery at each replicate date 26.9.96	10.10.96	18.10.96	25.10.96	Mean
P_p	14.5	26.0	30.5	49.0	30.0
P_{1mI}	9.5	9.5	13.0	39.0	17.8
P_{1mII}	11.5	28.0	33.1	35.0	26.0
P_{1mIII}	18.0	34.0	50.5	37.5	35.0
P_{1mIV}	17.0	35.5	36.5	24.0	28.4
C_i	17.5	40.0	30.5	38.0	31.5
C_{1mI}	7.0	26.5	48.0	22.0	25.9
C_{1mII}	22.5	30.5	37.5	46.0	34.1
C_{1mIII}	16.0	36.0	43.5	41.5	34.3
C_{1mIV}	8.5	33.0	40.0	40.5	30.5

Table A13 Aphid recovery from 1m potted plant experiment 1996 (section 5.2). See figure 5.1 for explanation of potted plant treatments and positions.

Potted plant	Total no. *Praon* spp. mummies at each replicate date 26.9.96	10.10.96	18.10.96	25.10.96	Mean
P_p	9	7	10	28	13.5
P_{1mI}	11	8	2	7	7.0
P_{1mII}	0	5	0	29	8.5
P_{1mIII}	16	6	3	4	7.3
P_{1mIV}	17	1	1	5	6.0
C_i	5	5	0	8	4.5
C_{1mI}	4	0	0	0	1.0
C_{1mII}	0	0	1	1	0.5
C_{1mIII}	5	0	1	0	1.5
C_{1mIV}	0	0	3	0	0.8

Table A14 Number of *Praon* spp. mummies recorded from plants from 1m potted plant experiment 1996 (section 5.2). See figure 5.1 for explanation of potted plant treatments and positions.

Potted plant	Total no. *Aphidius* spp. mummies at each replicate date 26.9.96	10.10.96	18.10.96	25.10.96	Mean
P_p	1	3	19	0	5.8
P_{1mI}	0	2	23	1	6.5
P_{1mII}	2	2	3	3	2.5
P_{1mIII}	1	1	13	2	4.3
P_{1mIV}	4	4	11	0	4.3
C_i	0	0	3	0	0.8
C_{1mI}	0	1	1	1	0.8
C_{1mII}	0	1	0	3	1.0
C_{1mIII}	3	0	0	0	0.8
C_{1mIV}	0	1	3	0	1.0

Table A15 Number of *Aphidius* spp. mummies recorded from plants from 1m potted plant experiment 1996 (section 5.2). See figure 5.1 for explanation of potted plant treatments and positions.

Potted plant	% Aphid recovery at each replicate date 12.7.97	15.7.97	19.7.97	25.7.97	2.8.97	6.8.97	Mean
P_p	45.0	28.0	26.5	26.5	44.0	68.0	39.6
P_{1mI}	42.5	31.0	23.5	23.5	46.5	59.5	37.8
P_{1mII}	38.0	38.5	19.0	16.0	54.0	57.0	37.1
P_{1mIII}	24.0	24.0	32.5	33.5	38.0	64.0	36.0
P_{1mIV}	39.0	25.5	14.5	27.5	40.5	57.5	34.1
C_i	26.5	33.0	22.2	22.2	55.0	61.5	37.6
C_{1mI}	23.5	22.5	10.5	25.5	53.0	67.5	33.8
C_{1mII}	61.5	29.5	33.5	32.5	38.0	53.0	41.3
C_{1mIII}	42.0	25.0	26.0	18.0	42.5	57.0	35.1
C_{1mIV}	46.0	31.0	20.5	35.5	39.5	50.5	37.2

Table A16 Aphid recovery from 1m potted plant experiment 1997 (section 5.2). See figure 5.1 for explanation of potted plant treatments and positions.

Potted plant	Total no. *Praon* mummies at each replicate date 12.7.97	15.7.97	19.7.97	25.7.97	2.8.97	6.8.97	Mean
P_p	14	16	14	10	32	15	16.8
P_{1mI}	4	8	18	6	12	5	8.3
P_{1mII}	0	4	2	6	2	3	2.8
P_{1mIII}	6	14	4	2	4	0	5.0
P_{1mIV}	6	0	12	6	4	0	4.6
C_i	10	0	0	4	8	5	4.5
C_{1mI}	12	4	0	0	0	2	3.0
C_{1mII}	6	4	0	0	0	0	1.7
C_{1mIII}	10	0	0	2	6	0	3.0
C_{1mIV}	10	6	0	0	2	4	3.7

Table A17 Number of *Praon* spp. mummies recorded from plants from 1m potted plant experiment 1997 (section 5.2). See figure 5.1 for explanation of potted plant treatments and positions.

Potted plant	Total no. *Aphidius* mummies at each replicate date 12.7.97	15.7.97	19.7.97	25.7.97	2.8.97	6.8.97	Mean
P_p	23	31	15	11	1	7	14.7
P_{1mI}	13	7	23	13	4	4	10.6
P_{1mII}	13	14	5	9	16	2	9.8
P_{1mIII}	11	16	11	9	8	1	9.3
P_{1mIV}	19	12	13	9	20	3	12.6
C_i	3	2	0	0	0	3	1.3
C_{1mI}	5	0	0	0	2	0	1.2
C_{1mII}	7	0	0	2	0	0	1.5
C_{1mIII}	3	0	0	4	4	0	1.8
C_{1mIV}	5	0	0	2	2	0	1.5

Table A18 Number of *Aphidius* spp. mummies recorded from plants from 1m potted plant experiment 1997 (section 5.2). See figure 5.1 for explanation of potted plant treatments and positions.

Potted plant	% Aphid recovery at each replicate date 15.8.97	5.9.97	12.9.97	18.9.97	26.9.97	Mean
P_p	49.5	35.0	56.0	43.5	55.5	47.9
P_{1mI}	56.5	40.5	49.0	28.0	43.5	43.5
P_{1mII}	39.0	46.0	51.0	39.0	45.5	44.1
P_{1mIII}	44.5	33.5	43.5	45.5	44.0	42.2
P_{1mIV}	51.0	44.0	49.0	41.5	46.5	46.4
C_i	48.5	43.0	49.5	47.0	51.5	47.9
C_{1mI}	60.5	45.5	54.5	38.5	47.0	49.2
C_{1mII}	48.0	39.5	43.5	42.0	53.5	45.3
C_{1mIII}	42.0	41.0	49.5	39.5	43.0	43.0
C_{1mIV}	46.0	39.0	40.0	49.0	45.5	43.9

Table A19 Aphid recovery from 3m potted plant experiment (section 5.2). See figure 5.1 for explanation of potted plant treatments and positions.

Potted plant	Total no. *Praon* mummies at each replicate date 15.8.97	5.9.97	12.9.97	18.9.97	26.9.97	Mean
P_p	15	21	46	11	49	28.4
P_{1mI}	7	3	19	4	26	11.8
P_{1mII}	5	2	13	1	20	8.2
P_{1mIII}	0	5	16	2	17	8.0
P_{1mIV}	3	11	19	3	6	8.4
C_i	0	9	17	2	17	9.0
C_{1mI}	10	3	7	9	13	8.4
C_{1mII}	0	5	12	6	27	10.0
C_{1mIII}	5	10	4	8	14	8.2
C_{1mIV}	1	5	13	5	23	9.4

Table A20 Number of *Praon* spp. mummies recorded from plants from 3m potted plant experiment (section 5.2). See figure 5.1 for explanation of potted plant treatments and positions.

Potted plant	% Aphid recovery at each replicate date 20.8.97	5.9.97	5.9.97	12.9.97	12.9.97
A	60.0	39.5	57.0	60.5	49.0
B	56.5	50.5	61.5	58.0	43.0
C	54.5	60.5	54.5	53.5	40.5
D	49.0	56.5	53.5	59.9	47.0
E	52.0	41.0	57.0	55.5	42.0

	% Aphid recovery at each replicate date (Cont.) 18.9.97	18.9.97	18.9.97	26.9.97	26.9.97	Mean
A	39.0	60.5	28.5	48.5	50.5	49.3
B	46.5	56.5	31.0	42.5	56.0	50.2
C	50.0	59.5	24.5	39.5	53.5	49.0
D	43.5	53.5	25.0	45.5	58.5	49.2
E	45.5	59.5	20.5	45.5	60.5	47.7

Table A21 Aphid recovery from synthetic nepetalactone experiment (section 5.3). Treatments; A= 98% (7R) nepetalactone, B= 99% (7S) nepetalactone, C= 50% (7S)/50% (7R) nepetalactones, D= plant-extracted nepetalactone, E= control

Potted plant	Total no. *Praon* mummies at each replicate date 20.8.97	5.9.97	5.9.97	12.9.97	12.9.97
A	18	3	4	8	20
B	45	1	32	85	41
C	1	0	0	30	18
D	2	11	23	19	43
E	2	0	0	8	19

	Total no. *Praon* mummies at each replicate date (Cont.) 18.9.97	18.9.97	18.9.97	26.9.97	26.9.97	Mean
A	20	1	3	26	33	9.6
B	19	77	12	8	67	39.0
C	4	4	1	26	41	7.3
D	4	50	10	30	44	20.3
E	13	0	5	10	16	5.9

Table A22 Number of *Praon* spp. mummies recorded from plants from synthetic nepetalactone experiment (section 5.3). Treatments; A= 98% (7R) nepetalactone, B= 99% (7S) nepetalactone, C= 50% (7S)/50% (7R) nepetalactones, D= plant-extracted nepetalactone, E= control

	Total no. *D. rapae* males and females captured in each trap							
	A		B		C		D	
Date	M	F	M	F	M	F	M	F
29.6-30.6.95	3	14	3	11	2	9	2	21
7.7-10.7.95	7	81	5	99	7	93	2	45
10.7.13.7-95	14	207	2	37	7	83	7	98
13.7-16.7.95	2	59	3	36	6	66	5	52
Mean	6.5	90.3	3.3	45.8	5.5	62.8	4.0	54.0

Table A23 Capture of *Diaeretiella rapae* in pheromone-baited water traps in 1995 (section 5.4). Site A. A= nepetalactone, B= nepetalactol, C= 1:1 ratio nepetalactone:nepetalactol, D= control.

	Total no. *D. rapae* males and females captured in each trap							
	A		B		C		D	
Date	M	F	M	F	M	F	M	F
29.6-30.6.95	4	16	3	12	3	7	1	0
7.7-10.7.95	4	72	2	68	5	94	2	20
10.7.13.7-95	3	50	3	38	2	28	1	26
13.7-16.7.95	1	25	0	12	0	12	0	7
Mean	3.0	40.8	2.0	32.5	2.5	35.3	1.0	13.3

Table A24 Capture of *Diaeretiella rapae* in pheromone-baited water traps in 1995 (section 5.4). Site B. A= nepetalactone, B= nepetalactol, C= 1:1 ratio nepetalactone:nepetalactol, D= control.

	Total no. *D. rapae* males and females captured in each trap							
	A		B		C		D	
Date	M	F	M	F	M	F	M	F
29.6-30.6.95	7	22	5	37	6	54	1	14
7.7-10.7.95	10	119	3	58	6	119	5	54
10.7.13.7-95	5	89	11	138	1	36	7	88
13.7-16.7.95	2	36	2	34	1	34	1	14
Mean	6.0	66.5	5.3	66.8	3.5	60.8	3.5	42.5

Table A25 Capture of *Diaeretiella rapae* in pheromone-baited water traps in 1995 (section 5.4). Site C. A= nepetalactone, B= nepetalactol, C= 1:1 ratio nepetalactone:nepetalactol, D= control.

	Total no. *D. rapae* males and females captured in each trap							
	A		B		C		D	
Date	M	F	M	F	M	F	M	F
8.8-12.8.96	4	11	1	4	3	12	1	6
12.8-14.8.96	2	8	1	9	2	2	0	0
14.8.16.8-96	6	29	7	23	3	21	4	16
16.8-18.8.96	5	29	4	16	1	13	5	15
Mean	3.8	19.8	3.3	13.0	2.3	12.0	2.5	9.3

Table A26 Capture of *Diaeretiella rapae* in pheromone-baited water traps in 1996 (section 5.4). Site A. A= nepetalactone, B= nepetalactol, C= 1:2 ratio nepetalactone:nepetalactol, D= control.

	Total no. *D. rapae* males and females captured in each trap							
	A		B		C		D	
Date	M	F	M	F	M	F	M	F
8.8-12.8.96	1	9	1	4	1	6	3	3
12.8-14.8.96	1	3	0	3	0	2	0	0
14.8.16.8-96	0	6	2	5	1	2	0	1
16.8-18.8.96	1	3	0	2	0	0	1	0
Mean	0.8	4.5	0.8	3.5	0.5	2.5	1.0	1.0

Table A27 Capture of *Diaeretiella rapae* in pheromone-baited water traps in 1996 (section 5.4). Site B. A= nepetalactone, B= nepetalactol, C= 1:2 ratio nepetalactone:nepetalactol, D= control.

Sample date	Semiochemical treatment in plot C	P1	P2	P5
1.6.96	1	7	1	0
10.6.96	1	9	0	0
17.6.96	4	17	7	5
24.6.96	73	85	12	63
1.7.96	360	406	379	311
10.7.96	1070	1228	1023	1040
16.7.96	3775	2841	2602	2794
24.7.96	4001	4296	3689	3860
30.7.96	393	332	260	367

Table A28 Total numbers of aphids recorded from winter wheat plots baited with 1,2 or 5 nepetalactone lures/plot, P1, P2,P5, and from unbaited control plots, C, in 1996 field trial (section 6.3).

Sample date	Semiochemical treatment in plot C	P1	P2	P5
1.6.96	0	0	0	0
10.6.96	0	0	0	0
17.6.96	0	0	0	0
24.6.96	1	1	0	0
1.7.96	0	0	5	4
10.7.96	2	10	9	20
16.7.96	7	21	14	20
24.7.96	14	31	31	29
30.7.96	14	26	22	23

Table A29 Total numbers of parasitoid mummies recorded from winter wheat plots baited with 1,2 or 5 nepetalactone lures/plot, P1, P2,P5, and from unbaited control plots, C, in 1996 field trial (section 6.3).

Sample date	Semiochemical treatment in plot				
	C	P	P/MS	MS	AF
3.6.97	24	13	76	42	31
10.6.97	146	173	174	179	147
17.6.97	471	611	511	575	401
24.6.97	969	1353	1223	847	944
1.7.97	1361	1508	1877	1362	1513
8.7.97	1701	3201	2595	1738	1892
15.7.97	1527	1095	1193	871	1199
22.7.97	108	68	41	58	69

Table A30 Total numbers of aphids recorded from plots of winter wheat treated with semiochemicals in 1997 field trial (section 6.3). C= control, P= nepetalactone, P/MS= nepetalactone + methyl salicylate, MS= methyl salicylate, AF= antifeedant (polygodial). No aphids were recorded before 3.6.97.

Sample date	Semiochemical treatment in plot				
	C	P	P/MS	MS	AF
3.6.97	0	0	0	0	0
10.6.97	3	0	2	1	3
17.6.97	1	5	0	1	3
24.6.97	5	5	10	1	3
1.7.97	12	14	9	4	6
8.7.97	24	25	24	18	15
15.7.97	24	27	32	23	21
22.7.97	19	23	22	18	19

Table A31 Total numbers of parasitoid mummies recorded from plots of winter wheat treated with semiochemicals in 1997 field trial (section 6.3). C= control, P= nepetalactone, P/MS= nepetalactone + methyl salicylate, MS= methyl salicylate, AF= antifeedant (polygodial).